DISTURBANCE AND TEMPORAL VARIABILITY IN INVERTEBRATE ASSEMBLAGES IN TWO SOUTH AFRICAN RIVERS

THESIS

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ABSTRACT

This thesis presents an examination of the relationship between floods as disturbances, the disturbance regime and the temporal dynamics of invertebrate assemblages, over the short term and at intra- and inter-annual time scales in the Molenaars and Berg Rivers in the Western Cape of South Africa.

Invertebrate responses to individual floods were investigated by a field study that links the displacement of river-bed stones by a flood to change in invertebrate densities and community and population structure from before to after flood events. The magnitude of the hydraulic force acting on each marked stone during the peak of each flood was also calculated, providing a second measure of physical disturbance. Multivariate analyses of similarity, hierarchical clustering and multidimensional scaling were used for analysis of invertebrate patterns before and after floods. Size frequency data for 28 species or genera were analysed to explore changes in population structure over the flood season.

Flood records were developed from the daily discharge hydrological record of both study rivers collected by the South African Department of Water Affairs and Forestry. Flood frequency analysis and descriptive statistics were combined with graphical methods to describe the flood regime and to test flood predictability in these rivers.

Quantitative monthly samples of invertebrates from the Molenaars River collected over 17 months were used together with a further 2 ½ years of semi-quantitative monthly data, to identify intra- and inter-annual patterns in communities. Multivariate analysis of community patterns was combined with a range of indices that reflect community persistence and stability over periods longer than one generation. Population dynamics of the common species were also studied. Life history attributes, specifically seasonality of life cycle stages and generation time, were explored using size frequency data from the samples.

The pre-flood data showed a significant negative linear correlation between invertebrate density and stone particle size ($r = -0.56$, $p < 0.0001$). Some 25 and 31 % of overall invertebrate density in the Berg and Molenaars Rivers respectively can therefore be explained by stone size, whilst individual taxa had individually stronger or weaker associations with stone size. This indicates that, contrary to what was expected, there was no selection by invertebrates of larger, stable stones at the start of the flood period.

Floods that do not move river-bed stones are associated with variable invertebrate population responses, including both reductions and increases in density. Floods that move some 40 % of the bed cause density reductions in all taxa. For events of such magnitude the movement of river stones is not the threshold for invertebrate disturbance, however, contrary to the findings of most other flood studies, since in this study almost all taxa were reduced in density on unmoved as well as moved stones. Whether or not a stone moved made a significant difference to post-flood assemblages on these stones, compared to the assemblage from the pre-flood sampling. Invertebrate community composition on stones that moved was 10 - 15 % more different from the initial condition than it was on stones that did not move. Unmoved stones thus provide a relative refugium for many species, with significantly lower population losses on these stones than on stones which moved. There is a wide difference in the relative refugium afforded to different taxa, with unmoved stones offering no advantage to some species.

Bed-moving floods occur in the Molenaars and Berg Rivers on average 7 or 8 times per year respectively. Inter-annual variability ranged from 3 to 15 floods annually. The maximum proportion of these floods in a year within any common 60-day window is 48 % for both the Molenaars and Berg Rivers, which is at the low end of the range of 44 – 96 % recorded in international studies. This indicates low predictability of flooding within the annual cycle. The period mid-June to mid-August (winter) is associated with the highest probability of flooding, whilst the period November to April is associated with a high probability of there being no bed-moving floods. The transitional months are associated with the greatest variability in flood occurrence.

Longer-term monthly sampling showed that invertebrate assemblages are highly variable over time scales of the order of months or less. Month-to-month changes in invertebrate assemblages during the flood season demonstrated that density reduction as a result of a flood is not long lasting. The concept that the winter flood season is characterised by continually declining species densities was not borne out by the results: reductions in densities during the flood season occurred only when samples were collected within seven to ten days of a flood. Increases in overall invertebrate density occurred in winter and spring months when samples were collected after a period of some 30 days without any flooding.

Correlation of monthly invertebrate community dynamics with biophysical variables yielded an unexpected result: the cumulative temperature in the 30 days preceding sampling had the strongest correlation with invertebrate community patterns, accounting for 56 % of the variability in the biota, and additional variables added little to explanatory power. However, a 48 % correlation was also achieved between the community patterns and the number of bed-moving floods that occurred in the 30- or 60-day period prior to sampling, which themselves were co-correlated.

Year-on-year, the winter months June, July and August are characterised by greater constancy in invertebrate community structure than were the spring or autumn months, suggesting that invertebrate assemblages during the major flood period are in fact fairly predictable, despite the low predictability in the flood regime when compared with international studies. Inter-annual variability in community structure is greatest in spring, as a result of the interaction of flood variability with inter-annual differences in the timing of increases in river temperatures. The summer period, when floods do not occur and temperatures are relatively constant, has highest month-to-month constancy in community structure.

Changes in population size structure and density illustrate some life-history attributes of a number of key taxa. Contrary to what was hypothesised, most invertebrate species in the Molenaars River are present year-round, even if winter densities are low, indicating that very few avoid the flood season. Only a small number of taxa appear to be restricted to the summer low-flow period or have life-history patterns where developmental stages are clearly timed so as to avoid the flood period altogether. The other two life-history patterns hypothesised to occur were indeed prevalent in the Molenaars River, namely winter seasonal development combined with avoidance of the mid-summer period, and a mix of asynchronous development and / or multivoltine life cycles. Contrary to expectations, a fourth life-history type was displayed by many taxa, namely a slow seasonal life history with synchronised development throughout the year.

The observation of limited short-term impacts of floods, or the continuous presence of species throughout winter indicates a larger degree of resistance to floods by some species than previously registered. In addition, the immigration of new individuals of some species, in the form of new recruits of small instars from refugia in the hyporheos or upstream reaches shows a resilience to floods rooted in life history adaptation. The dominance of species with a generation time of a full year, or whose life cycles included a winter generation, indicates that floods are but one of a suite of environmental variables that may influence

life histories. The heterogeneity of hydraulic features that supply refugia during floods and the investment in resistance and resilience traits by invertebrates appears to limit flood mortality to levels that can be overcome by large starting populations. Along with this, the poor predictability of the flood regime appears to be too noisy an evolutionary signal to be dominant in shaping life histories.

Nevertheless, this study shows that floods of all magnitudes affect the proportions of species that comprise invertebrate assemblages. This relative change might be as a result of one species' increase and another's decline or different degrees of population reduction, or simply changes in the relative proportions of different life stages. This may indeed be the crucial effect of floods, particularly where species compete for space or resources.

Similarly, the study showed that frequency of floods above the disturbance threshold is a far more important driver of community patterns than the degree to which a flood exceeds this threshold. Both periphyton and invertebrate densities were reduced by floods, but not in proportion to flood size. In contrast, the frequency of floods was strongly and linearly related to total invertebrate densities as well as densities of a number of individual species. The implications for managing modified flow regimes in dammed rivers are substantial. Whilst a few, large releases per annum may meet requirements for channel geometry and sediment movement, they are unlikely to be associated with adequate disturbance signals to maintain natural population dynamics in regulated rivers with flow regimes similar to these study rivers. These tentative findings require more research, since they are based on a small sample of pre- and post-flood data.

Finally, the importance and interactive effects of temperature and flow demonstrated in this study highlights a gap in current research. Further investigation of the ecological effects of temperature on both algal and invertebrate population and community dynamics is needed. Consideration of temperature effects must be an essential component of any flow management strategy. Long-term collection flow-linked temperature data is needed to support these needs.

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1 INTRODUCTION

1.1 AN INTRODUCTION TO THIS THESIS TOPIC

Spatial patterns and patchiness of invertebrate distribution partly reflect the layering of temporal dynamics on environmental gradients (Ward 1998). Ecologically meaningful temporal scales at which patterned change may be observed can range from days or weeks (e.g. colonisation of the stream-bed in intermittent streams) to geological time scales (Minshall 1988). At a time scale between these extremes, variability in physical conditions may have a pronounced effect on community composition. For example, the increased faunal diversity in the mid-reaches of rivers versus headwaters or lower river zones has been ascribed in part to the more temporally variable temperature regimes in these mid-reaches (Minshall *et al.* 1985) and more physically complex substrata (Downes *et al.* 1998b).

In explanation of the underlying processes that give rise to community attributes such as high species diversity, traditional equilibrium models assume a primacy of biotic interactions (e.g. competitive exclusion / resource partitioning) as determinants of community structure (Resh *et al.* 1988; Townsend 1989; Reice *et al.* 1990; Wu & Loucks 1995). More recently, the emphasis has shifted to attributing more importance to non-equilibrium and stochastic forces as agents of community structure (Townsend 1989). Non-equilibrium studies focus on the behaviour of populations or communities over time in relation to non-equilibrium forces, rather than the properties of communities in equilibrium. Central to this field of investigation is the notion of disturbance as a major driver of communities, and the concept that some communities may not ever reach an equilibrium state. At an extreme, such communities may be composed of species that are relatively independent of one another, and whose populations are limited by abiotic factors in a density-independent manner (Boulton *et al.* 1992; Jacobsen & Encalada 1998).

This thesis presents the results of studies into temporal variation in invertebrate assemblages in two rivers in the Western Cape Province of South Africa, over short-term time scales, associated with individual flood disturbances and over intra-annual and medium-term time scales (three years) associated with a winter flood regime. The first thrust of the thesis is an examination of invertebrate responses to flood events (natural disturbances) and the identification of thresholds of physical disturbance based on these ecological responses. The second component of the thesis is an examination, in one river, of temporal variability at the within-year time scale, in the light of the seasonality of flooding. Other temporally variable abiotic measures such as temperature are explored as alternative drivers of invertebrate patterns. The constancy of intra-annual patterns of invertebrate community dynamics over larger time scales, i.e. year-on-year, and the persistence of invertebrate assemblages are explored in the light of the predictability of flood disturbances in the natural flow regime. Following this, hypotheses are made and tested regarding life-history responses of invertebrate species to the flood regime.

The first, or flood-study, component of this thesis was undertaken in 2003 and 2004, funded by the Water Research Commission, to investigate the relationship between flood size and bed disturbance, and to describe the ecological response to floods and / or bed disturbance by invertebrate fauna. The research sites for this project were located on the Molenaars and Berg Rivers in the Western Cape Province of South Africa.

The second component, the investigation of temporal change in invertebrate assemblages, is based on data collected in the Molenaars River between 1991 and 1997, partly funded by the South Africa Roads Agency during its upgrade of the N1 national road through the Du Toitskloof valley between Paarl and Worcester, adjacent to the Molenaars River. As the Molenaars River is well gauged, these invertebrate data, together with hydrological data from 1960 to the present, formed the basis for an investigation into the relationship between the flow regime of a river and temporal variation in its macroinvertebrate fauna.

This chapter includes an introduction to major concepts in the field of ecological disturbance considered to be pertinent to this thesis, and that were used to develop hypotheses and guide the study design. The review focuses on ways in which disturbance may be defined, as well as methods for its quantification both as a physical force and an ecological response. These issues relate to the first component of the thesis, the flood-response study. The review also includes an examination of disturbance as a driver of species adaptations and community structure, which has informed the second component of the thesis. The chapter ends with a presentation of the objectives, hypotheses and structure of the thesis.

1.2 DISTURBANCE - A DEFINITION

Lake (2000) described three categories of disturbance. Floods represent the major form of natural "pulse disturbance" in lotic ecosystems (Lake 2000), whilst Lake defined "press disturbances" as acute change that is maintained, examples of which would include sediment load change as a result of landslides, and many anthropogenic impacts. Finally, he defined "ramp disturbances" as creeping change, for example drought or invasion by alien biota. The following section and the thesis as a whole are limited to examining concepts and research findings relating to pulse disturbances by flooding.

Minshall (1988) defined disturbance as a "destructive, rapid (e.g. spate) or prolonged (e.g. drought) change in the physical environment which exceeds the normal range of conditions experienced by a substantial number of organisms in a population, or the rate of their ability to adjust – resulting in their death and/or removal". Huston (1994) noted that the important to the notion of disturbance is that it should describe any process external to the natural physiology of living organisms that results in the sudden mortality of biomass (my emphasis) – disturbance may kill few, many or all the organisms of a population, or a part of an organism biomass. Resh *et al.* (1988) included in their definition of disturbance only those events that are outside of a predictable range, given that organisms "are adapted to predictable seasonal fluctuations of discharge, temperature, dissolved oxygen etc.", although they noted that a precise definition of what is predictable is not easily reached. In contrast, the definition advanced by Pickett & White (1985) did not make the definition of disturbance contingent on its predictability, but merely on a magnitude sufficient to disrupt community or population structure and alter resources. Townsend (1989) defined disturbance as "any relatively discrete event in time that opens up space which can be colonised by individuals of the same or different species". Similarly, Poff (1992) argued that a measure of predictability should apply only as an attribute of the disturbance regime, rather than as a defining criterion for disturbance *per se*. Poff (1992) argued that linking disturbance and predictability may result in using the magnitude of ecological response to define disturbance, rather than objective criteria related to the physical mechanism of a disturbance, for example movement of bed particles. Furthermore, the assumption that disturbance can only be unpredictable does not give due cognisance to the wide variation in frequency and seasonal predictability of hydrological events that "presumably…. creates noisy environmental selective forces that may not promote local adaptation" (Poff 1992).

Species traits (e.g. degree of weediness) or ecosystem attributes are likely to vary according to the heterogeneity of conditions, the frequency, intensity or magnitude and the timing of disturbance, both predictable and unpredictable, that constitute the disturbance regime. According to Poff (1992), "evolutionary adjustments to hydrological disturbance may be reflected in biological attributes that confer differential survival and persistence in the face of that regime". Rather than an *a priori* assumption of adaptation, these provide the basis of testable hypotheses regarding the effects of, *inter alia*, the predictability of a disturbance regime of a stream on its biota.

Lake (2000) argued that most definitions confuse the biological response to a disturbance with the event itself, and proposed a narrower definition of disturbance as the application of "potentially damaging forces to habitat space occupied by a population, community or ecosystem ... [the magnitude of which] may be such that organisms may be killed or displaced, consumable resources may be depleted and habitat structure may be degraded or destroyed" (Lake 2000).

The critical difference in this definition is the identification of a potential impact as a result of the application of a force. A definition of disturbance that is only response-based i.e. contingent on the biota being adversely affected - for example, the "opening up of space" in the definitions Resh *et al.* (1988) and Townsend (1989) which explicitly imply mortality or displacement of biota - would preclude the possibility, at least hypothetically, that some biotas may be perfectly resistant to a disturbance. Some groups, for example, might withstand even large floods, their populations remaining intact over the course of an event characterised by "the application of a force", as per Lake's (2000) definition of disturbance (e.g. Lancaster 1999; Lake 2000). Lancaster (1999) described disturbance as a "response phenomenon", with responses being species specific, so that "whether a species perceives an environmental event as a disturbance will also be species specific". Her laboratory study (Lancaster 1999) demonstrated variable responses by three taxa to increasing flow, these being either a) disturbance resistance - e.g. through physical attributes, such as clinging by hooks and claws - or b) changes in micro distribution - e.g. physical walking down a velocity gradient and / or accumulation in flow refugia. According to her definition of disturbance, species that show disturbance resistance through no net loss or movement of individuals will not have perceived the increase in flow forces as a disturbance. This description, whilst introducing a finer focus in the study of variable biological responses to disturbance, would require quantification of the response to environmental events before these may be pronounced 'disturbance events'. The definition of disturbance then becomes increasingly complicated as it may simultaneously be classified as both a disturbance event and a non-disturbance. In addition, the concept of resistance defines species attributes, or resistance traits, in relation to the species' response to disturbance, rather than as a definition of disturbance.

Poff (1992) argued that a disturbance should be defined in terms of the physical event, but that the specification of a disturbance is scale dependent, and needs to be ecologically relevant to the question at hand. In relation to flood disturbance, to measure disturbance forces and the responses of biota at the same spatial scale would mean identifying the size of the patch most appropriate to the measure of both. A scale that measures magnitude of forces that act directly upon substratum particles and their biota may be a better basis for developing an understanding of the mechanism by which a disturbance event elicits one or another biological response. Different thresholds in the magnitude of physical forces may represent different scales of disturbance, and may be accompanied by a variety of biological responses, including resistance, use of refugia and/or reductions in populations or biological fitness.

1.3 MEASURING DISTURBANCE AS A FORCE

Physical definitions of disturbance put forward by, for example, Resh *et al.* (1988) include hydrological indices representing events outside of a normal range of flows, defined by them as twice the median monthly flow, but such approaches are criticised by Poff (1992) as arbitrary statistical measures that do not necessarily represent a known level of physical disturbance or constitute ecologically meaningful forces acting on the biota. Debates on the definition of disturbance have led to increasing support in ecological studies for using the movement of substratum particles, as a function of discharge, as a criterion for defining and measuring disturbance (McElravy *et al.* 1989; Cobb *et al.* 1992; Lancaster & Hildrew 1993b; Death & Winterbourn 1994; Townsend *et al.* 1997b; Downes *et al.* 1998a; Biggs *et al.* 1999; Bond & Downes 2000; Gjerløv *et al.* 2003; Riseng *et al.* 2004).

Two main approaches have been used to determine bed movement - using theoretical relationships to calculate bed movement, and field-based measures, for example tracking the fate of marked bed particles after a flood. The latter approach has been adopted in the flood response component of this thesis (Chapters 3 and 4), as it provided empirical measurement of bed disturbance that could be linked to the measurement of invertebrate response. However, the flood study was undertaken in tandem with the development of a hydraulic model to quantify incipient motion of bed substrata during floods. That component of the study provided equations of bed particle entrainment that could be and were used to determine whether or not unmarked stones were likely to have moved. Thus some background to deriving estimates of bed movement from theoretical relationships is also provided below.

1.3.1 Calculating bed movement using theoretical relationships

A common approach to estimating bed-particle movement is the application of empirical relationships relating particle size to the forces required to move the particle. Incipient motion defines the beginning of sediment movement. In other words it encompasses all the factors involved at the threshold between non-movement and movement of a particle. A number of relationships have been proposed to link critical threshold parameters to the initial motion of a grain. The best known criteria are based on critical shear stress as originally developed by Shields (Cullis *et al.* 2008).

Most ecological studies have used equations defining critical shear stress to identify a particle size that would be at incipient motion at a given discharge, briefly described below (Downes *et al.* 1997; Lorang & Hauer 2003; Gordon *et al.* 2004). Shear stress represents momentum exchange across the planes along which water flows, and can be derived from the equivalency between forces acting in the various planes and momentum exchange. In open channels shear stress may be thought of as the frictional or drag force (per unit area) causing flow resistance along the channel boundary (Gordon *et al.* 2004), described by:

$$\tau = \rho gRS \qquad [1]$$

where ρ is the density of water (= 1000kg m^{-3} at 20 ˚C), g is acceleration due to gravity, *R* is the hydraulic radius (or average flow depth, D (m) in wide or rectangular channels) and *S* is the energy (or bed) slope, expressed as a fraction.

The value for critical shear stress, also termed critical tractive force, is derived from the assumption that at incipient motion, the shear force acting to overturn a particle is balanced with the submerged weight of the particle, which thus holds it in place. The equation describing incipient motion is known as the Shield's equation, where critical shear stress (τ_c in N m^{-2}) is:

$$\tau_c = \theta_c g d(\rho_s - \rho) \quad [2]$$

where θ_c is also called the dimensionless critical shear stress or dimensionless mobility factor, or Shield's parameter, d is a representative particle size (in m), g is acceleration due to gravity and ρ_s and ρ are particle and water densities, in kg m^{-3} (Armitage & McGahey 2003).

The onset of motion occurs when the shear stress along the bed (τ_0) exceeds some critical shear stress (τ_c) at which point the moment of the drag force (F_{drag}) about the pivot point of the relevant particle exceeds that of the immersed weight force of the particle (F_{iwt}) as shown in Figure 1.1.

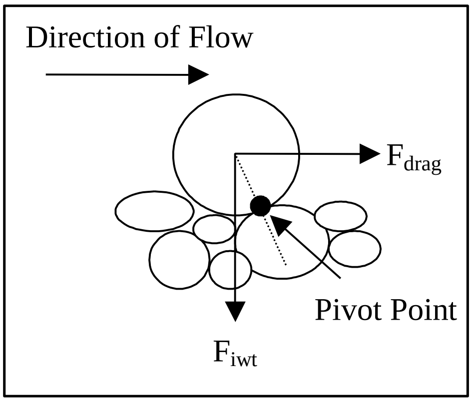

Figure 1.1 Schematic representing the hydraulic forces acting on a particle on the stream bed by flowing water (from Cullis *et al.* 2008).

The value of the constant, θ_c has been empirically defined as 0.04 - 0.06 for hydraulically rough conditions (Gordon *et al.* 2004).

This equation has been used to estimate disturbance intensity in streams in a number of ways. For a given flood size, the average depth is used in equation [1] to calculate reach-level shear stress, which is then substituted into equation [2] to predict the largest particle size to be moved under that discharge.

A number of studies have used shear stress equations to define a discharge at which bed movement would be initiated, as a threshold for disturbance. McElravy *et al.* (1989) used the discharge at which shear stress would be calculated to move the median size particle, as a threshold for defining disturbance in their inter-stream comparison (McElravy *et al.* 1989). The basis for this is the controversial concept of equal mobility (see Gordon *et al.* 2004, p190), which is based on the observation that in mixed alluvial channels, all particles may be moved at essentially the same average shear stress, and thus τ_c can be based on one grain diameter (the median) rather than computing individual values for each size fraction. A similar approach was used by Scarsbrook and Townsend (1993) who based their threshold shear stress equations on data gathered specifically in an upland gravel-bedded stream, in an attempt to improve the estimate of θ_c. This study compared stream stability on the basis of the theoretical shear stress required to effect movement of 50% of the bed material at each site (Scarsbrook & Townsend 1993).

Cobb *et al.* (1992) estimated the percentage of bed paving material at incipient motion (i.e. in motion) at low medium and high discharges, using the median diameter of bed particles as an estimate of the critical tractive force, or critical shear stress, required for incipient motion, and relating this to a particle-size frequency distribution for each site. They used this percentage of bed in motion to define a discharge-related average shear stress for a range of sites, which was then related to biological attributes.

Riseng *et al.* (2004) argued that although the Shield shear stress formula approach does not provide accurate predictions of sediment transport at a local scale, as it does not account for spatial variation in bed mobilisation nor streambed armouring and embeddedness, they nevertheless considered the utility of this approach for inter-stream comparisons of their index of rock movement at bankful flow. They defined this index as the difference between a) the diameter of rock expected to move at bankful flow (D_{bf}, from the Shield's equation for critical shear stress, where θ_c was estimated for different stream sites "based on streambed packing" and b) the size of the D_{84} particle. The D_{84} was chosen on the observation that entrainment of this particle size was associated with large-scale bed mobilisation.

Lake & Schreiber (1991), however, found that out of eight sites spanning four stream orders, the site with the highest bankful average shear stress had the lowest movement of tracer stone particles recorded over a period of two years. They found no relationship between bankful shear stress and particle movement.

Jonker (2002) highlighted the fact that the Shield's equation describes sediment entrainment for uniform sediment sizes, rather than describing sediment transport in natural channels, and makes no allowance for shielding or armouring effects, when in actual fact the critical shear stress for a specified grain size increases in natural channels with increasing roughness. Death & Winterbourn (1994) demonstrated that the critical tractive force was not a good indication of the extent of bed disturbance. Similarly, (Englund 1991) found only a weak relationship between particle size, by category, and whether or not a stone was overturned. Other authors (Downes *et al.* 1997; Townsend *et al.* 1997b, c; Downes *et al.* 1998a; Biggs *et al.* 1999; Duncan *et al.* 1999; Lorang & Hauer 2003) agreed that the assumption of a linear relationship between critical sheer stress and the movement of a given particle size is unlikely to hold up in all but the most uniform of channels. Downes *et al.* (1997) found that the spatial variation in the force required to move river bed stones, which they physically measured, depended highly on rock size, but also on bed packing, and varied more between rivers than between stream orders or sites on one river. Bed packing was defined qualitatively as loose-lying versus >50% embedded or wedged by surrounding rocks. They demonstrated that rocks in different size classes overlapped completely in the force required to move them, both between rivers and within sites, and concluded that methods of estimating disturbance using shear stress equations may be misleading given the sometimes order-of-magnitude differences in the forces required to move two particles of the same size but of different bed characteristics or particle embeddedness. Similarly, in their field study measuring displacement of stones marked in situ (Downes *et al.* 1998a), the movement of a stone was significantly related not only to its size category but also to its packing.

Thus, the same relative increase in discharge may result in considerable differences in applied forces and in actual bed movement within individual sites and between different streams. This difference is largely dependent on geomorphological features such as channel size, bed particle sizes and packing, heterogeneity (roughness) and gradient. In this regard, disturbance may need to be quantified in a locally specific manner, for example as the proportion of bed particles that are mobilised by a given discharge,. Approaches involving direct measurement of substratum movement are discussed in section 1.3.2.

Duncan *et al.* (1999) reviewed the deficiencies of the range of substratum-based approaches to the measurement of disturbance, including problems with theoretical approaches, as outlined above, accuracy and, not least, the effort involved in marking and following bed particles. These issues emphasise the continued importance for disturbance studies of finding general rules that describe the magnitude of particle movement and/or flow forces acting at a local (stone) scale during floods. Duncan *et al.* (1999) presented a modified version of the Shield’s equation, accounting for heterogeneous particle sizes, the effects of slope and small relative depths and Lorang & Hauer (2003) discussed the considerations that need to be incorporated into an assessment of stream stability based on flow competence equations such as critical shear stress.

Armitage & McGahey (2003) reported that, although shear stress may be the most commonly used method of determining incipient motion, probably the most serious criticism of its use is that the rate of sediment transport is not uniquely determined by shear stress (Armitage & McGahey 2003). In steep channels a large portion of the shear stress is manifested as turbulence around large objects, rather than frictional drag on bed particles. Equally, achieving localised threshold entrainment is more a function of the maximum instantaneous shear stress than the channel-averaged measures of shear stress used in incipient motion equations. Instantaneous shear stress values are affected by turbulence which is considerable during high flows in channels with high roughness (Lorang & Hauer 2003).

A number of researchers have preferred to use unit stream power as an indicator of sediment motion, although this application is not generally described in standard hydraulic introductory texts for ecologists (e.g. Gordon *et al.* 2004), where shear stress equations are suggested instead. However, Armitage & McGahey (2003) argued that the quantity represented by unit stream power is more directly related to the entrainment threshold than is critical shear stress, because it can be computed at any point in the water column, and because turbulence is directly related to dissipation of energy.

The movement of bed particles (or water) requires expenditure of energy, which is provided in streams by the release of potential energy as water travels down a slope. The stream power model of incipient motion equates the power made available through the loss of potential energy in a flowing stream with the power required to maintain the movement along the bed (applied power), and compares this to the amount of power required to entrain particles (required power). Incipient motion occurs when the applied power at the bed equals or exceeds the required power to lift the particle.

The stream power approach is considered to have advantages over other approaches to incipient motion in that it involves scalar quantities (unlike the momentum-impulse law used in shear stress models which use vector quantities), its terms are directly time-dependent and they account for the roughness (k) of the bed directly (Rooseboom 1998; Armitage & McGahey 2003).

1.3.2 Field based measures of stone movement

Given the problems with accuracy in the theoretical approaches, some researchers have used more intensive data collection techniques, rather than theoretically based equations for estimates of bed movement. For example, Townsend *et al.* (1997a, b) and Gjerløv *et al.* (2003) used painted stones of either the median diameter or a defined percentile of the stone particle size distribution, placed randomly on the stream bed, to measure bed movement at high discharges. Stone movement was assessed at 2 to 4-month intervals. The percentage of moved stones in any period was either regressed against maximum discharge in the preceding period, in order to calculate the discharge required to move 50 % of the particles as a threshold for disturbance (Gjerløv *et al.* 2003), or the percentage of particles moved, averaged across sampling times, was used as a basis to define the intensity of disturbance for each of a number of compared sites (Townsend *et al.* 1997a). In a different river type Matthaei & Townsend (2000) and Matthaei *et al.* (2003) mapped portions of gravel bed that were filled scoured or stable, using buried chains to determine depth of scour or fill.

1.3.3 Other empirical measurement of disturbance forces

Although the movement of bed particles undoubtedly constitutes a physical disturbance, it may under-represent the disturbance forces acting during the discharge event. Flow forces acting on river-bed materials increase with increasing discharge, and these may be as important as actual particle movement. This may be particularly true in heterogeneous cobble- and boulder-bed rivers, where strong hydraulic forces may be present long before bed entrainment occurs. Furthermore, the use of a statistic such as 40% bed movement as a threshold for disturbance (e.g. Townsend *et al.* 1997b; Gjerløv *et al.* 2003) was acknowledged by these authors to be arbitrary.

Lancaster & Hildrew (1993b) regarded bottom shear stress as the most meaningful measure of flow forces with respect to invertebrates, because they considered it to reflect the shearing force of water, which has the potential to dislodge organisms. They advocated this approach for comparing the hydraulic differences between streams. They characterised whole-reach hydraulic habitat through the creation of frequency distributions of shear stress, from spot measurements across the site made directly with FST hemispheres (Statzner & Muller 1989). They were able to repeat these measurements at the same point for a range of discharges to demonstrate how the distribution of shear stresses and the proportion of low and high shear stress areas changed with discharge at different sites and within different morphological units at a site (Lancaster & Hildrew 1993b). This approach was also followed by Gjerløv *et al.* (2003) to quantify the proportion of hydraulic refugia likely to be present at the elevated discharges that would occur under flood conditions. They modelled the two-dimensional distribution of shear stress over the stream bed in their study streams using macro-channel measurements as described by Lamouroux & Statzner (1992), and calibrated these results with field-based measurements of shear stress over the study sites, recorded at both base flow and moderately elevated discharges. This model was used to describe the distribution of areas of different shear stress at the same relative flood discharge in each of the study streams. Townsend *et al.* (1997c) used the percentage of low shear stress points, but measured only under base flow conditions, as one of three criteria for differentiating the refugium potential of different streams, based on the argument that some fraction of the low shear stress locations would remain during flood flows. This approach assumes that these relative proportions remain constant - that one stream with a higher percentage of low shear stress points than another stream, at base flow levels, would still have the same relatively larger proportion of low shear stress point at a flood discharge than the other stream.

1.4 FROM FLOODS TO FLOOD REGIME: DISTURBANCE REGIMES AS A BASIS FOR STREAM CLASSIFICATION AND THE PREDICTION OF BIOLOGICAL ATTRIBUTES

Five aspects of the flow regime are regarded as important regulators of ecological processes in river ecosystems: the magnitude, frequency, duration, the timing (or predictability), and rate of change (flashiness) of high and low flows (Poff *et al.* 1997). These are thought to represent firstly the extent of disturbance and time available for recovery and offer predictions about the disturbance response to different sorts of floods (see section 1.5.1 and Chapters 3 and 4). Secondly, these features determine the significance of periods of harsh or benign conditions, and the variability and rate of change on short-term, seasonal and inter-annual time scales, and thus offer predictions about species life cycles and behavioural adaptations that promote persistence and ultimately the characteristics of lotic communities (see section 1.5.2, 1.5.3 and Chapters 6 and 7) (Poff *et al.* 1997).

Poff & Ward (1989) classified streams across the USA into nine streams types, which were assessed using criteria such as the degree of intermittency, flow variability, and flood regime. They regarded this as a major axis of the habitat templet (*sensu* Southwood 1977) of lotic organisms (see section 2.5) and suggested that “the position of a stream in flow space [based on flow statistics] should provide some *a priori* basis for expectations of biotic attributes”. This is because the historical flow regime in any one stream should act as an evolutionary force, determining the success of species life-history attributes and, ultimately, of species populations. Their stream types were defined as ‘harsh intermittent’; ‘intermittent flashy’; ‘intermittent runoff’; ‘perennial flashy’; ‘perennial runoff’; ‘snowmelt’; ‘snow + rain’; ‘winter rain’; and ‘mesic groundwater’(Poff & Ward 1989).

Townsend & Hildrew (1994) argued that disturbance that is unpredictable is more likely to elicit a stronger biological response than the same magnitude event that is predictable, because the former may act as evolutionary drivers determining species adaptations and ultimately population characteristics. Gasith & Resh (1999), in developing hypotheses about the attributes of stream communities in Mediterranean climates (the ‘winter rain’ stream type of Poff & Ward 1989), suggested that the seasonal predictability of disturbance in these systems should result in assemblages that exhibit life history adaptations that maximise growth and reproduction during stable and mild periods. Also, the sequence of flooding and drying should select for life-history features (species traits) that favour resistance to dislodgement and allow rapid recolonisation responses.

These ideas regarding flow predictability are developed further in Chapter 5 of this thesis, which particularly differentiates between predictability of the flow regime overall, and that specifically related to the disturbance regime, as a driver of biological community attributes. This distinction has not been made in previous studies examining the predictability of disturbance.

The basis of a stream classification is that it allows one to make predictions, in this case about such features as the seasonality or not of biotic communities, the dominant sorts of behavioural and morphological adaptations that might be expressed by stream fauna traits and species life cycles. Such predications are made and tested in Chapters 6 and 7.

1.5 MEASURING THE DISTURBANCE RESPONSE BY STREAM BIOTA

Flood disturbance studies that measure or estimate both the magnitude of physical disturbance and its associated biological response have increased in volume over the past two decades. Many of the studies have focussed on macroinvertebrate assemblages and periphyton, as these are relatively immobile and also their response times are rapid. However, a key distinction must be made between important differences in temporal scale at which studies are conducted, as these frame some rather different questions regarding flood disturbance and community structure.

The first temporal scale of investigation relates to understanding the mechanisms of how floods act, and investigations at this level examine chiefly the magnitude of the event and the consequential impact on the biota and their recovery sequences. The focus here is on understanding the differential susceptibilities of species to flood disturbances, or their resistance attributes, and, post-flood, the resilience of species and communities, in other words, what recovery is possible and at what rates of change.

The second temporal scale views recovery trajectories on a wider scale, attempting to make generalisations about pattern and process on annual time scales. This scale of investigation encompasses some of the regime-type attributes of disturbance, such as average frequencies of floods, seasonality or predictability within annual cycles, and the duration of periods between floods, and the degree to which these drive the both the level of intra-annual variability in disturbance and the resulting biological patterns.

One aspect of this scale of investigation is the extent to which these longer-term trends in the disturbance regime are predictable. A key area of investigation in this regard is the degree of inter-annual variability in the disturbance regime, and its effect on the persistence, or lack thereof, and the stability of populations or assemblages.

These are introduced briefly in the sections that follow, whilst more detailed discussion of these topics is included in the relevant chapters of this thesis

1.5.1 The disturbance-response by stream biota to flood events

Field-based experimental studies using artificial disturbance of substrata have focused on measuring the biological response to disturbances of a range of magnitudes and or frequencies, allowing for investigation of concepts such as resistance, as measured by the different responses to floods of the same physical intensity, and recovery time, or resilience. For example, Boulton *et al.* (1988) overturned or acid-scoured rocks to demonstrate that reductions in invertebrate and epilithon densities were directly related to the magnitude of the disturbance: simply overturning stones, which was assumed to simulate the effects of small floods, had little effect on epilithon, whilst invertebrate numbers were significantly reduced by 10 – 100%, but not consistently for all taxa, and recovered rapidly, within four days. With acid-scoured stones - simulating large flood disturbance – the reduction in algae and invertebrate populations was total. Epilithon cover had not recovered to pre-disturbance levels after 32 days, whilst invertebrate species richness, but not densities, reached pre-disturbance levels in that time.

Lake *et al.* (1989) experimentally disturbed riffles to mimic a flood event, by kicking and raking 35 areas, each 1 m^2, at differing frequencies. The authors recognised the difficulty of relating experimental patch sizes to the size of areas disturbed by floods, and suggested that the experiment resembled more the sorts of disturbance that might occur with minor floods. There were marked decreases in the various parameters in the disturbed plots compared with control plots – reductions in the order of 54 - 72% for trapped organic material, 60% in invertebrate species richness, 80% in invertebrate numbers and 30% in invertebrate species diversity (H’) were recorded in disturbed plots. Smaller decreases (5 - 9%) in the number of invertebrate species, numbers of individuals and species diversity (H’) were recorded with the more frequently disturbed plots than with those disturbed only once, but only immediately following disturbance, on day 0 of the recovery process. This, and the fact that there were no differences in the composition of colonising fauna between treatments, led to the conclusion that disturbance frequency at this scale does not influence the rate of recolonisation or confer selective advantage or disadvantage on any species (Lake *et al.* 1989). After 33 days, there were no significant differences in the above community measures between control and treatment plots, indicating the rapidity of recovery from small-scale disturbance. The study, however, was somewhat confounded by the effects of three spates that occurred during the recovery period, measuring between five and six times base flow levels, but equally applied to both experiment and control treatments. Although no significant differences were detected over the study period in the mean number of species per sample in the control samples, the total number of individuals in these samples declined over the study period (Lake *et al.* 1989). Indeed, the control sites themselves could arguably be said to have undergone natural, repeated, small scale disturbance, the consequences of which were not significant for species assemblage structure but included a significant reduction in overall invertebrate density. Robinson and Minshall (1986) also found that significantly lower periphyton biomass on substrata that were frequently disturbed manually, although this effect was only significant in open-canopied streams. In both open-canopied and shaded streams, and in both summer and autumn, invertebrate densities and species density declined with increasing disturbance frequency, with the exception of one grazer taxon which showed a positive relationship with disturbance frequency during the summer experiment. However, they found no difference in the invertebrates assemblages 27 days or more after a disturbance, which led to their conclusion that equilibrium conditions may be established within 27 days of disturbance (e.g. Robinson and Minshall (1986) Boulton *et al.* (1988), Lake *et al.* (1989), McCabe & Gotelli (2000)).

One of the criticisms of artificial disturbance studies is that their relevance to real flood effects cannot be assessed. For example, the increased sediment load carried by flood waters may cause scouring of bed surfaces. Also, artificial disturbances may not measure natural responses as they would lack possible cues for refuge-seeking behaviour that natural floods might provide as discharges increase (Bond & Downes 2000, although see Downes *et al.* 1998a; Matthaei *et al.* 1999a for further discussion of the merits of these approaches).

Field studies of natural disturbance events are more difficult to undertake, in that the physical forces of floods are both difficult to measure at a scale at which the biota experience them, and their magnitude or frequency cannot generally be controlled. Nonetheless, a small number of such studies have been undertaken, examining biological responses to floods simultaneously with the measurement of the physical forces associated with the event, either by calculating shear stress during floods and using equations for critical shear stress to infer bed movement (Cobb *et al.* 1992; Lancaster & Hildrew 1993b; Scarsbrook & Townsend 1993; Townsend *et al.* 1997b; Nislow *et al.* 2002; Gjerløv *et al.* 2003) or, less commonly, involving the direct measurement of bed movement, such as through the placement and relocation after floods of large numbers of marked particles (e.g. Downes *et al.* 1998a; Bond & Downes 2000; Matthaei & Townsend 2000; Matthaei *et al.* 2000; Matthaei *et al.* 2003).

Most of these studies have demonstrated a temporally localised impact of floods of various magnitudes, particularly within the intra-annual to 1:2 year return period size ranges, i.e. those floods which occur on average once to many times per year. Perhaps one of the most profound consequences of small disturbances in streams, such as within-year flood events, is the subdivision of the habitat into patches. The concept of patch dynamics emphasises the role of disturbance in maintaining patches across the stream bed, each at a different point of recovery from disturbance, thus allowing for a mosaic-like aspect to the distribution of species at various stage of competitive exclusion (Pringle *et al.* 1988) and allowing for an increase in overall diversity compared with a uniform landscape (Townsend 1989). Changes in resource availability and community structure occur within patches as a result of competitive interactions. In freshwater environments, where the major components of the fauna are highly mobile, this has given rise to debate about the usefulness of the concept, or around the applicability of specific patch dynamics models of species coexistence (e.g. Townsend 1989; Downes 1990; Hildrew & Giller 1994; Barrat-Segretain &

Amoros 1996). Downes (1990) suggested that differential colonisation rates could be more significant than competition in determining species abundances where patches are available for short periods only. In cobble- or gravel-bed streams that are characterised by fauna with high mobility, patchiness in the effects of disturbance may therefore be only short-lived, although longer-term impacts have been reported (Englund 1991a; Giller *et al.* 1991; Matthaei & Townsend 2000; Matthaei *et al.* 2003).

These arguments emphasise the need to understand scale in relation to the patchiness of disturbance as well as in the response of the biota, both across the streambed and over time. A corollary of the notion that disturbance acts in a patchy manner is the idea that in-stream refugia should persist during the disturbance event. These may be defined as habitats or environmental factors that reduce disturbance effects or provide mechanisms that allow biota to survive, albeit in a depleted state, in disturbed environments, and may occur at a range of scales (e.g. Sedell *et al.* 1990). Examination of the impact of disturbance on communities and populations must therefore also examine the role of refugia in stream ecosystems in moderating these impacts.

Both the measurement of the disturbance force and the biological responses by stream invertebrates is the subject of the first part of this thesis as contained in Chapters 3 and 4. These chapters deal with issues of defining a threshold for disturbance, in terms of the response of invertebrate assemblages or individual species. The focus is on responses to individual disturbance events, and particularly the spatial scales at which local disturbance acts.

1.5.2 Intra-annual variability and the disturbance-response

Attributes such as frequency, duration, timing (e.g. seasonality) all contribute to intra-annual pattern in the flood disturbance regime (*sensu* Poff *et al.* 1997). Floods occur within the context of a temporally changing set of environmental conditions, usually seasonal shifts in ambient conditions and / or resources. This means that end-points of stream recovery, in terms of population densities and community structure, can seldom be expected to be the same as the starting point, i.e. the pre-flood state, and this makes understanding the effects of disturbance more complex (Ledger *et al.* 2006).

However, very few studies into the impacts of floods on invertebrate assemblages locate their results in the broader context of seasonal or intra-annual variation in ambient conditions, which might explain the still bewildering array of results described in the literature. For example, Giller *et al.* (1991) investigated an unseasonal and very large summer flood that massively reduced invertebrate densities and taxon richness, and disrupted a regular seasonal pattern in invertebrate densities for a three-year period. They compared the effects of the summer flood to one occurring in winter, concluding that recovery after the winter flood was more rapid. However, they did not examine hydrological or other drivers for the reported seasonal patterns in invertebrate densities before the summer flood. Furthermore, the winter flood occurred within the period where recovery from the initial summer disturbance was far from complete. The comparison of these floods, therefore, is made in the context of very different antecedent conditions, very different flood magnitudes and very different ambient conditions during and after the flood.

In the study of post-flood successional sequences investigated by Grimm & Fisher (1989), discernible patterns in algae, but no clear pattern in percentage reduction in invertebrates with flood size, and only weak correlations between post-flood invertebrate density and hydrological measures (days since disturbance) and nutrient and periphyton measures. Boulton *et al*. (1992), using a portion of the data set of Grimm & Fisher (1989), showed very strong seasonality in invertebrate assemblages, correlated not only with discharge, but with temperature and algal standing crop. They found no generalised relationship between peak discharge and either the degree of change in community structure or the percent reduction in individual species densities. Since the floods monitored, however, occurred during various times of the year, and since summer temperature range was some 10 °C higher than winter ones, seasonal differences themselves may have affected both the impact of the flood on the biota, as well as their post-flood population dynamics. Boulton *et al*. (1992) considered that the decline in density was probably related to the initial, pre-flood density, but this is itself a function of the time of year in their study stream. It is possible that the search for a common pattern in recovery trajectories was hampered, again, by combining the responses to floods in different environmental settings.

Many studies have demonstrated strong seasonal patterns in invertebrate assemblage composition and virtually all of these show a consistency in the seasonal signature of invertebrate assemblages that is only rarely overridden by the effects of floods or droughts (e.g. King 1981; Bunn *et al.* 1986; Meffe & Minckley 1987; McElravy *et al.* 1989; Feminella & Resh 1990; Boulton & Lake 1992; Boulton *et al.* 1992; Cobb *et al.* 1992; Jacobsen & Encalada 1998; Robinson & Minshall 1998; Wright & Symes 1999; Pardo 2000). There are some notable exceptions to this. For example, Thomson (2002) found no cyclical patterns of change in invertebrate assemblages in a temperate Australian river characterised by aseasonal flooding. Brewin *et al.* (2000) found pre-Monsoon declines in invertebrate abundances in rivers in Nepal that were clearly not associated with the floods that followed later.

In most of these studies on seasonality, the temporal scale of investigation has been a bi-annual one, namely sampling during wet versus dry periods, or the aggregation of data into these two categories. Viewed like this, flood-season effects could be simply one of the cyclical force driving biological pattern. If the disturbance period coincides with other periodic changes in the ambient conditions, then the causality of biological pattern is difficult to separate out. Clearly an important question is the degree to which floods on their own determine the nature of intra-annual variability in biological assemblages, versus other possible explanatory variables. In this regard, a useful approach is to examine the effect of floods (and / or droughts) through the course of an annual hydrological cycle (e.g. Silva Filho & Maltchik 2000; Suren & Jowett 2006), with a sampling strategy that is adequate in temporal scale (time interval) to separate out seasonality from disturbance impacts.

1.5.3 Inter-annual variability and adaptations to the disturbance regime

A final consideration in the attempt to explain patterns in stream biota and to understand life history adaptations is the constancy or variability environmental drivers over time scales of a number of years. The predictability in magnitude, frequency and timing of floods year after year determines whether or not one can describe general pattern in the cycle of intra-annual hydrological change in a river. This predictability will determine the efficacy of the flow regime as an evolutionary driver of life history adaptations that result in a distinct seasonality in the patterns of stream biota. If disturbances are predictable then specific life-history adaptations should evolve to cope with them (Lytle & Poff 2004). These may include, for instance, metamorphosis to a life stage that it outside of the stream, or located deep in the hyporheos, with this being timed to coincide with the onset of the flood season (Lytle 2002). Life cycles may also be geared to maximise growth and reproduction during stable periods of quieter flow (Gasith & Resh 1999). High levels of inter-annual variability in floods, where disturbance may not regularly affect stream populations, may otherwise be associated with a noisier signal, namely the disturbance signal to which species populations may be responding would be too variable to act as a directional evolutionary force, and other factors may be as important drivers of species attributes. There, adaptations such as asynchronous hatching of portions of eggs over an extended time may be more useful (Huryn & Wallace 2000), as would short life cycles that allow a quick response to favourable conditions. This may then be the strongest explanation for a lack of intra-annual pattern in biological communities. Inter-annual variability is greatest where floods are entirely aseasonal, but even within regions with strong seasonality of flow, high inter-annual variability may preclude the evolution of adaptive responses in the biota that result in a distinct seasonality in assemblage composition.

Measuring this requires that the predictability of disturbance or of periods of high or low environmental stress be related to the persistence, or lack thereof, and the stability of biological communities, and also to their life history patterns. Townsend *et al.* (1987) defined persistence as the extent to which the species complement of an assemblage remains unchanged over a time period encompassing at least one complete population turnover. Stability of natural populations or communities refers to the extent to which there is constancy in the numbers of organisms over time periods encompassing at least one turnover of generations (Connell & Sousa 1983), defined as the relative constancy of species densities over time, i.e. their ranked abundance relative to other species in an assemblage (Meffe & Minckley 1987).

Given the length of the datasets required for these sorts of trajectory studies, research papers that specifically examine community persistence, i.e. that measure persistence at one or a few sites, sampled repeatedly over a period of years, are few in the literature (Connell & Sousa 1983; Townsend *et al.* 1987; Lancaster *et al.* 1996; Bradt *et al*. 1999; Bradley & Ormerod 2001; Carter & Fend 2001; Scarsbrook 2002; Metzeling *et al.* 2002; Woodward *et al.* 2002). Indeed some of these examine persistence on only two time intervals, for example a decade apart. These studies indicate that in the medium term, communities tend to be generally persistent, albeit unstable with regard to absolute invertebrate abundance. In these studies, increased temporal environmental variability, and reduced habitat stability are associated with lower persistence.

In a review of long-term studies, Jackson & Fűreder (2006) found only 46 publications dealing with aquatic fauna where the temporal scale of investigation exceeded three years. A key finding of these studies was that insect populations show considerable inter-annual variability both in size and densities, community structure and life history attributes, that are the result of climatic conditions, chiefly hydrological characteristics.

1.6 OBJECTIVES AND STRUCTURE OF THIS THESIS

This thesis begins with an introduction to the major concepts that have been explored through analysis of the data collected as part of this thesis (this chapter). This is followed by a description of the approach to the study and the study rivers and sites (Chapter 2). Whilst the methods used for each of the data chapters (Chapters 4 to 7) are described in detail in the relevant chapter, the sampling schedule and descriptions of the various data-sets are presented in Chapter 2.

The central part of the thesis is an examination of the relationship between disturbance and the temporal dynamics of invertebrate assemblages, over short term, and at intra- and inter-annual time scales.

1.6.1 Invertebrate responses to floods on short-term time scales

Flood disturbance may be characterised as the application of hydraulic force, resulting in a biological response, which may vary between taxa, from ‘perfect resistance’ to total mortality, followed by variable recovery trajectories as a result of differences in resilience. Evidence suggests that the most appropriate way in which to conduct ecological studies of disturbance within an ecosystem may indeed be to attempt to understand what happens to fauna on disturbed patches versus undisturbed ones. The large scale roughness of cobble- and boulder-bed rivers of the Western Cape results in many areas that provide substantial hydraulic shelter, and thus in a range of physical disturbance effects associated with floods, both in terms of flow forces acting on bed particles and of particle movement. Whilst this provides a useful basis for the study of flood effects, it also necessitates completion of the difficult task of approximating what the magnitudes of flow forces are that act on individual bed particles during a flood, as well as the consequence of these forces in terms of whether or not the stone moved.

In this thesis section (Chapters 3 and 4), invertebrate responses to individual flood events are investigated through a field study of rock displacement by floods of different magnitudes, linked to the quantification of invertebrate community change and population shifts after flood events. Linking invertebrate response to disturbance of individual particles is fraught with difficulties, not least because of the heterogeneity in species assemblages typically found from one patch to another in streams. To what does one compare the biological composition of a ‘disturbed stone’, if not an average species composition from before the disturbance occurred? In this study an attempt has been made to repeat-sample marked stones before and after disturbances, allowing time after marking of stones before floods for reconditioning of sampled stones. The advantage of this approach is that it allows a direct comparison between physically identical patches (stones) before and after a disturbance, which can then be linked to an estimate of the hydraulic forces brought to bear upon the stones during the disturbance.

The spatial scale at which the disturbance force acts is examined as a key aspect of determining the effects of floods on stream communities. The results of this study component are used to define disturbance thresholds for the Molenaars and Berg Rivers.

Objective 1:

- To investigate invertebrate response to individual flood events of known magnitude, relative to the distribution of flow forces acting on bed particles as well as potential refugia; to define an ecologically relevant threshold of disturbance linked to discharge, for the study rivers.

Specific hypotheses to be tested for Objective 1:

a) There will be a positive relationship between the magnitude of a disturbance event, the proportion of the bed disturbed and the degree of reduction in invertebrate population size immediately after the flood.

b) Invertebrate response to floods above the disturbance threshold will vary across the suite of invertebrate species in the river, and these will be linked to life-cycle characteristics as well as to behavioural or morphological characteristics that provide greater or lesser resistance or resilience to flood disturbance. Some species are expected to show greater susceptibility to increased flows than others.

1.6.2 Describing characteristics of the flood regime

A further issue in disturbance studies is the frequency with which threshold disturbances (i.e. those with measurable biological responses) will affect stream assemblages. Aspects of the flow regime such as the frequency and predictability of disturbance can be gleaned from hydrological records and are posited to explain the extent to which stream fauna are persistent over intra-and inter-annual time scales.

Following from the ecological definition of disturbance levels, a detailed analysis of the hydrological regime of the Molenaars River is presented in Chapter 5, which describes variation in flow at seasonal and monthly time scales. Further, the long-term hydrological record is then analysed to provide information on the seasonal and by-month predictability of different disturbance events.

Objective 2:

- To describe the magnitude, frequency and predictability of hydrological disturbance as a characteristic of the natural flow regime, based on ecologically-defined thresholds of disturbance

Specific hypotheses to be tested for Objective 2:

a) Discernible patterns in seasonality of flows and in the periodicity of ecologically-defined hydrological disturbance are readily apparent in the Molenaars River, given its Mediterranean climate.

b) The predictability by month of flood disturbance is low for the rainy season and higher for the dry season.

1.6.3 Invertebrate responses to the flood disturbance regime at medium time scales

On the basis of being able to demonstrate the "predictably unpredictability" (*sensu* Davies *et al.* 1994) of the flood regime in the Molenaars River, Chapters 6 and 7 seek to examine temporal changes in invertebrate assemblages and to identify the abiotic factors that drive these changes. Both the flood regime and temperature are considered to be the key, seasonally variable, factors that might show relationships in this regard. Importantly, the extent to which the intra-annual patterns in community assemblages are repeatable year-on-year is examined, to establish the persistence and stability of invertebrate assemblages over inter-annual time scales. Additionally, the population dynamics and life history attributes of individual species are explored, by way of explaining these biological patterns.

Objective 3:

- To describe intra-annual temporal patterns in macroinvertebrate assemblages, and to investigate the links between these patterns and temporal gradients in abiotic variables.
- To examine persistence and population stability in the invertebrate community, especially in relation to hydrological disturbance and the seasonality of the flow regime

Specific hypotheses to be tested for Objective 3:

a) Temporal changes in invertebrate assemblages or densities should track most strongly changes in flow, and specifically will be driven by variations in the onset, intensity and duration of the flood season.

b) Invertebrate assemblages during the summer lowflow period, characterised by low levels of disturbance, should be more stable, and more persistent, than those assemblages subject to frequent flood events, where these measures should be influenced by inter-annual differences in flood intensity and frequency. Alternatively, if invertebrates are adapted to the flow regime, even to the lack of predictability in the timing or magnitude of floods, then their assemblages should be predictable and persistent, because at local or reach level they have evolved resistance to disturbance. In that case, persistence should be high year-on-year for all seasons, but stability may still vary as a result of short term climatic differences within each winter flood season.

Objective 4:

To explore the different types of life history exhibited by the invertebrate fauna of the Molenaars River, in the light of temporal variability in flow and abiotic conditions. Whilst the particularities of the flood regime, in combination with the temperature, which reaches seasonal peak values during the low-flow period would be expected to play an influential role in life-history adaptation, the data collected as part of the study of temporal change in invertebrate patterns precluded the testing of specific hypotheses regarding the causes of one or other life-history pattern. While simple survey data of larval cohorts provides far more limited information, few life-history data exist for South African invertebrates and this study did provide an opportunity for the larval cohort dynamics of a large number of species to be discussed, particularly in terms of their alignment with the seasonality of the disturbance regime. This chapter thus provides a preliminary sketch of differences in broad development periods and some indication of cohort patterns in the selected species.

Finally, Chapter 8 provides a synthesis of the major findings and conclusions of the thesis.

2 DATA OVERVIEW AND STUDY SITES

2.1 INTRODUCTION

This thesis effectively comprises four components, in addition to the literature review:

- characterisation of invertebrate responses to floods of different magnitudes, including resistance and resilience; an attempt to define a threshold of flood that constitutes a disturbance event, based on the responses of invertebrates to floods;
- an examination of the frequency, distribution and predictability of floods above a threshold constituting disturbance;
- an exploration of the relationship between floods and other seasonal phenomena to intra-annual and inter-annual dynamics in invertebrate assemblages; and
- an attempt to explain invertebrate community persistence in terms of both species-level resistance traits and life history attributes.

The field data collection that provided the material for this thesis was undertaken during two periods, some years apart. The flood-study component of this thesis (Chapters 3 and 4) was undertaken in 2003 and 2004. Data for the longer-term investigation of temporal change in invertebrate assemblages and the investigation of life histories (Chapters 6 and 7) were collected between 1991 and 1997. Because of the large time span, and the fact that the data used for this thesis were collected as part of two different projects, some of the field methods differed. An explanation of the different data sets is thus useful by way of orientation for the reader.

2.2 DESCRIPTION OF THE STUDY AREA AND SAMPLING SITES

The various components of the study took place on the upper reaches of the Molenaars and Berg Rivers, located in the Western Cape Province of South Africa. Both rivers have their origin in the same mountain range, but fall within different Primary catchments (Figure 2.1). The Molenaars River is one of the main tributaries of the Breede River, which is the largest river in the Western Cape and flows eastwards into the Indian Ocean. The Berg River is the second largest river in the Western Cape and flows west to the Atlantic Ocean. The area is characterised by winter rainfall with heavy rain (over 1000 mm per annum) in the mountain catchments. The fact that rainfall is produced by cold fronts that move across the country, producing one to five days of rain, means that the rivers are characterised by short, but intense flood events.

2.2.1 The Molenaars River

The study area comprises an approximately 14 km portion of the Molenaars River, within the Du Toitskloof valley, downstream of its confluence with the Elands River (Figure 2.1).

From its source in the Klein Drakenstein Mountains the river flows along a steeply graded, laterally confined valley for some 5km before combining with the Krom and the somewhat larger Elands River tributaries, from where the gradient flattens out for the *ca*. 14km length of the Du Toitskloof valley, the study reach for this thesis.

The segment of the Molenaars River upstream of its confluence with the Krom and Elands Rivers are third

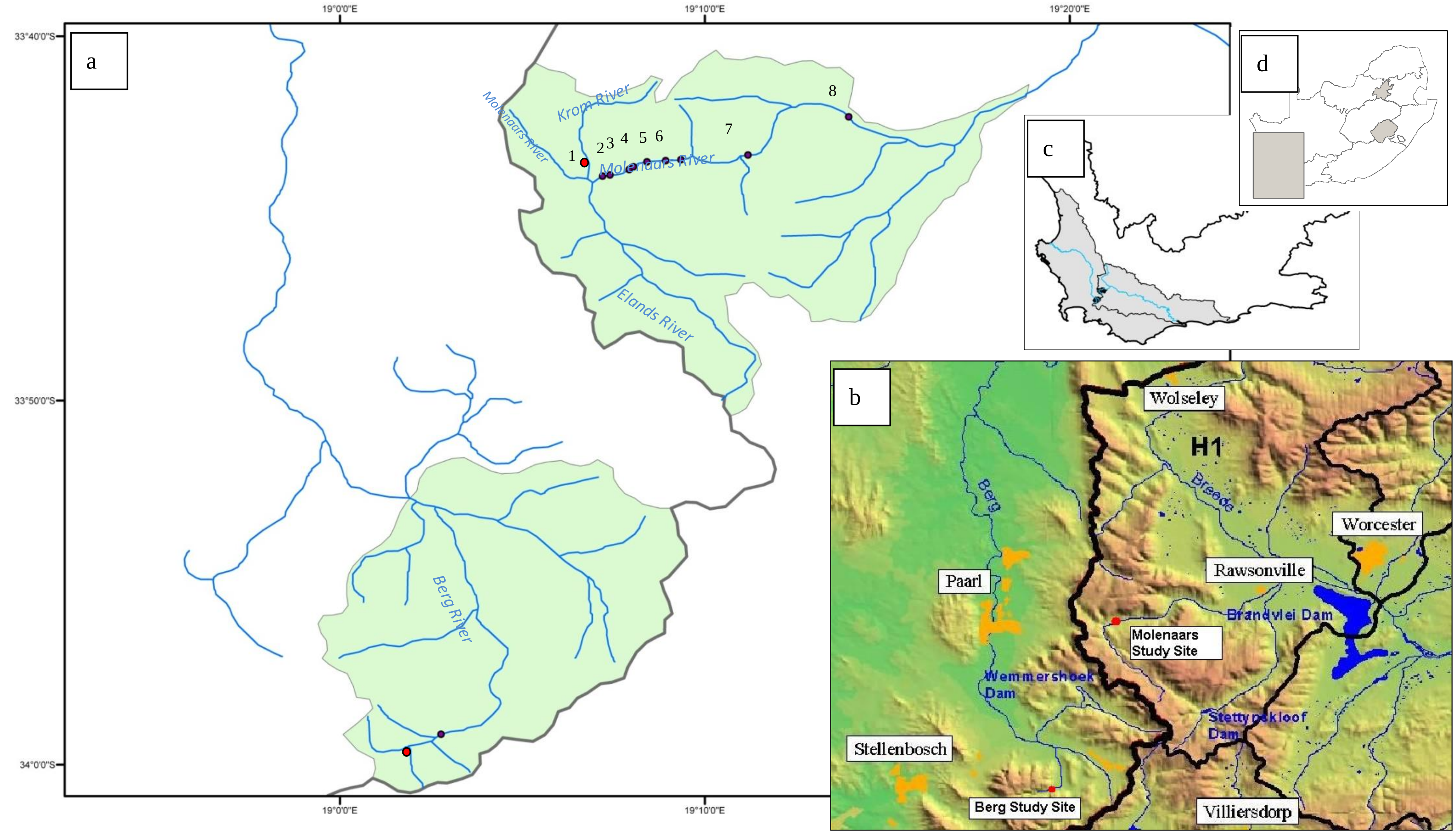

Figure 2.1. **Study area: a) upper reaches of the Berg River and the Molenaars River, showing the flood study site on each river (red) and eight sites for the longer-term temporal study on the Molenaars River. Quaternary catchments are shaded and the primary catchment divide between the Berg and Breede Rivers is shown; b) Relief map showing the study reaches, with flood study sites marked. The rivers are separated by the Klein Drakenstein Mountains. The primary catchment divide, major dams and local towns are shown; c) Location of the study area quaternary catchments (black) within the Western Cape, showing the mainstem Berg River flowing to the west coast and the Breede to the east coast; and d) location of the study area within South Africa.**

order mountain streams. The Molenaars River within the study area, downstream of its confluence with the Elands River is a fourth order, foothill river. Through the valley the river channel is mainly a single-thread, with gentle valley slopes. Where the valley widens, some braiding does occur, with lateral bars and flood terraces.

The minor tributaries of the upper Molenaars River generally have closed canopies, whilst the main river, the Krom and the Elands Rivers have open or partially closed canopies. Indigenous riparian vegetation was present, consisting of small woody trees and shrubs but some of the riparian zone, principally those areas where trout and protea farming occurs, had become infested with black wattle (*Acacia mearnsii*) and Australian long-leafed wattle (*Acacia longifolia*) at the time of the study.

2.2.2 The Berg River

Only a single site was located on the Berg River, for the flood response study. The catchment surrounding this site comprises fynbos and forestry plantations, with some areas invaded by alien trees. The river in this reach is characterised by a widening valley floor, relative to the incised channel reaches of the more confined mountain stream segments upstream. Valley side slopes remain steep, but alternating lateral bars and flood terraces are evident in places.

2.3 EXPLANATION OF DATA SETS

2.3.1 Flood response study

The flood-study component of this thesis was undertaken in 2003 and 2004 and was partly funded by the Water Research Commission (WRC). The project was a collaborative ecohydraulics study undertaken by the Universities of Cape Town and Stellenbosch. The hydraulics component was to refine hydraulic equations that describe the relationship between flood size and bed disturbance in rivers with heterogeneous substratum particle size. The ecological component, the basis for this thesis, was to describe the ecological response of invertebrate fauna to floods and / or bed disturbance. The research sites for this project were located on the Molenaars and Berg Rivers. The choice of site was aimed at ensuring as similar physical conditions as possible at both sites. The two study sites are located within 50 km of each other, but in different catchments (Figure 2.1). Hydraulic data collection and monitoring of the movement of river stones during floods was undertaken at both sites in 2003 and 2004. Biological flood response data were collected from the Molenaars River site in 2003 and the Berg River site in 2004.

2.3.2 Hydrological analysis

The second component of the study, the analysis of the flood disturbance regime, used the results of the flood study as a threshold for disturbance. The analysis of the regime characteristics of floods above this threshold was undertaken using long-term discharge data provided by the South African Department of Water Affairs and Forestry (DWAF). The flow gauge for the Berg River, G1H004, was located some 4 km downstream of the flood study site. The gauge on the Molenaars River, G1H018, was located in the middle of the study reach and some 5 km downstream of the flood study site.

2.3.3 Long-term temporal dynamics in invertebrate assemblages

The third component of the study, the longer-term investigation of temporal change in invertebrate assemblages presented in this thesis is based on data collected in the Molenaars River between 1991 and

1997. The collection of the data was funded by the South Africa Roads Agency during its upgrade of the N1 national road through the Du Toitskloof valley between Paarl and Worcester. The road alignment followed that of the Molenaars River for some 14 km, and its reconstruction necessitated an environmental monitoring programme.

During the first stage of the construction monitoring programme, associated with bridge building between 1991 and 1993, quantitative sampling of invertebrates and abiotic variables was conducted at nine sites along the Molenaars River study reach. The sites were selected up- and down-stream of two bridge construction sites, and thus some of the sites were impacted upon by bridge-building activities. Samples collected during this period were processed in the laboratory and invertebrates identified generally to family level, and enumerated.

During the second stage the construction monitoring programme, associated with road building, semi-quantitative invertebrate sampling was undertaken on a monthly to 6-weekly basis (depending on weather conditions), at a number of sites along the Molenaars River study reach from January 1994 to July 1997. Four of these corresponded to the monitoring sites used in the 1991 – 1993 study, viz. Sites 1,2,7 and 8. The identification of invertebrate taxa was undertaken in the field and ranked abundances ascribed through visual estimates. Given application of the sampling protocols consistently at all sites and all identification and abundance estimate made by the same field-worker, these data were considered to be a useful long-term semi-quantitative data set to examine temporal dynamics in invertebrate assemblages.

2.4 DESCRIPTION OF STUDY SITES

2.4.1 Study sites used for the flood study

The Molenaars River study site (Figure 2.2) had relatively well defined banks on both sides and consisted primarily of a riffle and rapid section ending in a large pool.

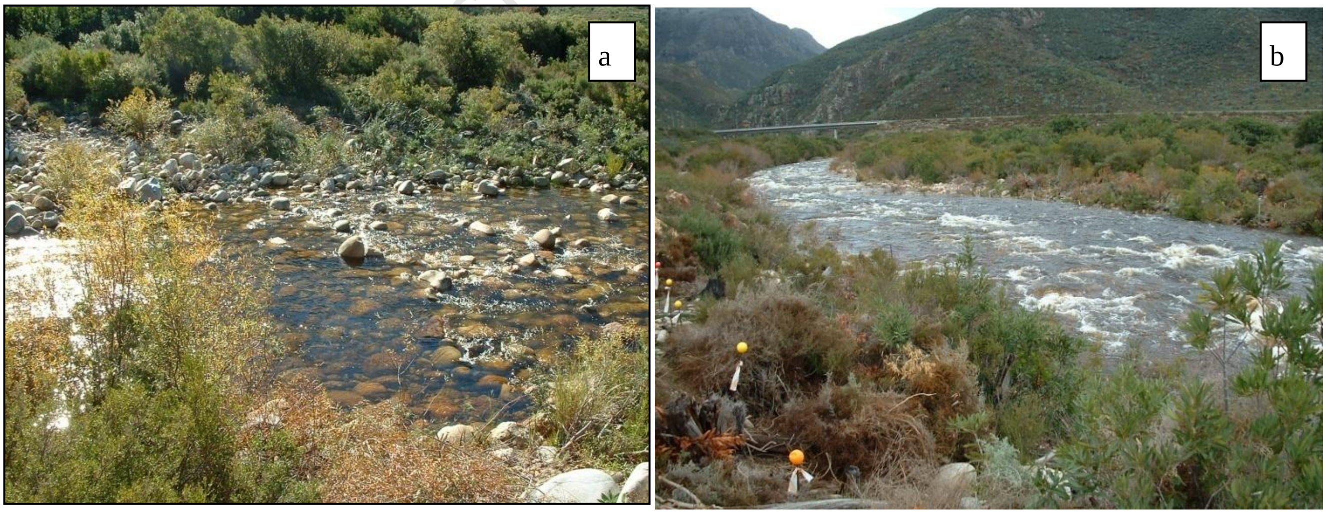

Figure 2.2. The Molenaars River flood-response study site a) at autumn low flow (baseline sampling) viewed from high up on the right bank and b) during a winter flood, view upstream from site.

The Berg River (Figures 2.3), on a slight bend in the river and has a steep bank on the outside (left hand) bank and a lateral bar of deposited cobbles on the inside bank. A small rapid section dominated the upstream half of the site, leading to a shallow run in the downstream half of the reach.

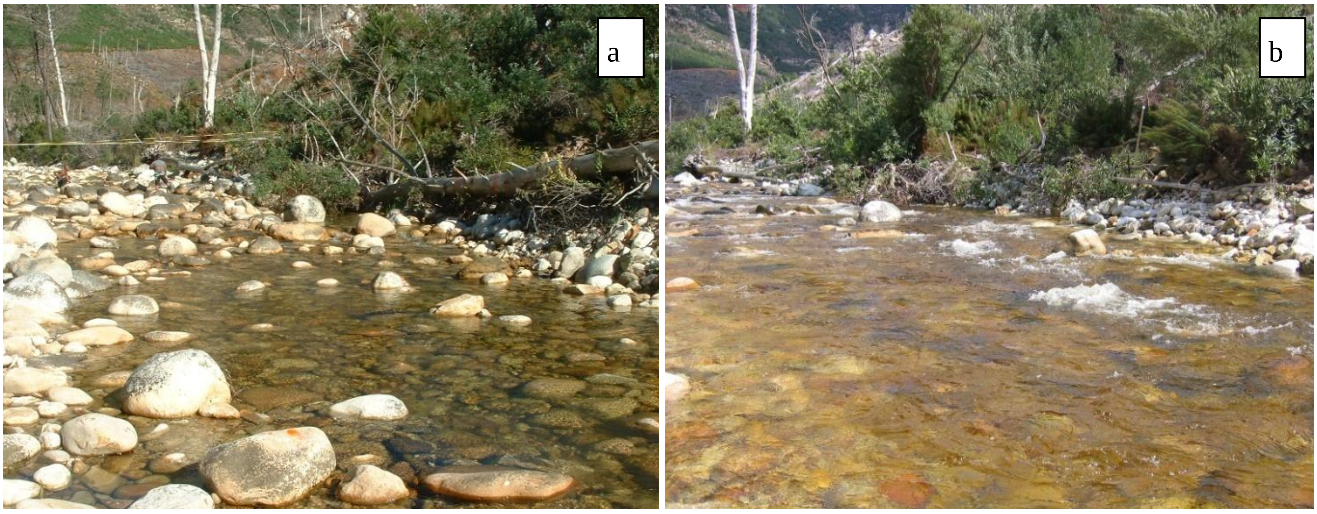

Figure 2.3 **The Berg River flood-response study site a) at autumn low flow (baseline sampling) and b) at winter base flow during post-flood sampling. Note that the fallen tree on the left bank has been removed during the floods.**

2.4.2 Study sites for the investigation of temporal dynamics in invertebrate assemblages

The eight sites used for the study of temporal patterns in invertebrate assemblages were chosen to monitor potential impacts of road construction. Because of the existence of trout farms along the river, as well as limited farming, sites were located in an attempt to separate out road construction impacts from other activities. Table 2.1 summarises these features of the sites.

Table 2.1. **Summary of the status of each of the eight sites used for the study of temporal dynamics in invertebrate assemblages.**

Site	Distance (km) from confluence of Molenaars and Elands Rivers	Motivation for site based on its initial inclusion for the road / bridge construction monitoring
1	0.23	Control site upstream of first bridge, potentially affected by trout farm effluent in upper catchment, although these are located > 5 km upstream
2	2.04	Impact site downstream of bridge construction, also impacted by small-scale fruit orchard cultivation
3	2.24	Control site upstream of second bridge, but existing impacts are farming and a trout hatchery
4	2.94	Impact site immediately downstream of second bridge
5	3.58	Additional impact site subject to existing impacts in the form of sediment loads from an eroding head cut on the slopes of the valley sides
6	4.45	Additional impact site, also impacted by trout farm and hotel
7	8.47	Unimpacted control site 4 km downstream of all anthropogenic activity
8	13.41	Additional unimpacted control site at the end of the valley

3 INVERTEBRATE RESPONSES TO FLOODS. PART 1: INTRODUCTION AND BASELINE (PRE-FLOOD) SURVEY OF INVERTEBRATE PATTERN

3.1 INTRODUCTION

A number of field studies have related the magnitude of flood discharges, expressed as a multiple of base flow discharge, to biological response. For example, Quinn & Hickey (1990) reported that invertebrate density after an autumn flood of ca. 60 times the median flow was 680 times lower than summer levels, but floods smaller than 20 times the annual median flow had no discernible effects. Robson (1996) recorded a 25 % reduction in invertebrate numbers on bedrock runs, but only a slight increase in the cobble riffles, in response to a spate reaching peak daily discharge of 15.5 times the preceding base flow. Biggs & Close (1989) reported that flood flows exceeding median flows by 6-fold always reduced periphyton biomass significantly. In these studies, only the relative increase in discharge was measured, not the physical forces acting on the biota associated with the flood discharge. The limitation of such approaches in that no clear relationship really exists between multiples of the annual median flow and the actual disturbance intensity across differing stream types. The physical forces exerted by a flood of the same magnitude relative to mean base flow or median flow may differ considerably between rivers, depending on channel and bed properties. This means that the biological responses described are difficult to compare. Useful comparisons of results can be made when biological responses are related to physical forces acting upon them, as this provides more insight into the actual mechanisms whereby some, or all, of the fauna are affected by flow forces.

Field studies that measure both the magnitude of natural physical disturbances and their associated biological response have shown relationships between flood magnitude or frequency and population reduction, through the agency of the movement of river bed stones. For example, Cobb *et al.* (1992) found a strong correlation between invertebrate density and the percentage of stones in motion. Insect densities started to decline with a displacement of about 10% of stones. Whilst they found a correlation between discharge and bed movement, the latter accounted for more variation in benthic invertebrate density than did discharge. This effect was variable among taxa, with the strongest relationships between bed movement and Plecoptera, Ephemeroptera and Trichoptera, and less so in the case of Chironomidae, Simuliidae or beetles (Cobb *et al.* 1992).

Matthaei *et al.* (2000) examined invertebrate responses to a flood that caused patchy bed particle movement. Stable and unstable stones prior to the flood were similar to each other in invertebrate species composition, but had completely different attributes after the flood. Invertebrate density and richness increased on stable stones after the flood and decreased on unstable stones, relative to their pre-flood condition. Stable and unstable stones returned to more similar states within 19 days. The results of this study suggest active use of refugia in the form of stable stones during floods.

If stone movement is determined by its size and/or its embeddedness (e.g. Downes *et al.* 1998b) then the distribution of less mobile or sessile organisms may be correlated with either large size or embeddedness of a stone, as a result of species selection for refugia. For example, (Englund 1991) found a strong relationship between the occurrence of moss and the embeddedness of small stones, but not of larger stones. Largest stones also had proportionally more moss than medium and small stones.

A corollary of the notion that disturbance acts in a patchy manner is the presence of instream refugia from the forces of flow. These may be defined as habitats that reduce disturbance effects or provide mechanisms that allow biota to survive, albeit in a depleted state, in disturbed environments, and may occur at a range of scales (e.g. Sedell *et al.* 1990). The short-lived nature of flood impacts may be as a result of either resistance (the capacity of a community, or a population to avoid change: Connell & Sousa 1983) or of resilience (the capacity of reduced or impacted populations or communities to recover after a disturbance: Hildrew & Giller 1994). Resistance involves the persistence intact of a population or species assemblage through a disturbance event, implying some attribute on the part of the species that affords it tolerance of the disturbance.

At a local scale, benthic assemblages recover from disturbance as a result of spates in time spans generally shorter than one generation time, an observation that suggests that there may be considerable flow refugia in or associated with stream channels (Lancaster & Hildrew 1993b) or 'within -habitat refugia', using the categorisation used by Lancaster & Beylea (1997). Indeed, since many recent studies indicate that disturbance, especially in floods that have a within-year return period, occurs at the scale of individual stones (e.g. Downes *et al.* 1998a; Matthaei *et al.* 1999a), recolonisation of denuded substrata may occur simply via the redistribution of fauna, rather than colonisation from a distant source (Matthaei *et al.* 1999b). An important caveat is the recognition that disturbance effects may be highly variable between rivers or sites, depending on the hydraulic characteristics of the site.

In this context, I define two fundamentally different types of instream flow refugia. The first may be defined as localised areas where hydraulic forces acting on the substratum remain low during a flood event, such as hydraulic dead zones, which prevent density-independent losses of animals and provide a source of colonists for recovery in denuded areas (Lancaster 1990; Lancaster & Hildrew 1993a, b; Rempel *et al.* 1999; Lancaster 2000). In this regard, Matthaei *et al.* (2003) also found scour, fill and stable patches in a stream bed that were not related to particle size, or in other words must have been related to the presence of patches where hydraulic forces remained low over the course of a flood. Most taxa utilising such refugia do so through active or passive movement with the onset of a disturbance event. At the scale of individual rocks facing high flow forces, these taxa cannot be considered to be resistant, but rather resilient, since they do not survive through a disturbance on that stone, but rather collect in refugia from where they can recolonise denuded patches of the stream bed after the disturbance.

The second type of instream refugium might be that of large stable particles (e.g. Townsend *et al.* 1997b; Francoeur *et al.* 1998). Very large particles may indeed be subject to considerable hydraulic stress during a flood, but for an organism that is well-adapted to resist such stresses, they may be preferable refugia to 'hydraulic dead zones' which could provide for other dangers, such as increased encounters with predators in these flow refugia (e.g. Thomson *et al.* 2002). Such refugia, by virtue of the fact that they do not move, allow taxa using them to resist disturbance at even a local stone scale.

Other instream refugia include 'microform bed clusters' (MBCs), which are defined as bedform micro-units consisting of a complex of several stones that are particularly resistant to entrainment during high flow, within which hydraulic refugia in the form of low shear stress spots could exist (Biggs *et al.* 1997; Francoeur *et al.* 1998, but see Matthaei *et al.* (2000) for a contrasting view).

The availability of in-stream flow refugia is related to the heterogeneity of channel and bed morphology, where roughness elements create resistance to flow.

One of the implications following from a) the well documented effects of floods in reducing invertebrate abundances and b) the importance of refugia in mitigating this effect is that, barring recruitment of new individuals from upstream reaches or smaller instars from the hyporheos, invertebrate densities on the streambed at a site scale should decline gradually over a period of repeated disturbance such as a flood season. The relative proportion of refugium patches in a stream should thus determine the overall magnitude of population losses during a flood of particular threshold intensity, and thus the rate or trajectory of this decline in abundance over successive floods.

3.1.1 Aims of Chapters 3 and 4

The aims of this thesis section were to investigate invertebrate responses to individual flood events of known magnitude; to link these to the magnitude of flow forces acting on river stones as well as to the availability of refugia; and to define an ecologically relevant threshold of disturbance linked to discharge for the study rivers.

Firstly, however, the relationships existing prior to the onset of floods, between the distribution of species at a local level and the hydraulic environment was examined.

The following hypotheses were tested:

a) Species distributions prior to floods will reflect varying preferences or tolerance of different hydraulic environments at a site level, rather than populations being randomly distributed over the site.
b) Some species may be disproportionately concentrated on larger, stable bed particles in the period immediately prior to the onset of floods, since these provide one sort of refuge during floods.
c) The magnitude of a flood will be proportional to the percentage of the bed disturbed and the degree of reduction in the invertebrate population, measured before and immediately after the flood.
d) Invertebrate response to floods will vary across species, such that some species will show greater resistance at a local (site) scale to floods than others. Thus intra-annual floods can be expected to act mainly by restructuring communities through their differential effects on populations of the species comprising the community.

3.1.2 Approach to this study

The movement of stones during floods is an empirical approach to measuring disturbance that has become popular in recent studies of invertebrates responses to floods (e.g. Townsend *et al.* 1997a, b, Matthaei *et al.* 1999a; Gjerløv *et al.* 2003). The spatial scale at which this movement appears to occur provides some insight into the scale at which the disturbance response by stream biota should be measured: Downes *et al.* (1998a) recorded 60 % cumulative bed movement of stones in the size range 40 – 200 mm over a six month winter period during which at least nine flood events occurred, including three floods estimated to have a return period >3 years. Their study showed that the spatial scale at which physical disturbance of stream substrata occurred was the scale of individual stones, rather than whole sites, with as much variation in disturbance within sites as between sites, even with large floods.

This approach to measurement of physical disturbance has been adopted in this study, as it provided a measurement of bed disturbance that could be linked to the measurement of invertebrate response, namely that of individual stones. The study was also undertaken in tandem with the development of a hydraulic model to quantify incipient motion of bed substrata during floods. That component of the study tested and refined equations of bed particle entrainment that could be used to determine whether or not unmarked stones were likely to have moved, given knowledge of their position within the surveyed stream. These equations were also used in this study, thus combining empirical and theoretical approaches to measuring disturbance.

Downes *et al.* (1998a), however, found that stones placed randomly on the stream bed moved half as often as those marked *in situ* and considered that the human-placed stones might be placed in positions where they were sheltered from shear stresses that would lead to their mobilisation, given that there may be only a small number of locations with sufficiently large ambient shear stress to move the sizes of stones used in their study. In addressing this, Gjerløv *et al.* (2003) repositioned all stones on each sampling visit to ensure that any stone in a sheltered position was not maintained there. The solution in this present study was to mark stones and relocate them exactly to the position in which they were deposited during prior, natural sediment transport processes, thus avoiding the human error in placing stones in sheltered locations.

A further consideration in measuring disturbance is that, although the movement of stones undoubtedly constitutes a physical disturbance, it may under-represent the force acting during the flood. Hydraulic forces acting on the bed increase with increasing discharge and these may be as important as actual bed movement. This may be particularly true in heterogeneous cobble- and boulder-bed rivers such as those in the Western Cape, where large hydraulic forces may be present long before stone movement occurs.

In this light, Bond & Downes (2003) cautioned against assuming that bed movement needs to occur to cause a measurable biological disturbance effect. In an experimental study (Bond & Downes 2000) they found that the densities of caddisflies on small and large bricks, both tethered to the bed or left unfixed and thus allowed to be moved by a flood, were not significantly different after a flood. This suggests that increased hydraulic forces acting on fixed substrata may play as great a role in disturbance as the overturning of stones (Bond & Downes 2000). This conclusion is reinforced by studies that demonstrate increases in drift with elevated discharge, where bed entrainment did not occur (e.g. Mathooko & Mavuti 1992; Imbert & Perry 2000; Bond & Downes 2003).

In this present study, therefore, in addition to the observation of whether or not marked stones were moved during consecutive floods, the magnitude of the hydraulic force acting on each marked stone during the peak of each flood was calculated from the hydraulic survey data. This provided a second measure of flood disturbance at the scale of individual stones, which was then related to biotic change from samples taken before and after the floods.

Complementary datasets of stone movement during each of a number of flood events and invertebrate assemblage data on these stones were thus obtained simultaneously during the study. This provided an empirical measurement of the intensity of disturbance, determined for each of a series of floods of varying size, as reflected by a) the percentage bed movement and b) the peak-flood hydraulic force acting on each sampled stone. Accompanying this, invertebrate assemblages before the flood, and on moved or unmoved stones after the flood, were compared to characterise the responses to disturbance.

Hydraulic and invertebrate data sets were collected from the Molenaars River in 2003. In 2004, hydraulic and biological data (invertebrates and periphyton) were collected from the Berg River, and hydraulic data only from the Molenaars River, to improve the hydraulic model.

3.2 METHODS

3.2.1 Hydraulic and stone movement data

3.2.1.i Sampling strategy

The approach to collecting data on the movement of stones, linked to flood events, had to satisfy the requirements for the calibration of the theoretically-based hydraulic model; provide information to calculate the hydraulic forces acting on each stone at different discharges; and simultaneously allow for biological data to be collected from the stones. This required:

a) a large enough sample of stones to be representative of the full range of hydraulic conditions likely to prevail during a flood;

b) the positioning of all stones along fixed cross-sections, to allow for the calculation of hydraulic indices from surveyed bed heights and water levels, and to facilitate finding the marked stones after each flood event;

c) a systematic basis for selecting stones along each transect, to provide a random sample for particle-size distribution analysis;

d) a sufficiently large sample to include at least 200 submerged stones from which biological data could be collected on any one occasion.

3.2.1.ii Baseline (pre-flood) hydraulic data

a <u>River bed survey and stone marking</u>

A detailed survey of each study site was performed prior to initial sampling of the individual stones for hydraulic and biological data. Thirty cross-sections were established at 2 m intervals at each site and marked with steel pegs on each bank. The bed level was surveyed at 1 m intervals along each cross-section. In the case of the Molenaars River a new survey was needed after the late, bed-moving floods of 2003 to ensure accuracy in the hydraulic data calculated from these bed levels, and thus the site was re-surveyed at the start of the 2004 study period. Similarly, following a large flood in 2004 (the second flood monitored during the 2004 study period) both sites were surveyed again to ensure accuracy in the hydraulic data calculated thereafter.

To select the stones, a tape measure was stretched along each cross-section, between the two steel pegs, and stones were randomly located at either 1 m or 1.5 m intervals, depending on the width of the river at that cross section, so that at least 10 stones were selected from each cross-section. In total, 345 and 435 stones were selected at the Molenaars and Berg River study sites respectively. All submerged and movable stones were sampled for invertebrates and, for the Berg River, invertebrates and periphyton (see section 3.2.2 for methods). Some submerged but immovable stones (boulders) were also sampled for invertebrates. All stones were measured, marked with an individual number using waterproof paint and pens, and replaced. If possible, stone size was measured along three perpendicular axes. Embedded stones that could not be lifted were measured according to their second longest axis (dy).

Lead-weighted fish markers were used to pinpoint the exact position of stones whilst they were being sampled and measured. Both the top and the bottom of the stones were marked to make it possible to determine if the stone had been turned over during a flood event. Submerged stones that could not be lifted were marked with putty into which an identity number was scratched. Initially, small magnets were attached to the stones as it was felt that this would allow for easier location of stones after floods. It was found, however, that there was too much natural magnetism in the rocks to make this a reliable method for locating stones after a flood and it was thus abandoned.

b Hydraulic parameters

After sampling, the stones were returned to their original positions, and water depth and average velocity (at 0.6 depth from the water surface) were recorded above each of the marked stones (Scientific Instruments Inc. Model 1205 Mini Meter Current Meter). Flow was measured as revolutions per 45 seconds and converted to velocity with the equation:

Velocity (metres per second) = ((Rev/Time x 0.977) + 0.028) x 0.3048

(Scientific Instruments Inc. 1980)

In addition to the measurement of stone size, depth and velocity, measures derived from these using local slope, viz. Froude number, Reynolds number, input stream power and bed shear stress were calculated to describe base flow hydraulic conditions. The first two parameters are commonly used in characterising hydraulic habitat, whilst the latter two are common measures of substratum entrainment used in flood studies.

Reynolds number represents the ratio of turbulent to laminar flow, defined as:

$$\mathrm{Re} = \frac{VD}{v}$$

where D is the depth, V is velocity and v is the kinematic viscosity.

Froude number, the ratio of inertial to gravitational forces acting on the bed, is defined as:

$$Fr = \frac{V}{\sqrt{gD}}$$

where V is velocity at each point, g = acceleration due to gravity and D the depth at each point. Froude number reflects the interaction of flow depth and velocity at a given point, related to the effects of bedform whereas Reynolds number is a better measure of conditions within the mass of water flowing over a point (Gordon 2004).

The third hydraulic parameter computed for each point in these analyses was input stream power, which was the variable used in the hydraulic model developed as part of this study. This index is defined as the rate of potential energy expenditure over a unit stream length:

$$W = \rho g V S$$

where ρ is water density, V is velocity, g =acceleration due to gravity and S is the energy slope over each point.

For these analyses, slope was taken as the average water surface slope between three consecutive cross-sections. There will be local fluctuations in the energy slope that are lost by using the average water slope, but this is a good first estimate of the average energy slope. For calculating stream power during floods, the average water slope for the full reach was used. This assumed that the flow was more-or-less uniform along the reach at high flows.

Finally, shear stress is defined as the frictional force causing flow resistance at the bed, and is the parameter used in most ecological studies of the effects of floods on river biota. Shear stress (τ) is calculated according to the following formula:

$$\tau = \rho g(D - y)s$$

where D is the distance from the water level, s is the energy slope, and y the distance from the bed, calculated from the bed-survey data.

c Hydraulic biotopes

River biologists have long recognised that different patches of stream may generally be associated with specific groups of invertebrate fauna as a result of the hydraulic conditions that prevail. The concept of the hydraulic biotope was introduced to provide some consistency in the description and naming of these categories of physical habitat, whilst concentrating on the flow-related aspects that define an organism's living space (Rowntree & Wadeson 1996). Hydraulic biotopes are defined according to the combination of flow types and substratum (Tables 3.1 and 3.2).

Table 3.1 Definitions of flow types (from Rowntree 2001)

Acronym	Flow type	Definition
NF	No flow	No water movement
BPF	Barely perceptible flow	Smooth surface, flow only perceptible through the motion of suspended objects
SBT	Smooth boundary turbulent flow	Water surface smooth; streaming flow takes place throughout the water profile; turbulence can be seen as the upward movement of fine suspended particles, or as boils on the surface in stronger flow
TR	Trickle	Shallow flow between bed elements with few submerged stones.
RSF	Rippled surface flow	The water surface has regular disturbances which form low transverse ripples across the direction of flow
FRF	Fast riffle flow	Shallow broken water flowing over cobbles
SF	Surging flow	Undular waves form on the surface, but they move downstream, breaking up
USW	Undular standing wave	Standing waves form at the surface, but there is no broken water
BSW	Broken standing wave	Standing waves present which break at the crest (i.e. white water)
FFF	Free-falling flow	Water falls vertically, without obstruction

Investigations into the biological distinctness of hydraulic biotopes (e.g. King & Schael 2001) has led to *inter alia* the recommendation that the RSF flow type used to identify run biotope may not adequately discriminate between units that are biologically different (Schael 2005). In this study, run biotope with RSF flow types were thus sub-divided where information was available. In the Berg River work the flow type on its own was used, as defined in Table 3.1. For the Molenaars River, the more detailed descriptions of where each stone was sampled allowed runs to be split into fast, medium or slow run.

The flow type was recorded at each stone location. Each stone was assigned to a substratum category based on the length of its second longest axis. Substratum categories were defined according to the Wentworth scale, but with the boulder and cobble categories modified to differentiate between small and large boulders or cobbles respectively (Table 3.3). Sand was observed but not sampled in this study. No silt or clay was recorded.

Table 3.2 **Definition of different hydraulic biotopes (King & Schael 2001; Rowntree 2001).** Flow type acronyms are defined in Table 3.1.

Hydraulic Biotope	General description	Flow type
Backwater	A morphologically defined area alongside but physically separated from the channel, connected to it at its downstream end; occurs over any substratum type	BPF or NF
Slackwater	An area of no perceptible flow within but hydraulically detached from the main channel; occurs over any substratum type	BPF or NF
Pool	Has direct hydraulic contact with upstream and downstream water; occurs over any substratum type	BPF
Trickle run	Cobble and boulder substratum; few bed elements submerged	TR
Run	Any substratum type apart from silt; relative roughness low; often in transitions zones between riffles and the downstream pool	RSF or SF
Glide	Occurs over any substratum type, where depth is sufficient to minimise relative roughness. Glides exhibit uniform flow with no convergence or divergence	SBT
Chute	Typically in boulder or bedrock channels where flow is funnelled between large bed elements. Chutes are generally short and exhibit flow acceleration, often due to flow convergence	SBT with acceleration
Riffle	Occur over coarse alluvial substrata from gravel to cobble; high bed roughness	FRF or BSW
Rapid	Occur over more or less fixed substrata, such as bedrock or boulders	USW or BSW
Cascade	Occur over bedrock or boulder substrata; small cascades may occur in cobble where the bed has a stepped structure due to cobble accumulations	FFF

Table 3.3 **Categories of substrata used in mapping, modified from the Wentworth scale.** Codes used to refer to each category are in parenthesis after each name.

Category (Wentworth)	Size range (mm)
Silt	< 0.063
Sand	0.063 - 2
Gravel (G)	2 – 64
Small Cobble (SC)	64 – 161
Large Cobble (LC)	161 – 256
Small Boulder (SB)	256 – 514
Large Boulder (LB)	514 - 1000

3.2.1.iii Flood measurement and stone-movement data

With the baseline data collected, flood events were then studied. Six flood events were studied the Molenaars River during the winter of 2003 and five floods each in the Molenaars and Berg Rivers in 2004.

Maximum water level was recorded during each flood at four points along each bank. This provided depth and water slope data required to calculate flood size and hydraulic conditions at each stone location. Water levels were measured using 2 m-long clear plastic pipes attached to metal y-sections that were concreted into the ground (Figure 3.1). Holes at the bottom and the top of each pipe allowed water and air to enter and leave the pipes. Prior to the flood, a small amount of sawdust, cork flakes or finely cut dry grass was placed in each pipe. These materials floated on the water surface inside the pipe, rising with the increasing water level. After a flood the level of the ring of sawdust / cork residue adhering to the inside of the pipe was measured as the highest water level reached under that flood. The inside of the pipe was then washed clean and the cork flakes replaced ready for the next flood.

The flood study consisted of revisiting each site after each flood, as soon as it was safe to enter the river, to record stone movement. For selected floods, biological data were collected immediately prior to and after the flood, as is described in section 3.2.2.

Cross-sections were searched for marked stones from downstream to upstream. Searches were done underwater using goggles in shallow - medium depth water that was still fairly turbulent. The movement of every stone was noted either in terms of whether it had simply turned over or had moved out of position. In the latter case, if the stone was located, the distance that it had moved was recorded. Sweep searches of the river bed up to 30 m downstream of the site were undertaken at each visit to locate stones that had moved some distance. Stones that had been turned over or moved were replaced in their original locations ready for the next flood event. Where marked stones could not be located, they were assumed to have been washed out of the study site reach, were recorded as moved, and were replaced by a new stone selected *in situ* at the same location along the cross-section, with a new individual number. The dimensions of any new stones were measured and used for the analysis of subsequent flood events.

The movement status of all 345 and 435 stones at the Molenaars and Berg River sites respectively was thus recorded after each flood.

Figure 3.1 Plastic stage pipe used to measure flood levels.

3.2.1.iv Flood analysis

In one of the recent holistic environmental flow assessment methods used in South Africa, DRIFT (King *et al.* 2003), flood flows are classified into eight flood classes based on analysis of the long- term average daily flows. Flood Classes 5 to 8 represent the inter-annual flood events with a return period of between two and twenty years. Classes 1 to 4 represent the spectrum of intra-annual flood events and the level of these floods is obtained simply by halving the two-year return period flood to obtain the Class 4 flood which is halved again to obtain the Class 3 flood, and so on.

The eight flood classes for the two gauges immediately downstream of the study sites were determined by Howard (2004) for G1H004 on the Berg River and Brown & Louw (2003) for H1H018 on the Molenaars River (Table 3.4). Howard (2004) noted that there was some concern about the reliability of the flow record for G1H004 prior to 1980. For this reason he determined flood classifications based on both the full record and a shorter record after 1980. In this thesis the classification based on the shorter record was used because of the greater level of accuracy of the flow record after 1980. This introduces a higher level of uncertainty, however, with regards to the size of the longer return period floods.

The maximum flow experienced at the study reach within each flood event was estimated by reducing the instantaneous flood peak observed at the downstream gauge by the ratio of the catchment area upstream of the study site to the catchment area upstream of the gauge. The estimated maximum flow rate, average daily flow rate, flood volume (including base flow) and class of flood as determined from the average daily flow rate at the downstream gauge for all the observed flood events during the invertebrate study periods are given in Tables 3.5 and 3.6 (invertebrates were not sampled in the Molenaars river in 2004).

Table 3.4 DRIFT Classification and magnitude if floods based on average daily flows for H1H018 and G1H004 (m^{3}s^{-1})

Class	Recurrence Interval	H1H018 (Molenaars)	G1H004 (Berg) (Short Record)	G1H004 (Berg) (Long Record)
I	Intra annual floods	5.0	3.6	4.3
II		16.0	7.2	9.5
III		31.0	14.5	19.1
IV		61.0	29.0	38.2
V	1 : 2 years	93.7	58.7	76.3
VI	1 : 5 years	146.0	75.3	118.0
VII	1 : 10 years	181.0	78.8	154.6
VIII	1 : 20 years	187.0	85.6	178.0

Table 3.5 Flood events studied in the Molenaars River.

Year	Event Number	Date	Duration (hours)	Max Flow (m^{3}s^{-1})	Avg. Daily Flow (m^{3}s^{-1})	Volume (Mm^3)	DRIFT Class
2003	1	10th July	33	5.6	3.7	0.41	I
	2	18th July	21	15.2	5.4	0.49	I
	3	25th July	14	8.6	4.2	0.28	I
	4	1st August	26	28.8	8.0	0.95	I
	5	8th August	26	36.5	14.5	1.74	II
	6	18th August	32	140.8	30.3	4.15	III

Table 3.6 Flood events studied in the Berg River.

Year	Event Number	Date	Duration (hours)	Max Flow (m^3s^{-1})	Avg. Daily Flow (m^3s^{-1})	Volume (Mm^3)	DRIFT Class
2004	1	5th June	28	7.3	2.1	0.40	I
	2	14th June	44	84.5	32.6	3.48	IV
	3	26th June	27	4.8	2.6	0.29	I
	4	3rd July	24	11.7	5.7	0.44	II
	5	23rd July	20	61.0	17.7	1.66	IV

3.2.2 Ecological data

3.2.2.i Sampling design

a Molenaars River

The sampling design for the investigation of invertebrate response to floods was the collection of initial baseline invertebrate data from 200 stones and then, following a period of reconditioning of these stones in the river, the collection of two flood-data sets each comprising 50 pre- and post-flood samples, illustrated in Figure 3.2.

Invertebrate collections from all stones were made at the start of the study (the Baseline survey) and the stones then returned to their precise location, following which recolonisation with stream fauna was possible. The timing of the Baseline study was aimed at minimising possible temporal differences in invertebrate assemblages that might not be due to flood disturbance, but also allowing the initially sampled stones sufficient time in the river to become 'reconditioned' before the onset of floods in the river. A criterion of 30 days after initial sampling was considered to represent the time required for re-establishment of the baseline condition.

The validity of this approach is based on the assumption a) that the sets of stones being compared were representative of the invertebrate assemblage in the river and b) that recovery of the invertebrate fauna had occurred prior to the first flood that was to be examined. These assumptions were tested by examining the extent to which the four sub-sets of the baseline stones shown in Figure 3.2 were representative of the whole community at the start of the study period. Secondly, the sampling of a subset of 50 of the baseline stones before each the two floods allowed for the establishment of a control for those sampled after the flood in the event that recovery from initial sampling was not complete.

Not all stones marked for the stone-movement monitoring and hydraulic model calibration were used for the invertebrate study, partly because many of the systematically selected stones were immovable and / or emergent boulders. Whilst the majority of samples were taken from whole (i.e. movable) stones, some samples were taken from the upper surfaces of large submerged boulders (i.e. immovable stones), as some taxa tend to dominate on these kinds of surfaces and their fate during a flood was also of interest. Each set of fifty stones repeat-sampled before or after a flood was randomly selected by generating random numbers from the individualised numbers assigned to each stone, within these two categories of 'whole' and 'immovable' stones.

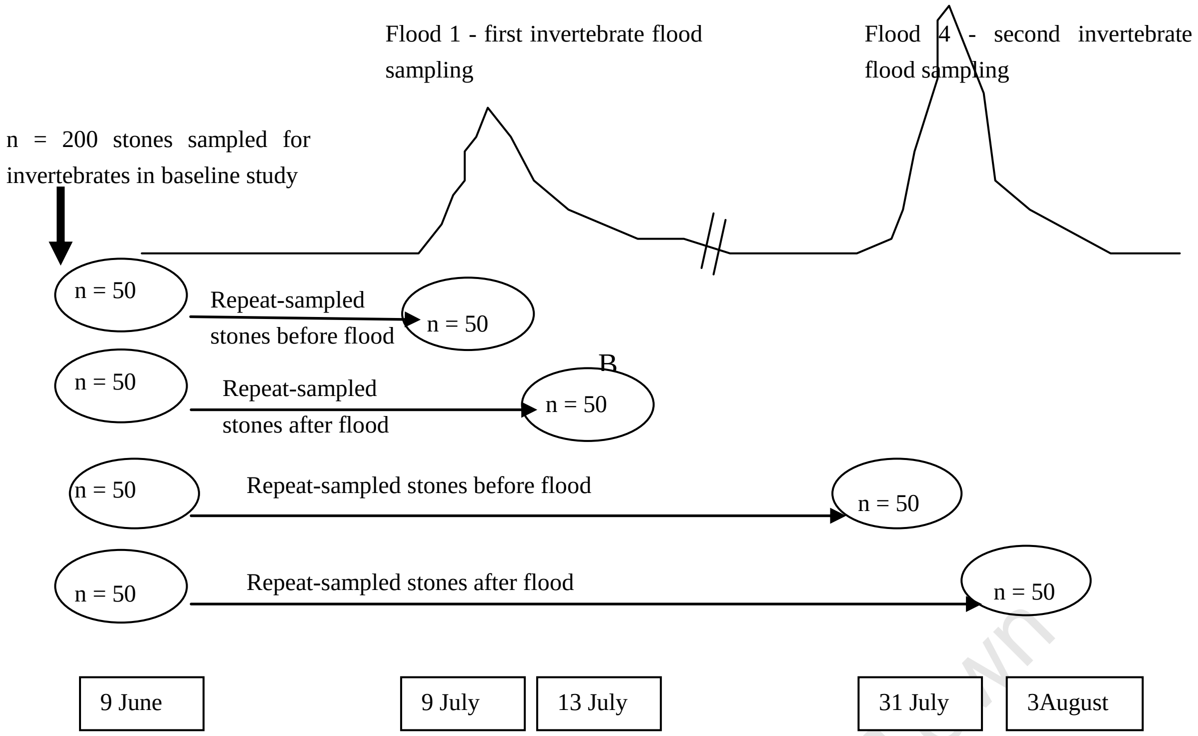

Figure 3.2 Design of invertebrate sampling in the Molenaars River flood response study in 2003.

For the Molenaars River study, 200 out of the 345 marked stones were sampled for invertebrate fauna in May 2003, the Baseline survey. This sample size allowed for two floods to be sampled for invertebrate responses, within the repeat-sampling, pre-and-post flood approach described above. The first flood of the season was a small flood which occurred on 10 July, one month following baseline sampling (Figure 3.3), in what was regarded as an unusually dry winter season. .

The second flood used to measure invertebrate responses was in fact the fourth of the winter season, as the forecasted rainfall for the second and third flood were not high and it was wished to obtain data for both a small and a larger flood event. Despite this, the fourth flood was also not substantial, with larger floods occurring only late into winter. By late winter, however, the Baseline stones had all been sampled either before or after Flood 1 or Flood 4, and no further data were collected.

Due to time limitations, only 30 stones from each set of 50 sampled stones were actually processed and analysed. Of the 200 Baseline stones collected, those that corresponded to the 30 stone numbers that were repeat-sampled before and after the first flood were processed. Of each subset of 30 stones processed, between three and seven stones were representative of stone tops (immovable boulders), with the remainder being whole sampled stones.

b Berg River

For the Berg River invertebrate study in 2004, a different design was necessitated, because of difficulties with field conditions. The initial Baseline sampling was conducted from 17 - 22 May 2004, earlier than that undertaken for the Molenaars River the previous year, in an attempt to ensure sufficient time for reconditioning and recolonisation of the stones, but nevertheless minimising possible temporal differences

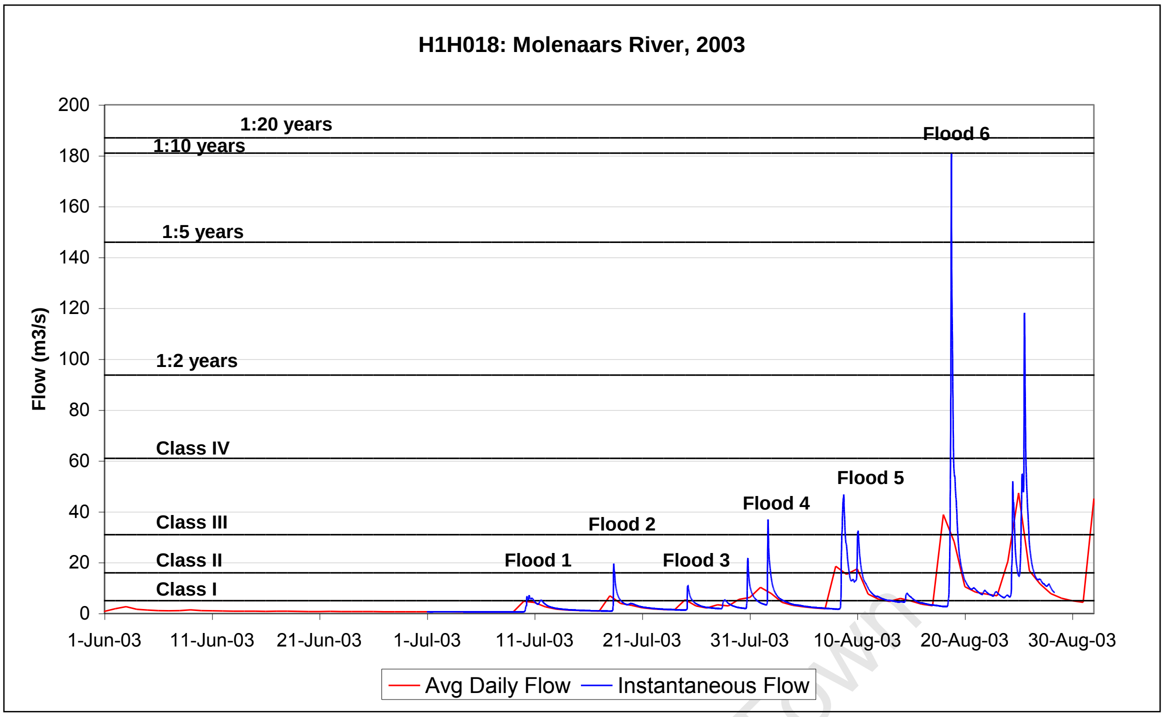

Figure 3.3 Flood events observed on the Molenaars River (H1H018) in 2003. Stone movement data were collected after all six floods, whilst invertebrate sampling was undertaken before and after Flood 1 and Flood 4. The equivalent DRIFT flood classes are indicated (see section 3.2.1).

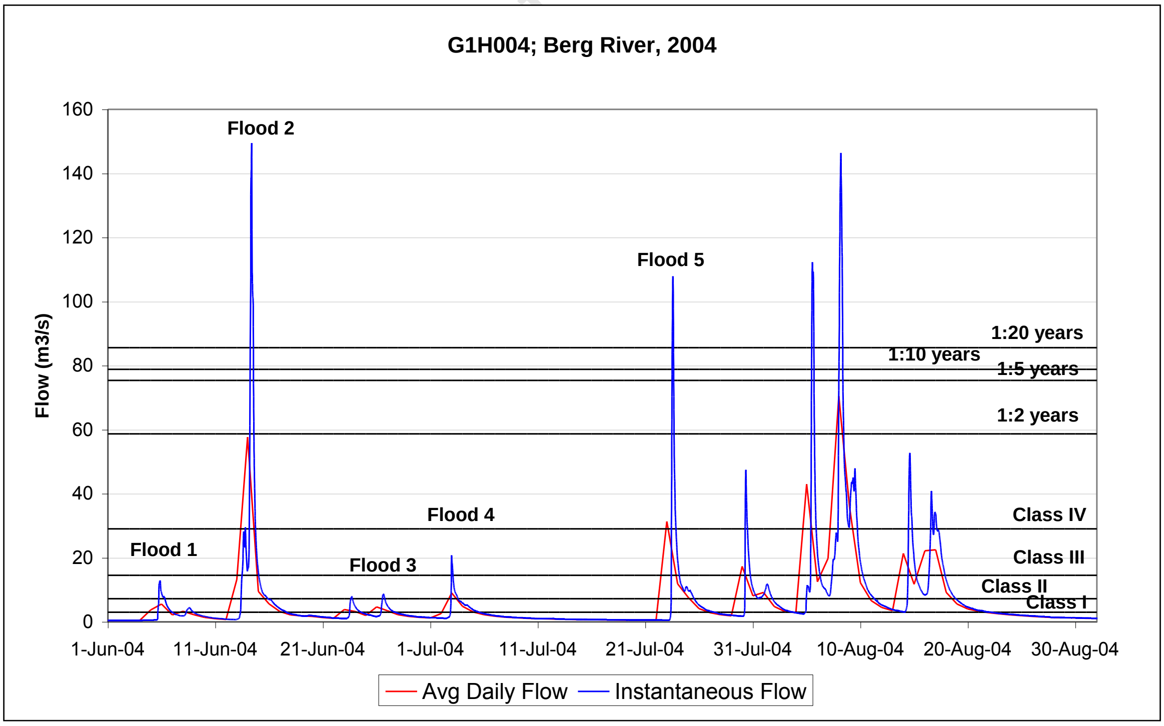

Figure 3.4 Flood events observed on the Berg River (G1H004) in 2004. Stone movement data were collected after all five floods, whilst invertebrate sampling was undertaken in May and again only after Flood 5. The equivalent DRIFT flood classes are indicated (see section 3.2.1).

in invertebrate assemblages that would otherwise result from sampling earlier in the season. Sampling in the Berg River also included whole stones and the upper surfaces of immovable stones, as in the Molenaars River.

A small flood occurred in the Berg River in early June 2004, followed immediately by a large one (DRIFT Class 4) on 14 June (Figure 3.4). This was judged as far too soon after the Baseline survey to allow for meaningful sampling to be conducted, as the effects of the initial denudation of stones would override or confound any flood-disturbance effects. A large proportion of stones were also washed away, thus reducing the biological dataset from 220 to fewer than 100 stones, only 61 of which were whole stone samples.

No pre- and post-flood sampling could thus be conducted on the data set, as was done for the Molenaars River, because of the reduced sample set of available stones and the fact that stones were deemed not to have been adequately reconditioned prior to the onset of floods. Instead, invertebrate sampling was conducted immediately following the next large flood, which was the fifth flood of the season (July 2004), two months after the Baseline survey. The final data set, therefore, comprised repeat-sampled stones with different disturbance histories, depending on whether or not they were moved by the floods. With few exceptions, which were excluded for the data analysis, stones either remained stationary, moved in the second flood (the largest for the study period), or moved in both the second and fifth floods.

An additional data set of invertebrate (and periphyton) samples from unmarked stones was also collected during the Post flood survey, with some 58 of these being whole stone samples and 20 representing stone top surfaces from immovable stones. This data set was intended to act as a control for the possible sampling effects associated with repeat-sampling the Baseline set of stones. This control set was necessary because the immediate Pre-flood control data could not be collected from the Berg River.

The dimensions of the additional stones, their position along each cross-section, and point depth and velocity at 0.6 m at each stone were recorded, so that the likely disturbance history of the stone could be determined based on the hydraulic model. Thus, the additional stones were identified as ‘probably unmoved’, ‘probably moved in Flood 2 only’ and ‘probably moved in Flood 2 and 5’, the same three categories of stone movement as identified for the Repeat-sampled stones. In addition, some of the additional stones fell into the ‘immovable stone tops’ category. Table 3.7 summarises the different stone categories identified for analysis.

3.2.2.ii Field methods

Invertebrates only, and both invertebrates and periphyton were collected from individual stones in the Molenaars and Berg Rivers respectively. A hand net (mesh size 125 μm), with aperture area of 300 X 300 mm was held downstream of the stone, close to the stream bed. The stone was then lifted free of the bed, and held in the mouth of the net, whilst the area upon which the stone had rested was gently brushed to collect invertebrates associated with the sampled stone that might have retreated to the stream bed as the stone was lifted. Brushing was done carefully to cause as little disturbance to the surrounding stones as possible, to avoid over-sampling the area upon which the stone had rested. A lead-weighted fish marker with a numbered floating cork was used to demarcate the position of the stone. Thereafter the net and stone were raised close to the water surface to prevent accidental sampling of any other animals dislodged from the bed. Invertebrates were removed from the stone as gently as possible by hand and with a toothbrush and / or forceps to minimise the amount of periphyton dislodged in the process. Sampling of

Table 3.7 Categories of stones sampled for invertebrates in the Berg River in 2004.

Stone category	Description of sampling frequency and nature of disturbance to stones	Number in category
Whole stones sampled		
1	Sampled in May Baseline survey; unmoved in all floods; re-sampled in July after 5th flood	26
2	Sampled in May Baseline survey; moved in Flood 2 but not thereafter; unmoved in 5th flood; re-sampled in July after 5th flood.	14
3	Sampled in May Baseline survey; moved in Flood 2 and subsequent floods, also moved in Flood 5; re-sampled in July after 5th flood.	21
4	Additional stones sampled for the first time after 5th flood - movement status derived from hydraulic model as unmoved	26
5	Additional stones sampled for the first time after 5th flood - movement status derived from hydraulic model as moved in Flood 2 but not thereafter	14
6	Additional stones sampled for the first time after 5th flood - movement status derived from hydraulic model as moved in Flood 2 and subsequent floods, also moved in Flood 5	18
Top surfaces of stones sampled (too large / embedded to sample fully)		
7	Paired stones, sampled in May Baseline survey; unmoved in all floods; re-sampled in July after 5th flood	24
8	Additional stones sampled for the first time after 5th flood - movement status in 5th flood derived from hydraulic model as probably unmoved	20

medium and large stones required two people. All stones on the Molenaars and on the Berg River respectively were sampled by the same person (this author) to minimise potential error associated with sampling effort. Notes were also made of the orientation and degree of packing around the stone, and every effort was made to replicate these conditions when the processed stone was returned to its position.

After removal of invertebrates, the stones were placed on large white plastic trays, scrubbed with a toothbrush and rinsed with river water in order to remove periphyton growth attached to the stone. The standard 2 minutes scrubbing time recommended for cobble sizes by Biggs & Kilroy (2000) was modified to take into account the large range in stone particle size at the site. Scrubbing time was thus scaled from 1 minute for small cobble (60 - 90 mm x-axis) to 18 minutes for small boulder (400+ mm x-axis). In all cases however, stones were scrubbed until no change in the colour of rinsing water was evident. The resulting periphyton slurry (usually approximately 200 ml) was stored on ice in the field and transferred to a freezer within 12 hours of collection. Periphyton samples were only taken from whole stones, not from the tops of immovable boulders.

The dimensions of each stone were measured as the longest axis (x), the longest horizontal axis perpendicular to x (y) and the longest vertical axis of the stone (z). The surface area of each stone was calculated using a regression equation relating the stone dimensions to stone area, derived from sixty stones collected from the Berg River. For these stones, both x, y, and z dimensions and the surface area were measured, the latter by covering them precisely with metal foil, which was then weighed. The foil area was calculated from the known weight per unit area of foil. The values obtained for surface area in cm^2 were converted to m^2.

The resulting regression ($r^2 = 0.93$) between the measured dimensions of a stone and its surface area was:

$$\text{Stone area (in cm}^2\text{)} = 0.014x + 33.819$$

where x = the sum of the multiples of measured axes, xy+xz+yz (in mm^2).

3.2.2.iii Laboratory methods

The invertebrate samples were fixed in 4% buffered formalin in the field. Upon return to the laboratory the samples were strained through a 250 µm sieve and transferred into 70% alcohol for preservation. Invertebrates were separated from the debris collected at the same time, and identified to a range of taxonomic levels, in part based on available expertise and on a presumption of the likelihood of different species-specific flood responses. Thus Trichoptera were identified to species or morpho-species, based on the best taxonomic keys available. Ephemeroptera were identified to genus (for the Baetidae) or species (other Families). Simuliidae and Blephariceridae (Diptera) were each represented by only one genus. Chironomidae were identified to sub-Family or Tribe, since these correspond generally with differences in size and feeding guild. Other Families, including Notonemouridae (Plecoptera) and all the Coleoptera, were identified to Family level only, based on morphological similarities between species and / or the difficulty of separating young instars.

Invertebrate identifications were performed using Nikon SMZ 1500 and SMZ 1B dissecting microscopes, an Olympus compound microscope and numerous field guides and taxonomic keys (de Moor 2002; Barber-James & Lugo-Ortiz 2003; de Moor & Scott 2003; Mansell 2003; Samways & Wilmott 2003). All animals were measured with a calibrated graticle inserted into the microscope eye-piece. The head width was used as the unit of measurement, as it is most often regarded as the most stable measure correlated with specimen age.

Each periphyton sample was divided into two equal portions, in order to determine two different measures of periphyton, the ash free dry weight (AFDW) of the periphyton on a rock, and the concentration of Chlorophyll *a*, both expressed as a density in mg m^{-2} of stone surface.

To obtain AFDW, the periphyton slurry of each half-sample was filtered onto Whatmann GFF 4 glass fibre paper, dried to a constant weight at 60°C and then ashed in an oven at 400°C for 4 hours. The difference between the ashed and dry weights represents the total organic content of the periphyton of each sample.

The eukaryotic photosynthetic component of periphyton is indicated by chlorophyll a mass (Biggs & Kilroy 2000). For each sample, chlorophyll was extracted with methanol, boiled at 70°C for 3 minutes to increase extraction efficiency and to fix the chlorophyll by destroying the enzymes (Biggs & Kilroy 2000). Absorbance was measured at a wavelength of 665 nm with a spectrophotometer. Background absorbance was measured at 750nm.

Finally, after invertebrates had been removed from the preserved samples in the laboratory for identification and enumeration, the remaining debris associated with each sample was dried to a constant weight at 60°C and burnt at 400°C for 4 hours, in order to determine the total content of organic and inorganic matter associated with each sample point. The values for organic / inorganic matter associated with the stone were divided by half the stone surface area to approximate an amount of matter per m^2 of the river bed associated with each stone.

3.2.3 Data analysis

3.2.3.i Overview

The first step in the analysis was to identify patterns in the Baseline (pre-flood) invertebrate data, and to quantify their relationship to stone periphyton, organic matter associated with stones and/ or and physical attributes at each stone. The extent to which these relationships were maintained or altered by floods was one of the key objectives of this study. A combination of uni- and multi-variate statistical methods was used. All multivariate statistics were produced using PRIMER Version 6. for Windows (Clarke & Warwick 1994) and the univariate statistics were produced using STATISTICA Version 7, 2007 (Statsoft Inc. U.S.A.).

In addition, a test was done to assess potential difference between sub-sets of the Baseline invertebrate samples from the Molenaars River, to establish the representativeness of the invertebrate community as a whole, of each sub-set of approximately 23 - 28 stones, which represented the size of each set of the whole stones sampled during the repeat-sampling on one of four future pre- or post-flood occasions. Also, recovery of invertebrate community structure on initially sampled stones, prior to flooding, was measured by comparing Baseline stones (9 June) with those taken during the first Pre-flood sampling (9 July).

The movement of stones during a flood was expected to constitute a higher level of disturbance to invertebrates than non-movement. This hypothesis was tested by examining the change in invertebrate composition on moved versus unmoved stones, relative to the Baseline or Pre-flood condition. Statistical methods for this analysis included correlation analysis, analysis of variance and multi-variate cluster analysis.

A second hypothesis was that the magnitude of force acting on individual stones would be related to the degree of population reduction of invertebrates, because areas experiencing lower applied power might act as refugia, and accumulate individuals eroded from areas experiencing high hydraulic forces during a flood. To explore this, a correlation was sought between the change in densities of invertebrate taxa on each stone after a flood and the applied stream power acting on that stone during the flood. It was further hoped that this might indicate various thresholds of applied forces at which different taxa become disturbed.

Finally, changes in population structure were investigated by comparing pre- and post-flood size frequency distribution data for selected individual taxa.

3.2.3.ii Multivariate statistics

A group of computer-based programs specifically developed for multivariate and statistical analyses of multispecies data was used to investigate the relationships between samples at each sample time, and between samples taken before and after floods. These programs collectively form the software package PRIMER (Plymouth Routines in Multivariate Ecological Research) Version 6, developed at the Plymouth Marine Laboratory, United Kingdom (Clarke & Warwick 1994). Multivariate analysis has the advantage over univariate methods (e.g. ANOVA) of maintaining much of the complexity of community-based biological data, as the comparisons of samples are based on the extent to which they have particular taxa in common and at the same levels of abundance. PRIMER is particularly useful as a tool to display the relationships between biological samples when the taxa-by-samples arrays are large, and thus patterns in community data not readily apparent (Clarke & Warwick 1994).

The PRIMER routines follow an initial computation of similarity coefficients between each pair of samples to produce a triangular similarity matrix. A 4th-root transformation of invertebrate data is recommended for comparison of samples from different sites or treatments, in order to dampen the effect of the most dominant taxa on the similarity matrix, whilst also being invariant to scale change (Field *et al.* 1982). For this flood study, the benthic invertebrate data were square-root transformed, because density differences were considered as important as species differences in before- and after-flood comparisons. The Bray Curtis coefficient of similarity does not take account of joint absences when computing similarity between pairs of samples, and, because many of the taxa recorded during this study were absent from many of the samples (e.g. as a result of seasonality in occurrence or because they occur only rarely in the benthos), this measure was chosen for the analysis (see Field *et al.* (1982) and Clarke & Warwick (1994) for a discussion on the relative merits of similarity coefficients).

A number of routines in the PRIMER package were used to test for significant differences between groups of samples identified *a priori* (ANOSIM), to represent relationships between samples/times (CLUSTER analysis and MDS ordination), to identify taxa contributing to these differences (SIMPER), and to examine the relationship between groups of invertebrates sampled and the environmental variables measured as they were collected (BIOENV). These are described briefly.

a Analysis of Similarities

ANOSIM is a multivariate approach to the analysis of similarities between groups of samples that are defined *a priori*, which compares the average similarity of all pairs of samples within a group to the average similarity of pairs of samples corresponding to replicates from different groups. The resulting test statistic, R ranges from -1 to 1, but usually falls between 0 and 1, since a negative R-value denotes that samples within the designated group are less similar to each other than to samples from other groups, which would be unlikely (Clarke & Warwick 1994). The closer to 1 the R-value, the greater the difference between groups, whilst an R = 0 denotes no difference between groups.

The probability of the R-value being significantly different from zero is computed by comparing the results computed with those generated from randomly defined combinations of the chosen groups. Clarke & Warwick (1994) caution against falsely rejecting the null hypothesis of no difference between groups in multiple tests (i.e. pairwise comparisons in ANOSIM), suggesting a conservative probability, or p-value be used to avoid potential Type 1 errors associated with multiple comparisons. In these analyses therefore, a p-value, of 0.01 is used as a threshold for significance, implying a 1% risk of false conclusion.

ANOSIM is a useful tool for testing the relevance of groupings assigned to the samples prior to any classification techniques such as CLUSTER or MDS. Examples from this study would be the testing of whether pre-flood and post-flood samples are significantly different, whether samples in different flow types represent distinct invertebrate assemblages, or whether assemblages on stones that have been physically disturbed are different from those on stones that have not. However, care should be taken when interpreting the biological relevance of very low R values, even should these be statistically significant, as they imply that the factor being tested (e.g. flood effect) is only a weak discriminator of these groups.

b Cluster analysis

Hierarchical cluster analysis fuses samples that have the highest similarities into distinct groupings, and then joins groups into larger clusters at progressively lower levels of similarity, to produce a plot of the natural similarities between groups of samples, irrespective of any group-identification assigned to them *a priori*. The results are represented in a dendrogram, with the x-axis representing the samples and the y-axis the level of similarity at which successive groupings are formed. Of the various hierarchical sorting strategies available, group-average sorting was used because it joins two groups of samples together at the average level of similarity between all members of one group and all members of the other, and so considers natural variability between samples (Clarke & Warwick 1994).

Although dendrograms have the advantage of clustering samples into distinct groups, the cut-off levels for the groupings are arbitrary and there are some disadvantages to this approach, for example, cluster analysis attempts to group samples into discrete clusters and may hereby tend to over-emphasize discontinuities, forcing a continuum or graded series into discrete classes. This makes it advisable to employ an additional method of presentation of the group relationships, e.g. ordination, which displays group inter-relationships on a continuous scale (Clarke & Warwick 1994).

c Ordination

The non-metric multidimensional scaling method of ordination (non-metric MDS) was performed, using the PRIMER program MDS (Clarke & Gorley 2001). The purpose of non-metric MDS is to construct an ordination of the sites or samples, in a small number of dimensions, in this instance 2 dimensions for ease of display, by interpreting some function of the similarity/dissimilarity measure between each pair of sites as Euclidean distance. It is an iterative procedure (nine iterations were used) where a starting map of the sites is constructed in the required number of dimensions, and the configuration is perturbed in a direction which decreases the stress to an acceptable minimum. A coefficient of stress provides an indication of the distortion involved in compressing the data: a stress coefficient < 0.05 gives an excellent representation of the data, with no prospect of misinterpretation; a stress value < 0.1 indicates a good ordination; stress <0.2 gives a potentially useful two-dimensional plot, although interpretation of the data should be complemented by an alternative technique. A stress coefficient > 0.2 indicates an unreliable representation of the relationships among samples.

d Determining discriminating taxa

The SIMPER (Similarity Percentage) routine in PRIMER was used to identify the taxa most responsible for the groupings of samples defined through the cluster and ordination techniques, or where ANOSIM identified significantly different groupings that were considered to be biologically relevant. SIMPER is computed on the initial triangular similarity matrix in PRIMER. This analysis produces a breakdown of average s*imilarity within* groups (i.e. representative taxa of each group), as well as a breakdown of average *dissimilarity between* groups, into differential contributions from the various taxa, ordered in decreasing contribution. Thus the n taxa contributing the first 50 % of dissimilarity between 2 groups could, for example, be identified in this way. The SIMPER results also include a term indicating, for each discriminating taxon, the ratio between the average dissimilarity of that taxon between the two groups, and the standard deviation of that dissimilarity. In other words, a taxon with a high ratio of average / standard deviation would indicate that it is consistently dissimilar (across all the samples within a group) and thus a good discriminating species between the two groups.

e Linking environmental and biological data

The BIOENV routine was used to identify the variable or combination of variables that is able to discriminate between samples in the same manner as was achieved using invertebrate data. The input files to this analysis consist of a set of environmental (e.g. physical or chemical) data, as well as the invertebrate similarity matrix with which the environmental variables are to be matched. A Euclidean distance similarity matrix is the general method used for environmental variables, compared to the Bray-Curtis similarity matrix for invertebrates.

The BIOENV output is a Spearman coefficient, or weighted Spearman coefficient, ρ_w, which lies in the range of –1 to 1, the value of which indicates the strength of the match between environmental variables and invertebrate samples. Values around zero indicate an absence of a match between any single or combination of environmental variables to the invertebrate matrix, whereas 1 indicates a complete match and –1 complete opposition. A negative value is unlikely, however, because of the constraints within similarity matrices. This procedure is similar to that of an iterative correlation between the biotic data and environmental variables that would best match the biotic distribution patterns (Clarke & Warwick 1994).

3.2.3.iii Univariate statistics

A number of basic comparative statistics were calculated to describe attributes of invertebrate assemblages, both before and after floods. Because invertebrate data were collected from stones of differing sizes, actual counts - total numbers of invertebrates or total number of species - were expressed as densities per unit area of stone surface, to take into account differences in available surface area.

In the case of species counts (total actual number of taxa on each stone) two comparative measures were calculated, to account for *either* the area of the stone, in which case species density per unit stone area was used, or the total abundance on a stone, in which case rarefied species richness, or number of species per 100 individuals, was used. The concept of rarefied species richness is recommended by McCabe & Gotelli (2001) as a better measure of true species richness than simple species density, since the greater the number of animals in the sample the more chance there is of finding additional taxa. Samples with large numbers will almost always have greater species densities, but lower rarefied species richness - the chances of one species encountering another will greatest where the rarefied species richness is highest. These measures therefore represent slightly different ecological ideas.

The difference in invertebrate attributes (for example density, richness) between categories of samples – e.g. biotope grouping or movement status during a flood - was tested for statistical significance using the non-parametric Kruskal-Wallis ANOVA by ranks. Non-parametric ANOVA tests for differences among groups or treatments, based on the ranking of pooled data between and across groups. Where means between only two groups were compared, Mann-Whitney rank sum test was used, where the null hypothesis is that the two groups come from populations with identical distributions. The test calculates a T-test statistic based on the sum of the ranks, and pairs were considered significantly different at a P-value of ≤ 0.05 as in all other statistical tests used throughout. These tests were chosen because the data did not meet one or both of the assumptions of normality (Kolmogorov-Smirnov test) or equal variances (Levene's test) that were required for the use of parametric statistical testing.

Where Kruskal-Wallis tests were significant, pair-wise differences between groups were explored using Dunn's Pairwise multiple comparison procedure.

Pearson correlation as well as regression analysis was used to assess relationships among variables. The former measures the strength of the linear relationship between two variables, and is compared with the null hypothesis that no linear relationship exists between them (correlation coefficient, r, is zero). The regression coefficient of determination (R^2) is a measure of how much the dependent (y) variable may be explained in terms of the independent (x) variable.

Correlation analysis requires that the assumptions of independence between variables as well as normality in distribution are met. In order to normalise data, 4th-root transformation (invertebrate data) and log transformation (periphyton and organic matter) of data was performed. Other physical data were not transformed, unless explicitly indicated.

Finally, population structure was investigated by comparing frequency distributions of individuals within invertebrate head width size classes. All individuals of each species or taxon were measured and included in the analysis. Population structure changes in the pre- and post- flood surveys were investigated, as well as potential differences in the size distribution of members of a species that remained on moved vs. unmoved stones after a flood. These size-frequency distributions were visually interrogated to interpret population characteristics - for example, to investigate whether floods might affect one portion of a population of a given species more than another. Statistical differences between data sets were examined using a Kolmogorov-Smirnov test for significant differences between frequency distributions.

3.3 RESULTS OF THE BASELINE INVERTEBRATE STUDY

3.3.1 Substratum characteristics

Overall stone size was somewhat larger in the Molenaars River (mean stone dy = 319 mm, median = 265 mm, n = 344) than in the Berg River (mean stone dy = 275 mm, median = 228mm, n = 432). This may have been influenced by the greater proportion of large boulders in the Molenaars River. The Berg River had more gravel and small cobble than the Molenaars River (Table 3.8 and 3.9). Immovable boulders, whose tops were sampled for invertebrates, were larger than in the Berg River (although the actual surface area sampled for invertebrates in each case was the same for most immovable stone samples).

Table 3.8 Bed particle characteristics for the Molenaars River site, Baseline survey 2003. G = gravel, SC = small cobble, LC = large cobble, SB = small boulder; LB = large boulder, as defined in Table 3.3.

Stone Size Classification	**Total**	**G**	**SC**	**LC**	**SB**	**LB**
Max size in class (mm)		64	161	256	514	1000
Total Number of Stones	344	4 (1%)	91 (26.5%)	73 (21%)	111 (32.5%)	65 (19%)

Table 3.9 Bed particle characteristics for the Berg River site, Baseline survey 2004. Definitions are as per Table 3.8.

Stone Size Classification	**Total**	**G**	**SC**	**LC**	**SB**	**LB**
Max size in class (mm)		64	161	256	514	1000
Total Number of Stones (%)	432	27 (6%)	120 (28%)	92 (21%)	139 (32%)	54 (13%)

Depth and velocity measured on each sampled stone were variable, but the Molenaars River was associated with higher velocities on both whole and immovable stones (Table 3.10). The amount of organic matter associated with sampled stones in the Molenaars River was less than half that in the Berg River, although variability was very high, an indication of the patchy distribution of this food resource. In the case of immovable boulder tops, this measure of associated organic matter reflects a density of organics associated with the upper surface area of the stone and not particulate matter trapped underneath the stone or in interstitial spaces as is the case with whole stones.

Table 3.10 Means (standard deviation) of physical characteristics and availability of organic matter and periphyton, associated with stones sampled for invertebrates in the Molenaars and Berg River Baseline surveys.

Attribute	**Molenaars River**		**Berg River**	
	Whole stones n=59	Immovable (tops only) n=9	Whole stones n=61	Immovable (tops only) n=24
Particle size (dy, in mm)	186.0 (96.0)	502.2 (182.2)	252.0 (90.5)	411.8 (140.1)
Water depth (mm)	0.21 (0.15)	0.06 (0.03)	0.09 (0.09)	0.14 (0.08)
Average water column velocity (m s^{-1})	0.24 (0.20)	0.35 (0.23)	0.16 (0.17)	0.16 (0.11)
Associated organic matter (g AFDW m^{-2})	4.08 (7.04)	0.12 (0.15)	10.32 (11.98)	0.92 (0.99)
Periphyton AFDW (g m^{-2} stone surface)	not sampled		0.47 (0.39)	not sampled
Periphyton as Chla (mg m^{-2} stone surface)	not sampled		1.96 (1.38)	not sampled

3.3.2 Invertebrate assemblages

3.3.2.i Comparison of the Molenaars and Berg River invertebrate samples

A range of invertebrate community measures is presented in Table 3.11. The mean invertebrate count per stone in the Molenaars River was double that in the Berg River. This measure does not take into account differences in available surface area, but since stone sizes in the two rivers were approximately the same, (barring differences in large boulders which were sampled separately from cobbles, and barring sand and gravel which was not sampled), total invertebrate **density** (number of individuals per unit stone area) was also significantly higher (Mann-Whitney test, $p<<0.0001$), on average more than double, in the Molenaars River than in the Berg River: trout farms in the headwaters of the Molenaars River have been implicated in low-level chronic nutrient enrichment of its waters (Brown 1996), whilst the Berg River reflects the natural oligotrophic state of Western Cape rivers. In addition, the invertebrate density on the top surface of immovable stones was considerably higher in the Molenaars than the Berg River (Table 3.11).

Despite this probable nutrient enrichment, the species count (total actual number of taxa on each stone) averaged approximately 18 in both rivers (Table 3.11), with somewhat higher species density in the Molenaars River. Species density on the top surfaces of stones was similar in both rivers and, expectedly, lower than that on whole stones, reflecting the fact that many benthic invertebrate taxa are only associated with the lower and under surfaces of river stones.

Table 3.11 **Means (standard deviation) of invertebrate community measures on whole stones and immovable boulder tops in the Berg and Molenaars Rivers during their respective Baseline surveys.**

Attribute	**Molenaars River**		**Berg River**	
	Whole stones n=59	Immovable (tops only) n=9	Whole stones n=61	Immovable (tops only) n=24
Invertebrate abundance (average no. indiv. per stone)	653.5 (489.5)	664.2 (642.0)	350.2 (248.2)	112.3 (71.5)
Invertebrate density (no. indiv. m^{-2} stone surface)	5119.5 (3209.1)	4734.1 (2032.2)	2625.2 (2502.9)	1425.1 (1201.3)
Species count (# taxa per stone)	18.2 (5.6)	9.0 (5.3)	18.3 (4.8)	6.5 (3.6)
Species density (# taxa m^{-2} stone surface)	199.1 (154.2)	81.1 (42.1)	158.9 (109.7)	88.6 (70.1)
Rarefied species richness (# taxa per 100 indiv.)	4.4 (3.5)	1.8 (0.8)	8.7 (7.6)	6.9 (3.6)

Rarefied species richness (species count per 100 individuals) was significantly higher in the Berg River than the Molenaars River (Mann-Whitney test, p<<0.0001). This indicates that even though animal densities in the Molenaars River could be expected to be double those in the Berg River, the chance of inter-specific encounters would be greater in the case of the Berg River.

In terms of their faunal composition, the most striking difference between the Molenaars and Berg Rivers from the baseline survey was the dominance of Baetidae in the former and of Chironomidae in the latter (Figure 3.5). Collectively, these two families comprised about 75% of the invertebrate density in each river. Other differences were the virtual absence of Blephariceridae in the Berg River, which situation persisted throughout the study period, and the greater proportion of Coleoptera, mainly Elmidae, in the Berg River than in the Molenaars River. The high invertebrate density on the tops of immovable boulders in the Molenaars River was in part a reflection of these taxonomic differences, since tops of immovable boulders were dominated by Baetidae and *Elporia* spp. (Blephariceridae) in that river.

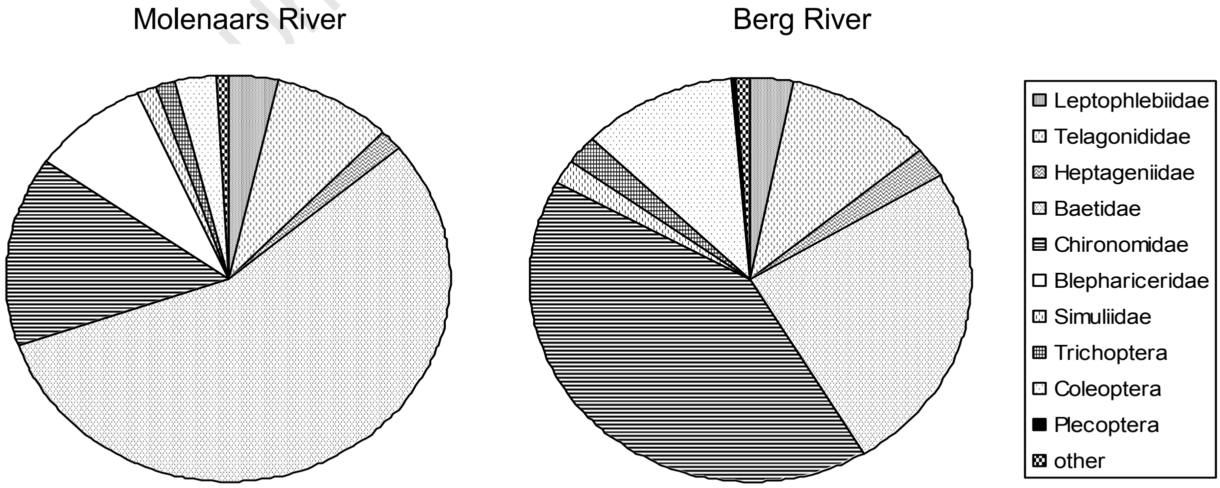

Figure 3.5 **Proportions by density of major insect Families and Orders in the Molenaars and Berg Rivers during the Baseline surveys.**

3.3.2.ii Multivariate analysis of the relationship between invertebrate assemblages and physical variables

Invertebrate assemblages in the Molenaars River at the start of the flood season, in June 2003, were fairly well differentiated by hydraulic biotope (Figure 3.6), with a gradation in assemblage structure from the slower-flowing pool, through shallow backwaters and slow runs, to fast runs and riffles. The single pool biotope sample was most dissimilar from the remaining samples, separating from these at 30% similarity (Figure 3.6a). The moderate stress level (0.15) on the MDS plot suggests that it is a reasonable representation of the relationship between samples. Nevertheless, the groupings identified from the hierarchical cluster analysis (Figure 3.6a) are superimposed upon the MDS plot (Figure 3.6b). The three categories of run biotope, usually described in terms of rippled surface flow, were subjectively determined according to their visual characteristics (how marked the ripples were) and differences between these sub-categories were most ambiguous, with the exception of fast run which grouped well with riffle biotope.

ANOSIM performed on the groupings of invertebrate samples, grouped according to their *a priori*-assigned biotope, indicated strong pairwise differences, particularly between backwaters and riffle / fast run biotopes (Table 3.12, italics), but less so between categories of run. Pairwise differences between biotopes that were represented by single samples were not examined (i.e. pool, vegetated run and vegetated backwater). Based on the cluster analysis and MDS, four main biotope clusters were identified, indicated by dashed lines in Figure 3.6b, and subjected to SIMPER analysis in order to identify those taxa most characteristic of biotope cluster, as well as those which best discriminated between biotope clusters. The species differences between the biotope clusters are presented in Table 5.6, both as the taxa most characteristic of each biotope cluster as well as those most consistently responsible for the dissimilarity between these clusters. The Dissimilarity / Standard deviation ratio in Table 5.6 indicates the consistency with which various taxa discriminate between biotopes (see section 3.4.2iv).

Table 3.12 Results of a 1-way ANOSIM testing for differences in invertebrate assemblages between *a priori*-defined hydraulic biotopes in the Molenaars River, June 2003 (baseline survey). Only significant pairwise comparisons are presented.

Groups	R Statistic	p-Value
GLOBAL SAMPLE STATISTIC	0.42	0.001
backwater - riffle	*0.941*	*0.001*
backwater - fast run	*0.669*	*0.001*
backwater - run	0.477	0.001
slow run - riffle	*0.747*	*0.001*
slow run - fast run	0.397	0.001
run - riffle	0.437	0.002
riffle - fast run	0.086	0.05

Biotope clusters had between 54 and 66% within-group similarity, with the best differentiated group comprising fast run and riffle samples (Figure 3.6, Table 3.13). Pairwise comparisons between biotope clusters showed low average dissimilarities overall, emphasising that invertebrate assemblages respond to a gradient of change in hydraulic conditions rather than forming discrete units. Differences between biotopes were represented by changes in species composition in some cases, but more often by differences in density of taxa over the range of biotopes. Biotope preferences for a number of species may be inferred

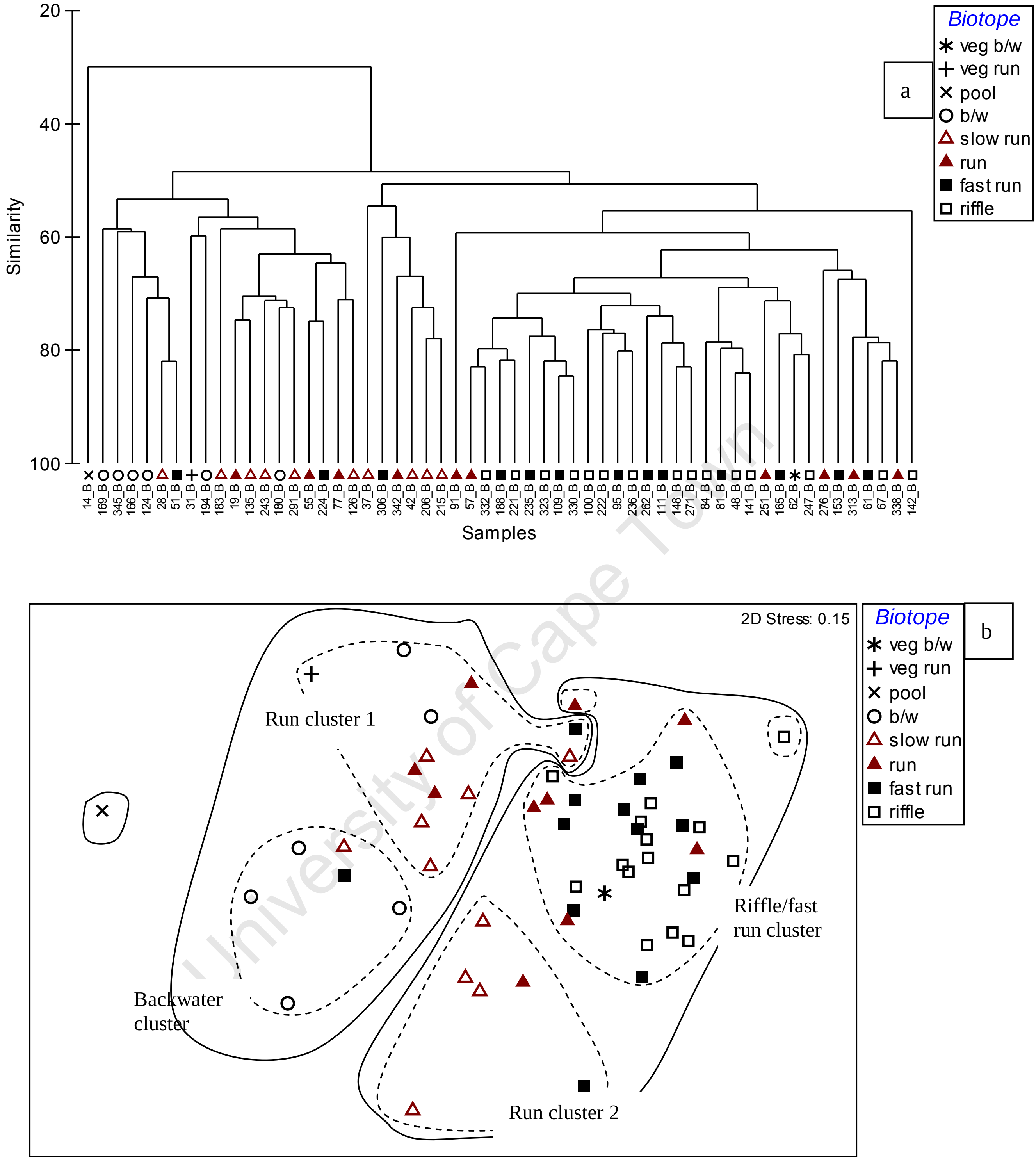

Figure 3.6 Molenaars River June 2003 invertebrate samples, a) hierarchical cluster dendrogram, based on Bray Curtis similarity coefficients, b) MDS plot with the main groupings defined by cluster analysis delineated by ellipses. Sample numbers in Figure 3.7a refer to actual numbers of individually marked and numbered stones; B- indicates Baseline sample.

from these results, for example, *Afroptilum* sp (Baetidae, Ephemeroptera), *Aprionyx* spp. and *Euthralus* sp. (Leptophlebiidae, Ephemeroptera) were largely restricted to backwaters and Run cluster 1, and poorly represented in fast runs / riffles; *Elporia* spp. (Blephariceridae, Diptera) and *Demoreptus capensis*

Table 3.13 Condensed SIMPER results for each biotope cluster from the cluster analysis and MDS of Molenaars River samples in June 2003. The taxa contributing to 60 % of within-group similarity are shown, as well as the taxa that were best discriminators between biotope groupings (high Diss / SD ratio). Densities are re-transformed from the square root used in the analysis to No. m^{-2}, for ease of interpretation. In the pairwise comparisons, the average density of discriminator taxa is provided in turn for each biotope cluster in the order in which it is listed. The pool biotope is not included in the analysis since it was represented by only one sample. *D. capensis* = *Demoreptus capensis*; *L. penicillata* = *Lestagella penicillata*.

Biotope cluster from MDS (average % similarity within biotope cluster)	**Taxa contributing to 60% of within-cluster similarity**	**Pairwise comparisons (average % dissimilarity between biotope-clusters)**	**Top discriminator taxa between pairs of biotope-clusters**	**Average density in each biotope cluster (# m^{-2})**	**Diss / SD ratio**
Backwater (53.5%)	*Afroptilum* sp. Orthocladinae *L. penicillata* *Euthralus* sp.	Backwater vs. Run 1 (49.15)	*Afroptilum* sp. *Aprionyx* spp. Tanypodinae *Afronurus* sp.	2039 / 117 34 / 0 33 / 8 56 / 33	2.03 2.07 1.90 1.70
Run 1 (62.48 %)	*Baetis* spp. *L. penicillata* *Afroptilum* sp. Orthocladinae *Euthralus* sp.	Backwater vs. Run 2 (46.66)	Orthocladinae *Aprionyx* spp. *L. penicillata* Tanytarcini	218 / 1589 34 / 1 205 / 673 5 / 67	1.75 1.72 1.63 1.58
		Backwater vs. Riffle/fast run (58.11)	*D. capensis* *Afroptilum* sp. *Elporia* spp. *Baetis* spp. *Aprionyx* spp. Tanypodinae	1 / 338 2131 / 37 4 / 662 175 / 2035 34/ 1 79 / 19	2.56 2.31 1.89 1.86 1.83 1.61
Run 2 (62.05 %)	*Baetis* spp. Orthocladinae *Afroptilum* sp. *L. penicillata*	Run 1 vs. Run 2 (52.80)	Athericidae Orthocladinae Tanytarcini Tanypodinae *L. penicillata* *Afronurus* sp.	8 / 3 70 / 1590 0.5 / 67 8 / 65 185 / 673 33 / 40	2.39 1.95 1.91 1.69 1.62 1.61
Riffle / fast run (66.32 %)	*Baetis* spp. *Elporia* spp. *D. capensis* *L. penicillata*	Run 1 vs. Riffle/fast run (49.31)	*D. capensis* *Elporia* spp. Athericidae Tanytarcini *Baetis* spp.	13 / 338 25 / 662 8 / 6 0.5 / 42 455 / 2035	2.26 1.84 1.68 1.61 1.56
		Run 2 vs. Riffle/fast run (48.26)	*D. capensis* *Elporia* spp. Orthocladinae	23 / 338 28 / 662 1590 / 308	1.81 1.68 1.57

(Baetidae, Ephemeroptera) dominated in fast runs and riffles. No taxa were exclusive to only one cluster, and only a few taxa were unrepresented in one or more biotopes. Examples of the latter are *Caenis* spp. (Baetidae, Ephemeroptera) absent from the fast run / riffle; and Simuliidae (Diptera), *Chimarra* sp., and *Athripsodes bergensis* complex (Philopotamidae and Leptoceridae, respectively, both Trichoptera) absent from backwaters.

Comparing the similarity between samples generated by the invertebrate data with that generated using seven continuous-data hydraulic variables calculated from field measurements (the BIOENV routine), revealed a fairly weak correlation, with the most relevant hydraulic variables being mean water column velocity, particle size (dy), shear stress and applied stream power (Table 3.14). The other hydraulic variables used were depth, Froude number (Fr) and Reynolds number (Re).

In the Berg River in May 2004, the most obvious trend associated with invertebrate patterns was that of a gradual shift in assemblage composition across hydraulic biotopes (here represented by flow type only; Figure 3.7), although this was not as clear as in the Molenaars River in June 2003. No distinct biotope groupings were suggested by the scatter plot, and although an ANOSIM to test for significant differences in assemblages between flow types was significant (Global R = 0.32, p = 0.001), few pairwise differences were strong (low R-statistic, Table 3.15) or significant below a p-value of 0.01 (see section 3.4.2i for conservative p-values required for pairwise tests in ANOSIM).

The moderate stress level (0.15) on the MDS plot (Figure 3.7) indicates a reasonable representation of the relationship between samples. Given the spread of samples and the lack of strong clustering within the data, only two groups are superimposed upon the MDS plot. These were identified at the 65% similarity level from the cluster analysis and the dendrogram is thus not shown. These two groups represent a mixed RSF / FRF grouping (i.e. equivalent to the fast run / riffle biotope in the Molenaars River analysis) and a mixed RSF / BPF grouping (i.e. equivalent to the run / slow run biotope in the Molenaars River analysis).

Table 3.14 **BIOENV results to examine which environmental variables best explain the MDS and cluster patterns based on invertebrate assemblages in the Molenaars River, June 2003.** V = mean water column velocity, d(y) = particle size (length of the second longest axis), Sh = shear stress, AP = applied stream power. The combinations of variables that resulted in the highest ρ_w values are in bold text.

Combinations of 2, 3 and 4 variables	Correlation coefficient ρ_w
d(y), V	0.263
d(y), AP	0.263
d(y), V, AP	**0.274**
d(y), V, Sh	**0.274**
d(y), V, AP, Sh	0.269

Table 3.15 **Results of a 1-way ANOSIM testing for differences in invertebrate assemblages between *a priori*-defined flow types in the Berg River, May 2004 (baseline survey).** Only significant (p<0.01) pairwise comparisons are presented. NF = no flow, RSF = rippled surface flow, FRF = fast riffle flow, BPF = barely perceptible flow.

Groups	R Statistic	p-Value
GLOBAL SAMPLE STATISTIC	0.32	0.001
NF - RSF	*0.525*	*0.001*
NF - FRF	0.366	0.008
BPF - FRF	*0.692*	*0.001*

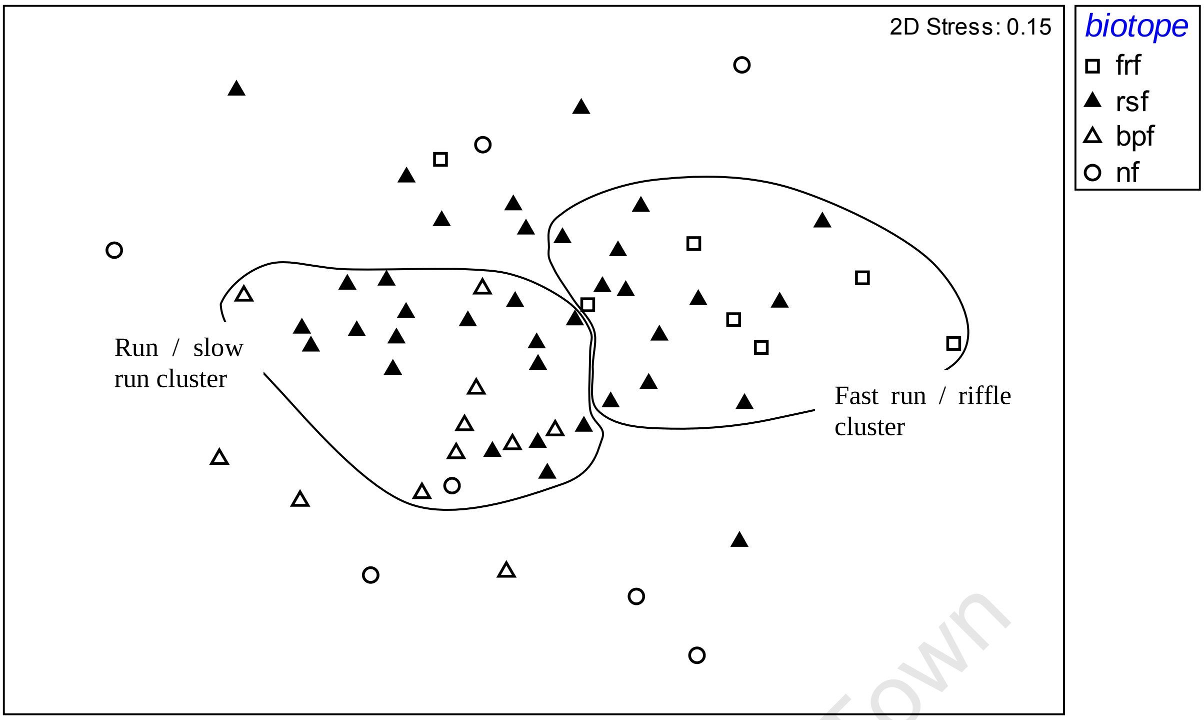

Figure 3.7 Berg River May 2004 invertebrate densities, 4th root transformed, 2-d MDS plot with the main groupings defined by cluster analysis delineated by ellipses.

A SIMPER analysis was run to examine species differences between these two groupings. The results (Table 3.16) show some similarities with the findings on the Molenaars River, although only two biotope clusters were compared in this latter instance. The discriminator taxa for the Berg River clusters were not strong, the relatively low Diss / SD ratios indicating that differences between biotopes were not highly consistent across samples. *Baetis* spp. (Baetidae, Ephemeroptera) and Orthocladinae (Chironomidae, Diptera), the most numerous of the taxa in the Berg River, were simultaneously major representatives of both biotope clusters, although the orthoclads occurred in higher densities in the RSF / FRF cluster (Table 3.16). Simuliidae (Diptera) were largely restricted to the fast run / riffle cluster, and a useful discriminator between the two groups, whilst the predator group Tanypodinae (Chironomidae) and *Adenophlebia* sp. were discriminator taxa dominant in slow run / run biotopes. *Elporia* spp. were absent from the Berg River samples, and *D. capensis* (Baetidae, Ephemeroptera) was present in very low numbers, unlike in the Molenaars River. Nonetheless, *D. capensis* and three Trichoptera taxa were between three and five times more numerous (if patchily so) in fast run / riffle biotopes than in slow runs (Table 3.16). As in the case of the Molenaars River, Leptophlebiidae (*Castanophlebia* sp., *Adenophlebia* sp. *Aprionyx* spp.) indicated a strong preference for slower-flowing biotopes.

The environmental variables used for BIOENV analysis of the Berg River May 2004 data included Chlorophyll a (Chla) and periphyton ash-free dry weights (AFDW), alongside the suite of hydraulic variables of depth, mean water column velocity, particle size (dy), shear stress, Froude number, Reynolds number and applied stream power. These variables individually and in combination were poorly correlated with the invertebrate assemblages: the best correlation from the BIOENV analysis was $\rho_w = 0.168$, using a combination of mean water column velocity, particle size, Chla and AFDW. Thus whilst the multivariate analysis identified a gradation of change in invertebrate species assemblages across visually-defined biotope groupings, there was a poor correlation (BIOENV) between these assemblages and hydraulic measurements associated with each sample.

Table 3.16 Condensed SIMPER results for each biotope cluster from the cluster analysis and MDS of Berg River samples of May 2004. The taxa contributing to 60 % of within-group similarity are shown, as well as the taxa that were best discriminators between biotope groupings (high Diss / SD ratio). Additionally, taxa that were numerically dominant in one or the other biotope cluster are listed. Densities are re-transformed from the square root used in the analysis to No. m^{-2}.

Biotope cluster from MDS (average % similarity within biotope cluster)	Taxa contributing to 60% of within-cluster similarity	Pairwise comparisons (average % dissimilarity between biotope-clusters)	Top discriminator taxa between pairs of biotope-clusters	Average density in each biotope cluster (# m^{-2})	Diss / SD ratio
BPF / RSF (64.9%)	*Baetis* spp. Orthocladinae Elmidae *L. penicillata* Tanypodinae	BPF / RSF vs. RSF / FRF (46.2)	Simuliidae Tanypodinae *Adenophlebia* spp. Orthocladinae.	1 / 88 86 / 20 25 / 0.3 231 / 1962	1.69 1.64 1.58 1.42
RSF / FRF (64.9 %)	Orthocladinae *Baetis* spp. *L. penicillata*	Taxa with 25 -100x higher densities in BPF / RSF	*Afroptilum* sp. *Aprionyx* spp. *Demoulinia* spp. *Castanophlebia* sp.		
		Taxa with 25 -100x higher densities in RSF / FRF	*D. capensis* *Chimarra* sp. *Cheumatopsyche afra* *Cheumatopsyche maculata*		

Univariate relationships between hydraulic biotopes and invertebrate assemblages

Hydraulic variables (depth, velocity, Froude and Reynolds numbers, shear stress and applied stream power), taken individually, did differentiate between some pairs of biotopes (Kruskal-Wallis analysis of variance), but they did not do so consistently. For example, significant differences between riffle/ run and run biotopes were identified on the basis of applied power and of Froude number, but these variables did not discriminate run biotopes from backwaters. Using shear stress, run and riffle/run biotopes were significantly different from backwaters, but not from each other. This inconsistency has also been reported elsewhere (Schael 2005).

The association between invertebrate density and species richness and the various biotopes or flow types was explored using non-parametric Kruskal-Wallis analysis of variance. Mean abundance of invertebrates per stone was higher in riffle and run biotopes and FRF flow types in the Molenaars and Berg Rivers respectively, than in other biotopes or flow types (Table 3.17). However, total abundance per stone does not take into account differences in available surface area. When this is accounted for, total invertebrate **density** (number of individuals per unit stone area) was not significantly different across biotopes.

The total species (or taxon) count per stone as well as species density per unit stone area was not significantly different across biotopes. However, the rarefied species richness was significantly higher in slow runs than riffle biotopes in the Molenaars River in June 2003 and higher in BPF and NF flow types than in FRF in the Berg River in 2004. This indicates that, although the total number of individuals on a stone was higher in riffle or FRF biotopes in both rivers, the chances of an individual of one species on a stone encountering an individual from another species was greater in slow run biotopes. Differences in total invertebrate density, species density and rarefied species richness across biotopes or flow types are shown in Figure 3.9 for the Molenaars and Berg Rivers.

Table 3.17 Kruskal-Wallis analysis of variance between invertebrate community attributes and biotopes or flow types, in the Molenaars and Berg River baseline surveys. H = test statistic. Significant post-hoc multiple comparisons are given, in order of the biotope with the higher value.

Density / richness measure	**H**	**p value**	**Significantly different post-hoc pairwise comparisons**
MOLENAARS RIVER, 2003 – comparison of biotopes			
Abundance (#indiv. on stone)	10.0	0.04	Riffles and runs had higher means but n.s.
Total density (#indiv. m^{-2} stone surface)		n.s.	
Species count (# taxa per stone)		n.s.	
Species density (#taxa m^{-2} stone surface)		n.s.	
Rarefied species richness (# taxa per 100 indiv.),	12.9	0.010	Slow run > riffle (p = 0.020)
BERG RIVER, 2004 – comparison of flow types			
Abundance (# individuals on stone)	9.77	0.020	FRF > BPF (p = 0.043) FRF > NF (p = 0.035)
Total density (#indiv. m^{-2} stone surface)		n.s.	
Species count (# taxa per stone)		n.s.	
Species density (#taxa m^{-2} stone surface)		n.s.	
Rarefied species richness (# taxa per 100 indiv.),	13.62	0.004	BPF > FRF (p = 0.006) NF > FRF (p = 0.020)

At the level of individual taxa, a number of taxa occurred in significantly higher densities in one biotope (Molenaars River) or flow type (Berg River) than in others (Kruskal Wallis analysis of variance and post-hoc pairwise comparisons, Table 3.18). Significant relationships are shown in Figure 3.10 and 3.11 for the Molenaars and Berg Rivers respectively. As with the multivariate analysis (SIMPER results, Table 3.13 and 3.16), discriminating taxa for slow biotopes included *Afroptilum* sp., *Demoulinia* sp., Tanypodinae and a number of Leptophlebiidae (*Aprionyx* sp., *Adenophlebia* sp.), whilst *Demoreptus capensis*, *Baetis* spp., *Cheumatopsyche afra*, Orthocladinae, and *Elporia* spp. were present in higher densities in fast runs and riffles in either or both the Berg and Molenaars Rivers (Figure 3.9 and 3.11). Even these, however, were distributed in gradually increasing or decreasing densities from slow to fast biotopes. For the majority of taxa, however, the overlap in densities across biotopes obscured any preferences.

Table 3.18 Kruskal-Wallis analysis of variance of invertebrate density among biotopes in the Molenaars River and flow types in the Berg River, for selected taxa. Only significant results are shown. H = test statistic. Significant post-hoc multiple comparisons are given, in order of the biotope with the higher densities of that taxon. n.s. = not significant; indiv. = individuals; b/w = backwater; sl. run = slow run.

Density (indiv. per m^2 stone surface area)	**H**	**p value**	**Significantly different post-hoc pairwise comparisons between biotopes or flow types**
MOLENAARS RIVER, 2003 – comparison densities across biotopes			
Aprionyx sp.	13.0	0.011	n.s. (highest mean in b/w)
Choroterpes sp.	10.8	0.029	n.s. (highest mean in b/w)
Adenophlebia sp.	13.2	0.010	n.s. (highest mean in sl. run + b/w)
Afroptilum sp.	28.1	0.000	b/w > fast run; riffle (p < 0.002) sl. run > fast run; riffle (p < 0.016)
Demoulinia sp.	11.7	0.020	n.s. (highest mean in b/w)
Demoreptus capensis	27.0	0.000	riffle > b/w; sl. run; run (p < 0.020)
Pseudopannota maculosa	10.8	0.029	n.s. (highest mean in fast run)
Chimarra sp.	16.2	0.003	n.s. (highest mean in riffle)
Elporia spp.	26.8	0.000	riffle > b/w; sl. run; run (p < 0.020) fast run > sl. run (p < 0.030)
Simuliidae	22.0	0.00	riffle > sl. run (p < 0.001)
BERG RIVER 2004 – comparison of densities across flow types			
Afroptilum sp.	19.3	0.000	BPF > RSF (p = 0.009) BPF > FRF (p = 0.026)
Baetis spp.	12.1	0.007	FRF > NF (p = 0.010
Demoreptus capensis	25.9	0.000	FRF > RSF (p = 0.001) FRF > BPF (p = 0.001) FRF > NF (p = 0.012)
Demoulinia spp.	21.4	0.000	BPF > RSF (p = 0.000) BPF > FRF (p = 0.003)
Aprionyx sp.	16.3	0.001	BPF > RSF (p = 0.007) BPF > FRF (p = 0.004)
Lithogloea harrisoni	14.8	0.002	BPF > RSF (p = 0.012) BPF > FRF (p = 0.014)
Cheumatopsyche afra	13.0	0.005	n.s.
Cheumatopsyche maculata	17.1	0.001	FRF > RSF (p = 0.018) FRF > BPF (p = 0.022)
Simuliidae	16.0	0.012	FRF > RSF (p = 0.045) FRF > BPF (p = 0.001)
Tanypodinae	15.6	0.001	BPF > FRF (p = 0.008) NF > FRF (p = 0.003)
Orthocladinae	14.3	0.003	FRF > BPF (p = 0.012)

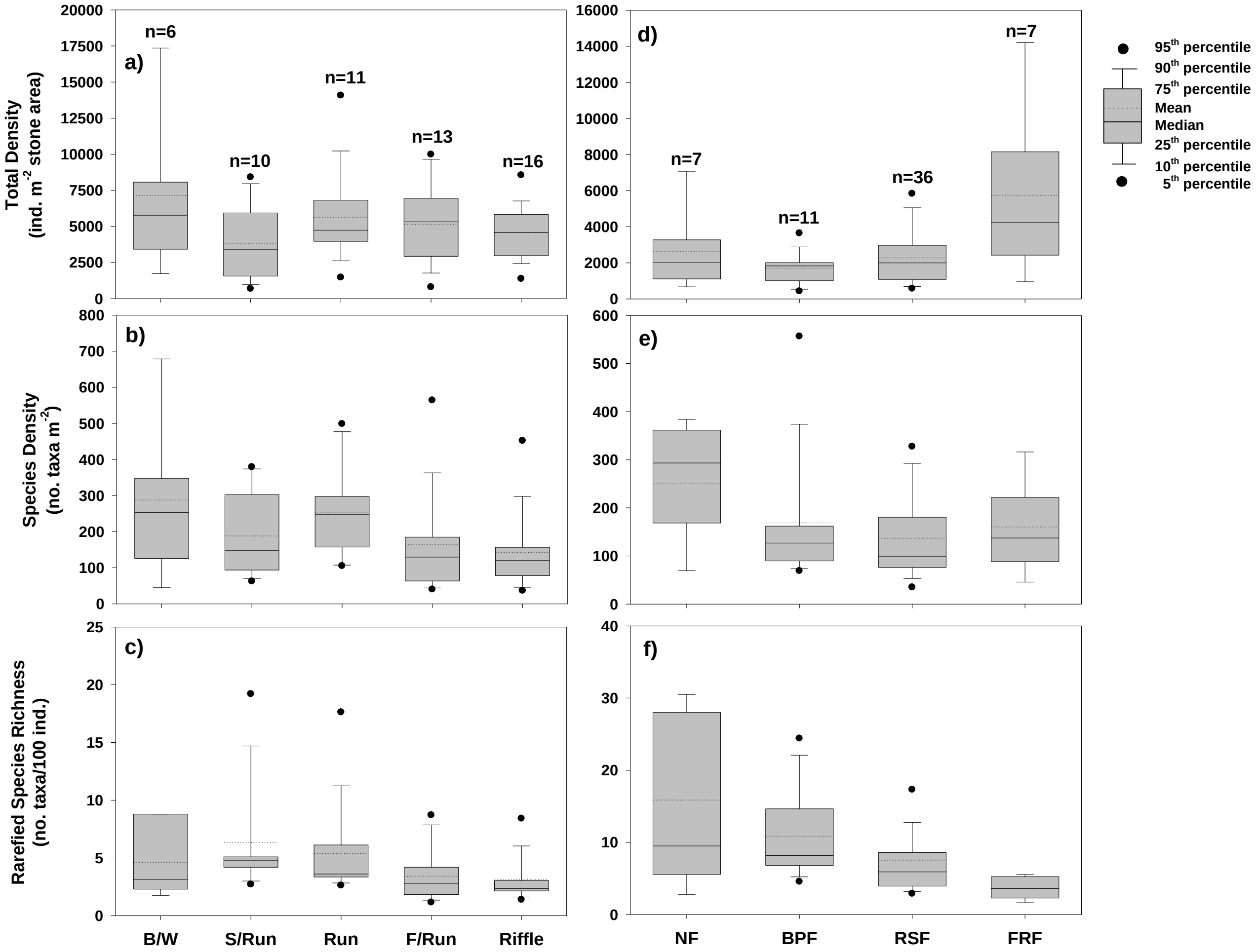

Figure 3.8 **Box plots of a) Total invertebrate density, b) species density and c) rarefied species richness in biotope clusters in the Molenaars and similarly d, e, f) for flow types in the Berg River Baseline surveys.**

Finally, periphyton density and organic matter associated with a sample point are also potentially important factors determining the distribution of invertebrates. Periphyton did not differ among biotopes in the Berg River (no collection was made in the Molenaars River). However, there were significant differences in the relative amount of organic matter between flow types (Figure 3.10) in the Berg River (Kruskal-Wallis H = 12.11, p=0.007), with the slower flowing biotopes having the greatest amount of organic matter. Only pair-wise comparisons between RSF flow and NF were significant (p = 0.03). A similar pattern was evident in the Molenaars River, despite the much lower overall availability or organic matter, although differences among biotopes were not significant, because of the high level of patchiness in distribution of organic matter, even within biotopes.

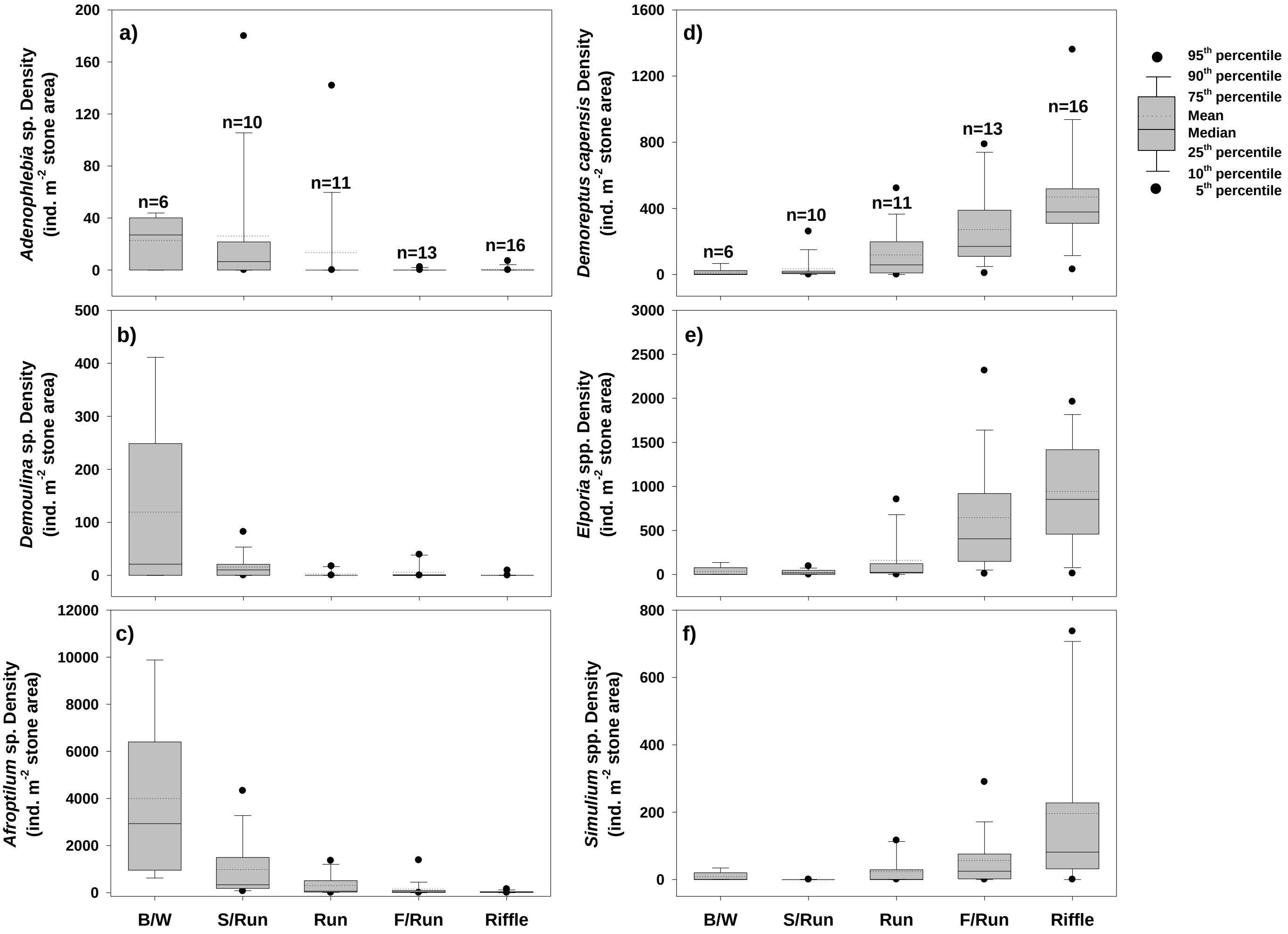

Figure 3.9 **Box plots showing the mean, median and range of densities (number of individuals per m^2 of stone surface) per biotope for selected taxa in the Molenaars River, at the Baseline survey in June 2003.**

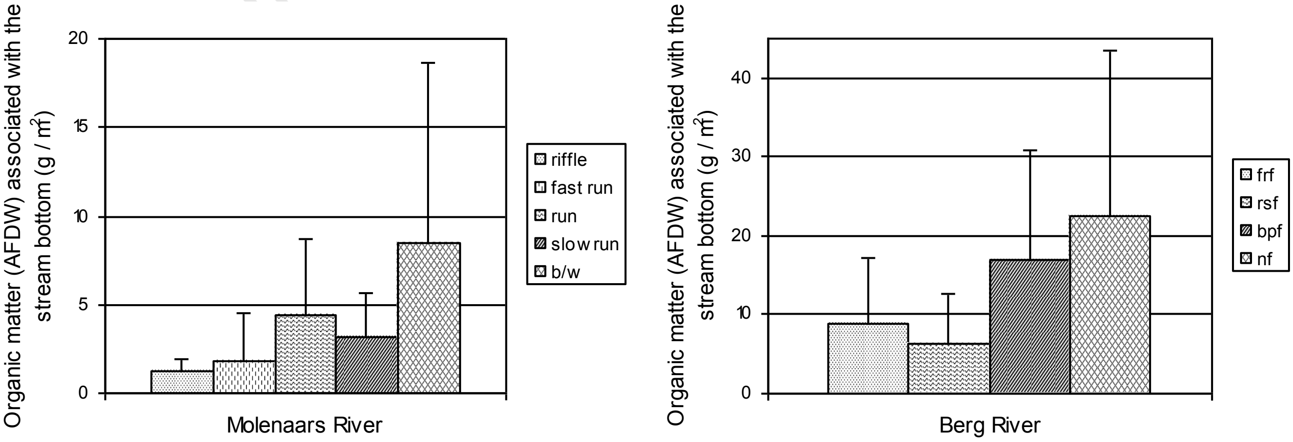

Figure 3.10 **Mean (standard deviation) organic matter associated with sampled stones (AFDW in g m^{-2} of river bed), in biotopes or flow types, in the Molenaars and Berg River baseline surveys.** Note scale differences between the Molenaars and Berg River plots.

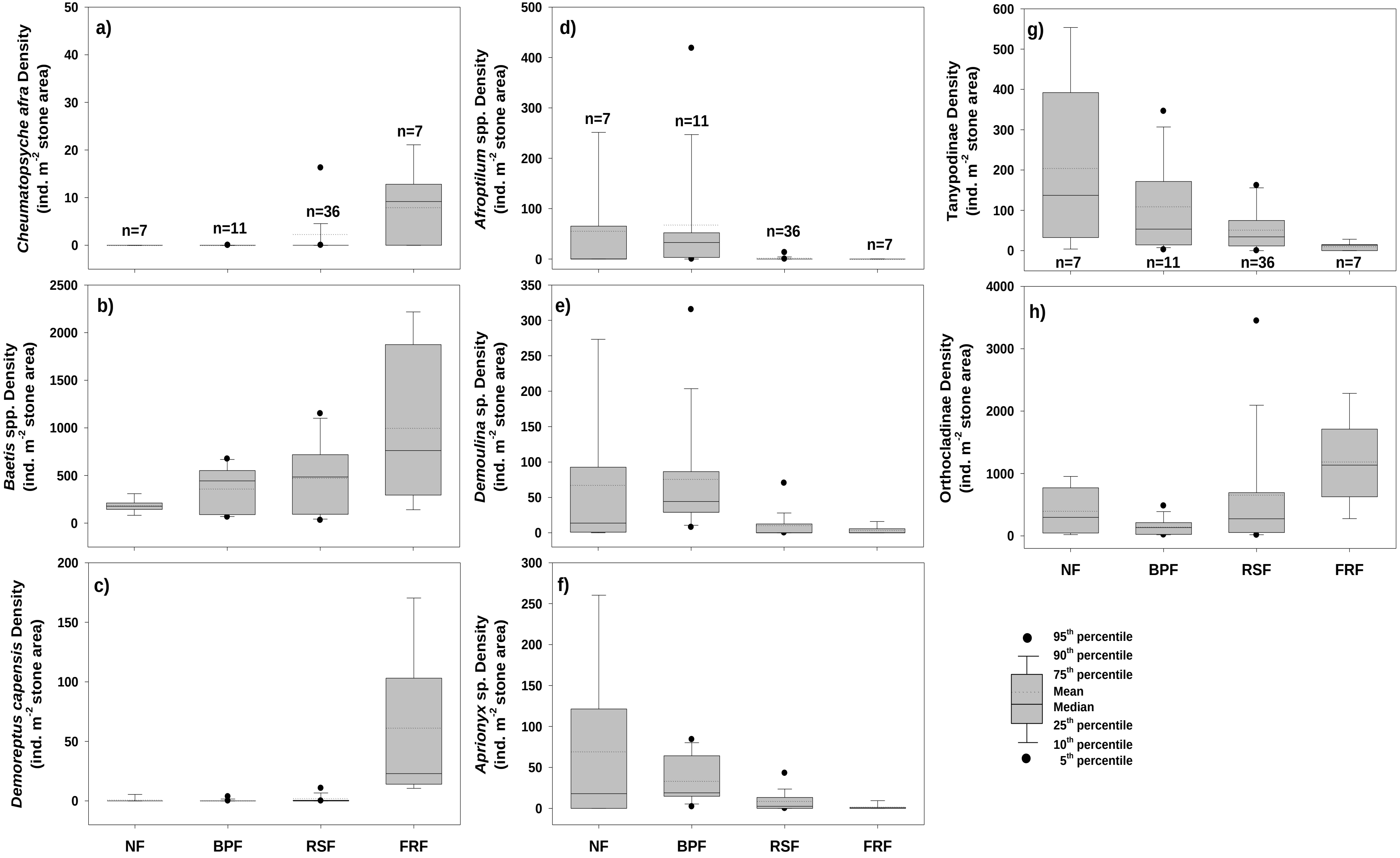

Figure 3.11 Box plots showing the mean, median and range of densities (number of individuals per m^2 of stone surface) per biotope for selected taxa in the Berg River, at the Baseline survey in May 2004.

3.3.2.iii Univariate relationships between invertebrate densities and hydraulic variables

Total invertebrate density was not correlated with either of the measures of periphyton density (Chla and AFDW, Berg River only), or with the amount of organic or inorganic matter associated with a stones, or indeed with any of the hydraulic variables related to flow and depth measured during the baseline surveys in each river in 2003 and 2004 (Pearson's Product Moment correlation on transformed normal data, all -$p > 0.05$).

However, different taxa might be expected to have different density patterns, in response to one or more sometimes opposite driving forces. Each taxon was thus examined separately in order to attempt to identify sets of relationships that might exist prior to flood disturbance, and which might then be used to measure a biological consequence or disturbance effect of each flood.

In the Molenaars River in June 2003, a number of taxa demonstrated density differences in response to velocity, both negative and positive. A second set of responses implicated depth, although among fewer taxa. Finally, the AFDW or organic material associated with stones was highly correlated with distribution differences among many taxa preferring slower flowing waters. In the Berg River survey, a great deal fewer relationships between invertebrate density and abiotic variables were significant than in the Molenaars River, particularly those implicating velocity and depth in affecting invertebrate density distribution.

Summary data reflecting the three main relationships typical of the fauna are indicated in Table 3.19 and Figure 3.12 for both the Molenaars and Berg Rivers. In general, densities of the Leptophlebiidae (*Choroterpes* sp., *Aprionyx* sp., *Adenophlebia* sp.) were negatively correlated with velocity or velocity-based hydraulic indices, and positively correlated with the amount of organic matter associated with the bed, in both rivers, whilst a similar relationship held for some Baetidae (*Afroptilum* sp., *Pseudocloeon* sp.) in the Molenaars River. Most chironomid groups were positively associated with organic matter availability and negatively associated with velocity, with the exception of Orthocladinae in the Berg River which showed a significant positive relationship with velocity. Many taxa that are known to occur on the upper surfaces of stones (i.e. *Demoreptus capensis*, *Baetis* spp., *Elporia* spp., *Agapetus agilis*, Orthocladinae) displayed either or both: a positive correlation with velocity; a negative correlation with organic or inorganic matter associated with a stone (Table 3.19).

Some taxa (e.g. Tanypodinae) displayed consistent relationships with the abiotic variables across both rivers, but others did not. Orthocladinae density in the Berg River, for example, was significantly correlated with velocity in the Berg River but not in the Molenaars River, despite the similar range in velocities on the stones on which they occurred. For many taxa, inadequate representation in one of the rivers precluded the identification of relationships (e.g. Blephariceridae in the Berg River).

Contrary to expectation, few of the individual taxon densities were correlated with either of the measures of periphyton density (Chla and AFDW), this analysis only possible with the Berg River survey. One or both of these measures were, however, positively correlated with overall species density, as well as the densities of the mayfly *Lithogloea* sp., elmid adults, and most chironomid grazers (Table 3.20), although the taxon-specific correlations were weak, with r values generally around 0.3. It is note-worthy that the Leptophlebiidae, which displayed a relationship toward organic matter in the Berg River (Table 3.19), did not do so in the case of periphyton density. This periphyton measure incorporates all organic matter attached and growing on stone surfaces (for example including both algal cells and bacteria), whilst the organic matter associated with the stream bed reflects, in the main, particulate organic matter such as leaf fragments and decaying plant debris.

An important abiotic variable, from the perspective of a study examining bed movement by floods, is that of the stone size. Thus the relationship between stone size and invertebrate assemblages is dealt with separately in section 3.3.2.

Table 3.19 Pearson's correlation coefficients of 4th root transformed invertebrate densities and abiotic variables, for taxa in the Molenaars River (M) in June 2003 and the Berg River (B) in May 2004. Only significant relationships are presented. Results are given as the Pearson's r-value. p-value is indicated as follows: * p <0.05; **p< 0.01; § p<0.001; † p< 0.000. Organic matter data were log-transformed to achieve normality; other variables not transformed.

Responses to depth		**Responses to velocity or hydraulic indices derived from velocity**		**Responses to organic matter**		**Responses to inorganic matter**	
M	**B**	**M**	**B**	**M**	**B**	**M**	**B**
Tanytarcini 0.32 *		Chironomini -0.34 *	Orthocladinae 0.47†	Tanypodinae 0.81 †	Tanypodinae 0.47 §	Tanypodinae 0.85 **	Tanypodinae 0.36 **
A. harrisoni -0.43 **		Tanypodinae -0.32 *	Tanypodinae -0.30*	Chironomini 0.78 §	Chironomini 0.40 **	Tanytarcini 0.34 *	Elmidae 0.39 **
Aeschnidae 0.78 **		*Choroterpes* sp. -0.54 *	*Adenophlebia* sp. -0.38*	*Choroterpes* sp. 0.85 †	*Adenophlebia* sp. 0.39 *	*Choroterpes* sp. 0.72 **	Scirtidae 0.41*
Hydroptilidae 0.56 *		*A. (bergensis)* sp. -0.47 *	*Aprionyx* sp. -0.4 *	*Aprionyx* sp. 0.90 *	*Aprionyx* sp. 0.55 §	*A. (bergensis)* sp. 0.62 *	Athericidae 0.40 *
Hydraenidae -0.61 *		*Afroptilum* sp. -0.56 †		*A. (bergensis)* sp. 0.93 †	Athericidae 0.58 †	*D. capensis* -0.49 *	
		Pseudocloeon sp. -0.37 *		*Afroptilum* sp. 0.76 †		*Baetis* spp. -0.51 *	
		D. capensis 0.65†		*Pseudocloeon* sp. 0.77 §			
		Baetis spp. 0.31 *		*A. harrisoni* -0.67 *			
		Elporia spp. 0.51†		*A. agilis* -0.69**			
				Elporia spp. -0.50*			

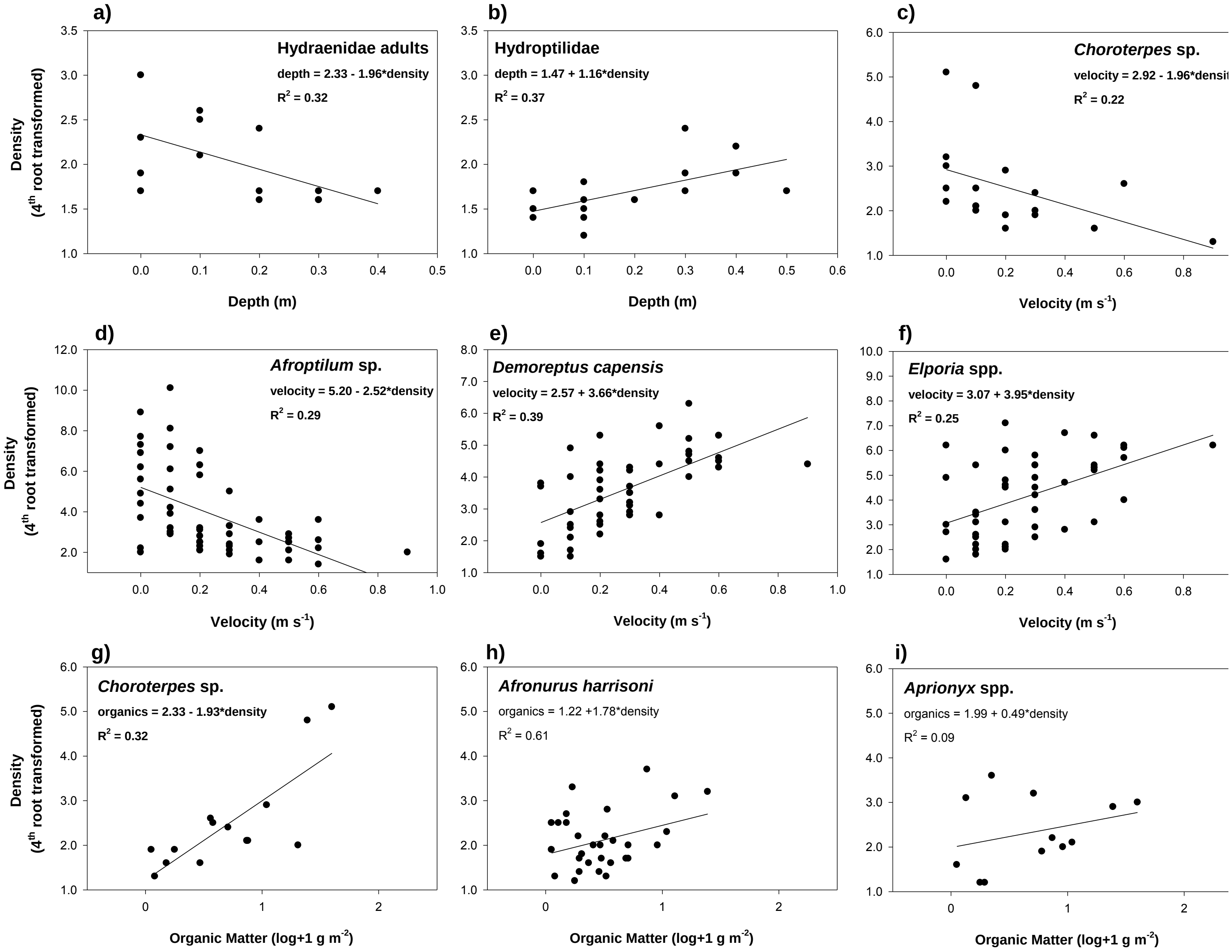

Figure 3.12 Correlation and linear regression of invertebrate density and four abiotic measures: depth, velocity, and the amount of organic and inorganic matter associated with the sample location. Plots a-m are for the Molenaars River in June 2003. Plots n-r are for the Berg River in May 2004 Transformations to obtain normality are as indicated on the graph axes. Statistical significance and correlation coefficients are provided in Table 3.19.

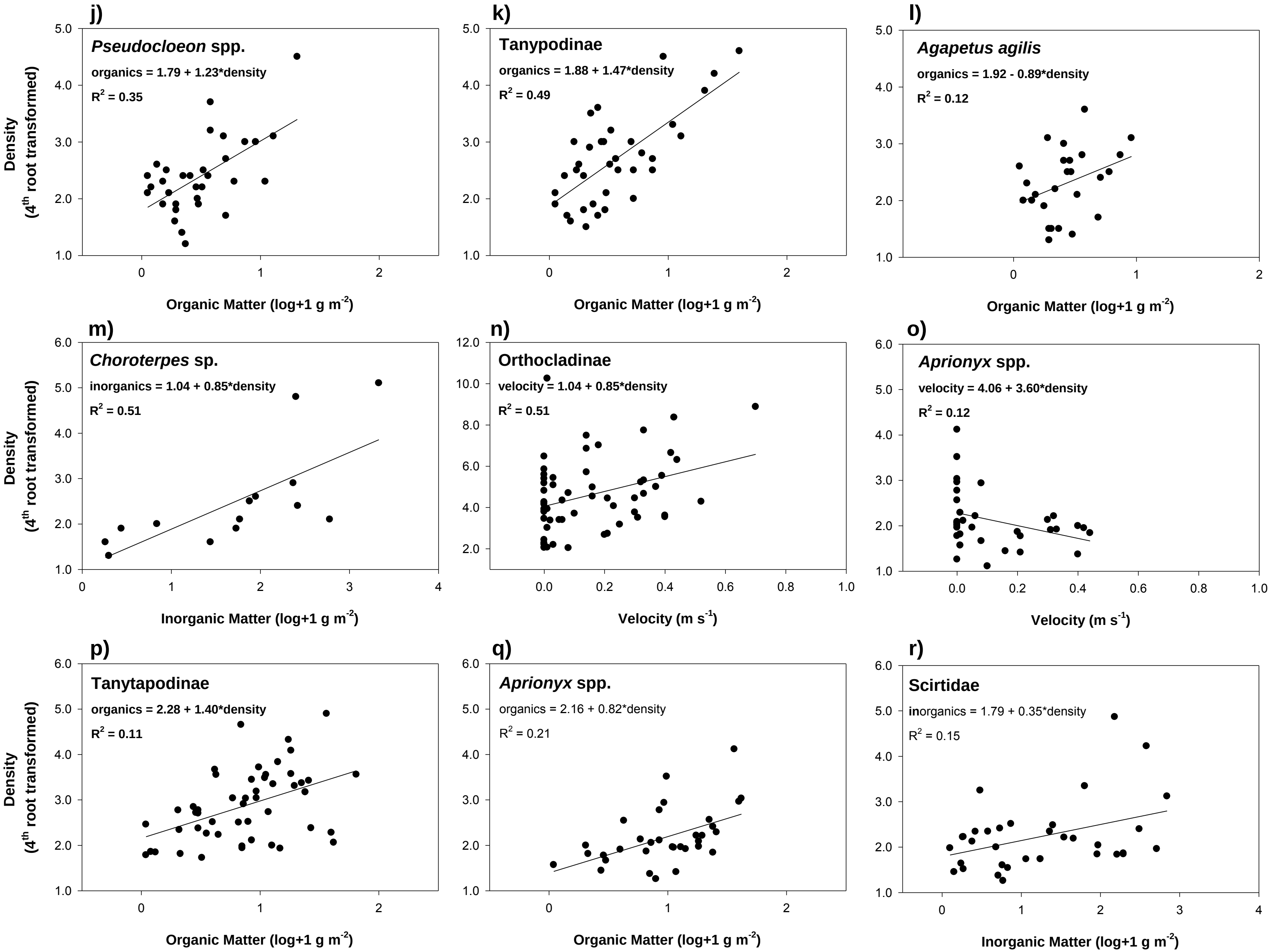

Figure 3.12 cont .Correlation and linear regression of invertebrate density and four abiotic measures: depth, velocity, and the amount of organic and inorganic matter associated with the sample location. Plots a-m are for the Molenaars River in June 2003. Plots n-r are for the Berg River in May 2004 Transformations to obtain normality are as indicated on the graph axes. Statistical significance and correlation coefficients are provided in Table 3.19.

Table 3.20 Pearson's correlation between invertebrate densities (per taxon), invertebrate species density, and periphyton density as Chlorophyll-*a* and as ash-free dry weight (AFDW). Density data were 4th root transformed; periphyton data were log+1 transformed. indiv. = individuals

	Correlation coefficient (r)	**Coefficient of determination (r^2)**	**p-Value**	**n**
Chlorophyll-*a* (mg m^{-2} stone surface)				
Species density (#taxa m^{-2} stone surface)	0.533	0.280	0.000	52
Lithogloea sp. (indiv. m^{-2} stone surface)	0.413	0.170	0.026	29
Elmidae adults (indiv. m^{-2} stone surface)	0.364	0.132	0.048	30
Orthocladinae (indiv. m^{-2} stone surface)	0.351	0.123	0.011	52
Chironomini (indiv. m^{-2} stone surface)	0.338	0.114	0.047	35
Tanytarcini (indiv. m^{-2} stone surface)	0.314	0.099	0.036	45
Periphyton AFDW (mg m^{-2} stone surface)				
Species density (#taxa m^{-2} stone surface)	0.495	0.274	0.009	24
Chironomini (indiv. m^{-2} stone surface)	0.406	0.165	0.015	35
Tanytarcini (indiv. m^{-2} stone surface)	0.333	0.111	0.025	45

3.3.2.iv Possible pre-flood selection of stable stones by invertebrates

Stable stones have been hypothesised to act as refugia for invertebrates during flood disturbance (e.g. Townsend *et al.* 1997c; Francoer *et al.* 1998). Since the baseline samples were taken from stones whose subsequent stability (or movement) during floods was recorded, it provided an opportunity to test whether invertebrates or specific taxa might congregate on stable stones in the immediate pre-flood period, thereby avoiding dislodgement as a result of bed particle movement.

Three categories of stones were identified for each river, based on the degree to which stones sampled during the Baseline survey subsequently moved during floods. In both rivers, stones either remained stable (Category 1), or moved once, in the largest flood of that year (Category 2), or else they moved twice - in the largest and second largest floods (Category 3). Kruskal-Wallis analysis of variance was used to test whether, in the period immediately before the flood season, differences in invertebrate densities on these categories of stones might suggest that they were selecting stable stone particles.

In the Molenaars River in 2003, average total invertebrate density on unmoved stones was 4283 indiv. m^{-2}, lower than the density on the most mobile stones where densities averaged 7171 indiv. m^{-2} (Figure 3.13), although this was not statistically significant (Table 3.21). In the Berg River, the difference between invertebrate densities on the Category 1-3 rocks was significant (Table 3.21) and substantial, with some 3250 indiv. m^{-2} on Category 3 stones, as opposed to 1833 indiv. m^{-2} on Category 1, unmoving stones (Figure 3.14). A similar pattern emerged comparing the species density (taxa m^{-2} stone surface), where Category 3 stones had a higher per unit richness than the larger stones of Category 1 (Figure 3.13) in both the Molenaars and Berg Rivers. The tops of immovable boulders had average densities of even less than this - some 1400 indiv. m^{-2} - although this density represents only that of invertebrates occurring on the upper surfaces of the stones and is not comparable with whole stone samples.

Category 3 stones represent those that would move during the winter period in each river. These were significantly smaller than Category 1 stones (Table 3.21), a large part of the reason that they were moved. Category 2 stones were not significantly different from either of the other stones groups, but nevertheless

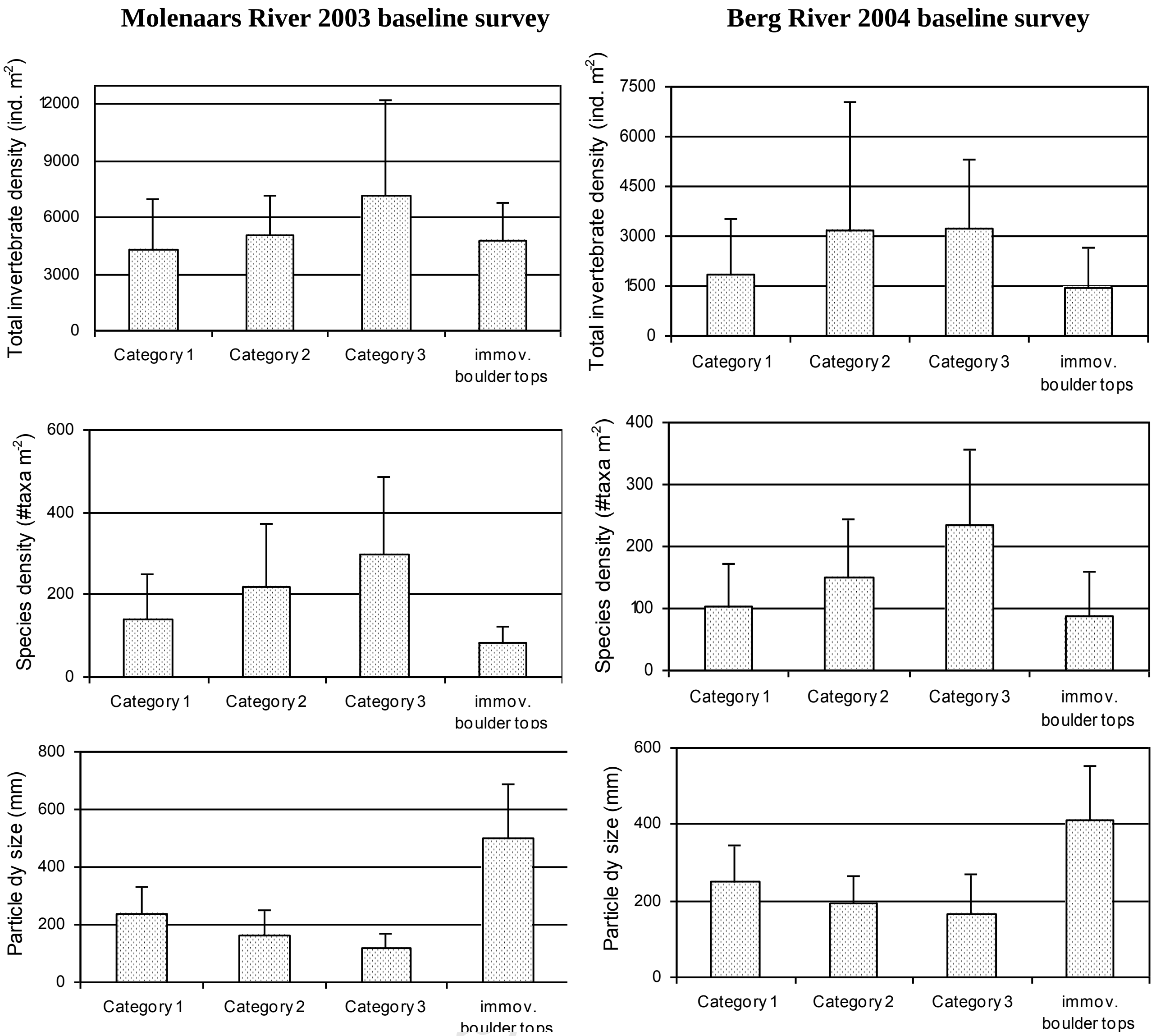

Figure 3.13 **Average (standard deviation) values for a) total invertebrate densities per unit stone area, b) species density per unit stone area and c) particle size dy, for the baseline condition in the Molenaars and Berg Rivers, and comparing four categories of stones.** Stone categories are as described in the text.

Table 3.21 **Kruskal-Wallis analysis of variance between total invertebrate density, species density and particle size, and stone movement category**. H = test statistic. Significant Post-hoc multiple comparisons are also presented.

	H	P value	Significantly different groups
MOLENAARS RIVER 2003			
Total density (# m^{-2})		n.s.	
Species density (# taxa/ m^{-2})	11.8	0.003	Category 1 vs. Category 3 (p = 0.004)
Stone d(y) (mm)	15.2	0.001	Category 1 vs. Category 3 (p = 0.027) Category 1 vs. Category 2 (p = 0.001)
BERG RIVER 2004			
Total density (# m^{-2})	8.0	0.018	Category 1 vs. Category 3 (p = 0.0001)
Species density (# taxa/ m^{-2})	17.0	0.002	Category 1 vs. Category 3 (p = 0.0001)
Stone d(y) (mm)	14.5	0.001	Category 1 vs. Category 3 (p = 0.0005)

moved only during the largest flood of the season in each river. This apparent preference of invertebrates for stones that were in essence more mobile is probably to a large extent a consequence of the fact that invertebrate densities were shown to decrease with increasing stone size and that larger stones generally corresponded to Category 1, unmoving stones. Figure 3.14 depicts the significant negative correlation between invertebrate density and stone particle size, a reasonably strong relationship with r-values of between -0.50 and -0.55. At the level of individual taxa, the majority of taxa in both the Berg and Molenaars Rivers followed this pattern of higher densities (per unit area) on smaller stones (Table 3.22). Figures 3.15 and 3.16 show this relationship for selected taxa.

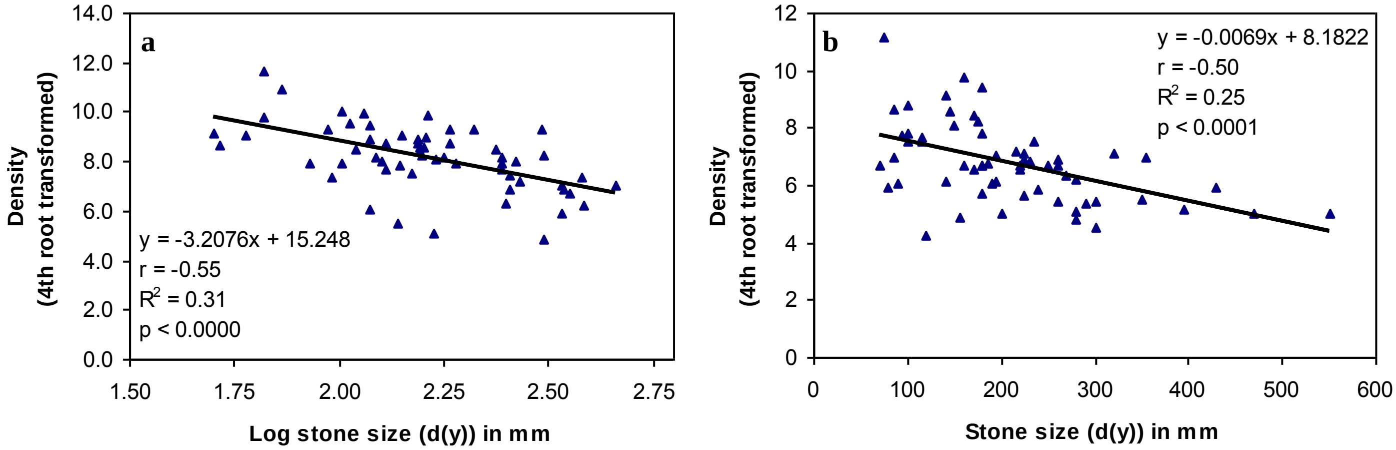

Figure 3.14 Correlation and linear regression of total invertebrate density and particle diameter a) in the Molenaars River in June 2003 and b) in the Berg River in May 2004, based on whole stone samples only. Transformations to obtain normality are as indicated on the graph axes.

Non-significant correlations were returned especially where individual taxa were not represented on many stones. The strength of this relationship varied among individual taxa, from a weak negative r value to a very strong relationship of (r values between -0.60 and -0.98, Table 3.22). The more abundant taxa were, interestingly, not the main drivers of this pattern, for example in the Berg samples, where weak correlations between stone particle size and both orthoclad midges and the most numerous of all the taxa, *Baetis* spp. were found (Table 3.22). Also, a number of taxa often observed to be prevalent on the upper surfaces of stones (e.g. *D. capensis*, *Elporia* spp., *Simulium* spp.) demonstrated a non-significant relationship with particle size. Stronger relationships were generally found in the Molenaars than in the Berg River, but this may in part be a reflection of the higher densities of all invertebrates in the former, making relationships easier to demonstrate.

Another significant relationship was that between stone particle size and the density of periphyton covering the stones surfaces in the Berg River (r = -0.66, p < 0.0001; r = -0.59 p < 0.0001 for Chla and AFDW respectively; Figure 3.17). This relationship appeared for the most part to be operating independently of invertebrate density, since non-significant correlations were returned between invertebrate total density, as a whole, and for most taxa, and either Chla or AFDW, with the exceptions of, *inter alia, Lithogloea* sp. and the grazer chironomids (Orthocladinae, Chironomini and Tanytarcini) as described in section 3.3.2*iv*. Even where a relationship between a taxon and periphyton density was established as significant, it was weak (Table 3.20).

Table 3.22 Results of correlation and linear regression of 4th root transformed invertebrate densities and particle diameter (dy), for most taxa in the Molenaars River in June 2003 and the Berg River in May 2004. Taxa are ordered by highest R^2 values for the Molenaars River. Particle size was log-transformed for the Molenaars River data to obtain normal distributions. P-values in bold font indicate a significant correlation.

	Molenaars River June 2003				**Berg River May 2004**			
Taxon	**n**	**r**	**R^2**	**p**	**n**	**r**	**R^2**	**p**
Aeschnidae	10	-0.98	0.97	**0.000**	sample size too small			
Parecnomina resima	10	-0.80	0.64	**0.006**	20	-0.67	0.45	**0.001**
Afronurus harrisoni	41	-0.78	0.60	**0.000**	44	-0.55	0.30	**0.000**
Notonemouridae	13	-0.77	0.59	**0.002**	31	-0.53	0.28	**0.002**
Hydroptilidae	15	-0.76	0.58	**0.001**	sample size too small			
Athripsodes (bergensis) sp.	23	-0.71	0.50	**0.000**	42	-0.37	0.14	**0.017**
Cheumatopsyche afra	16	-0.69	0.48	**0.003**	sample size too small			
Castanophlebia sp.	13	-0.65	0.42	**0.016**	37	-0.49	0.24	**0.002**
Chironomini	36	-0.62	0.39	**0.000**	43	-0.38	0.15	**0.011**
Tanytarcini	48	-0.61	0.37	**0.000**	54	-0.41	0.17	**0.002**
Lestagella penicillata	57	-0.61	0.37	**0.000**	61	-0.51	0.26	**0.000**
Choroterpes sp.	19	-0.60	0.36	**0.007**	not present			
TOTAL DENSITY	59	-0.56	0.31	**0.000**	61	-0.50	0.25	**0.000**
Lithogloea sp.	sample size too small				36	-0.53	0.28	**0.000**
Pseudocloeon sp.	39	-0.54	0.29	**0.000**	sample size too small			
Acarina	19	-0.52	0.27	**0.022**	26	-0.44	0.19	**0.026**
Aprionyx sp.	16	-0.51	0.26	**0.042**	36	-0.41	0.17	**0.013**
Scirtidae	20	-0.49	0.24	**0.028**	36	-0.32	0.10	0.060
Afronurus sp.	51	-0.48	0.23	**0.000**	51	-0.44	0.19	**0.001**
Orthocladinae	58	-0.47	0.22	**0.000**	61	-0.28	0.08	**0.028**
Tanypodinae	48	-0.45	0.20	**0.002**	53	-0.34	0.12	**0.012**
Elmidae	53	-0.42	0.18	**0.002**	55	-0.36	0.13	**0.007**
Baetis spp.	57	-0.38	0.14	**0.004**	61	-0.30	0.09	**0.019**
Afroptilum sp.	55	-0.36	0.13	**0.007**	18	-0.35	0.12	0.148
Athericidae	34	-0.34	0.12	**0.049**	38	-0.37	0.14	**0.023**
Adenophlebia sp.	15	-0.46	0.21	0.087	32	-0.28	0.08	0.127
Chimarra sp.	15	-0.45	0.20	0.096	18	-0.39	0.15	0.106
Elmidae adults	16	-0.29	0.08	0.285	36	-0.32	0.10	0.060
Agapetus agilis	34	-0.27	0.07	0.128	sample size too small			
Elporia spp.	48	0.26	0.07	0.078	sample size too small			
Euthralus elegans	55	-0.23	0.05	0.086	36	-0.37	0.14	**0.030**
Simuliidae	28	-0.15	0.02	0.450	36	-0.32	0.10	0.061
Hydraenidae adults	14	-0.06	0.00	0.842	12	-0.52	0.27	0.082
Demoreptus capensis	48	0.05	0.00	0.760	18	-0.43	0.18	0.075
Cheumatopsyche maculata	not present				16	-0.41	0.17	0.200

Stone size was negatively correlated with the amount of organic matter associated with each stone, only in the Molenaars River, and then weakly so (r = -0.32, p = 0.0.29).

Finally, particle size did not differ among four biotope clusters in the Molenaars River (Kruskal-Wallis analysis of variance, p= 0.07) or among the four main flow types in the Berg River (p= 0.41). In other words, the relationship between stone particle size and invertebrate density appeared to act independently of any affinity of particular species for, or assemblage differences across, biotope groupings, as described in section 3.3.2*iii*.

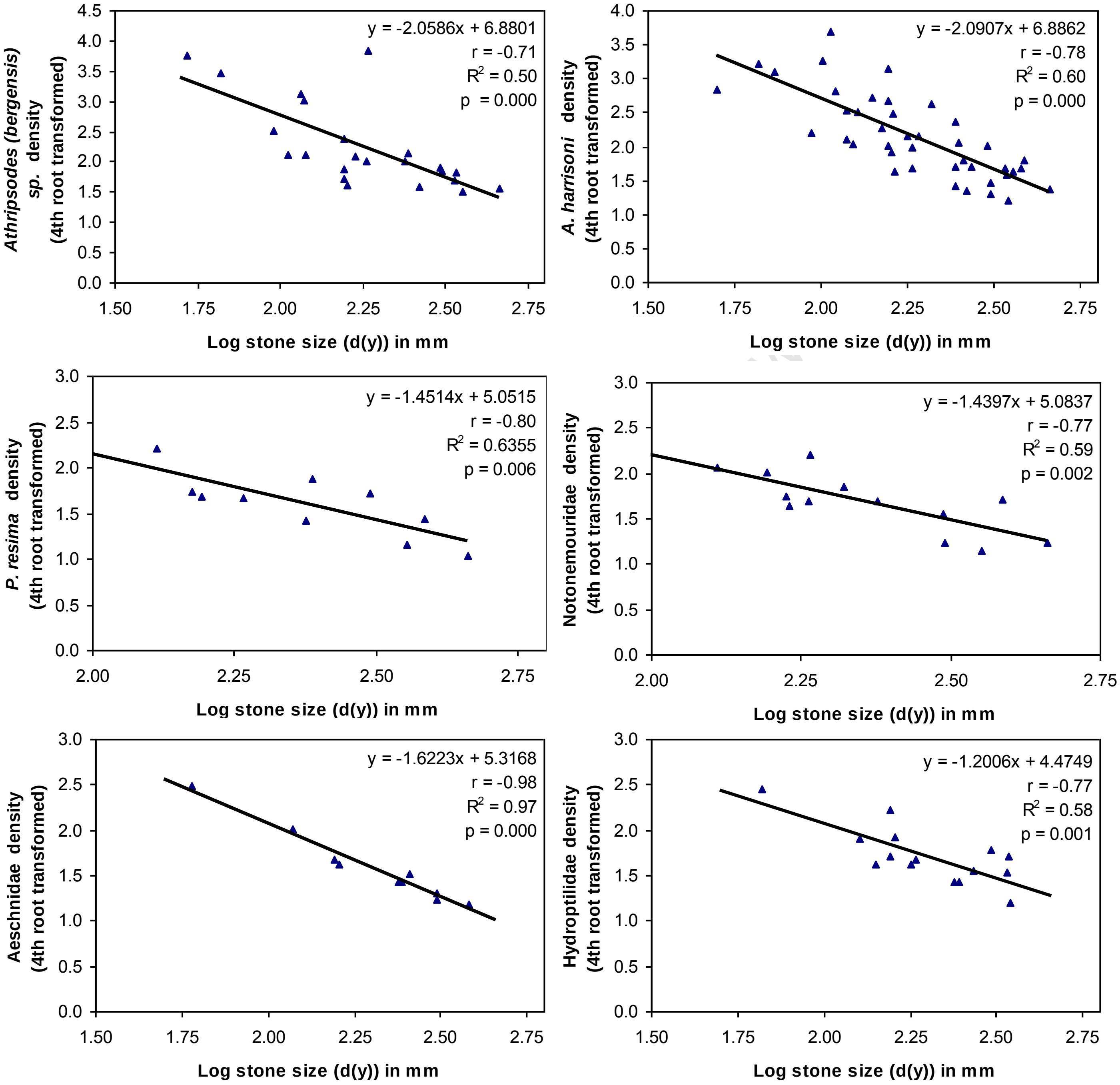

Figure 3.15 Correlation and linear regression of selected invertebrate taxon density and particle diameter in the Molenaars River in May 2004.

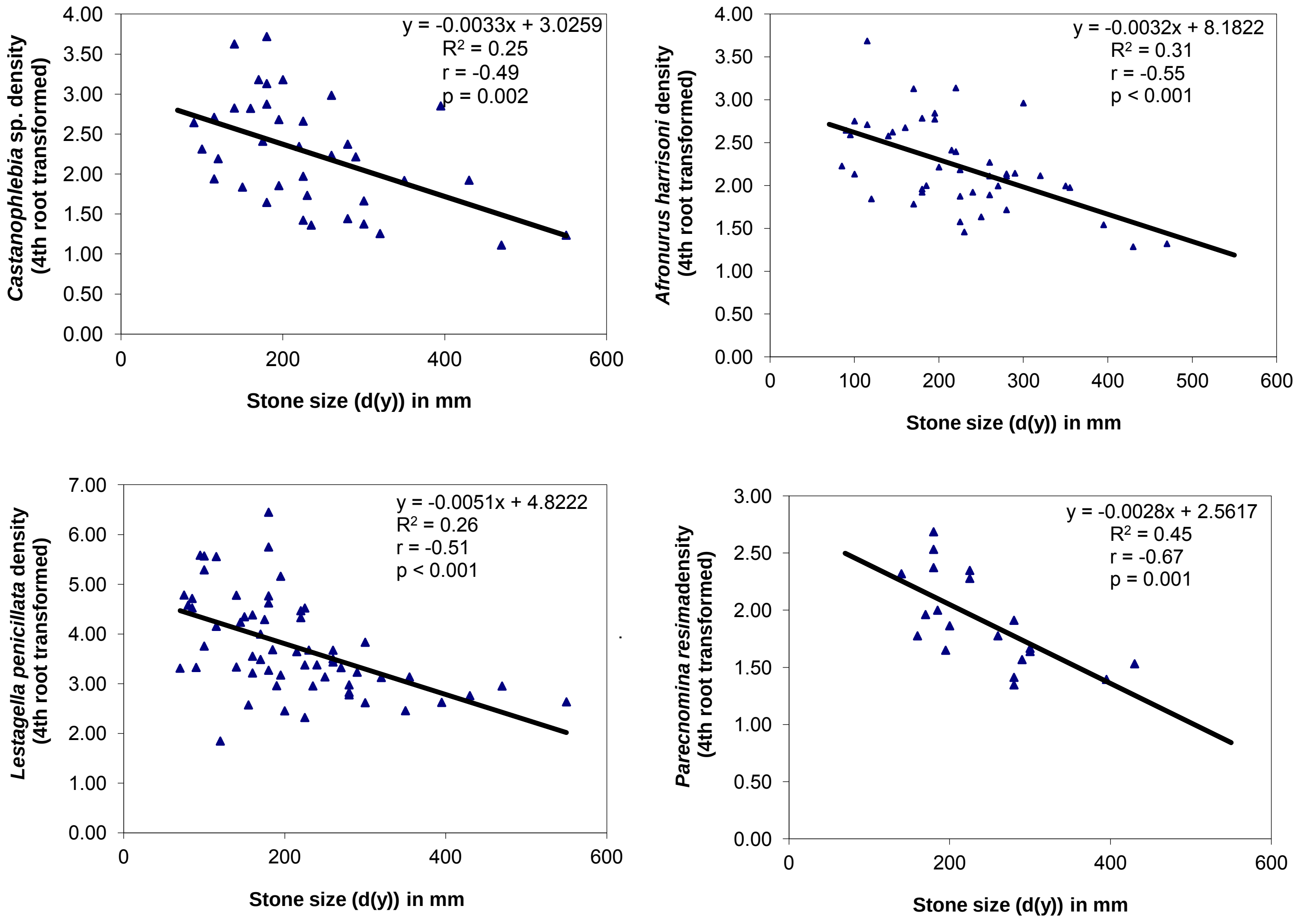

Figure 3.16 Correlation and linear regression of selected invertebrate taxon density and particle diameter in the Berg River in May 2004.

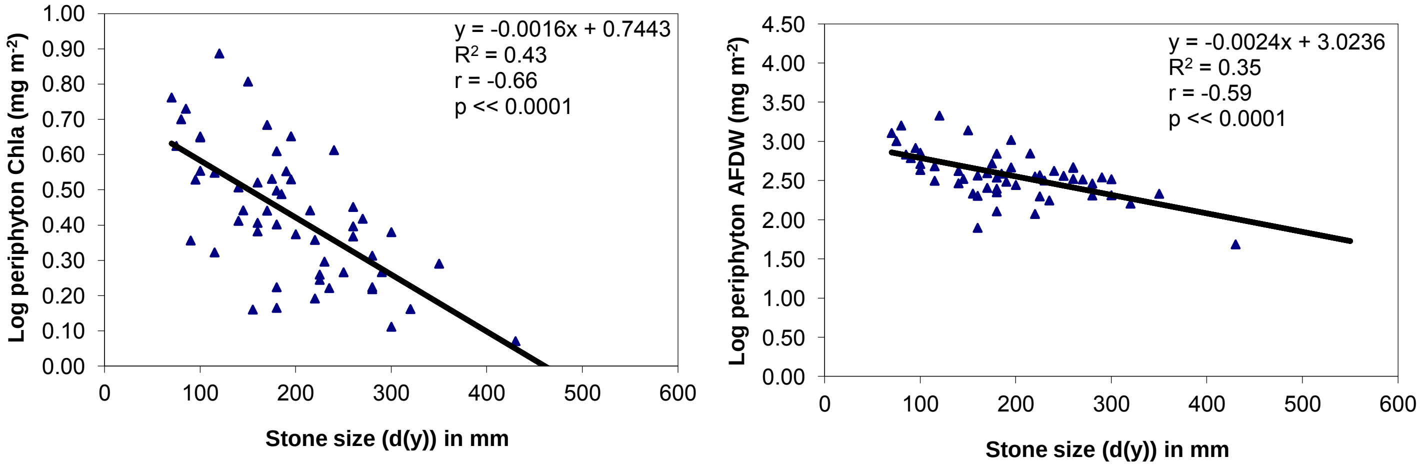

Figure 3.17 Correlation and linear regression of Chlorophyll a / periphyton ash-free dry weight (AFDW) and particle diameter (y-axis) in the Berg River in May 2004.

3.4 DISCUSSION

The late autumn / early winter invertebrate assemblages in the Molenaars and Berg Rivers, representing baseline conditions prior to floods, showed some interesting similarities as well as differences. Particle size differences were slight, the range of flow velocities, depths and other hydraulic variables were not dissimilar and the taxonomic overlap between the two rivers was very high - only a small number of taxa were well-represented in the Molenaars River but virtually absent from the Berg River, *Agapetus agilis* and *Elporia* spp. being the only two examples. On the other hand, the Berg River site was inhabited by more Hydropsychidae species than the Molenaars. The extremely low densities of *A. agilis* and *Elporia* spp. in the Berg River was not simply an artefact of possible inter-annual variation, but has been observed in other studies on this system. Both these species are grazers, have little mobility, and tend to colonise larger stones in the Molenaars River (personal observation). This observation is reinforced by the finding that both taxa were among the few in either the Berg or Molenaars River whose densities did not increase with decreasing particle size.

The most obvious and striking difference between the two rivers, however, was the substantially greater secondary productivity of the Molenaars River, as evidenced by invertebrate densities in the Molenaars River that were three or more times greater than those in the Berg River, particularly of the mayfly group, Baetidae. Baetidae made up over 50 % of the overall invertebrate density in the Molenaars River, but just under a quarter in the Berg River, which had highest densities of Chironomidae. Furthermore, Baetidae on the tops of immovable boulders in the Molenaars River were the main reason that densities on these stones and on whole stones were similar. In contrast, densities on immovable boulder tops in the Berg River were only half of that recorded on whole stones. These differences may be related to the combination of nutrient enrichment and the suspected relaxation of predation pressure on, particularly, baetid mayflies in the Molenaars River: evidence is beginning to emerge that links elevated baetid densities to the absence of indigenous minnows in many mountain streams of the Western Cape, a common feature of these rivers following invasion by alien trout and / or bass. A study by Lowe *et al.* (2008) investigating top-down predator effects on invertebrate assemblages up- and down-stream of a natural barrier to alien fish invasion in an adjacent catchment found larger densities of Baetidae in the sites without indigenous fish than with them (Lowe *et al.* 2008). Behavioural effects were also noticed: baetids at the downstream sites congregated on upper surfaces of river stones, whereas in the presence of indigenous fish, almost no top-dwelling baetids were observed (Lowe, Freshwater Research Unit, University of Cape Town, pers. comm.). Algal densities were substantially greater in the presence of indigenous minnows than where these were replaced by bass (Lowe, Freshwater Research Unit, University of Cape Town, pers. comm.). In the case of the Molenaars and Berg Rivers, the former has lost its complement of indigenous fish species, whilst the latter still supports populations of the Berg River redfin, *Pseudobarbus burgi* and the Cape kurper, *Sandelia capensis*. Reference has already been made to the fact that the Molenaars River receives enriched effluent from two trout farms in its headwater tributaries (Brown 1996). Thus the combined effects of increased productivity from effluents entering the Molenaars River and the possible relaxation of predation pressure on mobile grazers may account for much of the difference in faunal characteristics between the Molenaars and Berg Rivers as indicated by their Baseline invertebrate surveys. At present the evidence is fairly circumstantial, and more investigation of the energetic requirements of top predators in both systems would be required to illuminate these possible trophic cascades (Biggs *et al.* 2000).

Invertebrate assemblages in the Molenaars River appeared to have stronger relationships between their density distribution and hydraulic parameters than was the case in the Berg River, whether this be measured in terms of actual point measurements of velocity, depth and so on, or defined in terms of hydraulic biotopes (King & Schael 2001, Schael 2005). The taxa whose densities were correlated with faster velocities, or larger Froude number or stream power in the Molenaars River in June 2003 (e.g. *Baetis* spp., *D. capensis*, *Elporia* spp.) were also the most consistent discriminator species distinguishing between riffle / fast run and slower-flowing biotopes, as indicated by multivariate SIMPER analysis and Kruskal-Wallis analysis of variance. *D. capensis* showed no density changes in response to velocity increases, or any other variables, in the Berg River in 2004, despite being a good discriminator between biotopes. This might reflect the fact that Berg River in May 2004 experienced very low autumn flows, exacerbated by the failure of rains the previous year. Under such conditions, the average water column velocity may not adequately capture the flow is actually experienced by an organism, and here biotope category may be more accurate a descriptor of hydraulic affinity.

The amount of organic matter associated with a stone location proved to be important for many taxa, especially chironomid and leptophlebiid taxa. On the other hand, slow biotopes and / or low velocities were also correlated with increased availability of organic matter.

These results agree with other studies on habitat relationships. For example Whole *et al.* (1995) found differences in distribution of organic matter and invertebrate taxonomic and functional groups between depositional, bedrock and cobble-riffle biotopes (Wohl *et al.* 1995). Because species preferences overlap, and because each species' range of tolerances for one or another hydraulic variable will differ, however, invertebrate assemblages should not be expected to be represented as rigidly distinct entities. Nevertheless, the data do indicate that varying hydraulic characteristics across the stream bed influence the distribution of most species.

Probably one of the most pertinent relationships identified through analysis of the Baseline survey data was the significant linear correlation between invertebrate density and stone particle size. The high r^2 value for this relationship for both the Berg and Molenaars Rivers indicates that some 25 and 31 % of overall invertebrate density respectively can be explained by stone size, whilst individual taxa had individually stronger or weaker associations with stone size. More taxa were significantly correlated with stone size than any other variable. The importance of substratum size was hinted at by King & Schael (2001) in their evaluation of invertebrate communities within hydraulic biotopes, but could not be quantitatively demonstrated because their sampling was restricted to boulder and large cobble, and substratum data were categorical, not collected as individual measured stones. Quinn & Hickey (1990) found that both density and richness of collector-browsers were greatest on small cobble-boulder substrata, whilst filterers and facultative shredders had a strong preference for large cobbles and boulders. In this present study, over a stone size range, measured as the second longest stone axis, of between 80 and 550 mm, most taxa were found in higher densities on small stones. Important exceptions to this included the filter-feeding *Simulium* spp., and other taxa that are more strongly associated with the top surfaces of larger boulders in faster flow, for example *D. capensis* and *Elporia* spp. This finding contrasts with at least one other study which has examined differences in density on small and large stones. Bond & Downes (2000) found higher densities of two species of net-spinning cased caddis on larger stones than on small stones, inferring from this an adaptive advantage on larger and therefore more stable particles. In that study however, small stones were defined as those with the longest axis of 50-100 mm and these had lower densities than stones with the longest axis between 110 and 230 mm. In the Molenaars and Berg Rivers, large stones were those with a beta-axis of up to 550 mm, meaning that the longest axis would be in the order of 800 mm, whilst stones with a long axis of under 100 mm were not even sampled. It may well be that these findings are not contradictory at all, but merely that animals may congregate in higher densities on smaller stones to a point, after which very small stones are not attractive habitats, whether as a result of their disturbance history or some other feature related to resource use by invertebrates.

Periphyton biomass was also strongly negatively related to stone particle size. Similar results were found between algal biomass and stone size in the study of Cattaneo *et al.* (1997) who also found that cobbles supported a greater algal biomass per unit area than boulders. They also found that coarse and fine gravel supported increasingly lower densities of algae, which they attributed in part to the greater stability and better exposure of cobbles to flow and hence nutrient renewal than fine gravels.

The relationship between periphyton and stone size appeared to be independent of the influence of invertebrates: there was no co-correlation between the higher densities of invertebrate taxa on small stones and the greater availability of periphyton on small stones. The relationship between periphyton and invertebrate biomass was not clear, except for a weak positive correlation between the latter and the Chironomidae, elmid adults and *Lithogloea harrisoni*, and higher invertebrate species density on stones with greater periphyton biomass. Periphyton assemblage composition and particularly differences in life-form might offer some explanation of greater invertebrate biomass, at least of grazers, but this was not measured in the present study. In their study, Cattaneo *et al.* (1997) found strong differences in the proportion of loosely attached (removed by brushing or agitation) and firmly attached (not readily removable) life forms. They found that small substrata supported mainly the loosely attached algae, whilst the tightly attached forms increased in abundance with increasing particle size. The former life form was dominated by colonial cyanophytes, bacterial masses and motile diatoms, whilst the latter life form was dominated by adnate and filamentous algae. According to Steinman (1996) collector-gatherers such as mayflies tend to feed on the outer layers or loosely attached portions of the periphyton mat, comprising stalked or short filamentous algae plus their epiphytes. Preference for particular life forms is less a result of an ability to discriminate different algal taxa, but rather a consequence of the restrictions on feeding placed by each species' mouthpart morphology (Steinman 1996).

In relation to the study of flood effects, large stable particles have been suggested as potential refugia for invertebrates, from the effects of floods (Townsend *et al.* 1997c; Francoer *et al.* 1998). The findings of this study suggest that small particle size confers a relative advantage, perhaps associated with increased availability, better condition or type of food, or easier collection of food resources from within interstitial spaces, or perhaps associated with shelter from predation or hydraulic effects. The findings demonstrate clearly, though, that invertebrates are not selecting for large stable particles - at least not at the start of the flood season.

What did occur during floods, however, is the subject of the following chapter.

4 INVERTEBRATE RESPONSES TO FLOODS. PART 2: RESULTS OF THE FLOOD STUDY

4.1 INTRODUCTION

The aim of this thesis section (Chapters 3 and 4) was to investigate invertebrate response to individual flood events of known magnitude, relative to the distribution of flow forces acting on stones as well as potential refugia; to define ecologically relevant threshold of disturbance linked to discharge, for the study river.

Chapter 3 has provided detail of the study approach and methods, and a detailed characterisation of the pre-flood conditions in both study rivers. Since the stones sampled to determine the pre-flood conditions in the rivers were replaced and then monitored to see if floods moved them, this allowed an important question to be asked, and answered: do stream invertebrates select, at the start of the flood season, stones which end up remaining unmoved through a series of floods. The reverse appeared to be the case. Invertebrates generally selected smaller stones even at the start of winter, perhaps in order to maximise the benefit of better resources associated with smaller stones.

This chapter now turns to the results of the investigation into biological responses to floods. Central to the approach was the sampling, a month before floods, of a number of river stones, their replacement in the river in their original location to become re-conditioned to their original invertebrate complement, and then their further sampling after each flood event. Chapter 4 begins therefore with an assessment of the validity of this approach, which also provided unanticipated information on resistance and resilience abilities of the different invertebrate species in these ecosystems. Following this, the links between flood size and substratum movement, and between these and invertebrate responses, are presented and discussed.

4.2 REPEAT-SAMPLED STONES: VALIDITY OF APPROACH AND RECOVERY FOLLOWING INITIAL SAMPLING

4.2.1 Sample size and representativeness

The approach to evaluating flood effects in the Molenaars River (section 3.3.1*i*, Figure 3.8) was based on repeat-sampling river stones on two occasions: the baseline conditions, where all stones were sampled and then, either immediately prior to or immediately after flood events in mid and late July 2003, where ¼ of the baseline stones were repeat-sampled on each occasion. The validity of this approach is based on the assumption that the sample size of invertebrates on stones being compared was large enough to be representative of the invertebrate assemblage in the river. This was tested, using the data from the 49 whole stone samples that were processed from the Baseline survey set. These Baseline samples were divided into two sub-groups, of 23 and 27 stones, corresponding to the two groups of stones repeat sampled before and after the first flood.

A Mann-Whitney U non parametric T-test was used to identify differences in the two sub-groups. No significant differences were found, either in the total invertebrate densities, or in the densities of all but two of the 50 taxa taken individually. Those that were significantly different were Elmidae adults ($p = 0.009$) and *Choroterpes* sp ($p = 0.005$). The results suggest that a sample size of 23 or more is probably large enough to be considered representative of the overall invertebrate assemblage characteristics, with recognition of the caution required in interpreting comparison data for the two taxa which showed significant differences across subsets of the Baseline samples. In the same vein, each pre- or post- flood sample set was also considered to be representative of the invertebrate population at the site, allowing statistical comparison between each pre- or post- flood sample set with the overall Baseline dataset.

4.2.2 Recovery following initial denudation of repeat-sampled stones

A second requirement for comparing the Baseline survey data with post-flood surveys was a demonstration of adequate recovery of the invertebrate fauna after initial sampling, but prior to the first flood that was to be examined. This was evaluated by comparing Baseline data with the Pre-Flood 1 subset of samples.

Figure 4.1 shows the results of PRIMER cluster and MDS analysis of all Baseline and pre-Flood 1 samples collected on 9 June and 9 July respectively. The dendrogram groupings (Figure 4.1a) do not differentiate stones sampled on the two dates, but the major groupings (at 40 - 50% similarity) rather reflect the same sets of biotope clusters as were present during the baseline survey (refer to Figure 5.3). The scatter plot from MDS analysis (Figure 4.1b) confirms that this pattern of a gradual shift between biotopes, a feature of the baseline survey, was regained within a month after initial sampling. Indeed, a two-way ANOSIM (using date of sampling and flow type as factors) indicated no significant difference between assemblages between sampling date, but significant differences between flow types (ANOSIM Global R = 0.43, p=0.001).

In Figure 4.1b, repeat-sampled stones are indicated by the stone number, with suffixes representing Baseline (B) or Pre-Flood 1 (F1pr). The distance between the points in the plot indicates the extent to which the assemblage on each stone returned to its initial condition between the Baseline and Pre-Flood 1 sampling dates, i.e. a representation of its similarity.

The actual similarity coefficients between each pair of repeat-sampled stones were extracted from the similarity matrix. The average Bray Curtis similarity of the community on each stone on 9 June vs. 9 July (i.e. each re-sampled comparison) was 66.8 % (n=22), which was higher than within biotope similarity levels (Figure 4.1b).

Mann-Whitney U was used to test for differences in mean total invertebrate / per taxon densities between the Baseline and the Pre-Flood 1 periods. In only five out of 50 taxa were there significant decreases in densities at the latter time ($p > 0.05$), indicating a failure for full recovery to have occurred on repeat-sampled stones for these taxa (Figure 4.2). A number of other taxa show substantial decreases between these two surveys, but which were nevertheless not significant because of the high variance associated with patchily distributed populations. Examples include *Cheumatopsyche afra*, *Chimarra* spp. and *Elporia* spp.

Total invertebrate density was also lower after the initial sampling, albeit not significantly so. Other taxa recovered fully, or, in some instances (e.g. Simuliidae, *Afronurus* sp.) even increased in density, although these increases were again not significant.

Thus the community signature of samples taken during the Baseline survey was re-established a month later, on the same set of stones, as indicated by the restoration of biotopes assemblages in the Molenaars River over the time interval between the two sampling dates (PRIMER analysis). However, some species did not recover to full Baseline densities on the repeat-sampled stones. Recovery may have been affected by the population structure and life-history characteristics of individual species which determines the

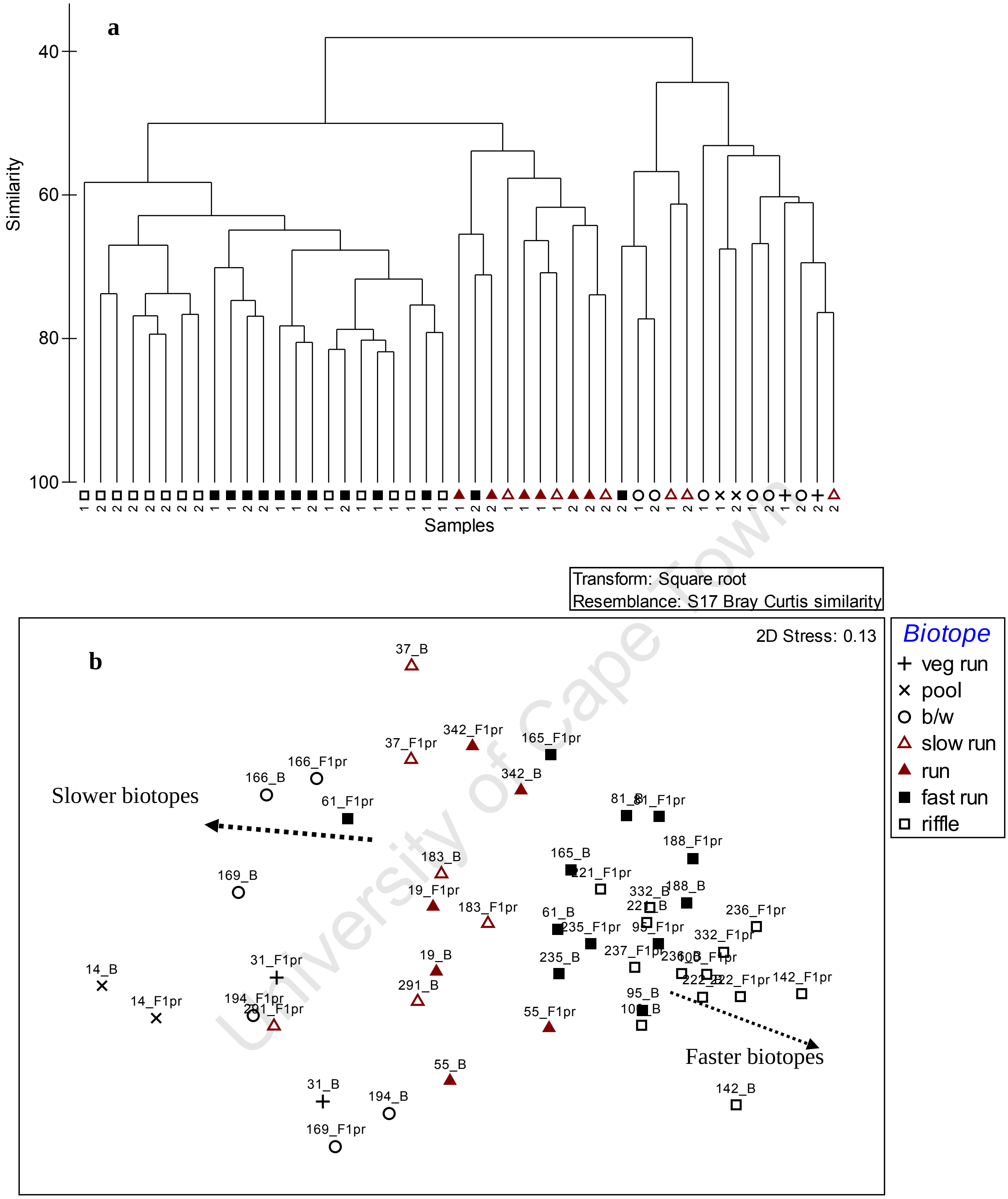

Figure 4.1 **Molenaars River Baseline and pre-Flood 1 invertebrate assemblages from June and July 2003, 4th root transformed, a) hierarchical cluster dendrogram, based on Bray Curtis similarities; b), a 2-d MDS plot with the biotope at each sample location indicated by symbols.** Codes: 1 = Baseline and 2 = Pre-Flood 1 sampling in (a); biotope symbols as indicated in the key; individual sample numbers in MDS plot are suffixed with a B (baseline survey) or F1pr (pre-Flood 1 sampling).

availability of colonists, and this is examined in more detail in section 4.4 on species-specific responses to floods.

These results suggest that, in terms of using repeat-sampled stones to measure possible flood effects on invertebrates, cognisance should be taken of the species differences in recovery following the Baseline denudation of stones (i.e. the sampling effect). In the light of these results, the Pre-Flood 1 data set was considered an alternative or additional baseline condition against which to compare the effects of the subsequent floods.

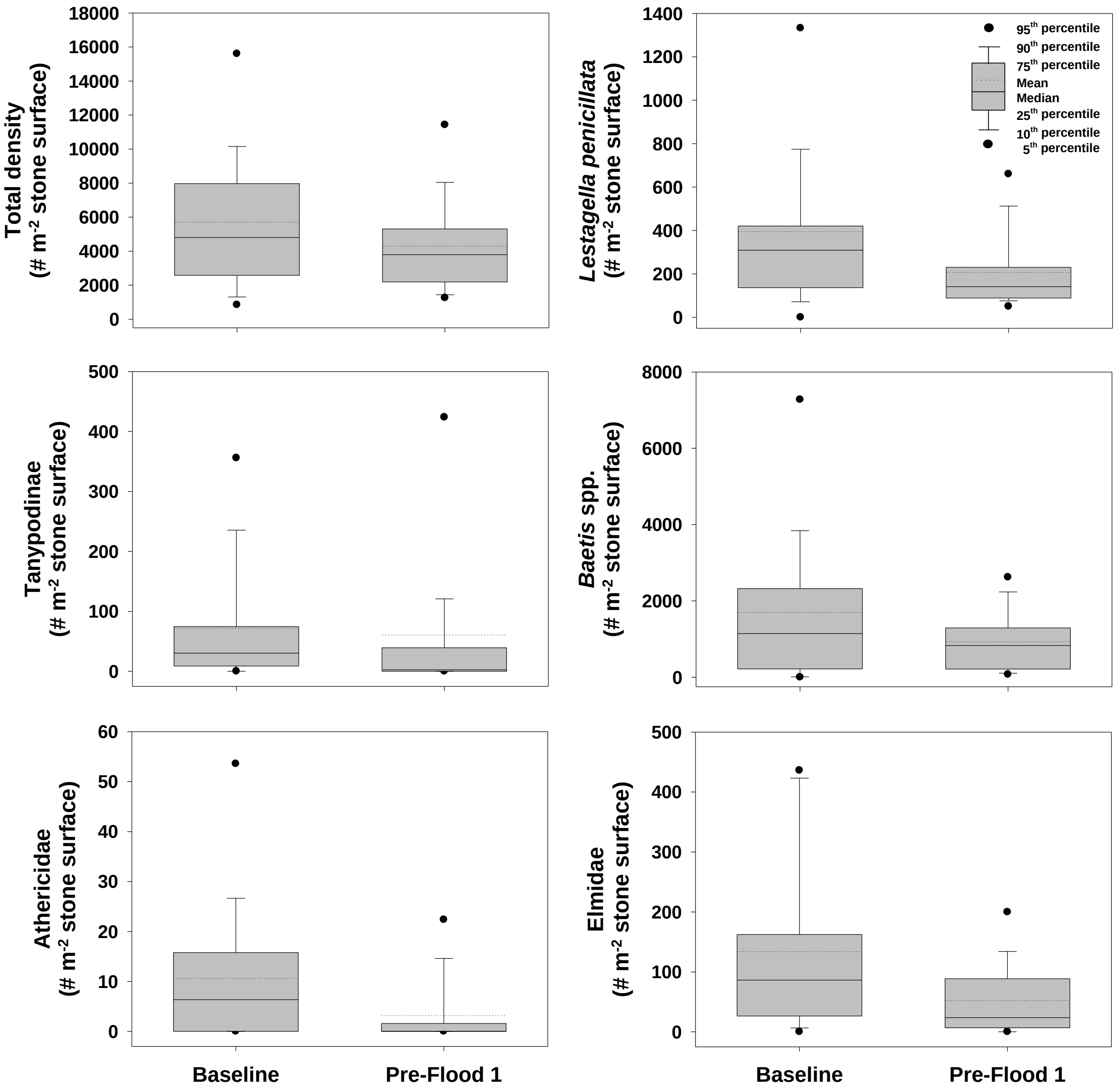

Figure 4.2 **Box plots of total invertebrate densities, and taxa demonstrating a significant decrease in density per m^2 stone surface area on repeat-sampled stones, from the Baseline to the Pre-Flood 1 sampling surveys (Mann-Whitney U tests).** Abundance data are untransformed to facilitate interpretation.

4.3 SUBSTRATUM MOVEMENT DURING FLOODS

The extent of particle movement, during floods in the Molenaars River in 2003 and the Berg River in 2004, is given in Table 4.1 and Table 4.2 respectively.

The first and fourth floods in the Molenaars River in 2003, that is, those for which invertebrate response data were collected, were associated with no stone movement and with 2.9 % overall stone movement respectively, roughly equally divided between small and large cobble (Table 4.1). The second and third floods were also associated with negligible bed movement (2 and 0.6 % respectively). All four early winter floods were DRIFT Class I floods, with larger floods occurring only late in the winter season as shown in the previous chapter (Figure 3.4). The later floods were Flood 5, DRIFT Class II in early August, with 7.6% bed movement; and Flood 6, DRIFT Class III in late August, with 33.4% bed movement, including 40% of large cobble and nearly 15% of small boulders (Table 4.1). Since all Baseline stones had been repeat-sampled by the fourth flood, no invertebrate sampling was possible for these latter two large floods.

In the Berg River in 2004 a large flood occurred in early June at the start of the winter season (Flood 2, a DRIFT Class IV flood, accompanied by 43.3% bed movement). This was followed by a period of small spates with negligible bed movement, and then five DRIFT Class III and IV floods in July and August. The first of these (Flood 5, just above the threshold defining a DRIFT Class IV, with 25.7% bed movement) was the final flood monitored during the study period, after which no further stone movement data were collected. The daily and instantaneous flow record over the study period is shown in Chapter 3 (Figure 3.5).

Class IV floods in the Berg River in 2004 moved between 25 and 50% of large cobble and around 15% of small boulders (Table 4.2), similar to the case of the Molenaars River in 2003. The Class IV floods in the Molenaars River in 2004 had similar levels of bed movement to those in the Berg River, although invertebrate data were not collected from the Molenaars River in that year.

Table 4.1 Stone Movements in the Molenaars River during 2003 flood events. Stone movement was defined as such even if a stone only turned over. G = gravel, SC = small cobble, LC = large cobble, SB = small boulder; LB = large boulder, as defined in Table 3.1.

Stone Size Classification		**Total**	**G**	**SC**	**LC**	**SB**	**LB**
Max size in class (mm)			64	161	256	514	1000
Total Number of Stones		344	4 (1%)	91 (26.5%)	73 (21%)	111 (32.5%)	65 (19%)
Flood 1	Moved Stones	0	0	0	0	0	0
	Movement %	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Flood 2	Moved Stones	7	0	7	0	0	0
	Movement %	2.0%	0.0%	7.7%	0.0%	0.0%	0.0%
Flood 3	Moved Stones	2	0	1	1	0	0
	Movement %	0.6%	0.0%	1.1%	1.4%	0.0%	0.0%
Flood 4	Moved Stones	10	0	6	4	0	0
	Movement %	2.9%	0.0%	6.6%	5.5%	0.0%	0.0%
Flood 5	Moved Stones	26	1	18	4	3	0
	Movement %	7.6%	25.0%	19.8%	5.5%	2.7%	0.0%
Flood 6	Moved Stones	115	4	65	29	16	1
	Movement %	33.4%	100.0%	71.4%	39.7%	14.4%	1.5%

Table 4.2 Stone movement in the Berg River during 2004 flood events. Stone movement was defined as such even if a stone only turned over. G = gravel, SC = small cobble, LC = large cobble, SB = small boulder; LB = large boulder, as defined in Table 3.1.

Stone Size Classification		Total	G	SC	LC	SB	LB
Max size in class (mm)			64	161	256	514	1000
Total Number of Stones (%)		432	27 (6%)	120 (28%)	92 (21%)	139 (32%)	54 (13%)
Flood 1	Moved Stones	1	1	0	0	0	0
	Movement %	0.2%	3.7%	0.0%	0.0%	0.0%	0.0%
Flood 2	Moved Stones	187	26	92	44	24	1
	Movement %	43.3%	96.3%	76.7%	47.8%	17.3%	1.9%
Flood 3	Moved Stones	10	2	3	3	2	0
	Movement %	2.3%	7.4%	2.5%	3.3%	1.4%	0.0%
Flood 4	Moved Stones	7	1	4	2	0	0
	Movement %	1.6%	3.7%	3.3%	2.2%	0.0%	0.0%
Flood 5	Moved Stones	111	10	58	28	14	1
	Movement %	25.7%	37.0%	48.3%	30.4%	10.1%	1.9%

4.4 EFFECTS OF FLOODS ON MACRO-INVERTEBRATE ASSEMBLAGES

4.4.1 Community patterns in the Molenaars River

For Flood 1, the average Bray Curtis similarity between pairs of stones sampled on 9 June and re-sampled on 9 July, prior to the first flood, was 66.8% (n=22). As indicated in section 4.1 this reflected the extent of recovery of stones prior to the floods. The average Bray Curtis coefficient of similarity between pairs of stones sampled on 9 June and 13 July, i.e. from Baseline to post Flood 1, was 64.6% (n=27). Thus the change in community structure, as measured by multivariate similarity values, was not significantly different from the Baseline to either before or after Flood 1 (Mann-Whitney U test, p > 0.68), suggesting that the flood had no significant effect on invertebrate assemblage similarities.

Cluster and MDS analysis of flood assemblages for all five sampling periods - Baseline and Pre- and Post-Flood 1 and Flood 4 - showed a substantial overlap in sampling periods, with no discreet groupings evident (Figure 4.3a and b). Most samples fell into a similarity range of between 50 and 85 % (Figure 4.3a). However, the MDS plot of all samples (Figure 4.3b) shows a gradient of change, with the Baseline samples on the opposite end of this gradient from most of the Post-Flood 4 samples.

An ANOSIM routine was used to test for differences by period. The Global R = 0.12, (p = 0.001), indicated that a significant difference was observable by period. Pairwise tests showed that the only significant difference in assemblage similarities was between the Baseline condition and the Post-Flood 4 survey (R=0.246, p = 0.001).

SIMPER analysis indicated an average dissimilarity of 53 % between samples from the Baseline and Post Flood 4 surveys (Figure 4.3). No taxa were highly consistent discriminators of the two assemblages, as indicted by low (<1.5) ratios of the average dissimilarity / standard deviation (Diss/SD) in the species comparisons between the two groups. The top five discriminator taxa are indicated in Table 4.3.

Almost all the Pairwise comparisons in the SIMPER analysis indicated reductions in density between the Baseline survey and the final, Post-Flood 4 survey (Table 4.3). However, four taxa were either unchanged (*Demoreptus capensis*, Orthocladinae) or increased significantly (*Simulium* spp., *Lithogloea harrisoni*) in density over this period. These and other species-specific responses to floods are examined in detail in section 4.5.

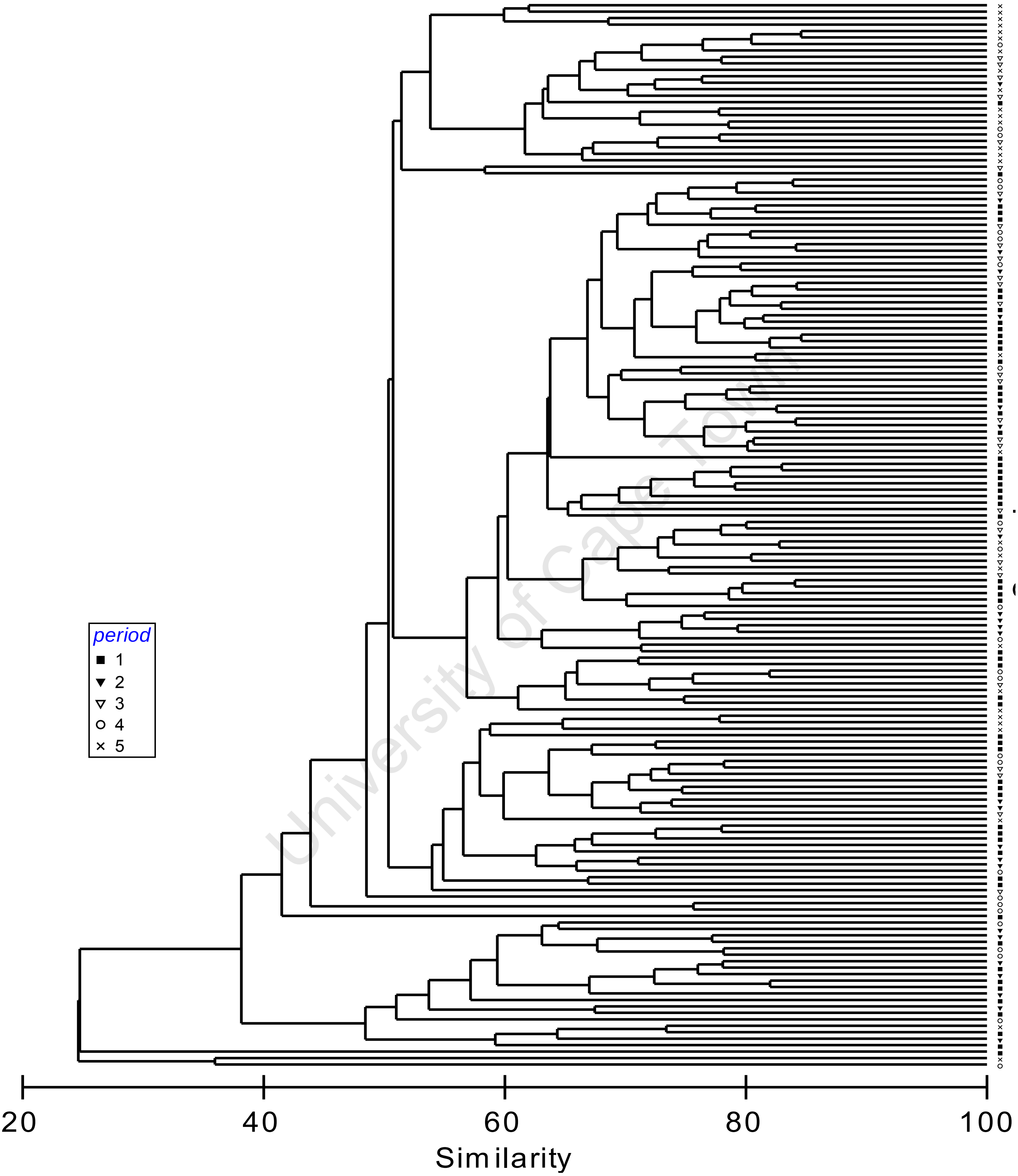

Figure 4.3 a) Molenaars River hierarchical cluster dendrogram of invertebrate assemblage changes over the five sampling intervals based on Bray Curtis similarity coefficients. Samples are reflected on the y-axis by symbols, numbered by date of sampling as follows: 1 = Baseline survey, 2 = Pre-Flood 1 survey, 3 = Post-Flood 1 survey, 4 = Pre-Flood 4 survey, 5 = Post-Flood 4 survey.

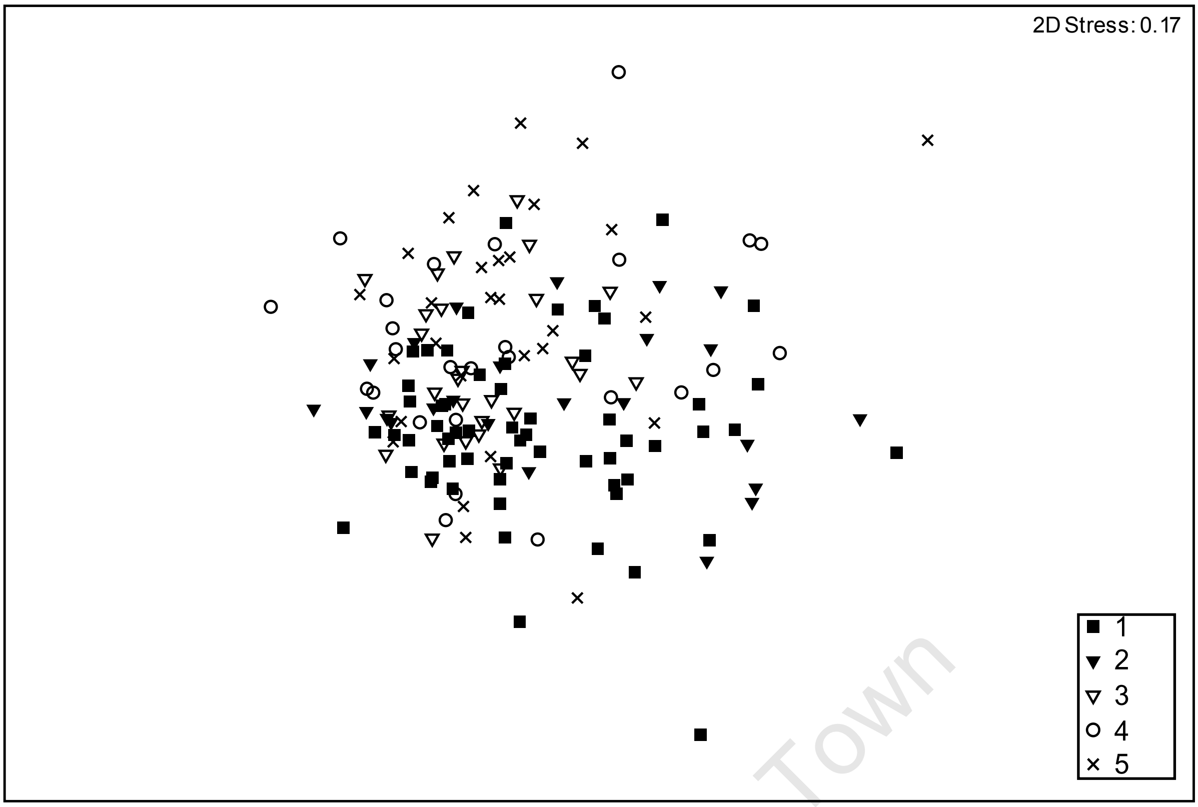

Figure 4.3 b) Molenaars River MDS plot of invertebrate assemblage changes over the five sampling intervals, based on Bray Curtis similarity coefficients. Samples are shown as symbols, numbered by date of sampling as follows: 1 = Baseline survey, 2 = Pre-Flood 1 survey, 3 = Post-Flood 1 survey, 4 = Pre-Flood 4 survey, 5 = Post-Flood 4 survey.

Table 4.3 Discriminator taxa in a SIMPER analysis comparing the dissimilarities between invertebrate assemblages from the Baseline and Post-Flood 4 surveys in the Molenaars River in 2003. Taxa that cumulatively made up 75% of the dissimilarity between groups are listed, in order of their individual contribution (%) to dissimilarity. Those with the highest Diss / SD ratio were the more consistent discriminator taxa. Densities are re-transformed from the square root used in the analysis to No. m^{-2}, for ease of interpretation.

Discriminator taxa	Average abundance (# m^{-2})		Average dissimilarity	Diss/SD	Contrib. %	Cumul. %
	Baseline survey	Post Flood 4 survey				
Baetis spp.	1407	637	6.69	1.33	12.64	12.64
Afroptilum sp.	324	104	4.62	0.85	8.73	21.37
Elporia spp.	248	78	4.36	1.16	8.24	29.61
Orthocladinae	416	403	4.06	1.20	7.66	37.27
Demoreptus capensis	134	139	3.26	1.31	6.17	43.44
Lestagella penicillata	347	158	3.09	1.00	5.84	49.28
Elmidae	86	46	2.27	1.15	4.29	53.57
Euthralus elegans	97	54	2.27	1.30	4.28	57.85
Simulium spp.	24	46	2.13	1.06	4.02	61.87
Afronurus sp.	43	2	1.82	1.36	3.45	65.32
Tanypodinae	34	3	1.65	1.15	3.13	68.44
Tanytarcini	31	15	1.38	1.31	2.61	71.05
Pseudocloeon spp.	15	1	1.13	1.04	2.13	73.18
Lithogloea harrisoni	0.1	16	1.10	0.75	2.08	75.26

Overlaying a bubble plot of total invertebrate density onto the MDS plot from sample similarities (Figure 4.4) shows a general, although inconsistent, decline in density over time since the Baseline survey, but without marked shifts in density before and after individual flood events.

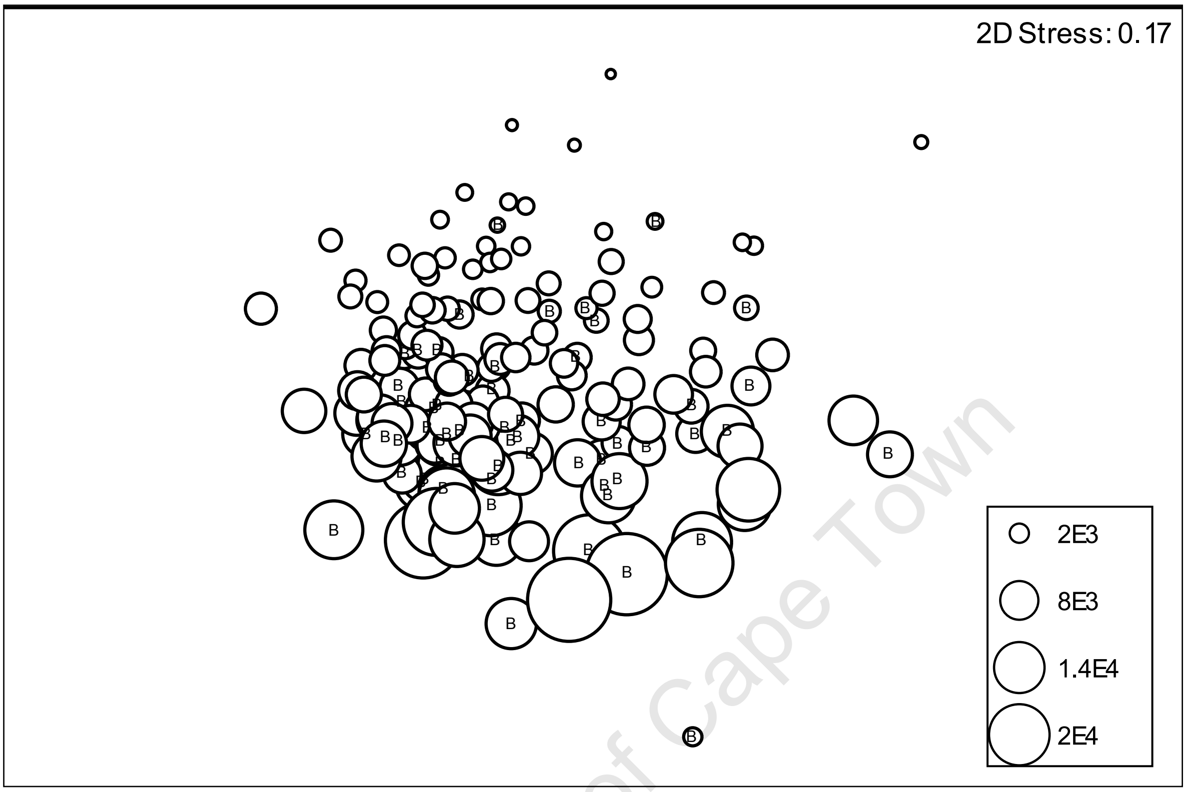

Figure 4.4 Molenaars River bubble plot of total invertebrate density per unit stone area, overlaid onto the MDS plot of invertebrate assemblages as in Figure 4.3b. Baseline samples are indicated with a B. Bubble size represents increasing orders or magnitude in density, where E3, E4 = x10^3, x10^4.

Kruskal-Wallis analysis of variance of total invertebrate density changes over the five sampling periods showed that the Baseline densities were not significantly different from the Pre-Flood 1 condition, but were significantly higher than all the following surveys (Figure 4.5a, Kruskal-Wallis analysis of variance, H = 28.40, p = 0.000, Dunn's Pairwise test significant for Baseline and F1-Pre surveys vs. F4-Po, $P < 0.05$). It should be noted here that the Pre-Flood 4 survey also reflects conditions in the river after the first three floods. Total density declined by 44 % from the Baseline survey to the end of the study, Post Flood 4. A comparison using the Pre-Flood 1 data with the post Flood 4 sample set, to account for potential sampling effects, indicated a 34 % decline in total invertebrate density, over the two month period associated with four DRIFT Class 1 floods (Figure 4.5a).

The amount of organic matter associated with each stone recovered fully after from the Baseline to Pre-Flood 1 sampling periods, but showed a non-significant decrease from before to after each flood.

Invertebrate densities on immovable stones whose tops only were sampled were approximately as high as densities on whole stones, although the species complement was different and comprised largely of Baetidae and *Elporia* spp. This total density declined by 70% over the study period (Figure 4.5b), although the Pre- and Post-Flood 4 surveys were represented by only four samples each on these immovable stones.

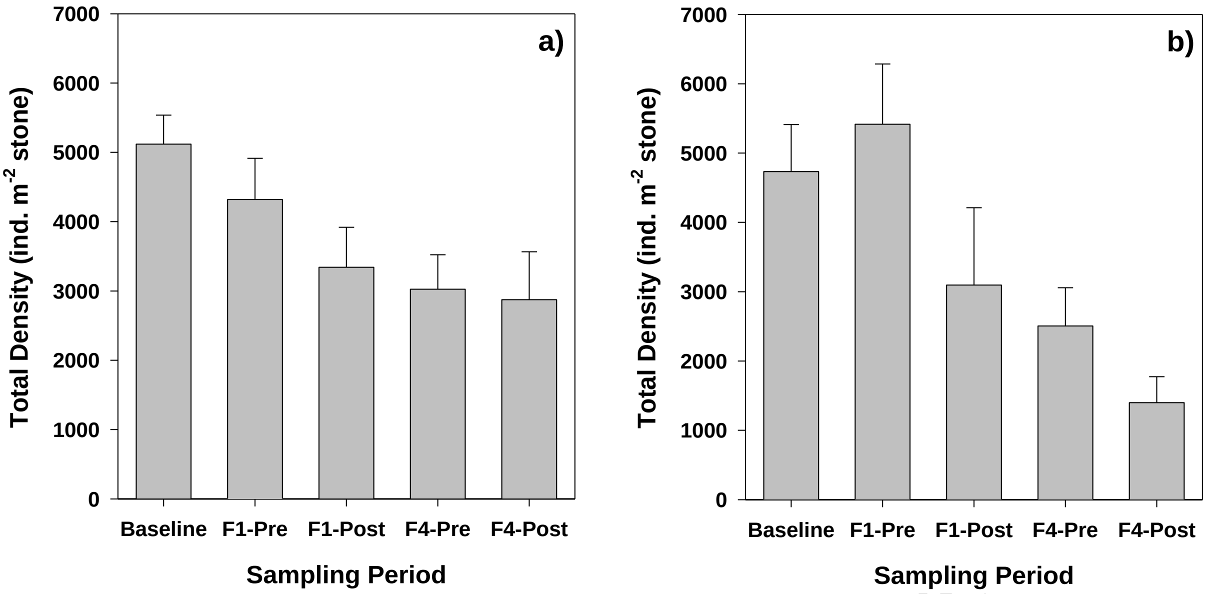

Figure 4.5 **Mean total invertebrate densities in the Molenaars River in 2003 a) on whole stones and b) on the tops of immovable stones, by sampling period.** F1, F4 = Floods 1 and 4 monitored over the season; Pre, Po = pre- or post-flood sampling survey. Bars denote standard error. Abundance data are untransformed to facilitate interpretation.

4.4.2 Community patterns in the Berg River

In contrast to the Molenaars River, invertebrate assemblages in the Berg River in 2004 were substantially different before and after the series of five floods that separated the Baseline and Post-flood survey. As indicated previously, these floods comprised two DRIFT Class 1 floods, one Class II and two Class IV intra-annual floods, the largest being on the 14th June and the second largest on the 23rd July, some four days before the Post-flood survey.

The results of multivariate analysis of Baseline and post-flood samples are indicated in Figure 4.6. The two-dimensional stress level associated with the MDS plot was 0.15, which makes the plot an adequate representation of the relationship between samples. There is a clear separation between Baseline samples collected in May and the Post-flood samples collected in July. The Baseline samples as a group were nevertheless only 56 % similar, whilst the Post-flood samples were more dispersed, with a 53 % similarity. However, the stones which were repeat-sampled were very similar in composition to the Additional set of stones sampled during the Post-flood survey, as is shown by the large overlap in their distribution within Figure 4.6. This Additional stones data set was collected to act as a control for the potential sampling effect associated with repeat-sampling the Baseline set of stones (see section 3.3.1*ii*). These MDS results suggest that any sampling effect was not significant.

To test this further, a 1-way ANOSIM was performed, based on the three sets of data - Baseline, Post-floods Repeat-sampled stones, and Post-floods Additional stones. The Global R-statistic = 0.234 (p = 0.001), indicating significant differences within the three groupings. Pairwise differences were assessed using a higher threshold for significance (a cut-off of $p < 0.01$), to avoid a Type 1 error. The results indicated that both Post-flood data sets were significantly different from the Baseline data (Baseline - Repeat sampled stones: R = 0.34, p = 0.001; Baseline - Additional stones: R = 0.37, p = 0.001), but not from each other (R = 0.02, p = 0.045). This result reinforces the conclusion that sampling effects on the

Repeat-sample stones were not significant in comparison to the other effects - either the effects of floods, or indeed, simply the shifts in seasonal signal over the two-month study.

SIMPER analysis was undertaken to identify the taxonomic differences between the Baseline survey data and Post-flood data. For the latter, both Repeat-sampled stones and Additional stones were combined, since the ANOSIM indicated they were not significantly different. There was a fairly low average dissimilarity, of 54 %, between the two periods, which was similar to the magnitude of change measured in the Molenaars River over the two-month period in 2003, despite the fact that floods were much smaller in that year, with only four Class 1 floods occurring over the 2003 invertebrate sampling period.

The relatively small SIMPER differences between the Baseline and Post-flood samples in the Berg River is a reflection of the fact that changes in species composition were largely the result of shifts in the densities of invertebrate taxa over the study period, rather than loss or gain of species. As with the Molenaars River study, no taxa were consistent discriminators of the two assemblages, indicted by generally low (< 1.5) ratios of average dissimilarity / standard deviation (Diss/SD) in Table 4.4. However, all taxa with the exception of *Lithogloea harrisoni* declined from the Baseline to the Post-flood sampling period.

A bubble plot of total invertebrate density superimposed onto the MDS plot from sample similarities showed a marked decline in total invertebrate density from the May Baseline samples to the Post-flood data set (Figure 4.7).

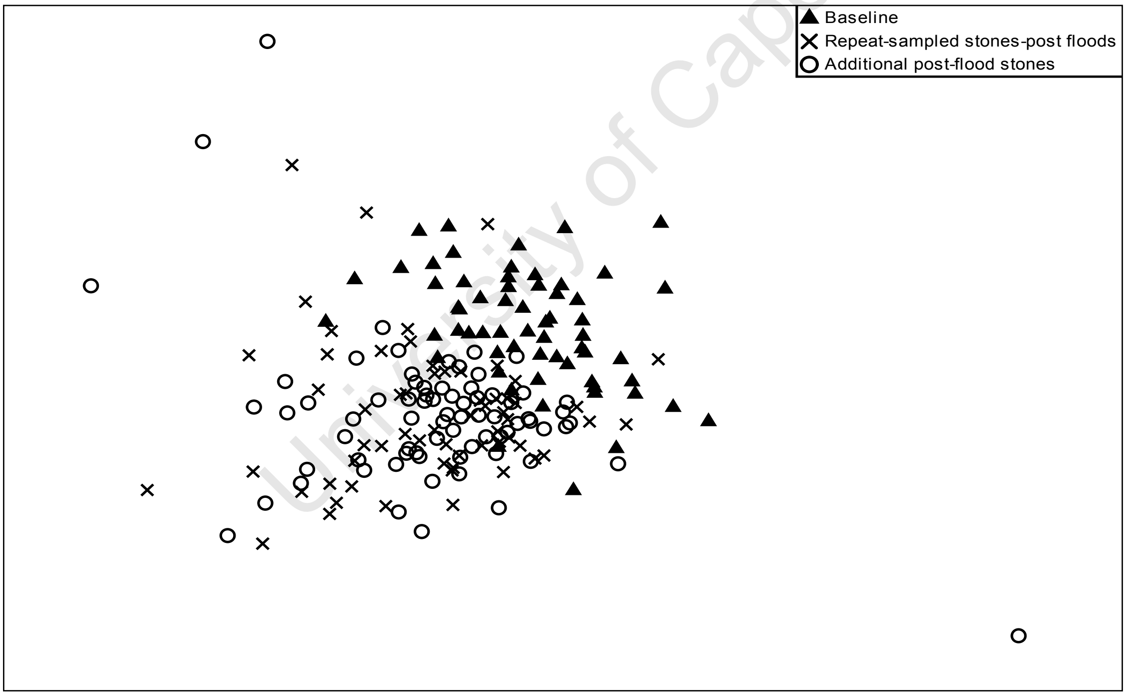

Figure 4.6 **Berg River MDS plot of invertebrate assemblage changes over the Baseline to Post-flood period based on Bray Curtis similarity coefficients.** Both repeat-sampled and additional stone (control) samples for the post-flood period were included in the analysis.

Table 4.4 **Discriminator taxa in a SIMPER analysis comparing the dissimilarities between invertebrate assemblages from the Baseline and Post-Flood surveys in the Berg River in 2004, whole stone analysis**. Repeat-sampled and Additional stones were pooled for the Post-flood data. Taxa that cumulatively made up 75% of the dissimilarity between groups are listed, in order of their individual contribution (%) to dissimilarity. Those with the highest Diss / SD ratio were the more consistent discriminator taxa. Densities are re-transformed from the square root used in the analysis to No. m^{-2}, for ease of interpretation.

Discriminator taxa	**Average abundance (# m^{-2})**		**Average dissimilarity**	**Diss/SD**	**Contrib.%**	**Cumul. %**
	Baseline survey	**Post Flood survey**				
Orthocladinae	575	315	6.61	1.09	12.30	12.30
Baetis spp.	518	350	4.79	1.26	8.91	21.21
Elmidae larvae	164	23	4.04	1.56	7.52	28.73
Lestagella penicillata	209	44	3.91	1.32	7.27	36.00
Tanypodinae	58	1	3.14	1.47	5.84	41.84
Afronurus sp.	31	2	2.17	1.45	4.03	45.88
Simulium spp.	18	10	2.05	1.02	3.82	49.70
Tanytarcini	35	6	2.03	1.35	3.78	53.48
Lithogloea harrisoni	7	17	1.73	1.13	3.22	56.70
Elmidae adults	14	1	1.63	0.99	3.03	59.73
Afronurus harrisoni	14	2	1.60	1.16	2.98	62.71
Athripsodes bergensis	11	1	1.51	1.12	2.81	65.52
Chironomini	14	6	1.50	1.18	2.80	68.32
Demoulinia spp.	11	0.5	1.49	0.85	2.77	71.09
Castanophlebia spp.	10	1	1.44	0.98	2.67	73.77
Scirtidae	10	2	1.42	1.04	2.64	76.41

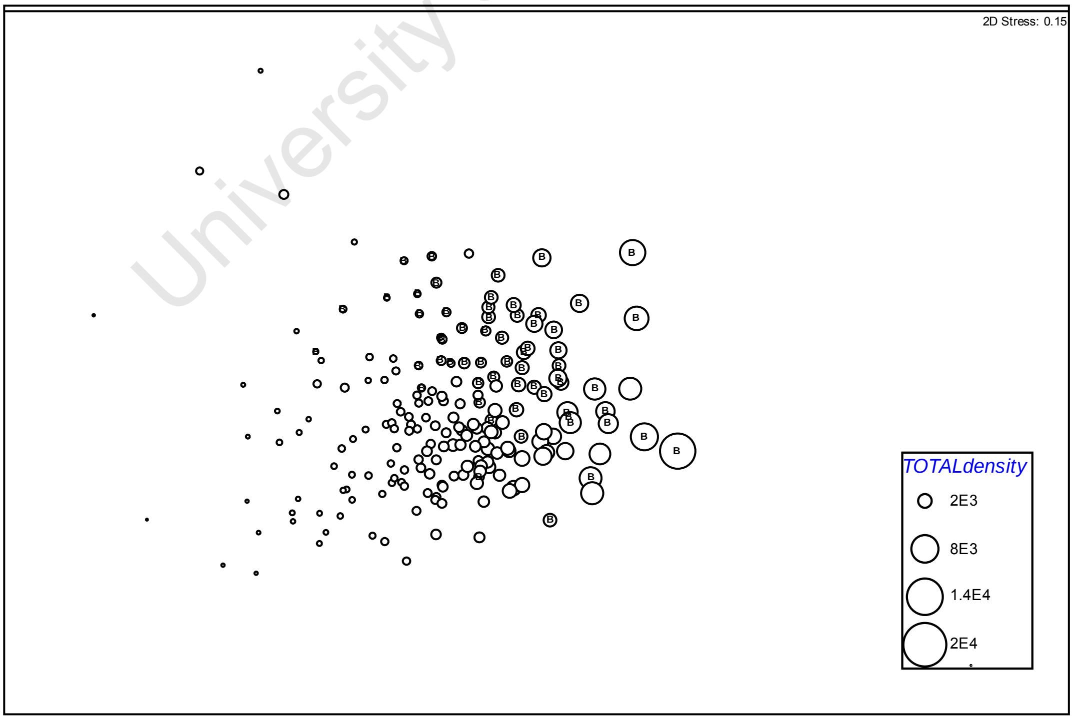

Figure 4.7 **Molenaars River bubble plot of total invertebrate density per unit stone area, overlaid onto the MDS plot of invertebrate assemblages as in Figure 4.6.** Baseline samples are indicated with a B. Bubble size represents increasing orders or magnitude in density, where E3, E4 = $x10^3$, $x10^4$.

Chlorophyll *a* per unit stone area, as well as the amount of organic matter associated with stones (per m^2 river bed) declined significantly in the Berg River from the Baseline to Post-flood period (Mann-Whitney U test, all $p < 0.001$). However, periphyton AFDW was not significantly different in the Post-flood period. Mean invertebrate density on whole stone surfaces declined from 2628 m^{-2} in the May Baseline sampling to some 1113 m^{-2} on Additional stones and to 1150 m^{-2} on Repeat-sampled stones (Figure 4.8a, Table 4.5), a decrease of 56 and 58 % respectively. This decrease was also substantially greater than the 34 % decrease recorded in the Molenaars River over a similar period, but with smaller floods. Table 4.5 presents the percentage reduction by taxon from the Baseline to the Post-flood period, but separately for Repeat-sampled and Additional stones. Only taxa whose Baseline and / or Post-flood densities were greater than 1 indiv. m^{-2} are represented. Significant differences (Mann-Whitney U test, adjusted for tied ranks) between Baseline - Additional stones are indicates in bold text. All but four invertebrate taxa declined significantly. Furthermore, for most taxa, the response on Repeat-sampled stones was similar to that on Additional stones, suggesting that the 'sampling effect' on the former was minor. Large differences in response were particularly noticeable on *Pseudocloeon* spp. *Lithogloea harrisoni* and *Nadinitella crassi*, three taxa that <u>increased</u> in densities over the study period (Table 4.5).

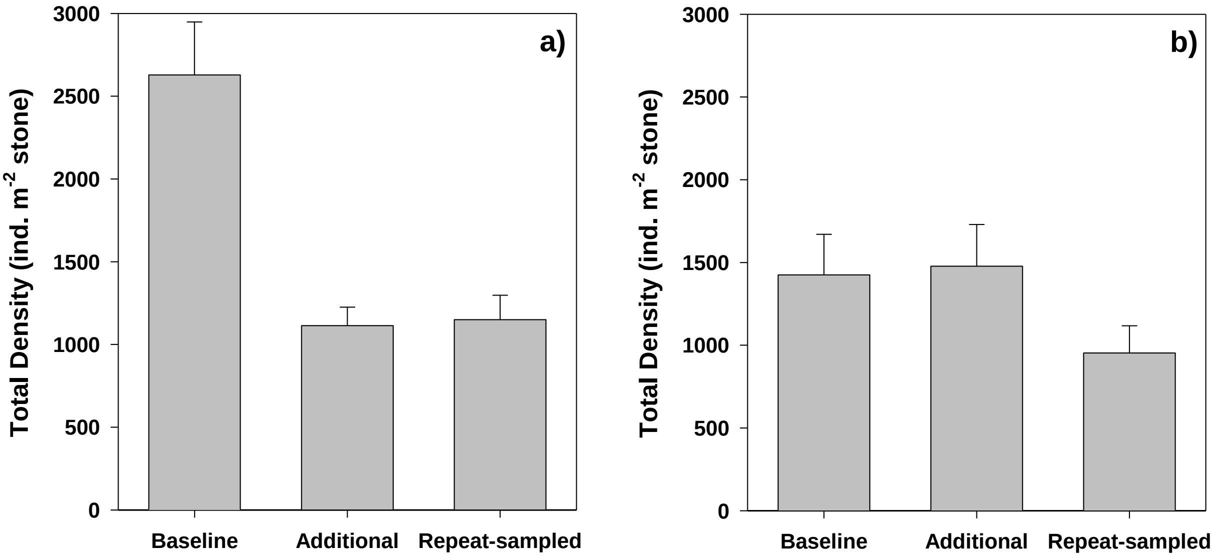

Figure 4.8 Mean total invertebrate densities in the Berg River in 2004 a) on whole stones and b) on the tops of immovable stones. Data for the post-flood sampling period are given separately for the Repeat-sampled stones and the Additional (control) stones. Bars denote standard error. Abundance data are untransformed to facilitate interpretation.

In contrast, densities on the tops of immovable stones (Figure 4.8b) showed no change in invertebrate densities between the Baseline sampling and post flood Additional stones, but a non-significant 30 % decline in the case of Repeat-sampled stones, considerably less than observed in the case of the Molenaars River data, where invertebrate densities on stone tops declined by some 70 %.

Although overall invertebrate densities on the top surfaces of immovable boulders were not dramatically altered, actual assemblage changes on stone tops from the Baseline to Post-flood periods were more pronounced than those on whole stones (Figure 4.9). Here there was a greater degree of separation between

Table 4.5 Percentage decrease from the Baseline to Post-flood periods, as represented by the Repeat-sampled stones and Additional stones data sets. All taxa are in densities per m^2 stone surface area. Negative values indicate increases in density. Significant differences (Mann-Whitney U test) between Baseline - Additional stones are indicated by p- values in **bold text**.

Taxon / variable	**% reduction on Repeat sampled stones, relative to Baseline**	**% reduction on Additional stones, relative to Baseline (Mann-U p value)**	
Chlorophyll-*a* (mg/m^2 stone)	64.8	38.7	**(0.002)**
Periphyton AFDW(mg/m^2 stone)	31.5	19.9	(0.746)
Associated organic matter (g/m^2 bed)	70.8	81.4	**(0.000)**
Associated inorganic matter (g/m^2 bed)	82.1	62.5	**(0.001)**
Total invertebrate density	56.3	57.6	**(0.000)**
Species density (# taxa /m^2 stone)	39.8	30.2	**(0.014)**
Demoulinia spp.	93.4	97.8	**(0.000)**
Afroptilum spp.	59.2	75.7	**(0.023)**
Demoreptus capensis	65.9	59.6	(0.232)
Pseudocloeon sp.	-19.0	-437.0	**(0.001)**
Baetis spp.	26.1	26.6	**(0.017)**
Lestagella penicillata	78.3	75.4	**(0.000)**
Lithogloea harrisoni	-49.3	-119.9	**(0.000)**
Nadinitella crassi	-120.7	100.0	**(0.000)**
Aprionyx spp.	88.5	93.6	**(0.000)**
Adenophlebia spp.	87.9	100.0	**(0.000)**
Euthralus elegans	77.0	90.5	**(0.000)**
Castanophlebia spp.	86.7	75.4	**(0.000)**
Afronurus harrisoni	65.5	81.4	**(0.000)**
Afronurus sp.	92.5	84.9	**(0.000)**
Cheumatopsyche afra	89.6	100.0	**(0.001)**
Cheumatopsyche maculata	77.0	86.3	**(0.002)**
Orthotrichia barnardi	65.4	35.5	(0.399)
Athripsodes bergensis	79.6	88.5	**(0.000)**
Chimarra sp.	95.1	94.0	**(0.000)**
Notonemouridae	4.8	22.4	(0.252)
Simulium spp.	21.5	61.2	(0.157)
Orthocladinae	53.0	54.9	(0.079)
Tanypodinae	91.4	93.6	**(0.000)**
Chironomini	36.9	58.9	**(0.018)**
Tanytarcini	71.2	77.5	**(0.000)**
Athericidae	92.0	97.6	**(0.000)**
Elmidae larvae	86.8	80.8	**(0.000)**
Elmidae adults	71.4	85.3	**(0.000)**
Scirtidae	69.9	67.7	**(0.005)**
Hydraenidae	100.0	100.0	**(0.026)**
Hydraenidae adults	85.7	100.0	**(0.000)**
Hydrachnellae	53.9	60.6	**(0.005)**
Aeschnidae	100	100	**(0.003)**

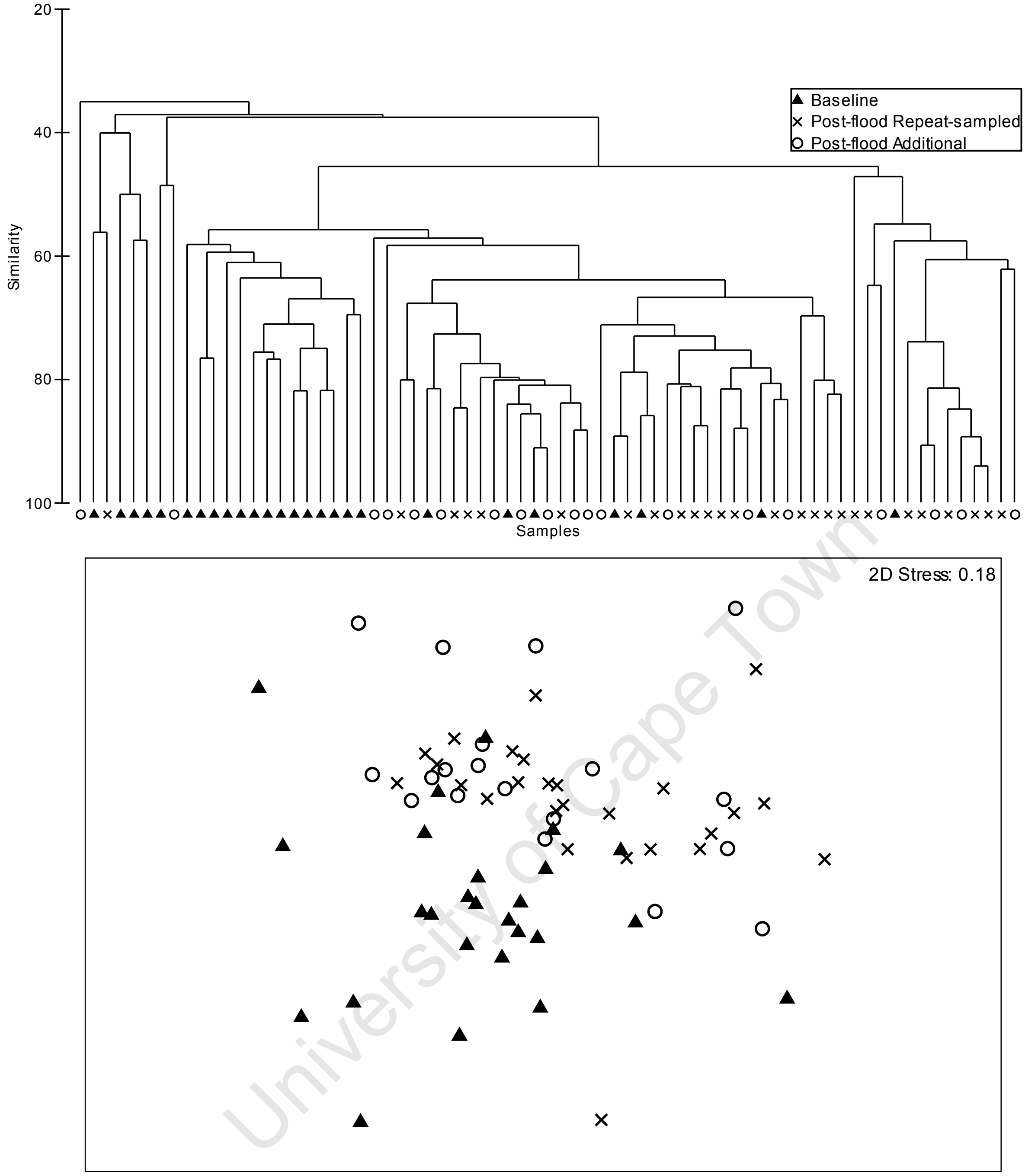

Figure 4.9 Berg River PRIMER results: a) Cluster analysis and b) MDS plots of invertebrate assemblage changes on the top surfaces of immovable boulders from Baseline to Post-flood period, based on Bray Curtis similarity coefficients. Both repeat-sampled and additional stones (control) samples for the post-flood period were included in the analysis.

samples according to period of sampling. As with the whole stone multivariate analysis, ANOSIM highlighted differences between Baseline and Post-flood sample sets (Global R = 0.147, p = 0.001, pairwise comparisons indicated in Table 4.6). Repeat-sampled stones were not significantly different from Additional stones (Table 4.6), despite the different mean invertebrate densities on these two groups of post-flood stones indicated in Figure 4.8b.

Table 4.6 **ANOSIM Pairwise tests for differences in invertebrate assemblages on the top surfaces of immovable stones over the Baseline to Post-flood period in the Berg River, 2004.**

Pairwise comparison	**R-statistic**	**Significance level**
Baseline vs. Repeat-sampled stones	0.214	p = 0.001
Baseline vs. Additional stones	0.176	P = 0.001
Additional vs. Repeat-sampled stones	0.033	P = 0.163 (n.s.)

Because of the difference in total invertebrate density between the two groups of stone tops data for the Post-flood survey, SIMPER analysis of the taxa contributing most strongly to the difference between assemblages was conducted separately for the Baseline - Repeat-sampled stones and the Baseline - Additional stones comparisons. The results show that, whilst many taxa declined in density over the flood period, as in the case of the whole stone analysis, a greater number of taxa showed an actual increase in density over the study period on this category of stones than on whole stones. Over one third of the discriminator taxa in the case of the immovable Repeat-sampled stones, and more than half the taxa on the Additional stones, either remained unchanged or increased in density, indicated by shading in Table 4.7a and b. The six most important discriminator taxa were the same in both Repeat-sampled and Additional stones comparisons.

Table 4.7a **Discriminator taxa in a SIMPER analysis comparing the dissimilarities between invertebrate assemblages on the tops of immovable stones, from the Baseline and Post-flood surveys in the Berg River in 2004: REPEAT SAMPLED STONES.** Taxa that cumulatively made up 75% of the dissimilarity between groups are listed, in order of their individual contribution (%) to dissimilarity. Those with the highest Diss / SD ratio were the more consistent discriminator taxa. Results are provided separately for the Baseline - Repeat-sampled stones and the Baseline - Additional stones comparisons. Densities are re-transformed from the square root used in the analysis to No. m^{-2}, for ease of interpretation. Taxa showing an increase in density over the study period are shaded.

Discriminator taxa	**Average abundance (# m^{-2})**		**Average dissimilarity**	**Diss/SD**	**Contrib. %**	**Cumul. %**
	Baseline survey	**Post-flood Repeat-sampled stones**				
Baetis spp.	701	302	11.46	1.24	22.79	22.79
Orthocladinae	190	420	11.22	1.44	22.32	45.11
Elmidae larvae	31	6	4.28	1.06	8.52	53.63
Simulium spp.	1	13	3.10	0.78	6.17	59.80
Demoulinia spp.	15	0.1	3.10	0.73	6.17	65.97
Lestagella penicillata	10	1	2.49	1.00	4.95	70.91
Tanypodinae	5	0.5	2.17	0.53	4.32	75.24
Chironomini	3	3	2.02	0.80	4.01	79.25
Tanytarcini	1	1	1.31	0.71	2.60	81.84
Lithogloea harrisoni	0.5	1	1.14	0.69	2.28	84.12
Afroptilum spp.	2	0	1.02	0.47	2.02	86.14
Elmidae adults	1	0.1	0.85	0.46	1.70	87.84
Demoreptus capensis	0.3	0.3	0.74	0.47	1.47	89.30
Athripsodes bergensis	1	0.1	0.73	0.50	1.45	90.75

Table 4.7b Discriminator taxa in a SIMPER analysis comparing the dissimilarities between invertebrate assemblages on the tops of immovable stones, from the Baseline and Post-flood surveys in the Berg River in 2004: ADDITIONAL STONES. Definitions are as per Table 4.7a.

Discriminator taxa	Average abundance (# m^{-2})		Average dissimilarity	Diss/SD	Contrib. %	Cumul. %
	Baseline survey	Post-flood Additional stones				
Orthocladinae	190	642	12.40	1.49	24.34	24.34
Baetis spp.	701	368	10.66	1.20	20.93	45.27
Elmidae larvae	31	33	4.18	1.15	8.21	53.48
Lestagella penicillata	10	13	3.30	0.85	6.48	59.96
Simulium spp.	1	14	2.84	0.77	5.58	65.54
Demoulinia spp.	15	0	2.78	0.71	5.45	70.98
Tanypodinae	5	1	1.74	0.64	3.42	74.40
Chironomini	3	2	1.67	0.74	3.29	77.69
Lithogloea harrisoni	0.5	2	1.44	0.62	2.82	80.51
Demoreptus capensis	0.3	2	1.15	0.74	2.25	82.76
Tanytarcini	1	1	1.09	0.71	2.14	84.90
Afroptilum spp.	2	0.1	1.08	0.52	2.11	87.02
Elmidae adults	1	0.3	0.92	0.52	1.80	88.82
Hydrachnellae	0.1	0.5	0.69	0.44	1.35	90.17

4.4.2.i Effects of stone movement

The results of MDS analysis of the Baseline and Repeat-sampled stones data for the Berg River are presented in Figure 4.10. The individual stone numbers are also indicated, and the distance between the points in the plot representing each paired Baseline and the Post-flood stone is representative of how similar its community structure remained, or the extent to which it changed over the study period. The results indicate again, the major axis of change being associated with the time period of sampling.

The individual Bray Curtis similarity coefficient for each repeat-sampled stone, from Baseline - Post-flood sampling, was extracted from these results. Stones were grouped according to their movement characteristics (refer to Table 3.7, section 3.2.2*ii*), and differences in the similarity coefficients across stone movement groupings were examined to test whether stones moved immediately before the post-flood survey were more different from their baseline condition than were those which had not moved over the full study period, or those which moved only once, during the early Class IV flood.

Since the data conformed to the assumptions for parametric testing, an ANOVA was performed on Bray Curtis similarities for the three movement groups, indicated in Table 4.8, resulting in a significant difference (F=5.53, p = 0.0065, d.f. = 2). A post-hoc Tukey test used to identify significant differences between pairs of samples showed that Movement Group 1 and 2 stones were not significantly different (p = 0.99), Movement Group 3 (i.e. moved in flood 5) was significantly different from both Group 1 and 2 stones (p = 0.009 and 0.025 for Group 1 vs. Group 3 and Group 2 vs. Group 3 respectively).

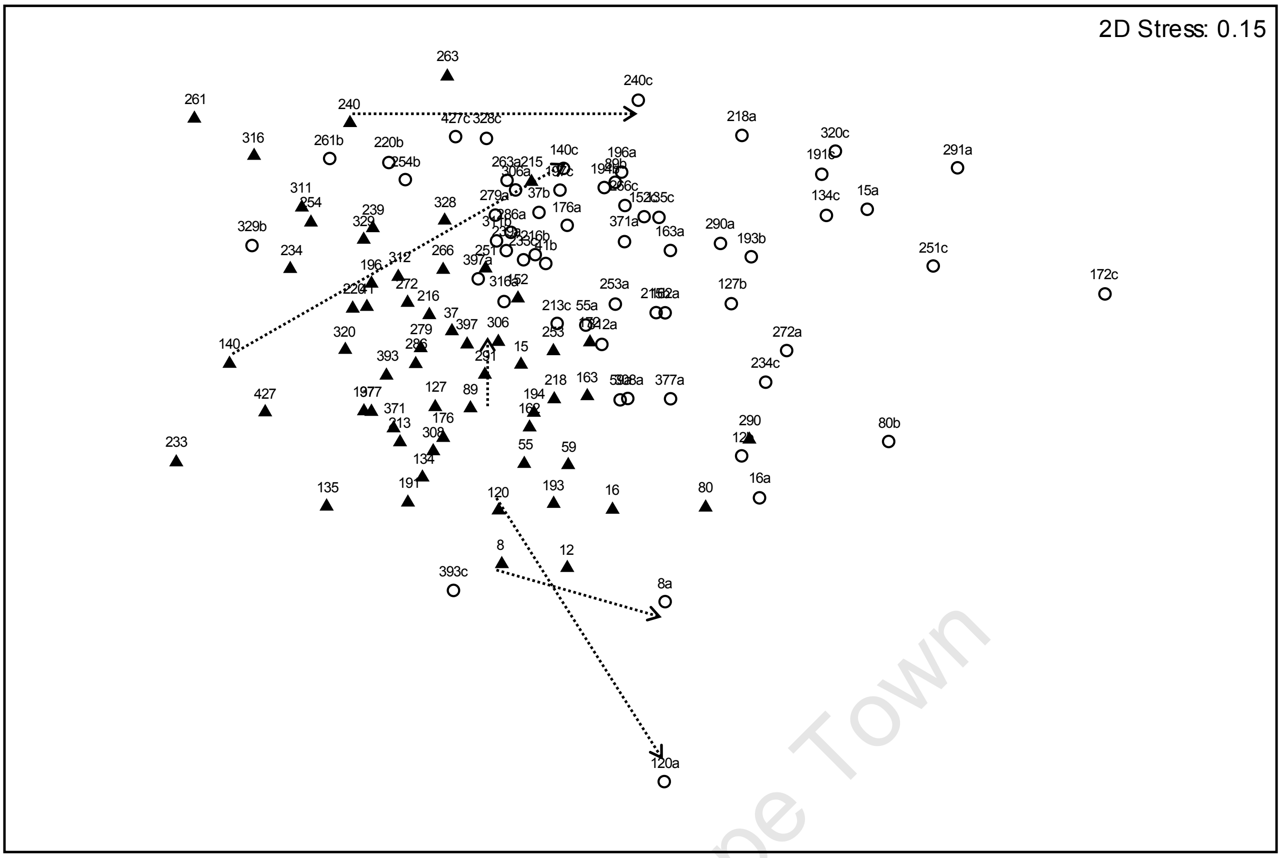

Figure 4.10 Berg River MDS plot of invertebrate assemblage changes over the Baseline to Post-flood period, based on Bray Curtis similarity coefficients, using Repeat-sampled stone data only. The distance between the points in the plot representing each paired Baseline and the Post-flood stone is representative of how similar its community structure remained, or the extent to which it changed over the study period. Examples of this are indicated by arrows from the Baseline to the Post-flood position for a few stones.

Thus stones that moved immediately before the post-flood sampling were some 10 - 15 % more different from their baseline condition than those that did not. The Group 2 stones which had moved in Flood 2, but then remained stationery for the month following their movement until the sampling date, after Flood 5, were not statistically different from unmoved stones, in terms of their overall assemblage similarities relative to their baseline condition.

Table 4.8 A comparison of the degree to which invertebrate assemblages on Repeat-sampled stones were altered from their Baseline condition, according to whether or not stones were moved during floods. The degree of change is represented by the Bray Curtis Similarity coefficient of each repeat-sampled stone, comparing its Baseline to Post-flood assemblage. In post-hoc pairwise comparisons, groups denoted by the same italic letter were not significantly different (Tukey's post-hoc test, p = 0.99)

Stone movement category	Average (median) Bray-Curtis similarity coefficient (%) of repeat-sampled stones, from the Baseline to the post-flood sampling	Significant differences (Tukey's post-hoc test)
1; unmoved in all floods	55.3 (59.2)	*a*
2; moved in Flood 2 but not thereafter	55.6 (58.0)	*a*
3; moved in Flood 2 and Flood 5	43.8 (43.5)	*b*

Despite the apparent effects of stone movement on the degree of change in assemblage composition, Cluster analysis and MDS of the Post-flood data showed no grouping of samples according to their movement category (Figure 4.11). An ANOSIM on these *a priori*-determined movement groups (1, 2, 3) revealed only weakly significant differences (Global R = 0.07 (significance level p = 0.04). Pairwise test results are indicated in Table 4.9 - at the more conservative significance level threshold of p = 0.01, suggested for pairwise tests in the ANOSIM procedure, none of the weak differences between movement groups (low R statistic) were significant.

Table 4.9 ANOSIM Pairwise tests for differences in invertebrate assemblages across stone movement groups. Group definitions are as per Table 4.8.

Pairwise comparison	R-statistic	Significance level
Group 1 vs. Group 2	0.041	p = 0.25 n.s.
Group 1 vs. Group 3	0.088	P = 0.04 n.s.
Group 2 vs. Group 3	0.079	P = 0.05 n.s.

The existence of possible relationships between applied stream power during the peak flood flows of Flood 2 and Flood 5, as well post-flood periphyton, flow and velocity variables and the post-flood invertebrate assemblages was tested by overlaying these variables onto the MDS plot. None of the physical or periphyton measures provided a good match that or suggested any gradients across the data. Total invertebrate density (inset in Figure 4.11) did show a gradient, but not one that compared well with the movement group of the samples. The BIOENV routine comparing these variables with the invertebrate patterns returned poor correlations.

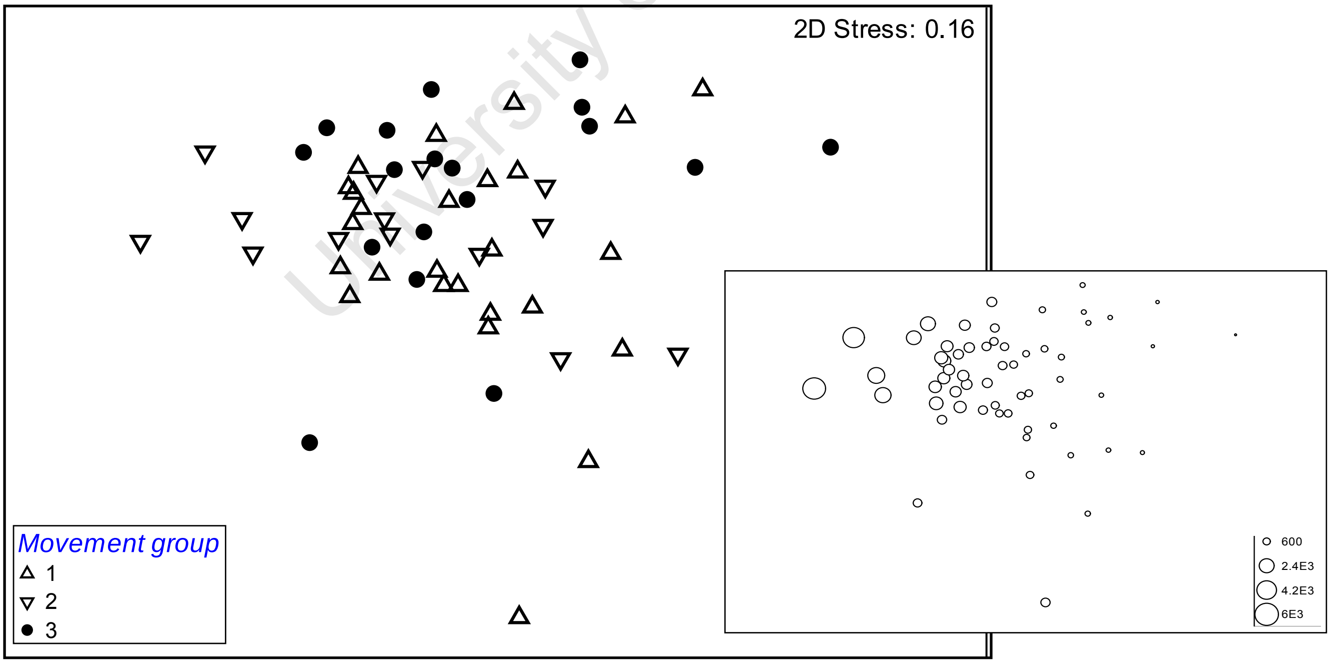

Figure 4.11 Berg River MDS plot of invertebrate assemblages on Repeat-sampled stones in the post-flood period, based on Bray Curtis similarity coefficients, categorised into stone movement groups. Groups 1-3 are defined as per Table 4.8. Inlay: bubble plot of total invertebrate densities superimposed over the MDS plot. The gradient of declining densities does not correspond to differences in stone movement category.

4.5 SPECIES-SPECIFIC RESPONSES TO FLOODS

The exploration community structure over the study period in the Molenaars and Berg Rivers indicated, firstly, that community structure changed gradually over a period characterised by numerous small intra-annual flood events, with significant levels of change only apparent as a cumulative effect of these small shifts (Molenaars River study). Secondly, a period characterised by substantially larger, bed-moving floods was associated with a more obvious alteration of assemblages (Berg River study). In both cases, community change was based largely on different degrees of reduction in the densities of the component species. Some apparently conflicting results were obtained regarding density decreases on top surfaces of large substrata between the two study rivers, with marked increases in many taxa in the Berg River, despite the larger floods there than in the Molenaars River. Evidence was also provided for community-level differences in the impacts of floods, based on the movement or not of stones, although community-level differences in this regard were not strong. Finally, the evaluation of species-level differences in recovery rates following initial denudation of stones suggested that some taxa recover rapidly, whilst others do not.

This section of the analysis seeks therefore to examine this aspect further, species by species where sufficient data were available. It therefore presents the results of an examination of the changes in densities and in population structure, associated with each sampling occasion in the Molenaars and Berg Rivers, relative to the magnitude of floods and the movement of individual stones.

The composite figures that follow present bar graphs of the mean density (+ standard error) in the Molenaars River from each of the five sampling occasions, Baseline, and Pre- and Post- Floods 1 and 4, as well as the frequency distributions describing population size structure at each of the five sampling occasions. For the Molenaars River, only whole stone samples were included in this analysis, since the sample sizes for pre- and post- flood datasets for stone tops were too small for meaningful comparisons. Each composite figure also provides the same density and population structure data for the Berg River, for the two sampling periods (May and July) in that study, but separately for each of the stone movement categories, as outlined in Table 3.7 (section 3.2.2*ii*), and including tops of immovable stones.

Significant invertebrate density changes from Kruskal-Wallis analysis of variance in densities over the five sampling periods on the Molenaars River are presented in Table 4.10. The percentage increase or decrease was calculated for both the Baseline - Post Flood 4 statistic and the Pre-Flood 1 to Post-Flood 4 statistic. Since recovery in many species was only partial, the latter measure is more conservative as it takes into account the potential error associated with initial sampling of stones, referred to as the ‘sampling effect’. In Table 4.10, where sample densities at the Pre-Flood 1 survey were lower than the Baseline, the more conservative value for the percentage change has thus been used.

Analysis of variance results for the Berg River invertebrate samples, comparing Baseline and Post-flood stones, for unmoved stones and those moved in both Flood 2 and Flood 5 are indicated in Table 4.11, for both Repeat-sampled stones and Additional stones. The results of analysis of invertebrate changes on tops of immovable boulders (Category 7 and 8 stones) are also included. The changes in density on stones that moved only once (Category 2 and 5 stones) were not included in the analysis, as separating out flood impact from possible post-initial sampling recovery proved to be too difficult.

Significant differences in population size frequency distributions based on Kolmogorov Smirnoff tests for pairwise differences between each of the five periods for the Molenaars River study are presented in Tables 4.12 and 4.13 and between the two periods for the Berg River, in Table 4.14.

4.5.1 Ephemeroptera:

4.5.1.i Baetidae

Figures 4.12 to 4.16 show the results of this analysis for five Baetidae species or genera. The response of each species, in terms of seasonal changes and flood effects on population density and size structure, is discussed separately in the following passages.

a ***Demoulinia*** spp.

Demoulinia spp. was characterised during the baseline condition by a dominance of relatively small instars (Figure 4.12b in the Molenaars River, Figure 4.12 e for the Berg River), with very few mature nymphs. Mature nymphs (black wing buds) were only associated with head width size classes from around 0.8 mm for all the Baetidae. There was a fairly good recovery in densities from the Baseline to the Pre-Flood 1 sampling period (Figure 4.12a): the difference in density was not significant between these periods. However, the recovering population was significantly different with regard to its size distribution, with an increase in mean head width from 0.32 mm to 0.41mm (Table 4.12, frequency distributions for the Baseline and Pre-Flood 1 data shown in Figure 4.12b and c). This appeared to reflect developmental growth of the population, with the modal head width size class in the Baseline survey of 0.3 mm being replaced by a mode of 0.4 mm head width a month later in the Pre-Flood 1 survey (Figure 4.12b and c).

Although the differences in density in the Molenaars River were not significantly different over the study period (Kruskal-Wallis, $p > 0.05$, Table 4.10), this, as with many invertebrate data is at least in part a reflection of the patchiness of distribution even prior to the onset of floods. Overall, there was a decline in density of some 53 % (Table 4.10; Figure 4.12a), and the significant frequency distribution changes from the Baseline to subsequent periods suggest that the population that remained was simply maturing, without any new instar recruitment over the study period (Table 4.12 and 13).

In the Berg River the population in May 2004 was more mature than that in the Molenaars River in June 2003 (modal head width size class in the Berg River = 0.5 mm vs. 0.3 mm in the Molenaars River), but nevertheless still comprised of immature nymphs. Over the flood period, this species was dramatically and significantly reduced in density by between 90 and 100%, as previously indicated in Table 4.5. This reduction was on all categories of stones (refer back to Table 3.7 for stone category definitions), irrespective of whether or not they moved (Figure 4.12e, Table 4.11). The remaining population was not significantly different with regard to size distribution (Table 4.14).

b ***Afroptilum*** spp.

In the Molenaars River, the Baseline density of *Afroptilum* spp. was far greater than that of *Demoulinia* spp. (Figure 4.13a vs. Figure 4.12a), and recovery following initial sampling was characterised by a non-significant increase on stones. Overall, the population of Afroptilum showed a 58 % decrease from the Baseline condition to the end of the study period (Table 4.10). The effect of floods on population structure seemed to emphasise losses of smaller instars: post-flood populations were significantly different from the pre-flood populations in both Flood 1 and Flood 4 (Figure 4.13b,c,d; Table 4.12) and were comprised of a greater proportion of older / larger nymphs than the pre-flood populations, with significantly larger individuals

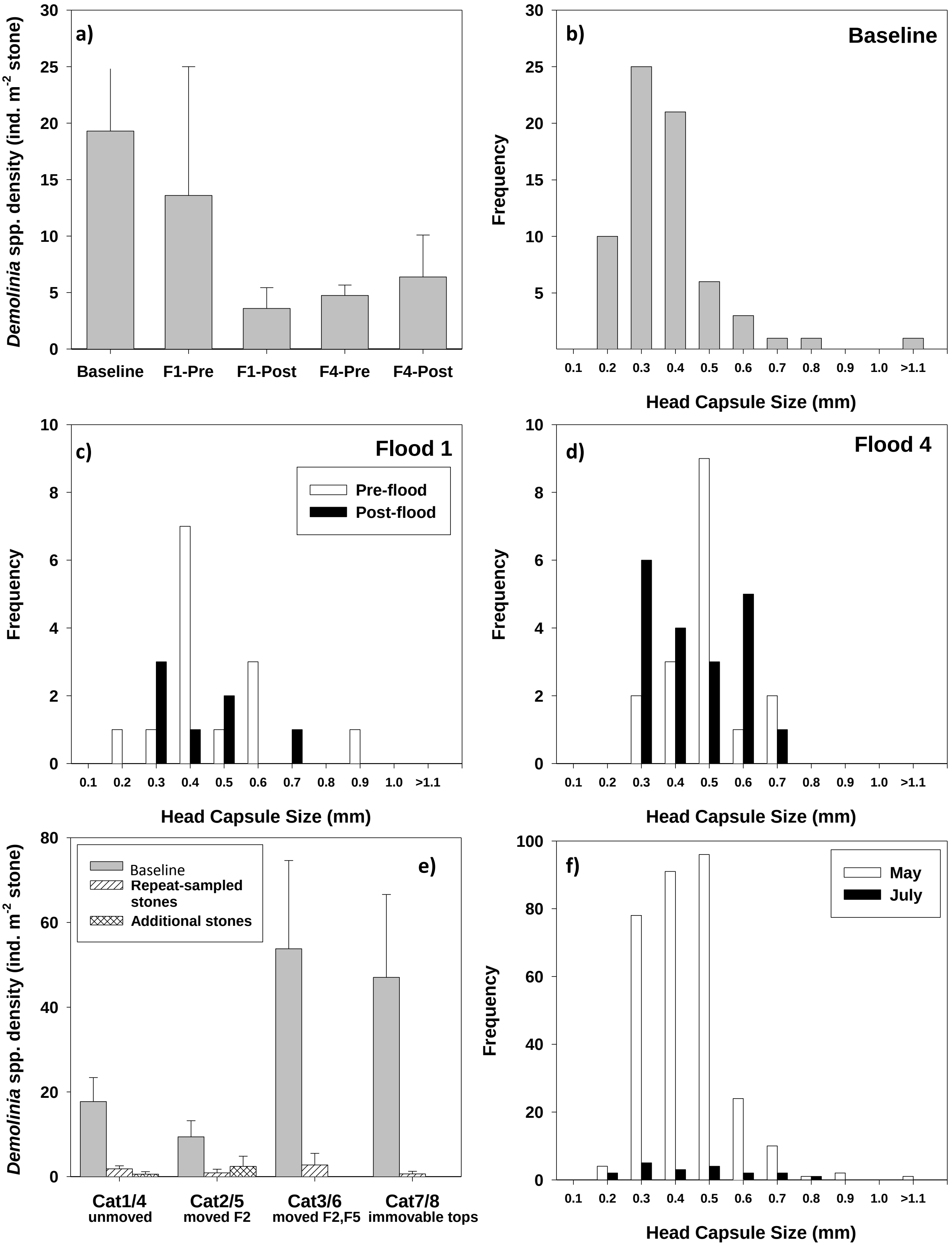

Figure 4.12 a) Mean density (+ standard error) and (b-d) population size frequency distribution of *Demoulinia* spp. in the Molenaars River, on each of the five sampling occasions during the 2003 study, and (e) mean species density and (f) population size frequency distributions for the two sampling surveys in the Berg River in 2004. Density data show each of the stone movement categories separately for the Berg River study.

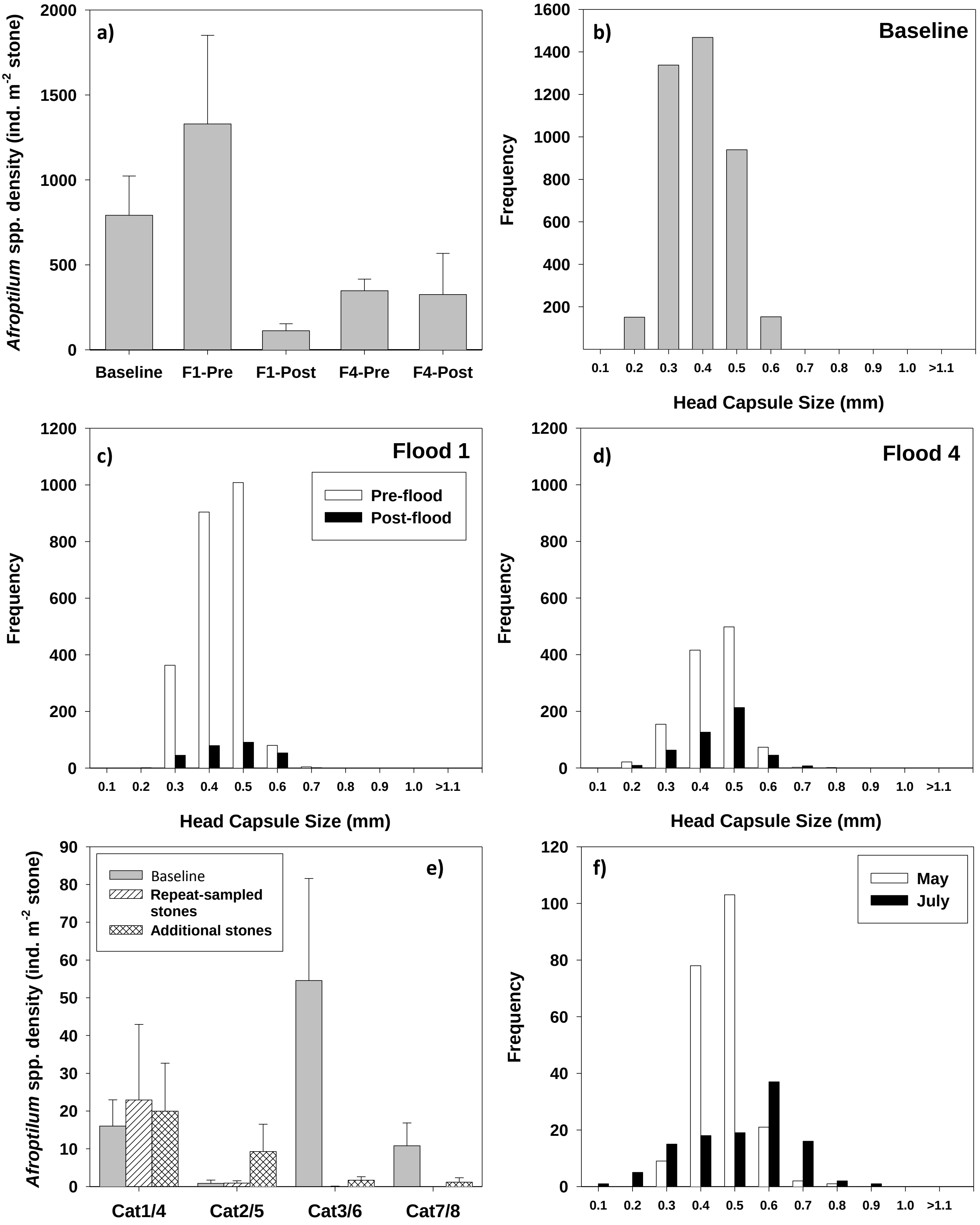

Figure 4.13 a) Mean density (+ standard error) and (b-d) population size frequency distribution of *Afroptilum* spp. in the Molenaars River, on each of the five sampling occasions during the 2003 study, and (e) mean species density and (f) population size frequency distributions for the two sampling surveys in the Berg River in 2004. Density data show each of the stone movement categories separately for the Berg River study.

In the Berg River, there was an overall decrease in density of some 60 - 75 % (Table 4.5), which was not substantially higher than in the Molenaars River, despite the difference in flood sizes, although in the case of the Berg data this decrease was significant. Importantly, however, unmoved stones provided a substantial refugium for *Afroptilum* spp. This refugium is demonstrated by the finding that post-flood densities on Category 1 and Category 4 (unmoved) stones were higher but not significantly different from the Baseline condition, whilst those on moved stones (Category 3 and 6) showed a significant decrease by between 97 and 100 % (Table 4.11). The species was not found on the top surfaces of immovable stones after the Class IV flood (Category 7 and 8 stones), these clearly not providing the same refugium as Category 1 and 4 stones. The Post-flood population structure was significantly different from the Baseline situation (Table 4.14), where the frequency-distributional changes suggest both a relative decline in small instars, as a result of flood mortality, and a maturation of the remaining population over the two-month period, with no new recruitment.

c ***Demoreptus capensis***

There was very little change in *D. capensis* densities in the Molenaars River over the study period, with a non-significant increase in population size overall (Table 4.10, Figure 4.14a). Population structure, however, did change significantly over the study period, reflected in a gradually increasing mean head width, with significant differences in size-frequency distribution between the Baseline and all other surveys (Table 4.12). However, pre- to post-flood populations for both Flood 1 and Flood 4 were not significantly different (Table 4.12). Despite the increase in mean head width over the study period, the continuous dominance of the smallest size classes suggests an ongoing recruitment of new instars during the season, unlike *Demoulinia* spp. or *Afroptilum* spp.

In the Berg River, the Baseline population of *D. capensis* was comprised of a somewhat more mature cohort than the Molenaars River (0.5 mm modal head width vs. 0.3 mm in the Molenaars River; Figure 4.14e). *D. capensis* was one of the four taxa demonstrating a non-significant change in overall density, with a decline of between 60 and 66 % (Table 4.5), the former based on comparing Baseline and Additional stones, and the latter comparing Baseline with Repeat-sampled stones. The analysis per stone category, however, showed a somewhat confusing picture - a relative decline on unmoved stones, whilst on the other hand an increase on moved stones and on immovable boulder surfaces - although once again none of these changes were significant (Figure 4.14e, Table 4.11). Analysis of the change in size-frequency distribution from the Baseline to Post-flood periods indicates a strong shift from a normal size distribution dominated by medium sized instars (head width 0.5 mm), to a bimodal distribution with peaks at 0.3 and 0.8 mm, a significant change in frequency distribution (Figure 4.14f, Table 4.14). The results indicate that this species maintained its population density in the face of floods by a combination of continuous recruitment of new instars, as well as through the resistance to floods shown by larger instars, some of which had reached maturity by the end of the study period in July. Despite the apparent resistance, there was no indication of a relative refugium provided by unmoved stones in the case of *D. capensis*.

Table 4.10 Percentage decrease in density (unless increase specified) and results of Kruskal-Wallis analysis of variance between invertebrate density and the five sampling periods in the Molenaars River, for selected taxa. The % change in density is given as the Baseline - Post Flood 4 % change, unless recovery after initial sampling was not adequate, in which case it is given as the Pre-Flood 1 to Post-Flood 4 % change. B = Baseline; F1, F4 = Floods 1 and 4, Pre-, Po- = Pre- and Post- Flood sampling periods. H = test statistic. Significant Dunn's post-hoc multiple comparisons are also presented. n.s. = not significant

	% change over study period	**H**	**P value**	**Dunn's significantly different groups (p-value)**
Demoulinia spp.	53 %	n.s.		
Afroptilum spp.	58 %	13.6	0.009	B - F1Po (0.005)
Demoreptus capensis	10 % increase	n.s.		
Pseudocloeon sp.	82 %	26.3	0.000	B - F4Pre (0.002) B - F4Po (0.002)
Baetis spp.	16 %	16.3	0.003	B - F4Pre (0.034) B - F4Po (0.018)
Lestagella penicillata	No change F1Pre to F4Po	25.3	0.000	B - F1Pre (0.021) B - F1Po (0.006) B - F4Pre (0.004) B - F4Po (0.001)
Lithogloea harrisoni	2500 % increase	45.0	0.000	B - F4Pre (0.001) B - F4Po (0.001) F1Pre - F4Pre (0.011) F1Pre - F4Po (0.001)
Aprionyx spp.	99 %	9.3	0.054	n.s.
Euthralus elegans	No change F1Pre to F4Po	17.2	0.002	B - F4Pre (0.000)
Afronurus harrisoni	78 %	19.8	0.001	B - F4Po (0.002)
Afronurus sp.	84 %	51.6	0.000	B - F1Po (0.014) F1Pre - F1Po (0.014) B - F4Pre (0.000) B - F4Po (0.000) F1Pre - F2Pre (0.001) F1Pre - F2Po (0.000)
Cheumatopsyche afra	Recovery to 66 % of Baseline over study period	14.6	0.006	n.s.
Chimarra sp.	Recovery to 64 % of Baseline over study period	n.s.		
Athripsodes bergensis	93 %	13.6	0.001	B - F4Po (0.043)
Agapetus agilis	47 %	13.4	0.010	B - F4Po (0.018)
Notonemouridae	756 % increase	n.s.		
Elporia spp.	4 %	n.s.		
Simulium spp.	100 % increase	11.19	0.025	B - F1Po (0.015)
Orthocladinae	3 %			
Tanypodinae	72 %			B - F1Po (0.017) B - F4Pre (0.031) B - F2Po (0.000)
Chironomini	14 %	17.0	0.002	B - F1Po (0.049)
Tanytarcini	14 %	10.4	0.035	n.s.
Elmidae larvae	Recovery to 83 % of Baseline over study period	9.5	0.049	n.s.
Elmidae adults	31 %			
Scirtidae	10 % increase			
Hydrachnellae	51 %	n.s.		

Table 4.11 Percentage reduction (+ = increase) and results of Kruskal-Wallis analysis of variance between invertebrate density, from the Baseline condition to the Post-flood sampling on the Berg River, for each stone category, for most taxa. H = Kruskal-Wallis test statistic, p = significance level n.s. = not significant. Dunn's pairwise significant differences are indicated as follows: * p < 0.05; ** p< 0.01; ***p>0.001.

	% decrease on Category 1 / 4 stones (unmoved)				**% decrease on Category 3 / 6 stones (moved F2 and F5)**				**% decrease on Category 7 / 8 stones (immovable boulder tops)**			
	Repeat-sampled (Cat1)	**Additional (Cat4)**	**H**	**P value**	**Repeat-sampled (Cat3)**	**Additional (Cat6)**	**H**	**P value**	**Repeat-sampled (Cat7)**	**Additional (Cat8)**	**H**	**P value**
Demoulinia spp.	90**	97**	17.6	0.000	95	100**	29.9	0.000	99	100	17.6	0.001
Afroptilum spp.	+43	+25	n.s.		100	97*	16.8	0.000	100	89	n.s.	
Demoreptus capensis	87	80	n.s.		+120	+580	n.s.		+27	+209	n.s.	
Pseudocloeon sp.	1	+367**	16.6	0.003	Not present in Baseline samples				100	+5	n.s.	
Baetis spp.	34	11	n.s.		46*	29	7.0	0.030	57***	41**	15.7	0.000
Lestagella penicillata	67***	64***	21.6	0.000	89***	84***	36.8	0.000	82	+32	n.s.	
Lithogloea harrisoni	+100	+261**	10.7	0.005	22	1	n.s.		+24	+148	n.s.	
Aprionyx spp.	74**	85**	12.9	0.002	100**	98***	25.6	0.000	Not present on top surfaces of boulders			
Euthralus elegans	71*	77	8.9	0.012	92***	95***	27.3	0.000				
Afronurus harrisoni	54	88**	12.6	0.002	75	89***	20.4	0.000				
Afronurus sp.	89***	79***	23.2	0.000	97***	93***	42.2	0.000				
Cheumatopsyche afra	100	100	n.s.		-	100	n.s.		100	100	n.s.	
Cheumatopsyche maculata	91	75	7.0	0.030	100	89	n.s.		100	n.s.	n.s.	
Orthotrichia barnardi	92	69	n.s.		27	4	n.s.		100	100	n.s.	
Athripsodes bergensis	72*	91**	14.5	0.007	97*	93*	14.7	0.001	n.s.	72	n.s.	
Chimarra sp.	92	51	n.s.		100	99	8.8	0.012	Not present on top surfaces of boulders			
Notonemouridae	28	56	n.s.		16	+57	n.s.					
Simulium spp.	55	73	n.s.		+5	8	n.s.		+635*	+664	8.0	0.018
Orthocladinae	42	28	n.s.		60	50	n.s.		+40	+124**	9.8	0.008
Tanypodinae	94***	94***	35.8	0.000	90***	94***	29.5	0.000	52	81	n.s.	
Chironomini	17	24	n.s.		14	50			35	41		
Tanytarcini	76**	62	10.4	0.005	85***	84***	21.7	0.000	+5	+28	n.s.	
Elmidae larvae	84***	75**	26.2	0.000	91***	82***	25.0	0.000	75	27	7.3	0.026
Elmidae adults	73*	87*	11.6	0.003	95	79	7.5	0.023	95	70	n.s.	
Scirtidae	43	27	n.s.		95	87	n.s.		Not present on top surfaces of boulders			
Hydraenidae	100	100	n.s.		100	100	n.s.					
Hydraenidae adults	100	100	n.s.		100	100	n.s.					
Hydrachnellae	46	43	n.s.		81	25	n.s.		31	+55	n.s.	

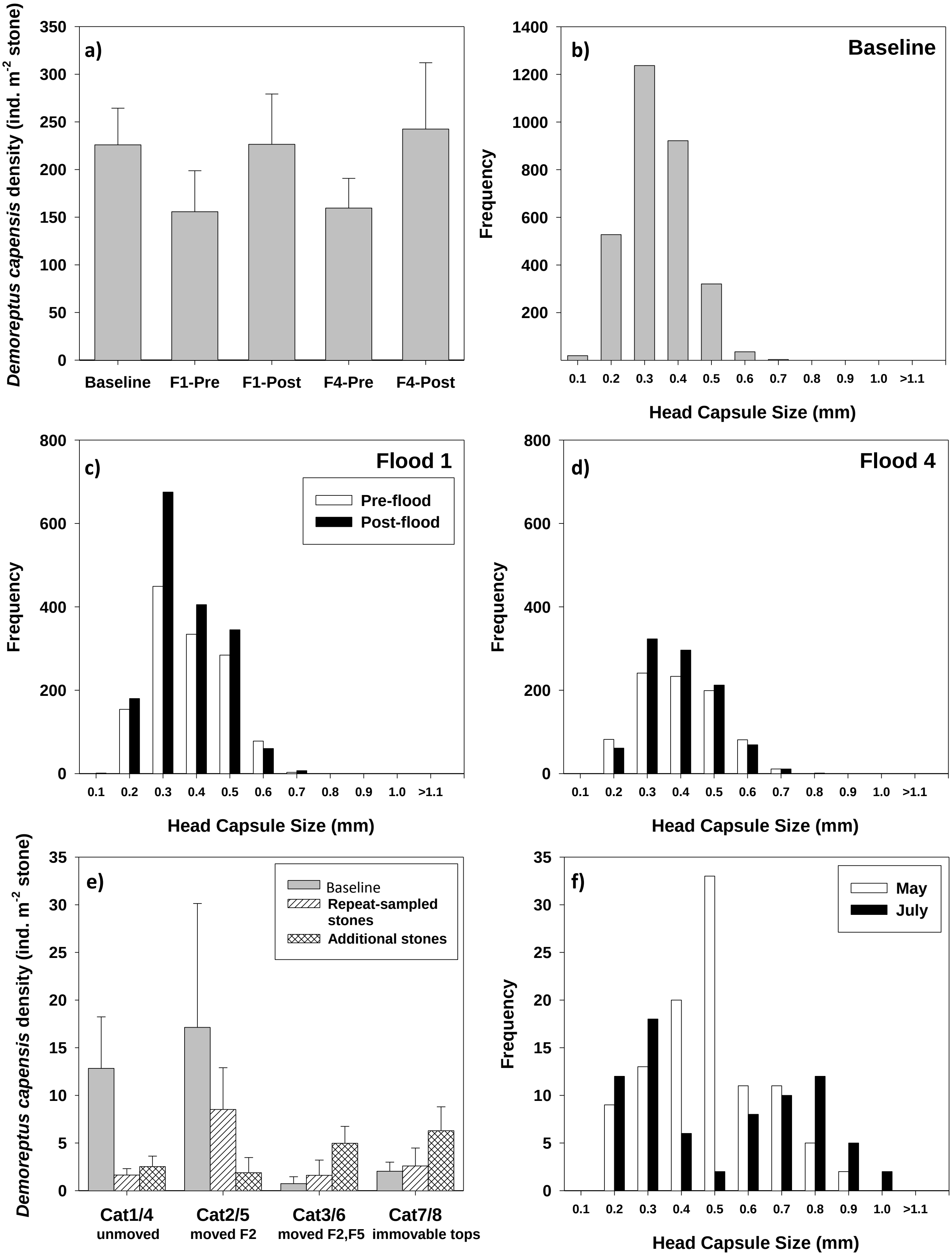

Figure 4.14 a) Mean density (+ standard error) and (b-d) population size frequency distribution of *Demoreptus capensis* in the Molenaars River, on each of the five sampling occasions during the 2003 study, and (e) mean species density and (f) population size frequency distributions for the two sampling surveys in the Berg River in 2004. Density data show each of the stone movement categories separately for the Berg River study.

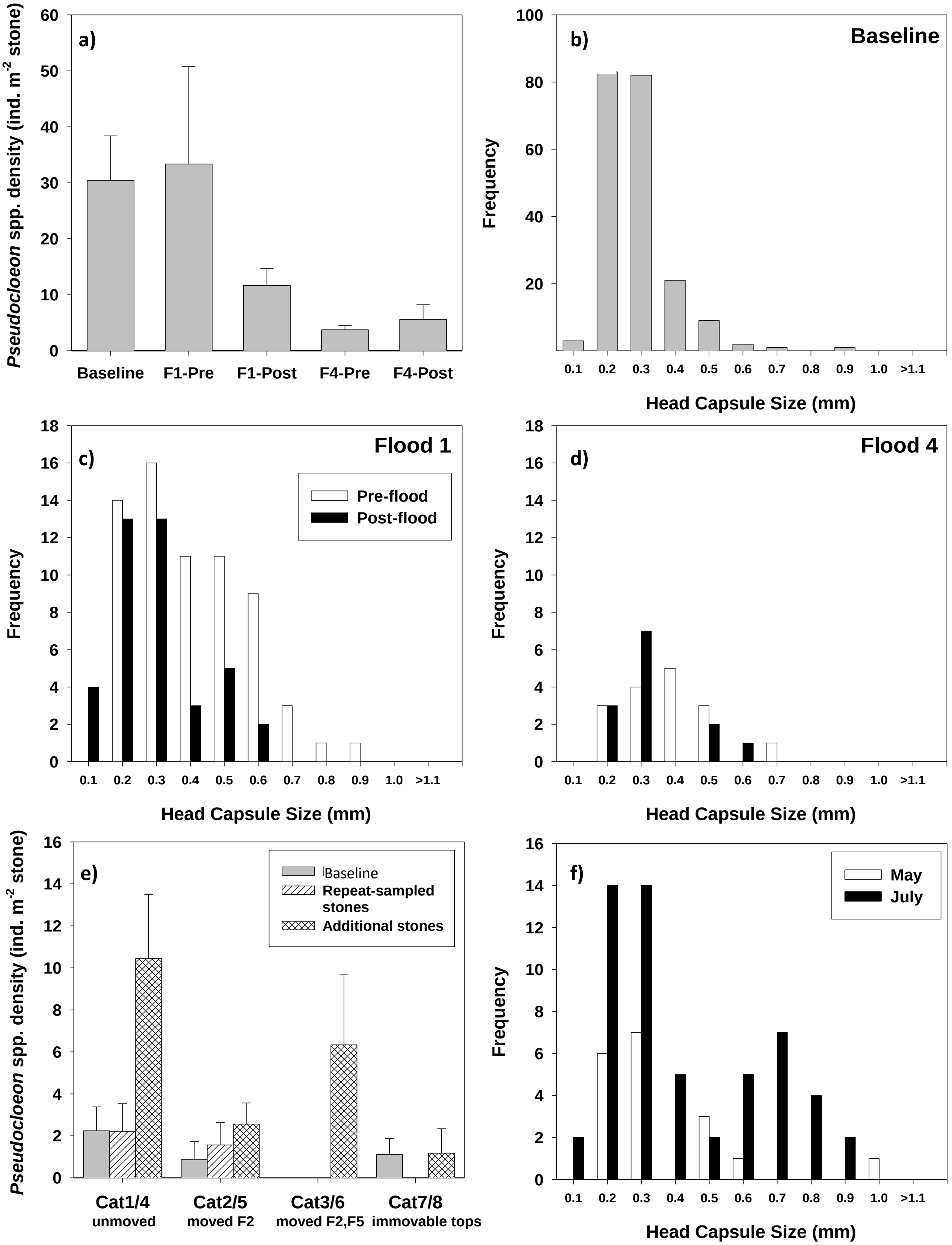

Figure 4.15 a) Mean density (+ standard error) and (b-d) population size frequency distribution of *Pseudocloeon* spp. in the Molenaars River, on each of the five sampling occasions during the 2003 study, and (e) mean species density and (f) population size frequency distributions for the two sampling surveys in the Berg River in 2004. Density data show each of the stone movement categories separately for the Berg River study.

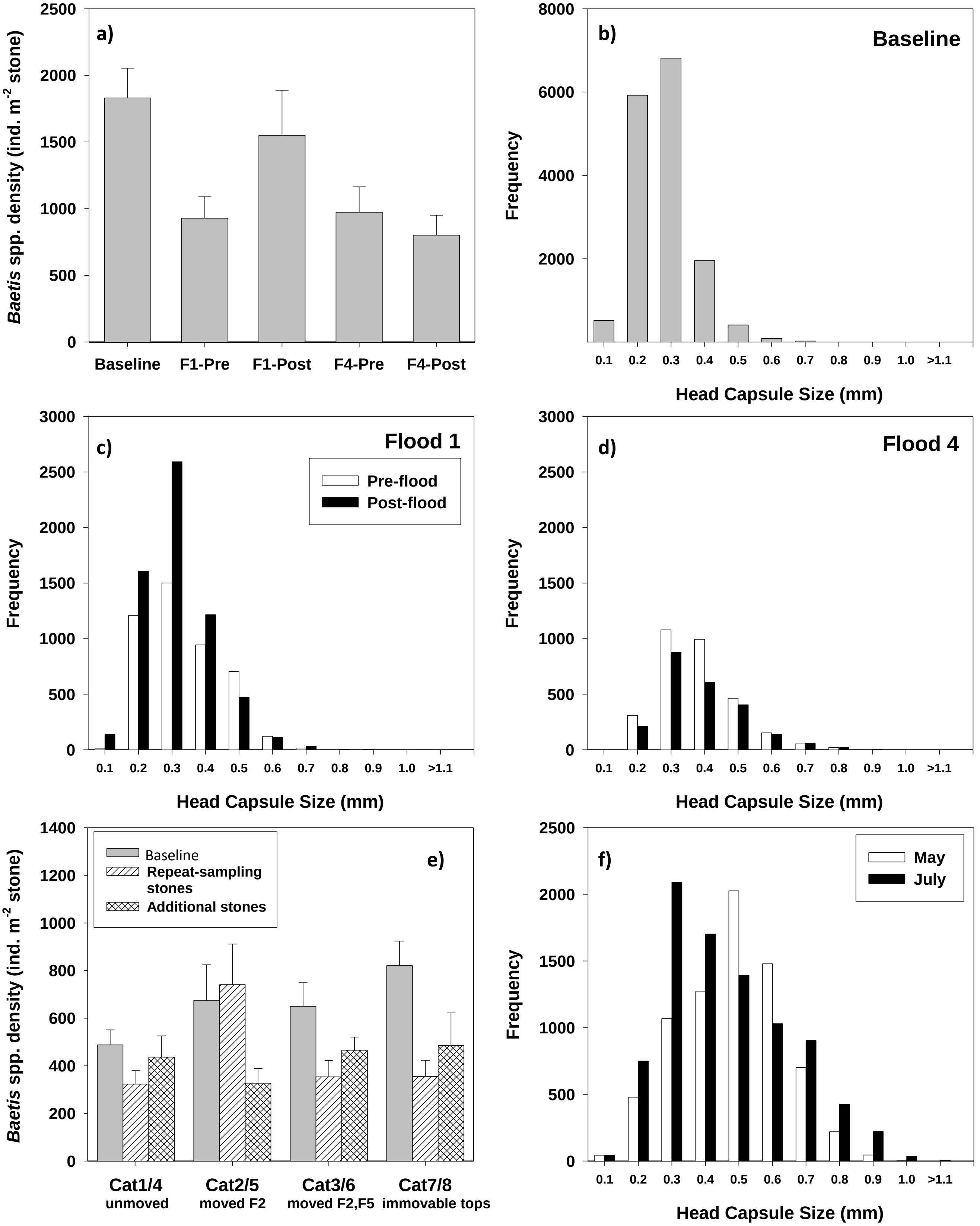

Figure 4.16 a) Mean density (+ standard error) and (b-d) population size frequency distribution of *Baetis* spp. in the Molenaars River, on each of the five sampling occasions during the 2003 study, and (e) mean species density and (f) population size frequency distributions for the two sampling surveys in the Berg River in 2004. Density data show each of the stone movement categories separately for the Berg River study.

d *Pseudocloeon* spp.

Pseudocloeon spp. showed very different responses to the floods in the Molenaars River from its responses in the Berg River, one of the few cases in this study that demonstrate such a contradiction. In the Molenaars River, despite a strong recovery from the Baseline to Pre-Flood 1 period, the series of small floods had the cumulative effect of reducing population size by some 82% (Figure 4.15a, Table 4.10). Floods appeared to affect larger individuals disproportionally, as demonstrated by changes in frequency distributions and reductions in mean head width pre- and post- floods (Figure 4.15b-d, Table 4.12).

In the Berg River, however, *Pseudocloeon* spp. densities increased significantly from a low base in May (Table 4.5), and increased in density on all categories of stones over the flood study period (Table 4.11; Figure 4.15e), with the exception of top surfaces of immovable boulders. Furthermore, as with *D. capensis*, the population size-frequency distribution changed from a normal to a bimodal distribution, indicating both recruitment and survival (resistance) of larger instars (Table 4.14). This result was not statistically significant, although that is probably because of the very low starting densities of this species.

e *Baetis* spp.

Baetis spp. (largely *B. harrisoni*) was the dominant baetid mayfly in both the rivers, although in the Molenaars River this taxon occurred at three-times the density of that in the Berg River. In the Molenaars River, *Baetis* spp. did not recover on previously sampled stones after initial sampling, being one of the few species to show significant differences in Baseline - Pre-Flood 1 sampling (refer to Figure 4.2). However, accounting for this by using the Pre-Flood 1 to Post-Flood 4 data sets, there was a 16 % decrease in population size associated with the series of small floods (Table 4.10).

Despite possible flood mortality, small floods in the Molenaars River may also have acted in some way to increase recruitment of young instars: not only were densities of *Baetis* spp. after Flood 1 higher (Figure 4.16a), Post Flood 1 mean head width was also smaller than that prior to the flood, and there was a significant difference in the pre- to post flood population size-frequency distribution (Table 4.12, Figure 4.16c and d), with an increase in the dominance of small size classes suggesting recruitment of smaller individuals. The fact that population declined over the study period despite recruitment of new instars, and the fact that the proportion of near-mature instars did not increase suggests that this species is not particularly resistant to floods, unlike *D. capensis* for example, but that continuous recruitment offsets population losses that occur even with small floods.

In the Berg River, the population of *Baetis* spp. at the May 2004 Baseline survey was also more mature than its counterpart had been in the Molenaars River in June 2003 (modal head width 0.5 mm in the Berg vs. 0.3 mm in the Molenaars River; Table 4.11, Figure 4.16e). Even though the floods were far greater than those on the Molenaars River, the population reduction was only some 26 % (Table 4.5). Unmoved stones provided a small refugium - an 11 - 34% decline occurred on unmoved stones vs. a 29 - 46 % decline on moved stones (Table 4.11; Figure 4.16e). The change in population structure over the two month period was characterised by a considerable shift towards small instars (Figure 4.16f, Table 4.14), suggesting that massive recruitment offset population losses during floods. Because of the large sample size, a comparison of the size frequency distribution on unmoved (Category 1 and 4) and moved (Category 3 and 6) stones was undertaken (Figure 4.17). This showed a strong tendency for mid-sized to large individuals to be associated with unmoved stones, whilst new instars recruited readily (and quickly) onto moved stones. Thus not only did unmoved stones represent a relative refugium for *Baetis* spp. but also this refugium acts to protect larger individuals from flood-induced mortality. Nevertheless, it is noteworthy that even moved stones were inhabited by large, mature nymphs (0.8 mm and larger), as indicated in Figure 4.17.

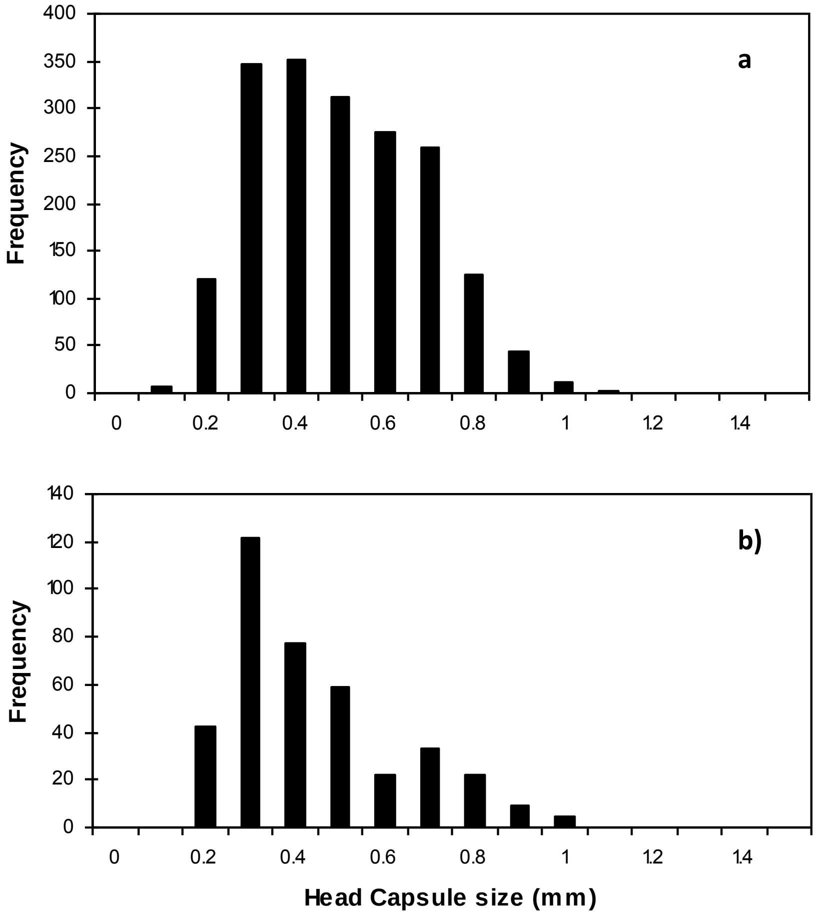

Figure 4.17 Post-flood population size frequency distributions of *Baetis* spp. in the Berg River a) on unmoved stones (Category 1 and 4), and b) on moved stones (Category 3 and 6). Note the scale change on the y-axes of the two graphs.

4.5.1.ii Telagonodidae

Three species of Telagonodidae were present in the Berg and Molenaars Rivers, *Lestagella penicillata* (Figure 4.18), *Lithogloea harrisoni* (Figure 4.19) and *Nadinitella crassi*. The latter two species responded to floods in the same manner, but since *N. crassi* was represented in low densities, only data for *L. harrisoni* are presented.

a *Lestagella penicillata*

As with *Baetis* spp. this species also showed a significant decline from the Baseline to Pre-flood 1 survey, where only 47 % of the Baseline density was recorded (see Figure 4.2), indicating the failure of this species to recover from initial sampling. Thereafter, no change in density was observed over the duration of the study in the Molenaars River (Figure 4.18a; Table 4.10). In other words, accounting for the sampling effect of initial Baseline sampling, by comparing the Pre-Flood 1 densities with those of subsequent surveys, the data in Figure 4.18a show no change in density from the Pre-Flood 1 to Post-Flood 4 surveys, and this can be considered to be the species' response to the series of Class I floods. However, significant differences in population structure were observed between the Baseline population and all other surveys (Table 4.12, Figure 4.18b-d), with increasing head capsule width indicating the maturation of this species over the season.

In the Berg River, the population of *L. penicillata* was, once again, generally more mature (larger modal head capsule width) than the Molenaars Baseline population. However, it showed an average decline in population density of 75 - 78 % over the study period (Table 4.5), the former based on comparing Baseline and Additional stones, and the latter comparing Baseline with Repeat-sampled stones. However, there was a substantially higher loss from stones that moved than from those that did not (Table 4.11, Figure 4.18e). Unmoved stones were associated with a 64 - 67 % decline whilst moved stones were associated with an 84 - 89 % decline in density, both of which were highly significant (Table 4.11). This difference of some 20% represents a relative refugium afforded by unmoved stones for *L. penicillata*. The significant difference in population structure between the Baseline and the surviving population in the Berg River, post-floods (Figure 4.18f, Table 4.14) indicates maturation of *L. penicillata,* with no recruitment of new instars.

b ***Lithogloea harrisoni***

In contrast to *L. penicillata*, densities of *Lithogloea harrisoni* in the Molenaars River in June (Baseline survey) were very low, but increased exponentially over the study period (Table 4.10, Figure 4.19a). The low numbers precluded adequate testing of changes in population structure, although the pre- to post-Flood 4 period was characterised not only by a large increase in densities, but also by a significant change in population structure (Table 4.12), suggesting that the population at the end of the study period was comprised of more than one cohort (Figure 4.19d).

In the Berg River, the higher densities of *L. harrisoni* provided for a clearer pattern. There was also an overall increase in densities over the flood period, of between 49 and 120 % (Table 4.5), the former based on comparing Baseline and Repeat-sampled stones, and the latter comparing Baseline with Additional stones. Examination of changes associated with different stone movement categories, however, shows that these increases were only on stones that did not move (Category 1 and 4 stones), or which moved once but then had remained stable for a month prior to sampling (Category 2 and 5 stones). Moved stones (Category 3 and 6) were associated with a non-significant decrease in *L. harrisoni* density (Figure 4.19e, Table 4.11). The population size-frequency analysis indicated a significant difference in population structure from the Baseline to Post-flood period (Table 4.14). These differences were associated with an increased representation of larger size classes, whilst the modal size class remained 0.3 mm, as with the Baseline condition (Figure 4.19f; Table 4.14). This indicates a substantial and continuous recruitment over the study period, combined with the maturation of the population. These results therefore also suggest that larger individuals were highly resistant to floods.

4.5.1.iii Leptophlebiidae

Five genera of Leptophlebiidae were present in the Molenaars River, of which four were also present in the Berg River. However, two somewhat different responses were identified in relation to changes in density and population structure over the study period and are presented below. The first of these was best reflected by *Aprionyx* spp. which embodied the more characteristic leptophlebiid response of three of the five genera,

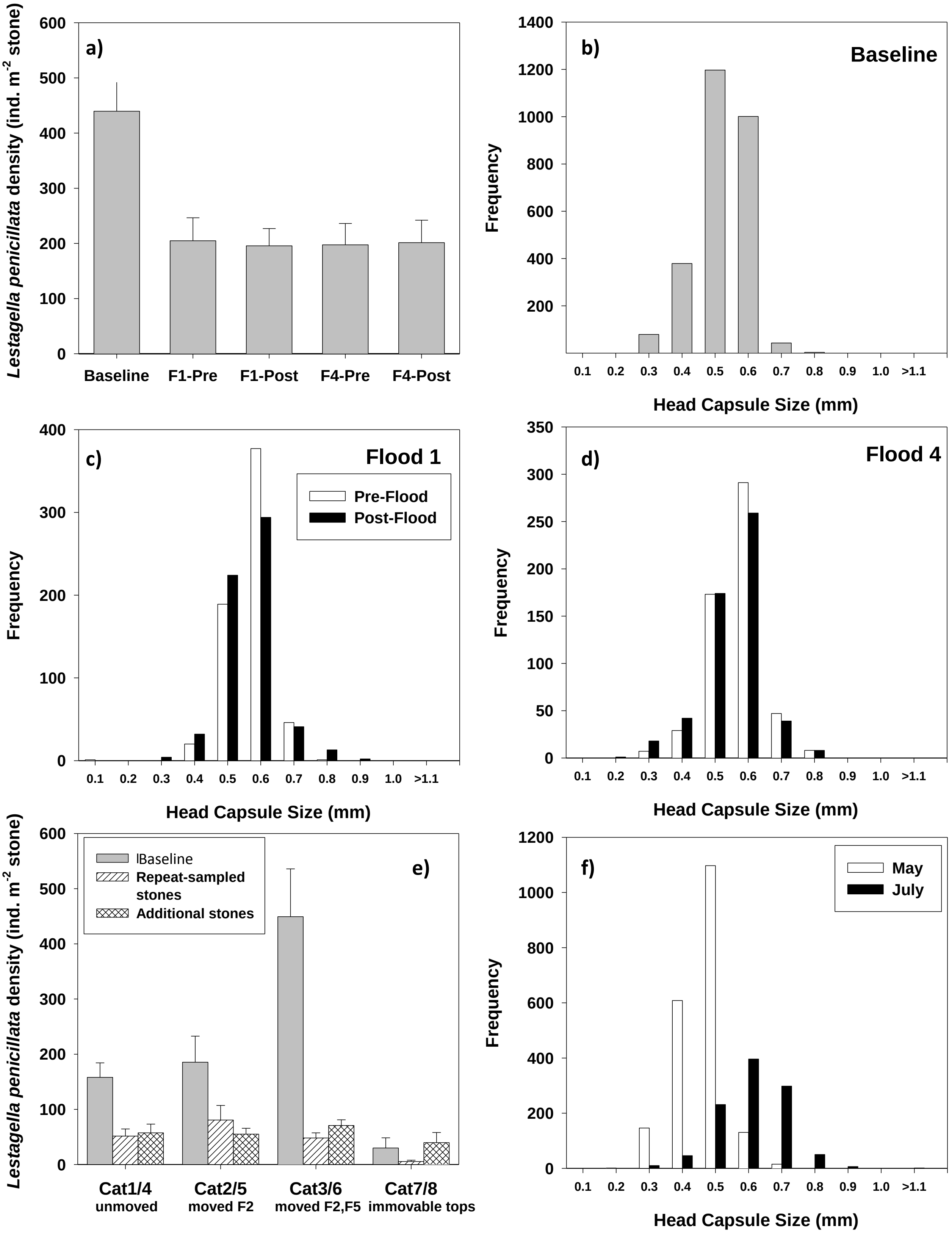

Figure 4.18 a) Mean density (+ standard error) and (b-d) population size frequency distribution of *Lestagella penicillata* in the Molenaars River, on each of the five sampling occasions during the 2003 study, and (e) mean species density and (f) population size frequency distributions for the two sampling surveys in the Berg River in 2004. Density data show each of the stone movement categories separately for the Berg River study.

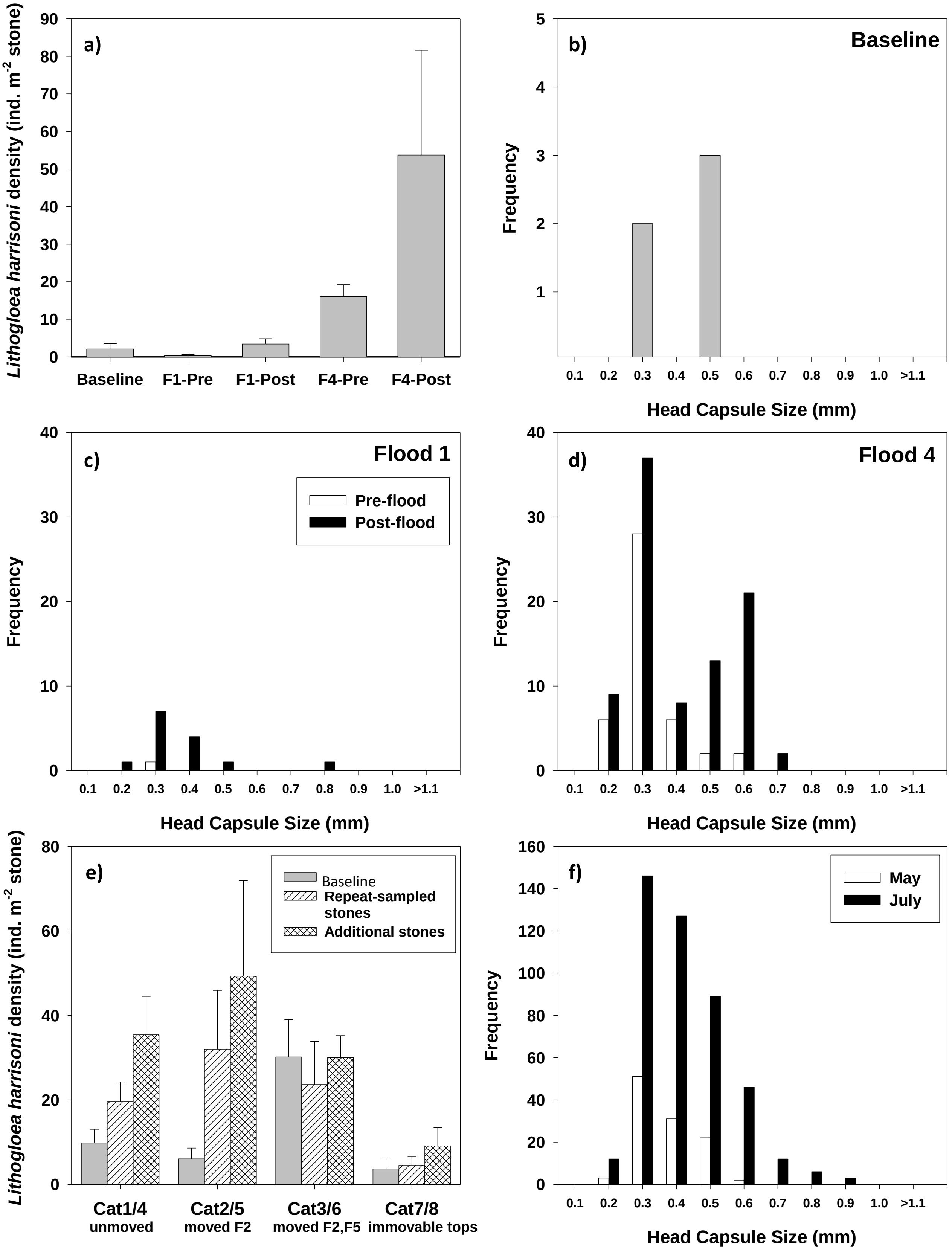

Figure 4.19 a) Mean density (+ standard error) and (b-d) population size frequency distribution of *Lithogloea harrisoni* in the Molenaars River, on each of the five sampling occasions during the 2003 study, and (e) mean species density and (f) population size frequency distributions for the two sampling surveys in the Berg River in 2004. Density data show each of the stone movement categories separately for the Berg River study.

Choroterpes spp., *Castanophlebia* spp. and *Adenophlebia* spp. The second was *Euthralus elegans*, which demonstrated higher resistance to floods than the others.

a *Aprionyx* spp.

Aprionyx spp. in the Molenaars Baseline survey (Figure 4.20b) was represented by a range of size classes including nymphs nearing maturity, estimated as head capsule size classes from approximately 1.3 mm and above. Population density at the Pre-Flood 1 survey was close to that of the Baseline condition, but many of the larger individuals were not well represented within the population (Figure 4.20a and c), probably representing an autumn emergences of mature nymphs during the month between these sampling dates. Despite this, the number of individuals in all sample sets after the initial Baseline sampling was very low and this reduces confidence in the population structure results. Overall, the Pre-Flood 1 population was reduced by 99 % over the study period (Table 4.10, Figure 4.20a).

In the Berg River, Baseline samples were again suggestive of two size cohorts within the population (Figure 4.20f), but where most of the population comprised younger instars. Despite an overall 89 - 94 % reduction in the population (Table 4.5), based on comparing the Baseline densities with both Repeat-sampled and Additional stones, there were no significant differences in size structure in the Berg River (Table 4.14) from before to after the floods. Whilst decreases on moved and unmoved stones were both significant (Table 4.12), *Aprionyx* spp. were reduced to a lesser degree on unmoved stones - a difference of between 13 % (using the Baseline vs. Additional stone data set) and 26 % (using the Baseline vs. Repeat-sampled stone data set).

b *Euthralus elegans*

This species was represented by a comparatively young population during the Baseline sampling in both the Molenaars and Berg Rivers (modal head capsule width 0.6 mm in both cases; Figure 4.12i). In the Molenaars River, although there was a dip in the population density in the Pre-Flood 4 survey, there was no change from the Pre-Flood 1 density to the end of the study period (Figure 4.21a, Table 4.10). The population structure varied statistically over the study period (Table 4.12), but the size distribution plots (Figure 4.21b-d) suggest that this was associated with the maturation of the population, with no new recruitment over the winter period.

Comparing the Baseline densities with both Repeat-sampled and Additional stones, *E. elegans* in the Berg River was reduced by the series of floods by between 77 and 91 % (Table 4.5). As with Aprionyx spp., unmoved stones provided a substantial relative refugium, with losses on these stones some 18 - 21 % lower than on moved stones (Table 4.11; Figure 4.21e). The size frequency data suggest, as with the Molenaars River, that maturation of the study period took place with no new instar recruitment (Figure 4.21f).

4.5.1.iv Heptageniidae

Of the two species of Heptageniidae present in both rivers, *Afronurus* sp. (probably mostly *A. barnardi*) was numerically dominant over *Afronurus harrisoni*, although the latter, both in the Molenaars River in June 2003 and in the Berg River in May 2004, was represented by a more mature population than *Afronurus* sp., as indicated by the greater normality in the frequency distribution as well as the larger modal head width (Figures 4.22 and 4.23, Table 4.10).

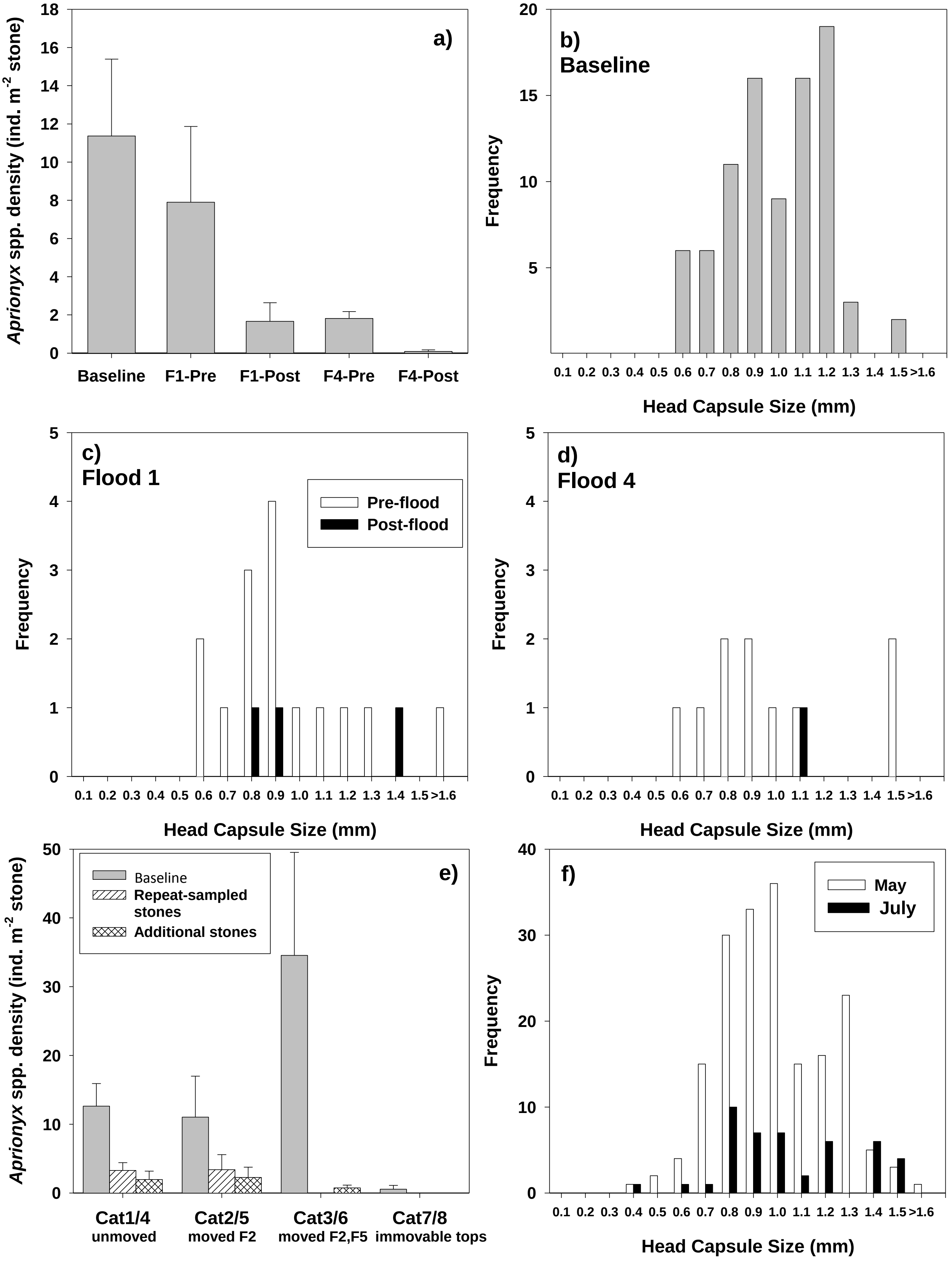

Figure 4.20 a) Mean density (+ standard error) and (b-d) population size frequency distribution of *Aprionyx* spp. in the Molenaars River, on each of the five sampling occasions during the 2003 study, and (e) mean species density and (f) population size frequency distributions for the two sampling surveys in the Berg River in 2004. Density data show each of the stone movement categories separately for the Berg River study.

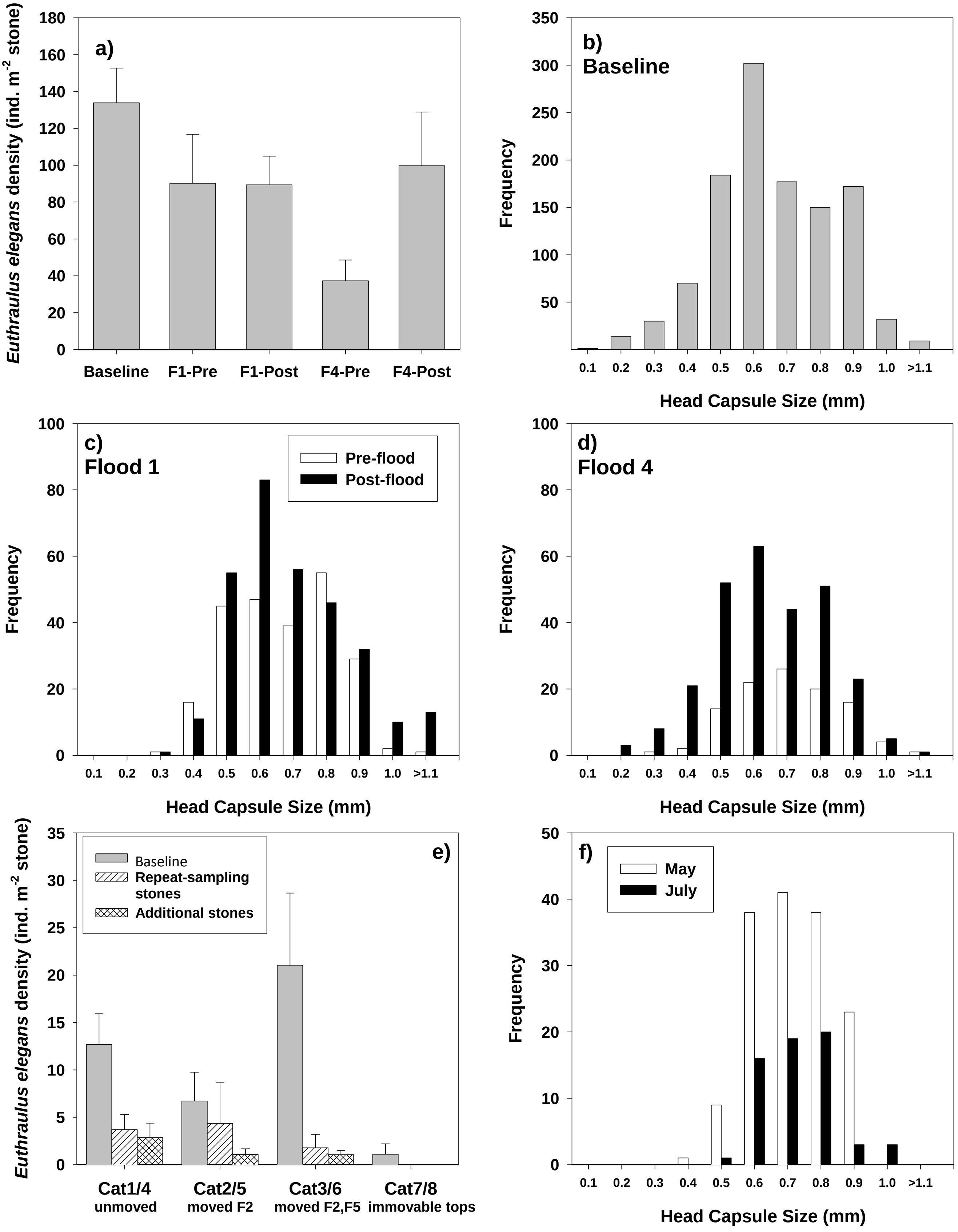

Figure 4.21 a) Mean density (+ standard error) and (b-d) population size frequency distribution of *Euthralus elegans* in the Molenaars River, on each of the five sampling occasions during the 2003 study, and (e) mean species density and (f) population size frequency distributions for the two sampling surveys in the Berg River in 2004. Density data show each of the stone movement categories separately for the Berg River study.

Table 4.12 Results of Kolmogorov Smirnov test for differences in size-frequency distributions over the study period, for taxa on the Molenaars River: sample size, mean head width ± standard error (SE) for each of the five sampling periods. Significance levels are provided in Table 4.13.

	Baseline		**Pre-flood 1**		**Post-flood 1**		**Pre-flood 4**		**Post-flood 4**	
Taxon	**N**	**Mean ± SE**	**N**	**Mean ± SE**	**N**	**Mean ± SE**	**N**	**Mean ± SE**	**N**	**Mean ± SE**
Demoulinia spp.	68	0.32 ± 0.018	14	0.41 ± 0.045	7	0.38 ± 0.063	17	0.43 ± 0.029	19	0.40 ± 0.030
Afroptilum spp.	4052	0.33 ± 0.001	2359	0.37 ± 0.001	270	0.40 ± 0.006	1165	0.38 ± 0.003	463	0.40 ± 0.005
Demoreptus capensis	3060	0.28 ± 0.002	1302	0.32 ± 0.003	167	0.31 ± 0.008	847	0.34 ± 0.003	97	0.34 ± 0.011
Pseudocloeon spp.	202	0.22 ± 0.008	66	0.34 ± 0.021	40	0.23 ± 0.021	16	0.32 ± 0.033	13	0.27 ± 0.036
Baetis spp.	15726	0.22 ± 0.001	4500	0.28 ± 0.001	6171	0.26 ± 0.001	3072	0.32 ± 0.002	2316	0.33 ± 0.002
Lestagella penicillata	2703	0.47 ± 0.002	634	0.52 ± 0.003	610	0.52 ± 0.004	555	0.52 ± 0.004	541	0.51 ± 0.004
Lithogloea harrisoni	5	0.36 ± 0.049	1	0.27	14	0.33 ± 0.037	44	0.28 ± 0.012	90	0.36 ± 0.016
Aprionyx spp.	88	0.95 ± 0.022	15	0.90 ± 0.077	3	0.98 ± 0.185	10	0.95 ± 0.101	1	1.07
Euthralus elegans	1141	0.62± 0.005	235	0.62 ± 0.016	307	0.64 ± 0.010	106	0.65 ± 0.016	271	0.58 ± 0.010
Afronurus harrisoni	132	1.62 ± 0.039	40	1.62 ± 0.054	26	1.39 ± 0.086	26	1.38 ± 0.065	9	1.72 ± 0.120
Afronurus spp.	379	0.85 ± 0.021	180	1.17 ± 0.037	55	1.02 ± 0.080	67	0.78 ± 0.056	20	0.93 ± 0.127
Cheumatopsyche afra	45	0.86 ± 0.042	1	1.02	2	0.71 ± 0.198	3	0.90 ± 0.087	8	0.79 ± 0.092
Chimarra spp.	206	1.09 ± 0.040	10	0.81 ± 0.221	11	0.62 ± 0.093	10	0.89 ± 0.180	27	0.92 ± 0.098
Athripsodes bergensis	98	0.48 ± 0.020	14	0.55 ± 0.048	22	0.41 ± 0.038	15	0.33 ± 0.028	2	0.56 ± 0.141
Agapetus agilis	183	0.25 ± 0.007	74	0.26 ± 0.012	43	0.29 ± 0.018	23	0.34 ± 0.029	18	0.25 ± 0.019
Notonemouridae spp.	23	0.58 ± 0.044	3	0.45 ± 0.110	10	0.28 ± 0.057	16	0.41 ± 0.073	48	0.46 ± 0.025
Simulium spp.	1075	0.54 ± 0.006	827	0.44 ± 0.007	522	0.54 ± 0.010	937	0.52 ± 0.008	380	0.50 ± 0.011
Elporia spp.	7716	0.24 ±0.002	1525	0.26 ± 0.003	1272	0.25 ± 0.003	1717	0.28 ± .003	778	0.31 ± 0.005
Tanypodinae spp.	392	0.41 ± 0.009	121	0.49 ± 0.022	53	0.33 ± 0.023	62	0.51 ± 0.029	29	0.33 ± 0.022
Orthocladinae spp.	3589	0.19 ± 0.001	1697	0.18 ± 0.001	1918	0.17 ± 0.001	1988	0.19 ± 0.002	1544	0.19 ± 0.002
Tanytarcini spp.	340	0.21 ± 0.003	74	0.22 ± 0.007	60	0.22 ± 0.008	53	0.22 ± 0.008	72	0.22 ± 0.007
Chironomini spp.	175	0.19 ± 0.007	29	0.25 ± 0.026	12	0.22 ± 0.026	19	0.31 ± 0.021	23	0.19 ± 0.019
Elmidae spp. larvae	894	0.24 ± 0.003	136	0.23 ± 0.009	189	0.21 ± 0.006	238	0.22 ± 0.005	262	0.21 ± 0.004
Elmidae spp. adults	81	0.35 ± 0.009	5	0.23 ± 0.049	18	0.33 ± 0.009	13	0.34 ± 0.014	10	0.37 ± 0.016
Hydrophilidae spp.	17	0.18 ± 0.007	14	0.16 ± 0.005	37	0.16 ± 0.007	18	0.15 ± 0.007	5	0.17 ± 0.011
Hydraenidae spp.	98	0.32 ± 0.004	49	0.33 ± 0.007	2	0.36 ± 0.057	5	0.30 ± 0.004	13	0.33 ± 0.017
Scirtidae spp.	71	0.57 ± 0.020	9	0.54 ± 0.047	18	0.49 ± 0.033	13	0.52 ± 0.028	17	0.54 ± 0.024
Hydrachnellae spp.	69	0.20 ± 0.010	39	0.21 ± 0.010	39	0.21 ± 0.006	22	0.17 ± 0.015	16	0.21 ± 0.015

Table 4.13 Results of Kolmogorov Smirnov test for differences in size-frequency distributions over the study period, for the Molenaars River: significance level for pairwise comparisons between the Baseline and all other periods, and between pre- and post Flood 1 and Flood 2... Significant differences are indicated in **bold** text.

Taxon	Baseline v Pre 1	Baseline v Post 1	Baseline v Pre 4	Baseline v Post 4	Pre 1 v Post 1	Pre 4 v Post 4
Demoulinia spp.	**p < 0.050**	p > 0.100	**p < 0.001**	**p < 0.025**	p > 0.100	p > 0.100
Afroptilum spp.	**p < 0.001**	**p < 0.001**	**p < 0.001**	**p < 0.001**	**p < 0.001**	**p < 0.001**
Demoreptus capensis	**p < 0.001**	**p < 0.001**	**p < 0.001**	**p < 0.001**	**0.050 > p < 0.010**	P > 0.100
Pseudocloeon spp.	**p < 0.001**	p > 0.100	**p < 0.010**	p > 0.100	**p < 0.005**	p > 0.100
Baetis spp.	**p < 0.001**	**p < 0.001**	**p < 0.001**	**p < 0.001**	**p < 0.001**	**p < 0.010**
Lestagella penicillata	**p < 0.001**	**p < 0.001**	**p < 0.001**	**p < 0.001**	**p < 0.010**	p > 0.100
Lithogloea harrisoni		p > 0.100	p > 0.100	p > 0.100		**p < 0.010**
Aprionyx spp.	p > 0.100	p > 0.100	p > 0.100		p > 0.100	
Euthralus elegans	**p < 0.050**	p > 0.001	**p < 0.025**	**p < 0.001**	**0.050 > p < 0.010**	**p < 0.025**
Afronurus harrisoni	p > 0.100	**p < 0.005**	**p < 0.005**	p > 0.100	p > 0.050	p > 0.100
Afronurus spp.	**p < 0.001**	p > 0.100	**p < 0.001**	p > 0.100	**p < 0.025**	p > 0.100
Cheumatopsyche afra		p > 0.100	p > 0.100	p > 0.100		p > 0.100
Chimarra spp.	**p < 0.050**	**p < 0.025**	p > 0.100	p > 0.100	p > 0.100	p > 0.100
Athripsodes bergensis	p > 0.100	p > 0.100	**p < 0.025**	p > 0.100	p > 0.100	p > 0.100
Agapetus agilis	p > 0.100	p > 0.100	**p < 0.050**	p > 0.100	p > 0.100	p > 0.100
Notonemouridae spp.	p > 0.100	**p < 0.005**	p > 0.100	**p < 0.050**	p > 0.100	**p < 0.050**
Simulium spp.	**p < 0.001**	**p < 0.001**	**p < 0.001**	**0.050 > p < 0.010**	**p < 0.025**	p > 0.100
Elporia spp.	**p < 0.001**	**p < 0.001**	**p < 0.001**	**p < 0.001**	**p < 0.050**	**p < 0.001**
Tanypodinae spp.	**p < 0.001**	**p < 0.001**	**p < 0.001**	**p < 0.001**	**p < 0.025**	**p < 0.001**
Orthocladinae spp.	**p < 0.001**	**p < 0.001**	**p < 0.001**	**p < 0.001**	**p < 0.001**	**p < 0.050**
Tanytarcini spp.	p > 0.100	p > 0.100	**p < 0.001**	p > 0.100	p > 0.100	**p < 0.005**
Chironomini spp.	p > 0.100	**0.050 > p < 0.010**	**p < 0.025**	**p < 0.050**	p > 0.100	p > 0.100
Elmidae spp. larvae	p > 0.100	**p < 0.001**	**p < 0.001**	**p < 0.001**	**p < 0.025**	p > 0.100
Elmidae spp. adults	**0.050 > p < 0.010**	p > 0.100	p > 0.100	p > 0.100	p > 0.100	p > 0.100
Hydrophilidae spp.	p > 0.100	p > 0.100	p > 0.100	p > 0.100	p > 0.100	p > 0.100
Hydraenidae spp.	p > 0.100	p > 0.100	**0.050 > p < 0.010**	p > 0.100	p > 0.100	p > 0.100
Scirtidae spp.	p > 0.100	p > 0.100	p > 0.100	p > 0.100	p > 0.100	p > 0.100
Hydrachnellae spp.	p > 0.100	p > 0.100	p > 0.100	p > 0.100	p > 0.100	p > 0.100

Table 4.14 Results of Kolmogorov Smirnov test for differences in size-frequency distributions for taxa on the Berg River: sample size (N), mean head width ± standard error (SE) and significance level for comparison between the Baseline and Post-flood periods. Significant differences are indicated in **bold** text.

	May (Baseline)		July (Post-floods)		
Taxon	**N**	**Mean ± SE**	**N**	**Mean ± SE**	**P-value**
Demoulinia spp.	307	0.40 ± 0.007	19	0.40 ± 0.039	>0.100
Afroptilum spp.	**214**	**0.43 ± 0.031**	**114**	**0.46 ± 0.014**	**<0.001**
Demoreptus capensis	**104**	**0.44 ± 0.017**	**75**	**0.47 ± 0.030**	**<0.025**
Pseudocloeon spp.	18	0.31 ± 0.054	55	0.36 ± 0.032	>0.100
Baetis spp.	**7333**	**0.44 ± 0.002**	**8594**	**0.41 ± 0.002**	**<0.001**
Lestagella penicillata	**1997**	**0.42 ± 0.002**	**1038**	**0.56 ± 0.003**	**<0.001**
Lithogloea harrisoni	**109**	**0.33 ± 0.009**	**441**	**0.38 ± 0.006**	**<0.001**
Aprionyx spp.	184	0.95 ± 0.016	45	0.99 ± 0.042	>0.100
Euthralus elegans	150	0.57 ± 0.010	62	0.57 ± 0.014	>0.100
Afronurus harrisoni	**220**	**1.54 ± 0.033**	**89**	**1.68 ± 0.052**	**<0.100**
Afronurus sp.	**418**	**0.80 ± 0.022**	**91**	**1.15 ± 0.067**	**<0.001**
Cheumatopsyche afra	36	0.75 ± 0.058	1	0.75	
Cheumatopsyche maculata	**84**	**0.61 ± 0.036**	**22**	**0.83 ± 0.064**	**<0.001**
Chimarra spp.	**140**	**0.78 ± 0.041**	**18**	**0.98 ± 0.085**	**<0.005**
Athripsodes bergensis	**237**	**0.26 ± 0.003**	**102**	**0.16 ± 0.005**	**<0.001**
Notonemouridae spp.	**80**	**0.58 ± 0.020**	**67**	**0.56 ± 0.035**	**<0.025**
Simulium spp.	**496**	**0.54 ± 0.009**	**568**	**0.55 ± 0.009**	**<0.010**
Orthocladinae spp.	**6986**	**0.19 ± 0.001**	**9180**	**0.22 ± 0.001**	**<0.001**
Tanypodinae spp.	**816**	**0.34 ± 0.005**	**105**	**0.31 ± 0.016**	**<0.010**
Chironomini spp.	**251**	**0.23 ± 0.005**	**136**	**0.20 ± 0.006**	**<0.010**
Tanytarcini spp.	433	0.25 ± 0.004	106	0.24 ± 0.009	>0.100
Elmidae spp. larvae	**2046**	**0.23 ± 0.002**	**281**	**0.25 ± 0.004**	**<0.001**
Elmidae spp. adults	**330**	**0.32 ± 0.004**	**88**	**0.34 ± 0.006**	**<0.100**
Scirtidae spp.	**232**	**0.37 ± 0.007**	**106**	**0.50 ± 0.010**	**<0.001**
Hydrachnellae spp.	**91**	**0.25 ± 0.009**	**35**	**0.31 ± 0.019**	**<0.100**

There was a significant reduction in both *A. harrisoni* in the Molenaars River over the study period, of some 78 %, and of *Afronurus* sp. by 84 % (Table 4.10), although these were not significant when comparing the Baseline with either Post Flood 1 or Post Flood 4 conditions, again presumably because of high variances associated with small numbers of patchily distributed animals. In the case of the more mature *A. harrisoni*, the decreased density was reflected as a loss of larger individuals (Figure 4.22a-d). However, with *Afronurus* sp., the post-floods frequency distribution of size data (Figure 4.23c,d) suggests if anything a loss of small to medium-sized nymphs.

In the Berg River *A. harrisoni* was also associated with a more mature population (compare Figure 4.22f with Figure 4.23f), and showed a slightly lower population reduction than *Afronurus* sp. (Table 4.5). *A. harrisoni* enjoyed a relative refugium on unmoved stones (Figure 4.22e; Table 4.11), although this was only true comparing Baseline densities against Repeat-sampled stones, and not when the Additional stones data set was compared with the Baseline data.. Nevertheless, on Repeat-sampled stones, there were non-significant population reductions of only 54 % on unmoved stones vs. 75 % on moved stones over the study period (Table 4.11). This was also a far smaller reduction than that measured in the floods in the Molenaars River in 2003, a somewhat puzzling result given the much larger floods in the Berg River study.

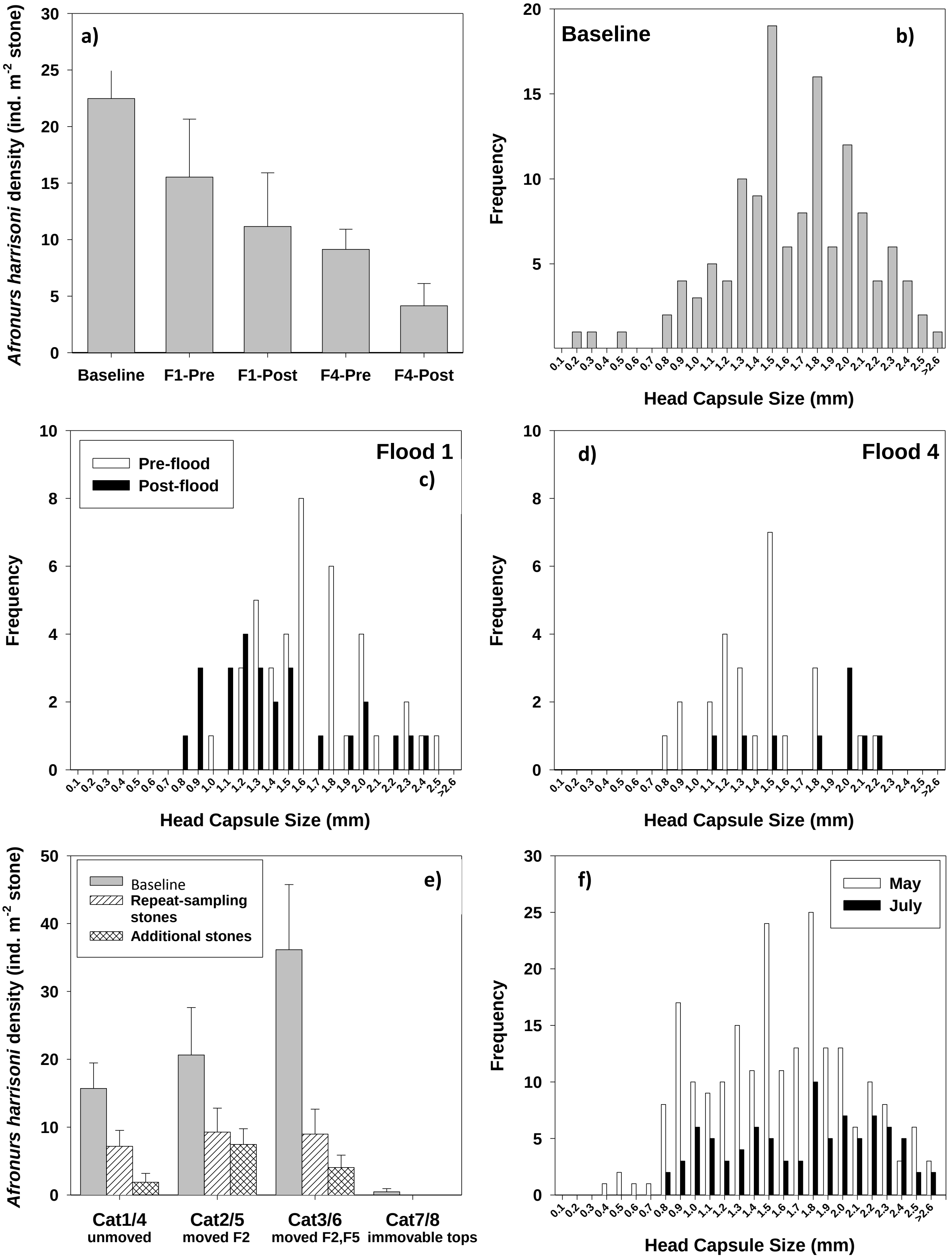

Figure 4.22 a) Mean density (+ standard error) and (b-d) population size frequency distribution of *Afronurus harrisoni* in the Molenaars River, on each of the five sampling occasions during the 2003 study, and (e) mean species density and (f) population size frequency distributions for the two sampling surveys in the Berg River in 2004. Density data show each of the stone movement categories separately for the Berg River study

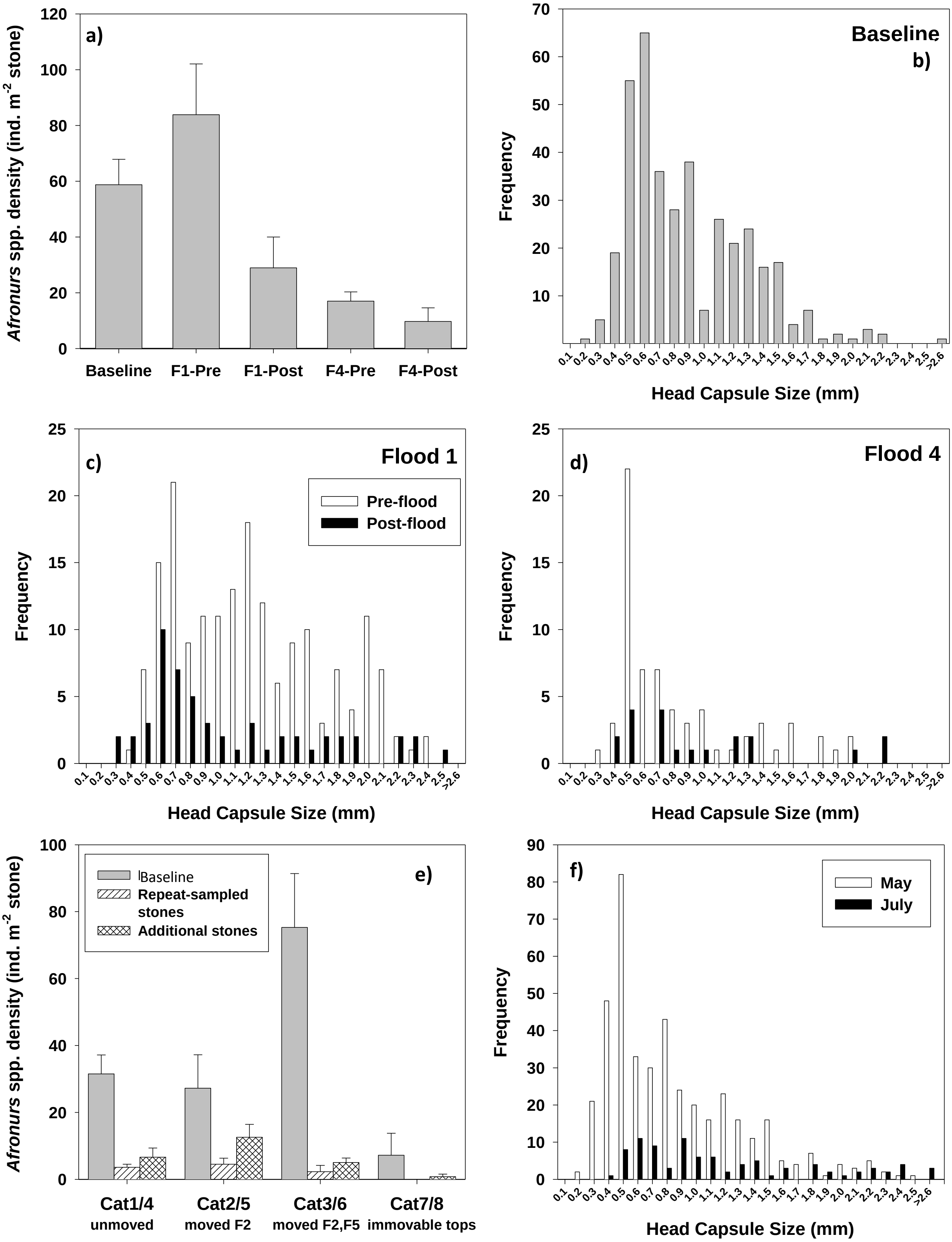

Figure 4.23 a) Mean density (+ standard error) and (b-d) population size frequency distribution of *Afronurus* spp. in the Molenaars River, on each of the five sampling occasions during the 2003 study, and (e) mean species density and (f) population size frequency distributions for the two sampling surveys in the Berg River in 2004. Density data show each of the stone movement categories separately for the Berg River study.

Based on the Additional stones data set, however, the reduction in density over the flood season was much larger - between 88 (unmoved) and 89 % (moved stones).

In the case of *Afronurus* sp., there was more agreement between the results based on Baseline vs. Repeat-sampled stones and Baseline vs. Additional stones (Table 4.11). *Afronurus* sp enjoyed an 8 - 14% relative refugium on unmoved stones vs. moved stones.

Size frequency analysis showed very little change in population structure in the case of *A. harrisoni*, suggesting a combination of slow growth over the study period, no new recruitment and a tendency for smaller individuals to succumb to floods (Figure 4.22f). There was a significant difference, however, in the frequency distribution of *Afronurus* sp. before and after the floods, with a substantially increased mean head width (Table 4.14) which, combined with visual examination of the plot in Figure 4.23f, is strongly suggestive of higher mortality rates for younger instars.

4.5.2 Trichoptera:

Six families of Trichoptera were present in the Berg and Molenaars Rivers at densities that allowed for analysis of changes over the study period. Low genus / species richness within the Families, at least for the autumn / winter assemblages, meant that most of these were represented by only one or two species.

4.5.2.i Hydropsychidae - Cheumatopsyche afra and *C. maculata*

Cheumatopsyche afra occurs in both the Berg and Molenaars Rivers, although in the Berg River it co-occurs with *C. maculata*. In the Molenaars River, *C. afra* was very patchily distributed, as indicated by the high standard error in the Baseline density in Figure 4.24a The size-frequency distribution indicates that there was a range in the maturity level at this time (Figure 4.24b), skewed toward more mature instars.

The Post-Flood 1 densities of *C. afra* were indicative of a complete failure of this species to recover on previously denuded stones within a month of initial sampling, although the patchy distribution of the Baseline data precluded statistically significant results. By the Post Flood 4 survey, i.e. notwithstanding the four small floods, densities had gradually recovered to 34 % of the Baseline condition. This recovery was as a result of the colonisation of stones by mature individuals, not from new recruitment, as evident from the size frequency data in Figure 4.24c and d. Excluding the initial sampling effect, therefore, it can be considered that Class I floods did not cause a change in *C. afra* densities in the Molenaars River.

In the Berg River, the size frequency data for *C. afra*, although different from that in the Molenaars River in 2003, had the a similar modal size class - 0.8 mm - which is assumed to correspond to mid- late instars, given the broader spread of size classes within the population. However, in nearly 200 Post-flood samples, only one individual was collected (Figure 4.24e), illustrating a different fate of this species in the face of larger floods than those which occurred in the Molenaars River in 2003.

In the Berg River *C. maculata*, on the other hand, was represented by a substantially larger population dominated by early instars (Figure 4.25a,b). A reduction in density over the study period in the order of 86 % was observed when comparing Baseline with Additional stones. Repeat-sampled stones were not used for comparison, given the strong sampling effect evident in the Molenaars study. The difference in density decreases on moved and unmoved stones (Table 4.11, using Baseline - Additional stones only) was 14 %, which represents the relative refugium offered to this species on unmoved stones. Finally, population losses appeared to affect small instars disproportionally, with a significant decline over the study period in

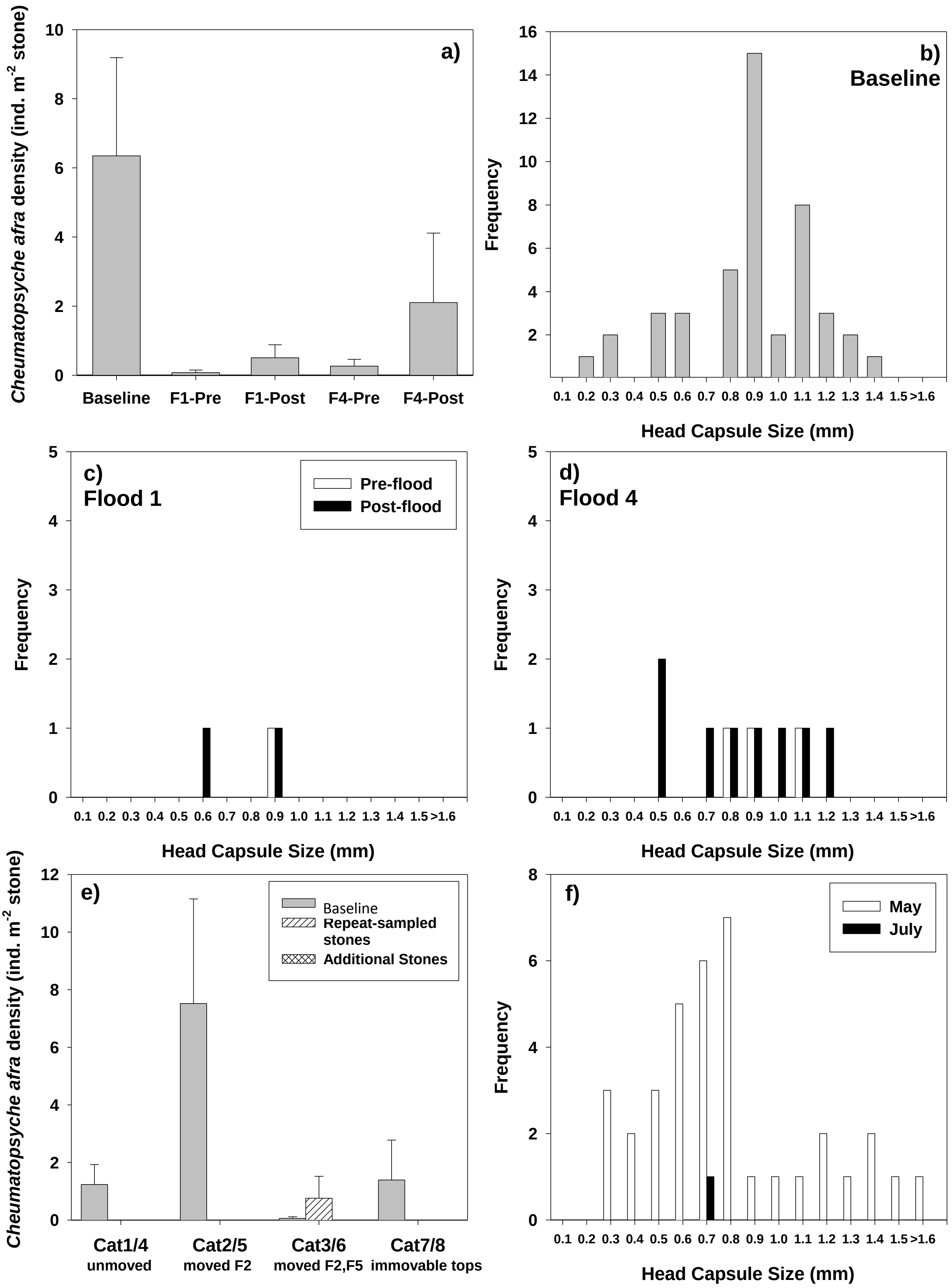

Figure 4.24 a) Mean density (+ standard error) and (b-d) population size frequency distribution of *Cheumatopsyche afra* in the Molenaars River, on each of the five sampling occasions during the 2003 study, and (e) mean species density and (f) population size frequency distributions for the two sampling surveys in the Berg River in 2004. Density data show each of the stone movement categories separately for the Berg River study.

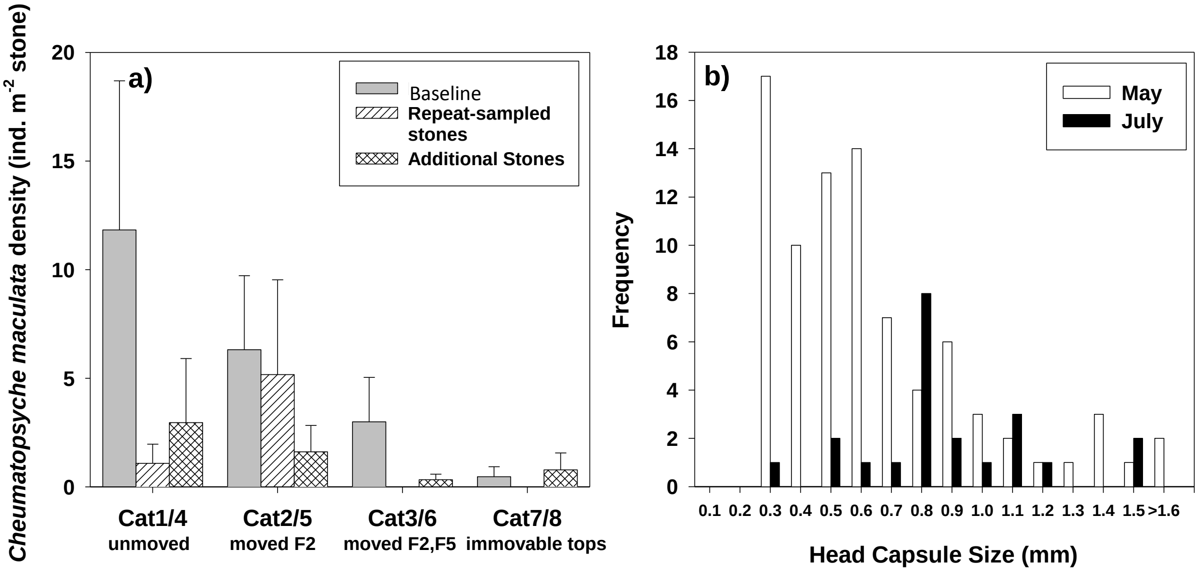

Figure 4.25 a) Mean density (+ standard error) and (b) population size frequency distributions of *Cheumatopsyche maculata* for the two sampling surveys in the Berg River in 2004. Density data show each of the stone movement categories separately.

mean head capsule width, accompanied by a shift in the modal size class from 0.3 mm to 0.8 mm (Table 4.14), a change that is unlikely to represent mere developmental growth.

4.5.2.ii Philopotamidae - Chimarra spp.

Chimarra spp. displayed a similar response to initial sampling as *C. afra*, with a slow recovery over the sampling period. Interestingly, densities after Flood 1 and Flood 4 were marginally higher than the pre-flood densities, suggesting that small floods might have aided, rather than inhibited, recolonisation (Figure 4.26a).

In the Berg River study, only Additional stones were used in the analysis because of the sampling effect demonstrated in the Molenaars River study. Table 4.11 indicates a large difference in the extent to which density decreased on unmoved (51 %) vs. moved (99 %) stones, thus indicating the relative refugium potential of unmoved stones for this species (Figure 4.26e). Although the sample size in the post-flood survey was very small (Figure 4.26f), comparison of the head capsule size-frequency distribution for the two periods indicates the larger effect of the floods on small instars, with a significant change in distribution (Table 4.14) and larger mean head width in the later survey.

4.5.2.iii Glossosomatidae - Agapetus agilis

Recovery of *Agapetus agilis* on denuded stones was slow following Baseline sampling in the Molenaars River, but increased after the first flood (Figure 4.27a), as with *Cheumatopsyche afra*, another species with low mobility. However, the population on the re-sampled stones thereafter declined over the study period. The Baseline sample was dominated by young instars (Figure 4.27b), and shifts in head capsule size frequency distribution from unimodal to bimodal over the study period (Figure 4.27c, d, Table 4.12) suggest simultaneously recruitment of new instars and developmental growth of the starting population, despite the overall decrease in density of some 47 %.from Pre-Flood 1 to Post-Flood 4. The density data for the Berg River were too low to allow for size-frequency analysis or statistical analysis of difference. The bar graphs are included, however, simply to indicate that in this river, *A. agilis* increased over the study period, especially on unmoved stones (Figure 4.27e).

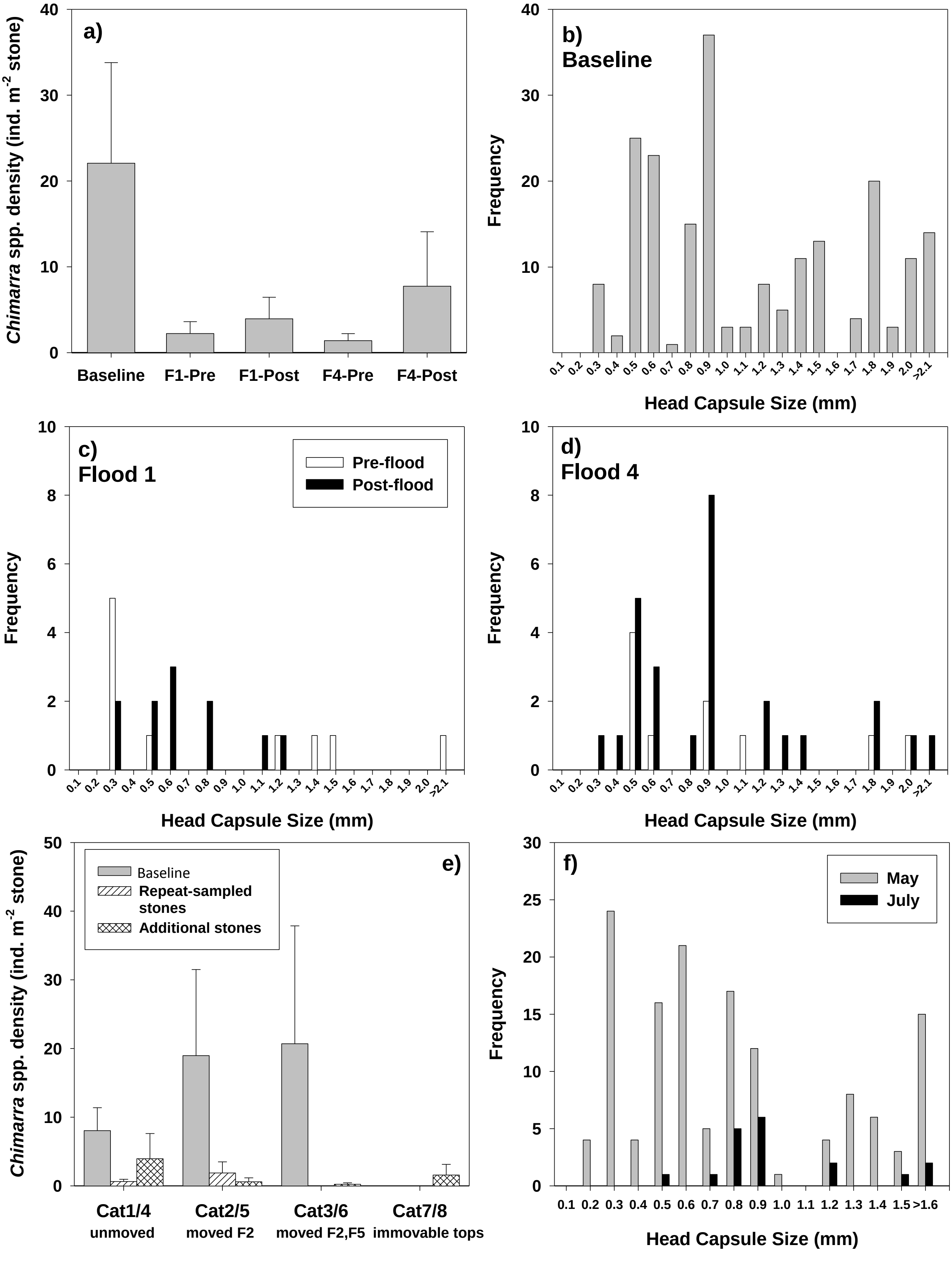

Figure 4.26 a) Mean density (+ standard error) and (b-d) population size frequency distribution of *Chimarra* spp. in the Molenaars River, on each of the five sampling occasions during the 2003 study, and (e) mean species density and (f) population size frequency distributions for the two sampling surveys in the Berg River in 2004. Density data show each of the stone movement categories separately for the Berg River study.

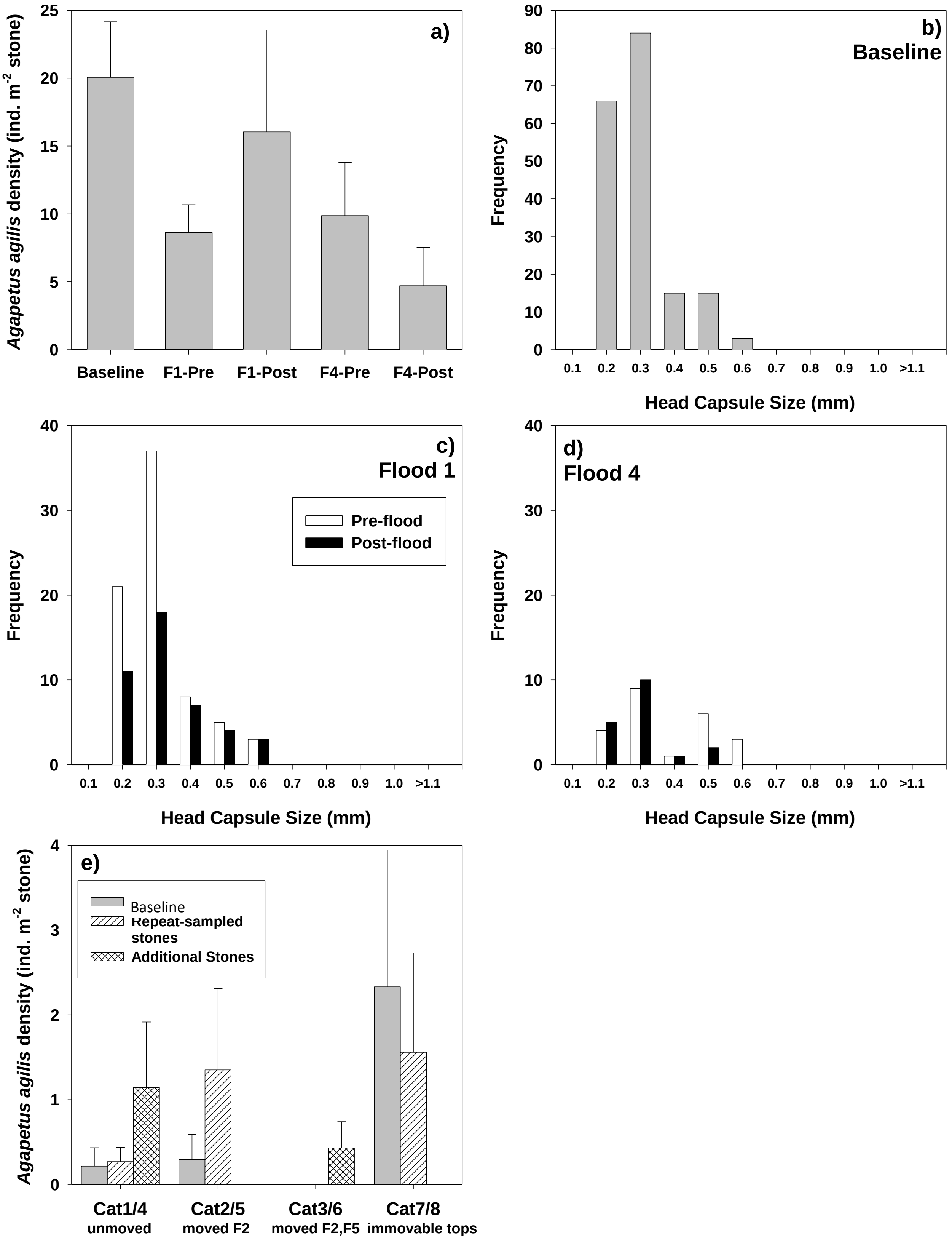

Figure 4.27 a) Mean density (+ standard error) and (b-d) population size frequency distribution of *Agapetus agilis* in the Molenaars River, on each of the five sampling occasions during the 2003 study, and (e) mean species density for the two sampling surveys in the Berg River in 2004. Numbers in the Berg River were too low for size frequency analysis. Density data show each of the stone movement categories separately for the Berg River study.

4.5.3 Notonemouridae:

This family is represented up to five species in the Molenaars River. Figure 4.28a indicates a large increase over the study period in the Molenaars River - an impressive 750 %, albeit off a very low standing crop. However the large standard deviation in the Post-Flood 4 data set, and the fact that this increase was not significant (Table 4.10) suggests that the sample size may not be sufficiently large to reflect actual increases in the notonemourid population accurately, and the results should be thus treated with caution. The size frequency data suggest new instar recruitment in autumn and early winter (Figure 4.28b-d).

In the Berg River, Notonemouridae was one of the four taxa demonstrating a non-significant change in overall density, with a decline of between 5 and 22 % (Table 4.5), the former based on comparing Baseline and Repeat-sampled stones, and the latter comparing Baseline with Additional stones. Although mean Post-flood densities on Repeat-sampled stones were nearly identical for all stones categories, unmoved stones were associated with a larger percentage decline in Notonemouridae density than those which moved during floods (Figure 4.28e), although none of the changes were significant. There was a significant difference in head width frequency distributions between the pre- and post-flood population (Table 4.14), and the shift again from a single peak at 0.6 mm to a bimodal distribution, with peaks of 0.3 and 0.9 mm suggests a combination of resistance to floods and recruitment of new instars over the winter season (Figure 4.28f). Of course, the resistance by the existing population and recruitment of new instars may reflect not only one species, but a staggering of the appearance on the stream bed of co-existing species.

4.5.4 Diptera:

4.5.4.i Simuliidae - *Simulium* spp.

Even with the effect of initial sampling of stones in the Molenaars River, *Simulium* spp. increased by 100 % over the study period (Figure 4.29a). This genus was extremely quick to recover after initial denudation of stones, and also demonstrated remarkable microhabitat specificity: a Wilcoxon's Test for dependent samples was conducted on the data collected from Repeat-sampled stones from the Pre-Flood 1 survey and their original assemblages collected during the Baseline survey - a set of 24 whole stones. There was no significant difference in *Simulium* spp. densities (Wilcoxon's Z = 1.53, p = 0.125), indicating complete recovery. More than this, *Simulium* spp. densities actually increased, but the recolonising animals were largely restricted to almost exactly those stones which were colonised in the initial Baseline survey (Figure 4.30). In addition, the densities of recolonising *Simulium* spp. followed the initial Baseline survey densities very closely. In the Baseline survey 11 of 24 stones were bare of *Simulium* spp. Of these 11, only two stones (numbers 183 and 235 in Figure 4.30) were colonised by *Simulium* spp. during the month interval until the second sampling date, and then only at very low densities. Both these results indicate the enormous resilience and strong micro-habitat specificity in this group. Size-frequency analysis suggests ongoing recruitment throughout the study period in the Molenaars River (Figure 4.29b-d).

In the Berg River, *Simulium* spp., as with the Notonemouridae and *D. capensis* already mentioned, exhibited a non-significant overall decrease in density over the flood period of between 22 and 61 % (Table 4.5), the former based on comparing Baseline and Repeat-sampled stones, and the latter comparing Baseline with Additional stones. Examination of the changes in relation to stone movement categories (Table 4.11) indicated that *Simulium* spp. fared better on moved stones, where negligible decreases and even increases in density were observed in the Post-flood survey (Figure 4.29e). In this regard, Category 2

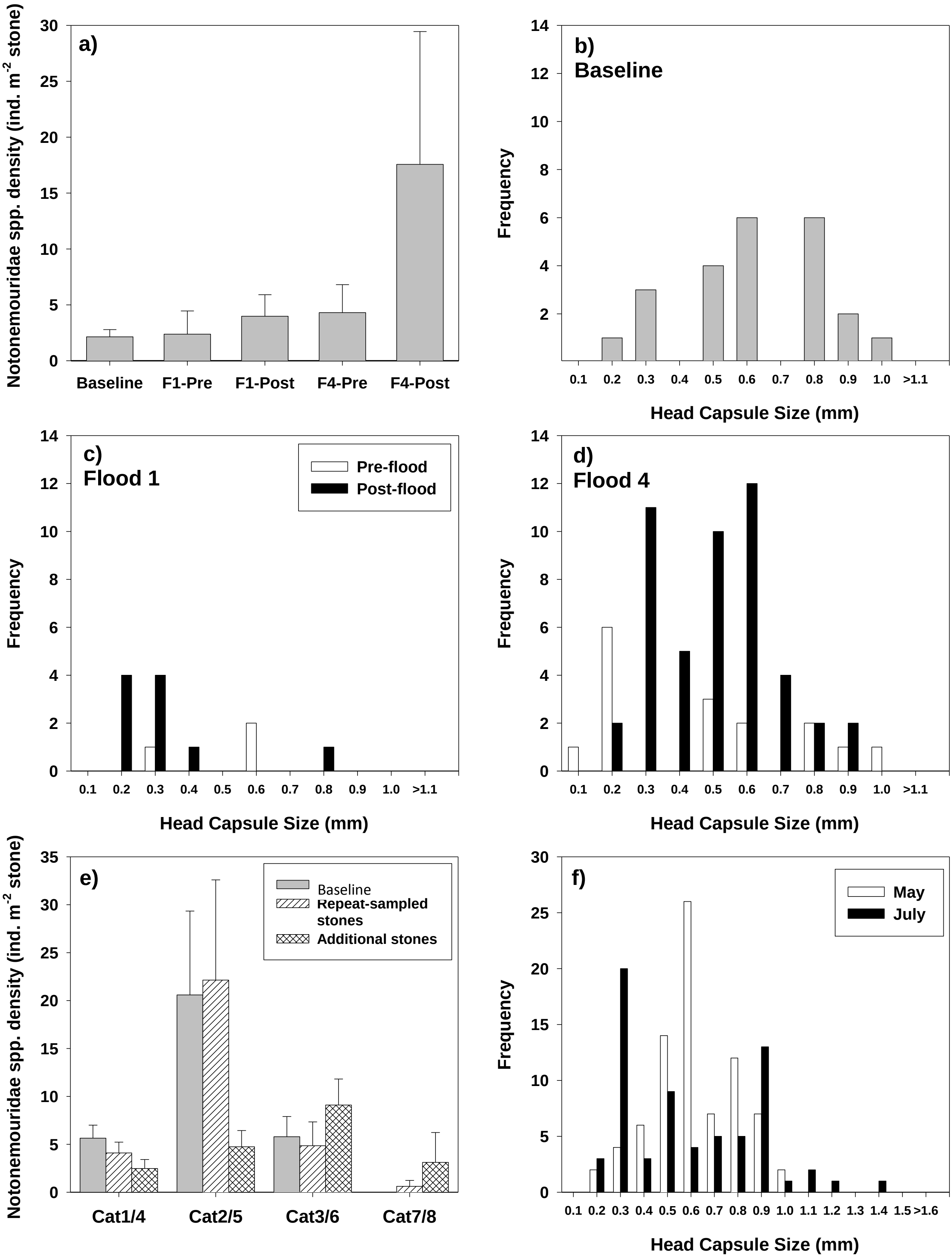

Figure 4.28 a) Mean density (+ standard error) and (b-d) population size frequency distribution of Notonemouridae spp. in the Molenaars River, on each of the five sampling occasions during the 2003 study, and (e) mean species density and (f) population size frequency distributions for the two sampling surveys in the Berg River in 2004. Density data show each of the stone movement categories separately for the Berg River study.

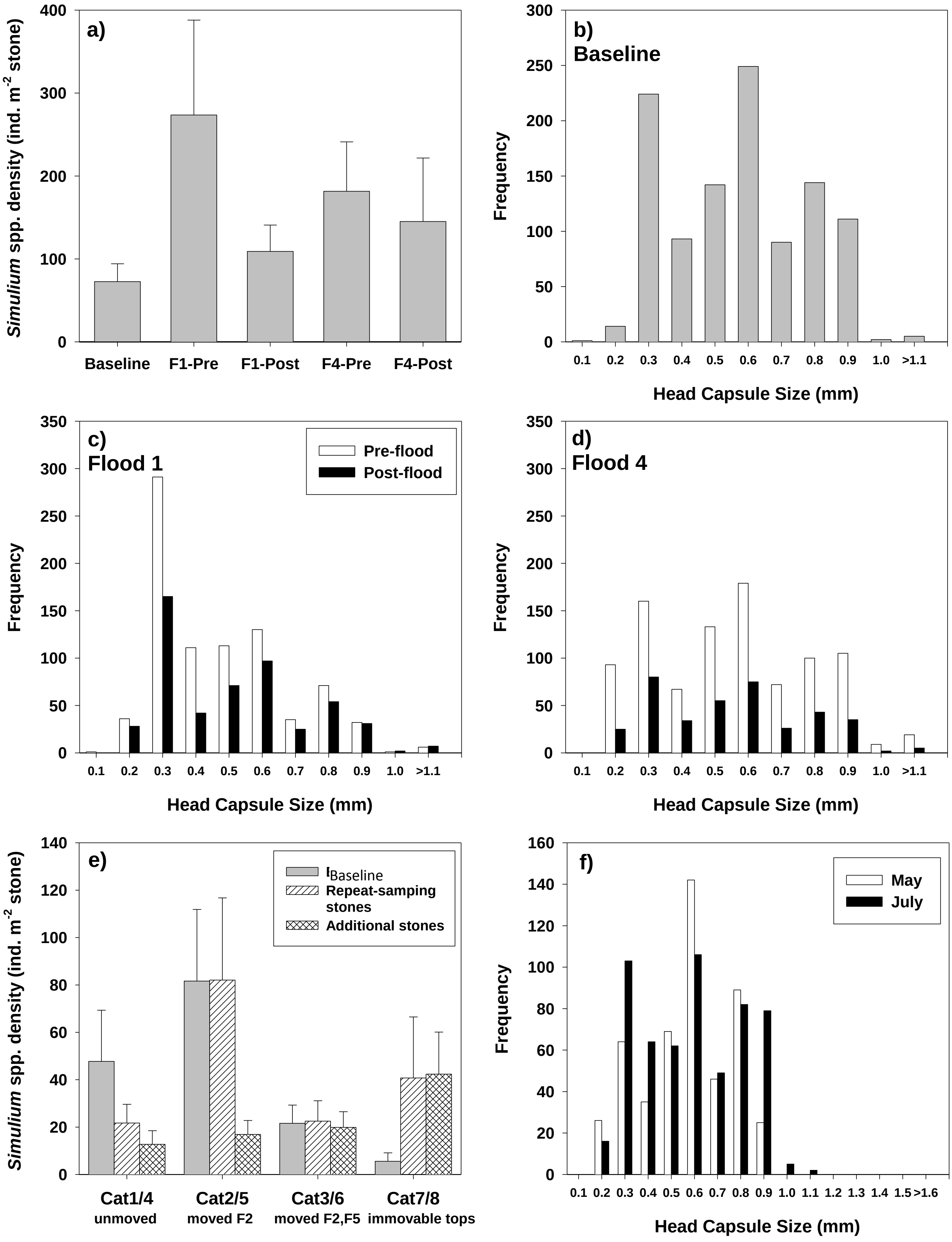

Figure 4.29 a) Mean density (+ standard error) and (b-d) population size frequency distribution of *Simulium* spp. in the Molenaars River, on each of the five sampling occasions during the 2003 study, and (e) mean species density and (f) population size frequency distributions for the two sampling surveys in the Berg River in 2004. Density data show each of the stone movement categories separately for the Berg River study.

stones - repeat-sampled stones which moved in Flood 2, but remained stable thereafter - supported substantially greater densities of *Simulium* spp. than any other stone category. Of equal importance was the significant, large percentage increase in *Simulium* spp. densities on the tops of immovable boulders (Figure 4.29e, Table 4.11). There were no obvious differences in the size-frequency distribution of *Simulium* spp. on these different stone categories after the floods, unlike the case for *Baetis* spp. Size-frequency analysis comparing the pre- and post-flood periods indicated a very slight increase in mean head capsule width (Table 4.14) but the distribution in Figure 4.29f suggests both developmental growth and recruitment over the study period.

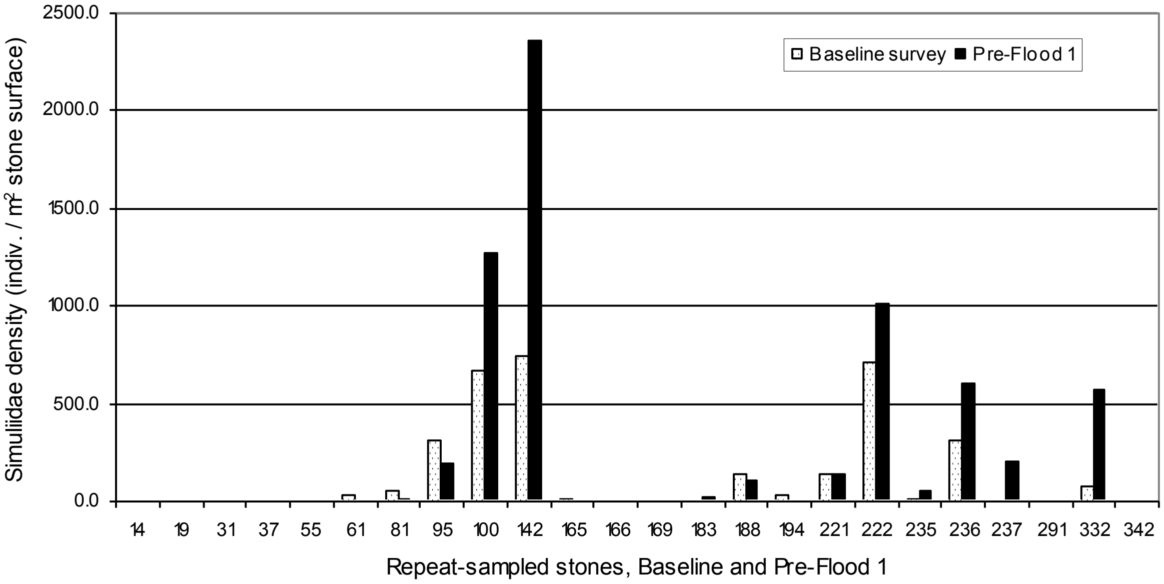

Figure 4.30 Molenaars River Baseline and Pre-Flood 1 *Simulium* spp. densities on individual repeat-sampled stones, demonstrating the recovery after initial denudation of stones during the Baseline survey, and illustrating the strong microhabitat specificity of this taxon. Individual stone numbers are listed along the z-axis.

4.5.4.ii Chironomidae

The major sub-Families of Chironomidae in both rivers are the Orthocladinae, the predatory Tanypodinae and the Chironominae, which are easily differentiated into two Tribes: the tube-dwelling Tanytarcini and the Chironomini. Responses to floods were separately examined for each of these four groups.

a ***Tanypodinae***

Tanypodinae in the Molenaars River recovered fairly well after the Baseline survey, but the population declined over the flood season by 70 % of their starting density (Table 4.10, Figure 4.31a), with the major losses occurring on smaller instars (Figure 4.31b-d)). A similar pattern was evident in the Berg River, although in this case density reductions by the larger floods amounted to between 90 and 94 % of the Baseline condition, with no refugium offered by unmoved stones (Table 4.11, Figure 4.31e).

b ***Orthocladinae***

Orthoclad density in the Molenaars River remained remarkably constant over the full study period (Figure 4.32a, Table 4.10), and although there were statistically significant differences in size-frequency distributions (Table 4.12, 4.13), mean head capsule width was unchanged. There were no more than five pupae collected in all 10 000 animals examined over the Molenaars River study, indicating that the population either remained static in terms of growth, or that any losses were matched by new recruitment.

In the Berg study, the Orthocladinae were the last of the four taxa already mentioned that exhibited a non-significant overall decrease in density over the flood period, of between 53 and 55 % (Table 4.5), the former based on comparing Baseline and Repeat-sampled stones, and the latter comparing Baseline with Additional stones. Examination of the changes in density by stone movement categories (Table 4.11; Figure 4.31f) indicated that Orthocladinae enjoyed an approximate 20 % relative refugium from flood-induced density reductions on unmoved stones, with immovable boulder tops representing a particular haven from the effects of floods. On the latter, orthoclad densities increased by 40 % on Repeat-sampled stones and 124 % on Additional stones, the latter being significant (Table 4.5). The significant difference in size-frequency distributions over the flood period (Figure 4.32f, Table 4.14) suggests both recruitment of young instars and developmental growth.

c *Tanytarcini*

Tanytarcini did not recover completely on sampled stones between the Baseline and Pre-Flood 1 sampling periods, although the differences in density between these periods was not significant (Figure 4.32a; Table 4.10). Over the remaining study period however, there was only a small decrease in density of 14 %. An interesting feature was the size-frequency distribution of this group, since very few large animals (0.4 - 0.5 mm) were present throughout the study period, even by the time of the fourth flood, where stones were dominated by animals of 0.3 mm (Figure 4.32b-d).

In the Berg River, Tanytarcini were substantially reduced by floods, of between 71 and 77 % (Table 4.5), the former based on comparing Baseline and Additional stones, and the latter comparing Baseline with Repeat-sampled stones. Examination of the changes in relation to stone movement categories (Table 4.11; Figure 4.32e) indicated that Tanytarcini enjoyed 10 - 20 % relative refugium from flood-induced density reductions on unmoved stones, with immovable boulder tops representing a haven from the effects of floods, as was the case with orthoclads, although the Tanytarcini were represented in very low densities on these boulders. The population structure showed a more even spread of size classes than in the case of the Molenaars River (Figure 4.32f), but was unchanged over the sampling period (Table 4.14).

d *Chironomini*

This group of chironomids did also not recover well on previously denuded stones, and was represented unevenly over the study period, although seemingly not in response to flood events (Figure 4.33a). Population size frequency distribution analysis indicated significant changes between the Baseline and Pre-flood 4 surveys that were suggestive of new recruitment as well as developmental growth (Figure 4.33b-d).

The picture in the Berg River study was somewhat confusing. Small decreases in density was observed for both Category 1 / 4 stones (unmoved) and Category 3 / 6 stones (moved in Floods 2 and 5), with very little difference in the degree of reduction between these groups and no relative refugium on the former (Figure 4.33e). Somewhat surprisingly therefore, Category 2 / 5 stones, which moved in Flood 2 but remained stationary over Flood 5, were associated with significant and substantial decreases in density. Differentiating between movement vs. non-movement flood effects is thus not possible for this chironomid group. Overall, the reduction in densities over the flood period (Table 4.5) was between 37 % (comparing Baseline and Repeat-sampled stones) and 59 % (comparing Baseline and Additional stones).

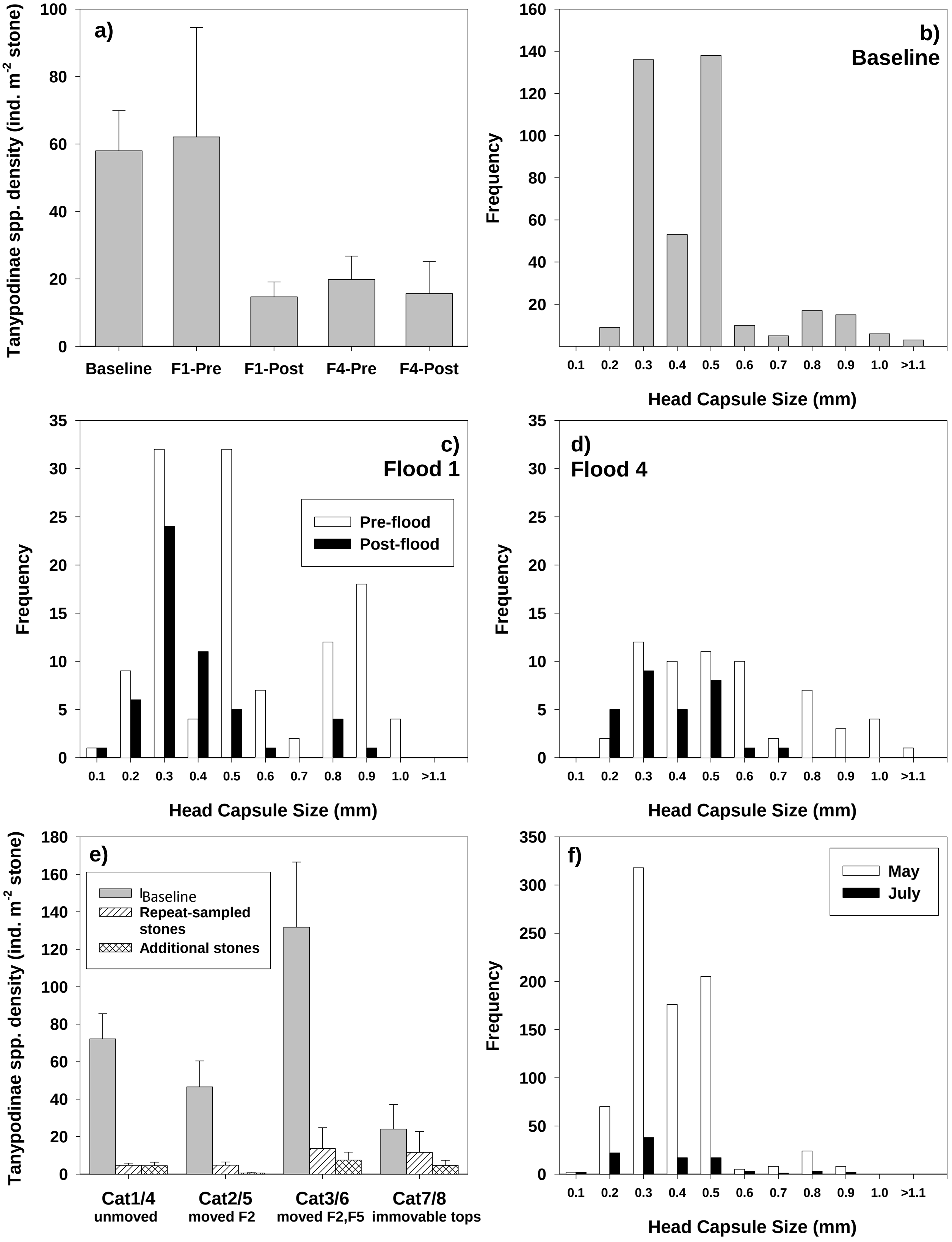

Figure 4.31 a) Mean density (+ standard error) and (b-d) population size frequency distribution of Tanypodinae spp. in the Molenaars River, on each of the five sampling occasions during the 2003 study, and (e) mean species density and (f) population size frequency distributions for the two sampling surveys in the Berg River in 2004. Density data show each of the stone movement categories separately for the Berg River study.

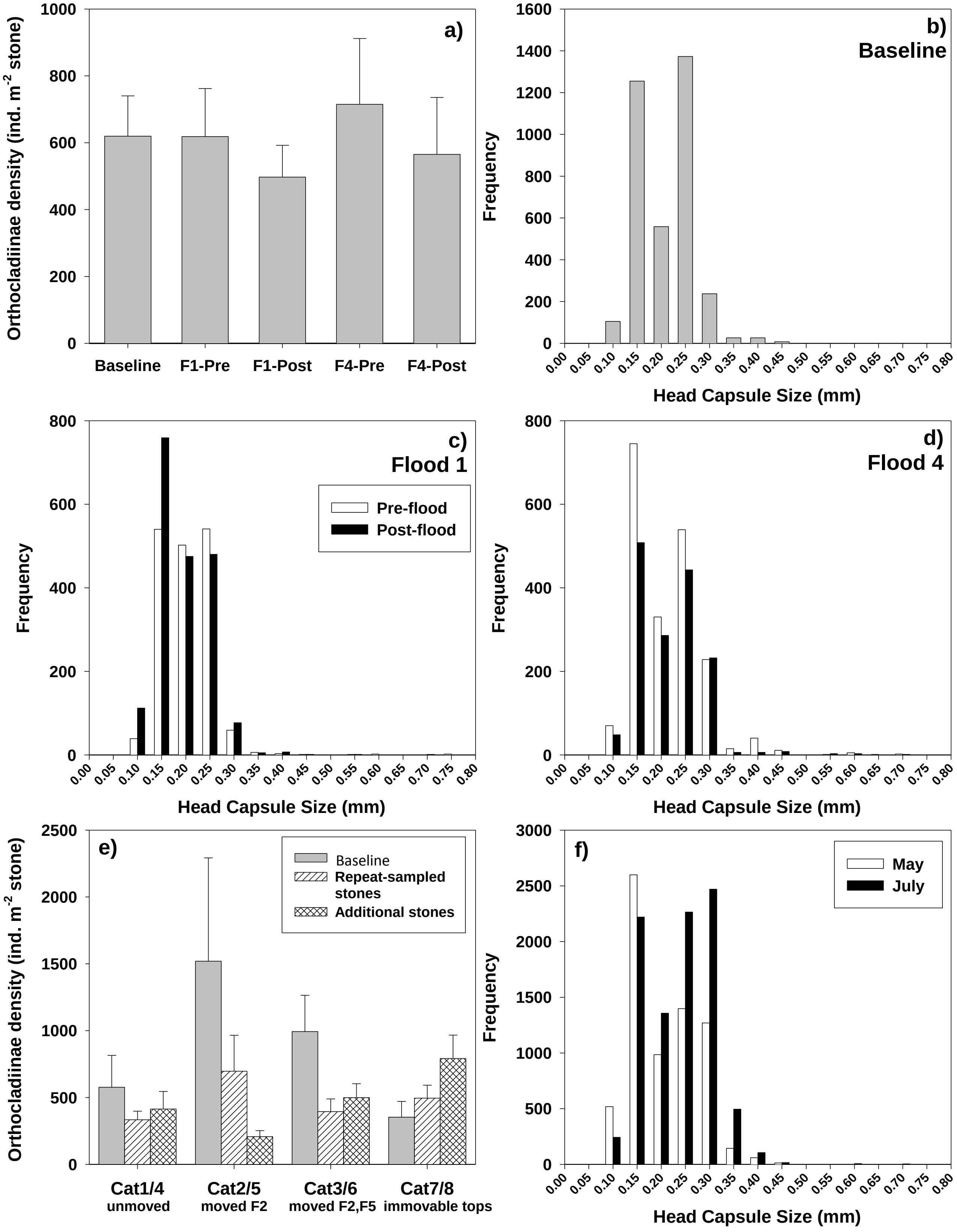

Figure 4.32 a) Mean density (+ standard error) and (b-d) population size frequency distribution of Orthocladinae spp. in the Molenaars River, on each of the five sampling occasions during the 2003 study, and (e) mean species density and (f) population size frequency distributions for the two sampling surveys in the Berg River in 2004. Density data show each of the stone movement categories separately for the Berg River study.

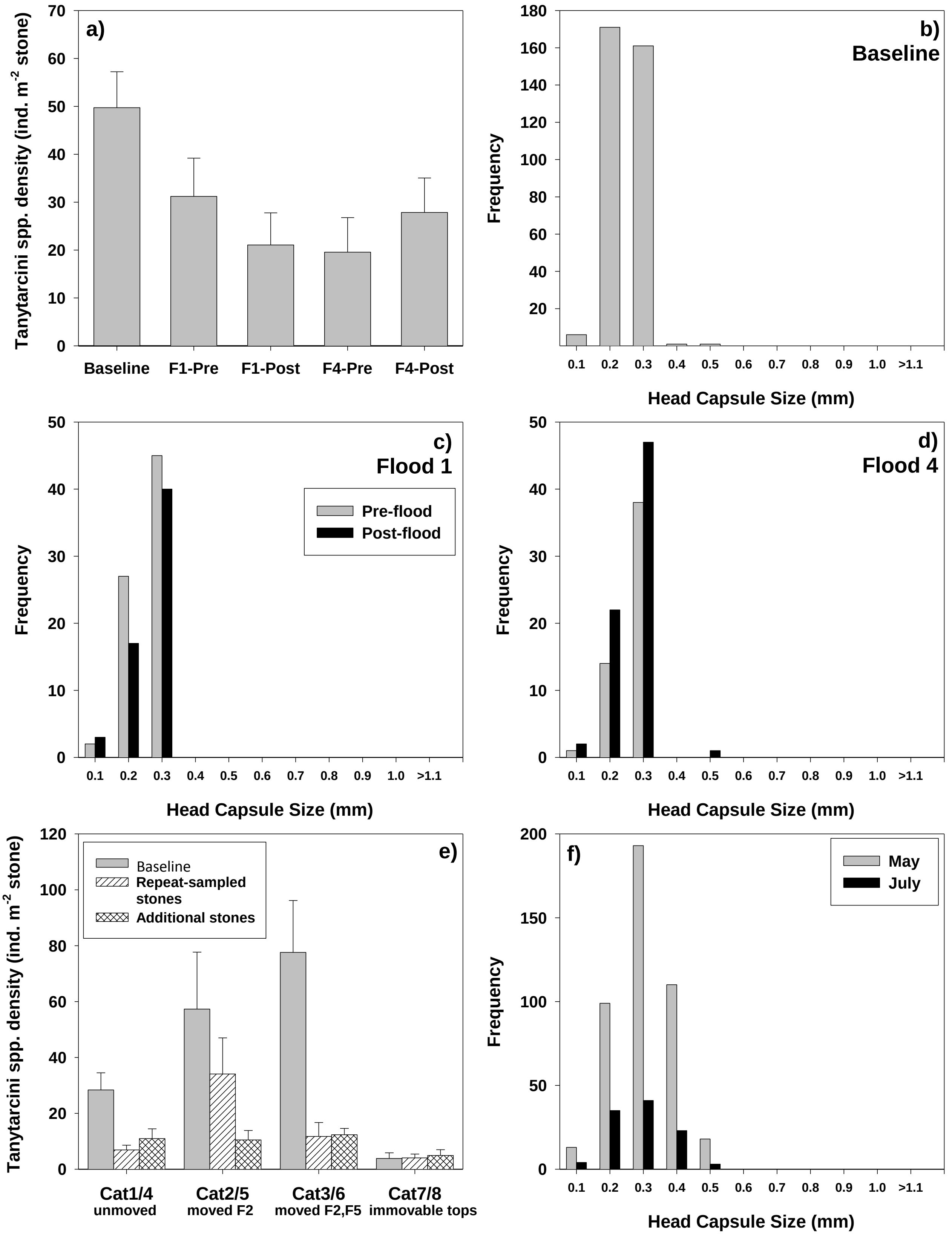

Figure 4.33 a) Mean density (+ standard error) and (b-d) population size frequency distribution of Tanytarcini spp. in the Molenaars River, on each of the five sampling occasions during the 2003 study, and (e) mean species density and (f) population size frequency distributions for the two sampling surveys in the Berg River in 2004. Density data show each of the stone movement categories separately for the Berg River study.

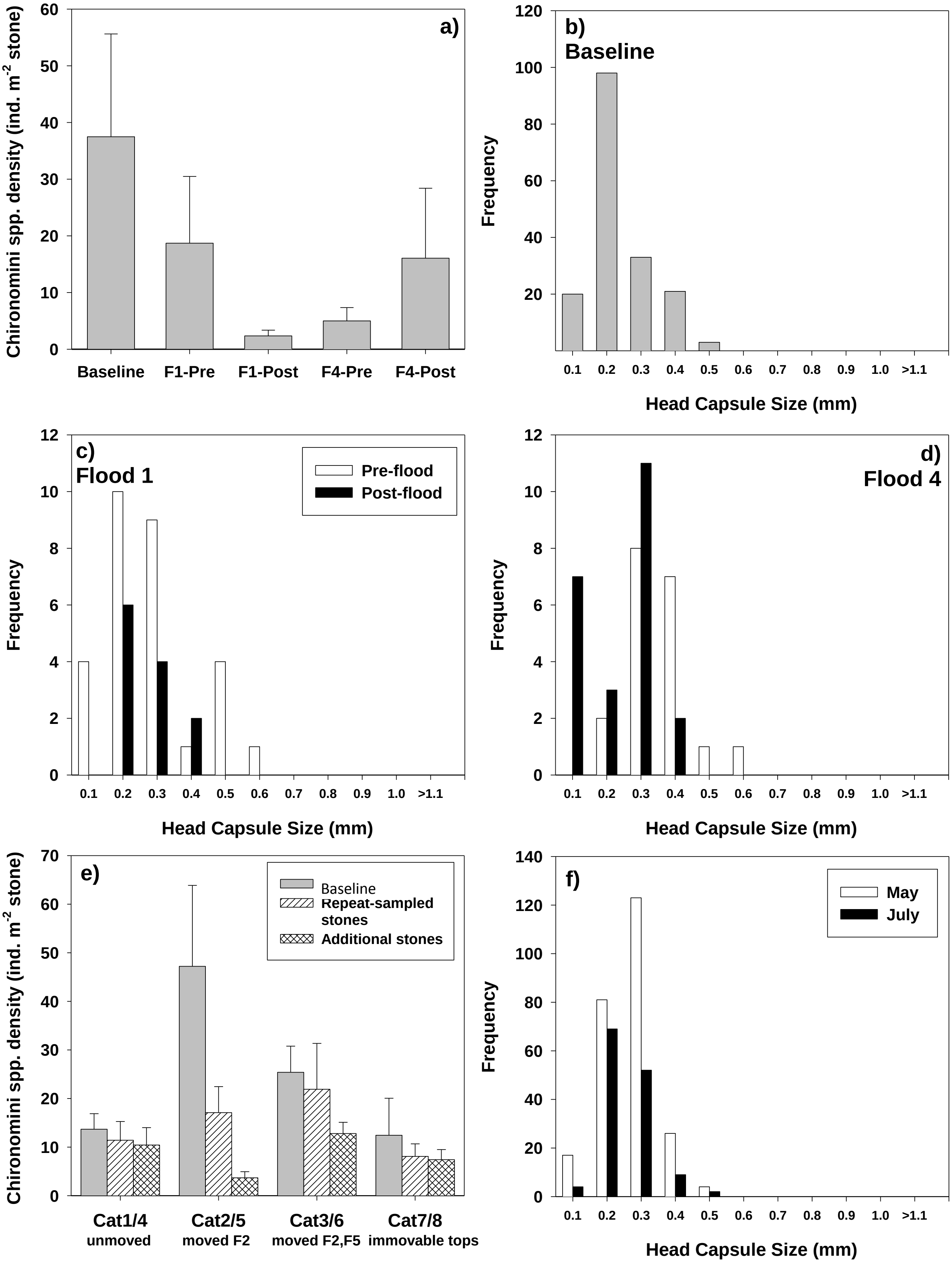

Figure 4.34 a) Mean density (+ standard error) and (b-d) population size frequency distribution of Chironomini spp. in the Molenaars River, on each of the five sampling occasions during the 2003 study, and (e) mean species density and (f) population size frequency distributions for the two sampling surveys in the Berg River in 2004. Density data show each of the stone movement categories separately for the Berg River study.

4.5.4.iii Elporia spp.

Elporia spp. were inadequately represented in the Berg River at any time over the study period to allow for flood effects to be examined. In the Molenaars River, as with the mayfly *Lestagella penicillata*, *Elporia* spp. recovered to only 34 % of the Baseline density in the month following initial sampling, whereafter no change in the population as a result of floods was observed (Figure 4.35a). There were significant changes in size-frequency distribution over the full study period, which suggested continuous strong recruitment of young instars as well as developmental growth (Figure 4.35b-d).

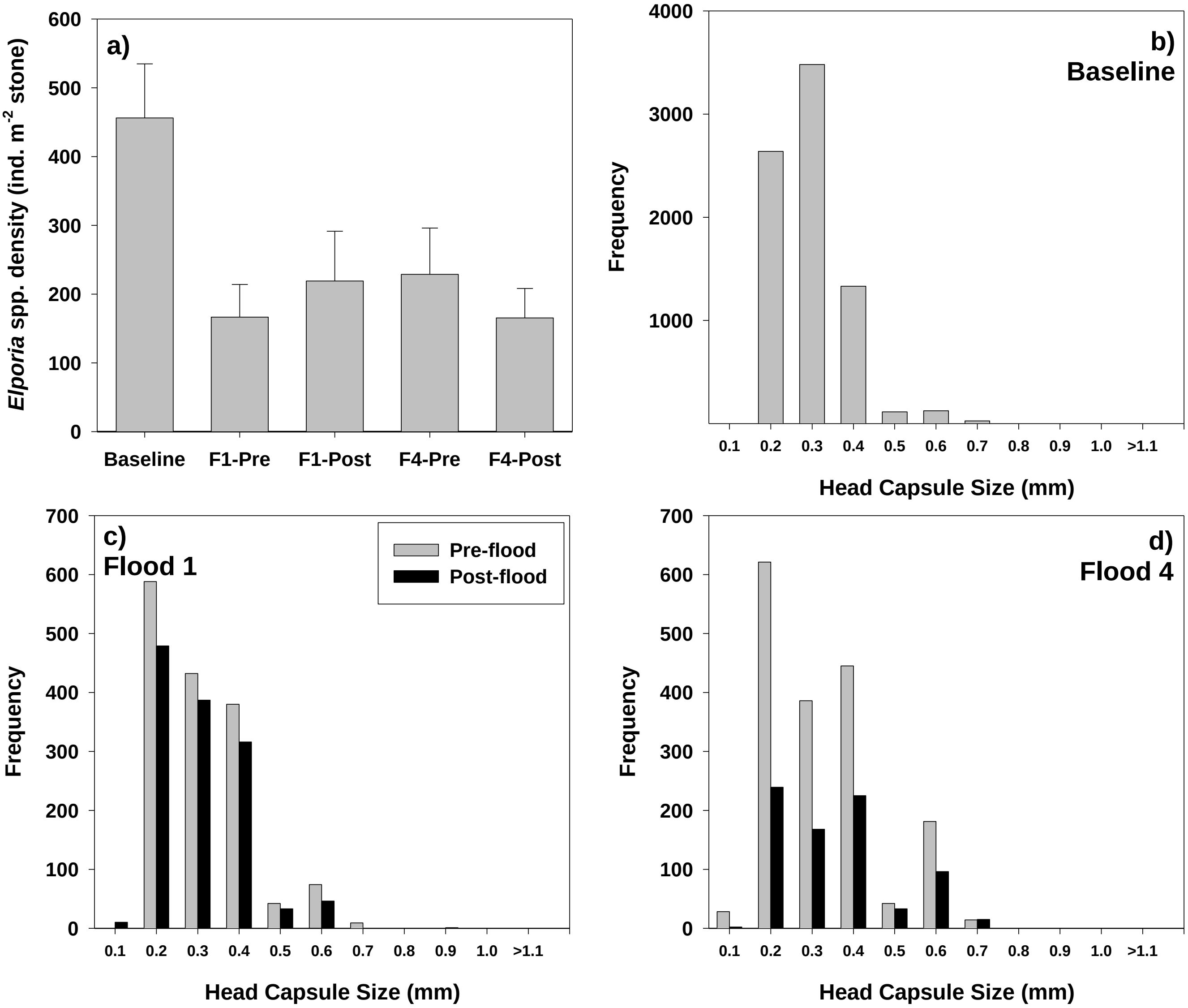

Figure 4.35 a) Mean density (+ standard error) and (b-d) population size frequency distribution of *Elporia* spp. in the Molenaars River, on each of the five sampling occasions during the 2003 study, and (e) mean species density and (f) population size frequency distributions for the two sampling surveys in the Berg River in 2004. Density data show each of the stone movement categories separately for the Berg River study.

4.5.5 Coleoptera:

4.5.5.i Elmidae

As reported in section 4.2.2, larval Elmidae was one of the few groups that were significantly lower on re-sampled stones at the Pre-Flood 1 sampling than the Baseline (Figure 4.2). Thereafter, no effects of floods were apparent. In fact, this group increased to 63 % of the initial Baseline density, despite the floods over the study period (Figure 4.36a). Elmidae adults followed a similar pattern of inadequate recovery following the Baseline sampling, but their numbers declined slightly over the study period, even from the Pre-Flood 1 densities, with an overall recovery of only 31 % of the Baseline density (Figure 4.36a).

In the Berg River both larvae and adults decreased on all categories of stones, with unmoved stones providing a small (7 %) relative refugium for larvae, but no consistent refugium was apparent in the case of adults (Figure 4.36e and 4.37e). The head capsule width data (Table 4.14) suggest slow growth of the population that did remain.

4.5.5.ii Scirtidae

Whilst Scirtidae densities were variable over the study period, the Baseline and Pre-Flood 1 densities were similar, and there were no significant changes by the end of Flood 4 (Figure 4.38a). Size-frequency distributions also did not change significantly (Table 4.12, Figure 4.38b-d).

In the Berg River Scirtidae decreased by between 68 and 70 % overall, but were substantially more affected by floods on stones that moved (Figure 4.38e; Table 4.11), with more than 50 % difference in density reductions between these two categories of stones, indicating a high relative refugium on unmoved stones. The change in size-frequency distribution was significant, and represented developmental growth, with an increase in average head capsule width from 0.37 mm in May to 0.50 in July (Table 4.14).

4.5.6 Acarina: Hydrachnellae (Order: Acariformes)

Hydrachnellae (water mites) was the only non-insect group included in the study of invertebrate responses to floods. In the Molenaars River there was little change in density for much of the study period, although densities were non-significantly reduced immediately after Flood 4 (Figure 4.39a; Table 4.10). No significant differences in population structure were found over the study period in either river. In the Berg River there was a significant decrease of between 54 and 61 %. However, with regard to differences in responses on moved vs. unmoved stones, the results were somewhat contradictory (Table 4.11), with higher densities being found on Additional stones that had moved (Category 6 stones) than any other.

4.6 THE INFLUENCE OF APPLIED STREAM POWER ON INVERTEBRATE DENSITIES

The relationship between applied stream power and post-flood density of invertebrates was examined only on unmoved stones, since the analysis was aimed at testing whether, outside of disturbance associated with particle movement, there was a relationship between the magnitude of forces acting on a particle and the ‘erosion’ of animals from that particle.

The range of applied stream power values calculated for unmoved stones, at the peak of each of the monitored floods, was as follows: 2000 - 10 000 Watts m^{-3} on those stones sampled immediately after Flood 1 on the Molenaars River; 4000 - 20 000 Watts m^{-3} on Flood 4 sampled stones. In Flood 5 on the

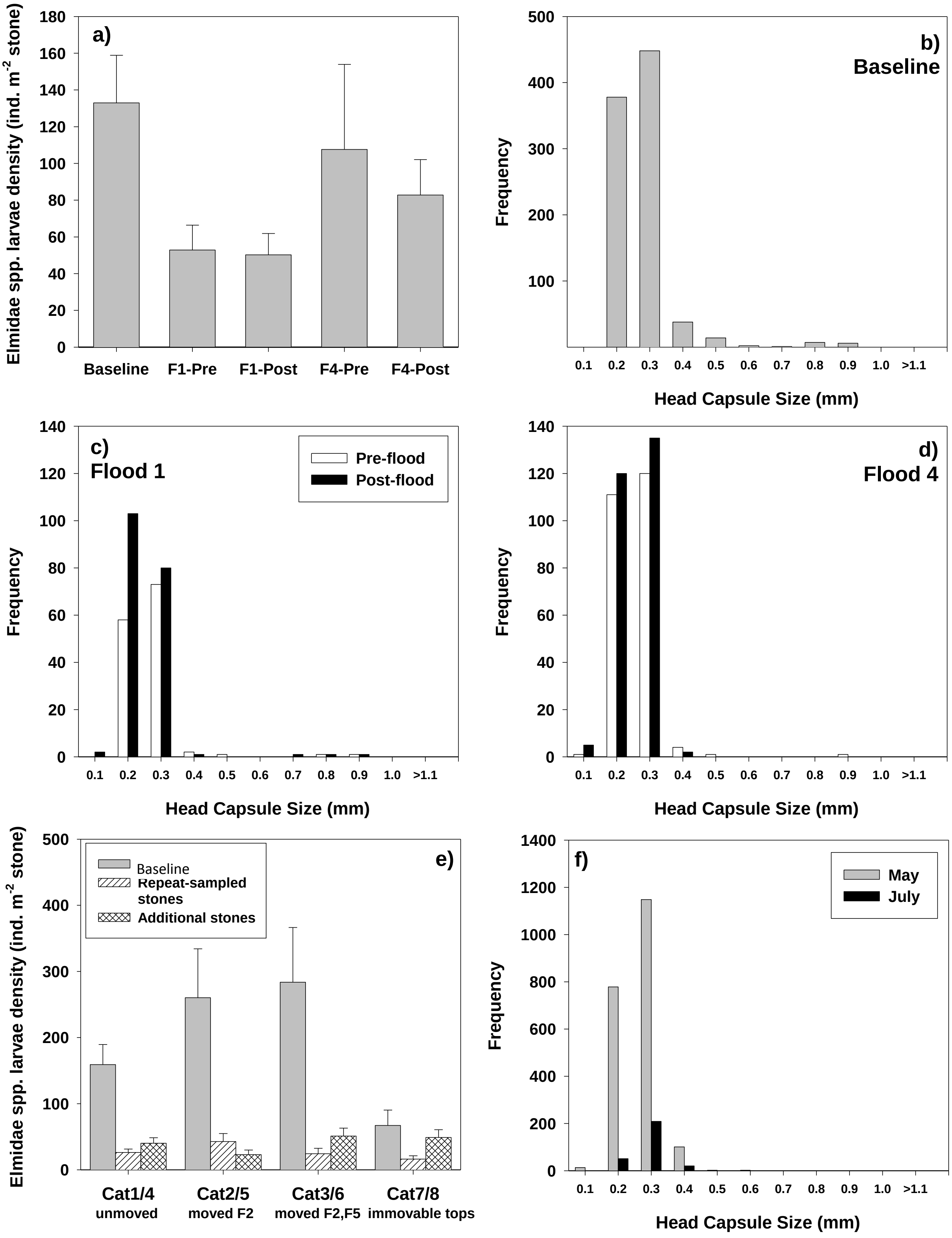

Figure 4.36 a) Mean density (+ standard error) and (b-d) population size frequency distribution of Elmidae spp. larvae in the Molenaars River, on each of the five sampling occasions during the 2003 study, and (e) mean species density and (f) population size frequency distributions for the two sampling surveys in the Berg River in 2004. Density data show each of the stone movement categories separately for the Berg River study.

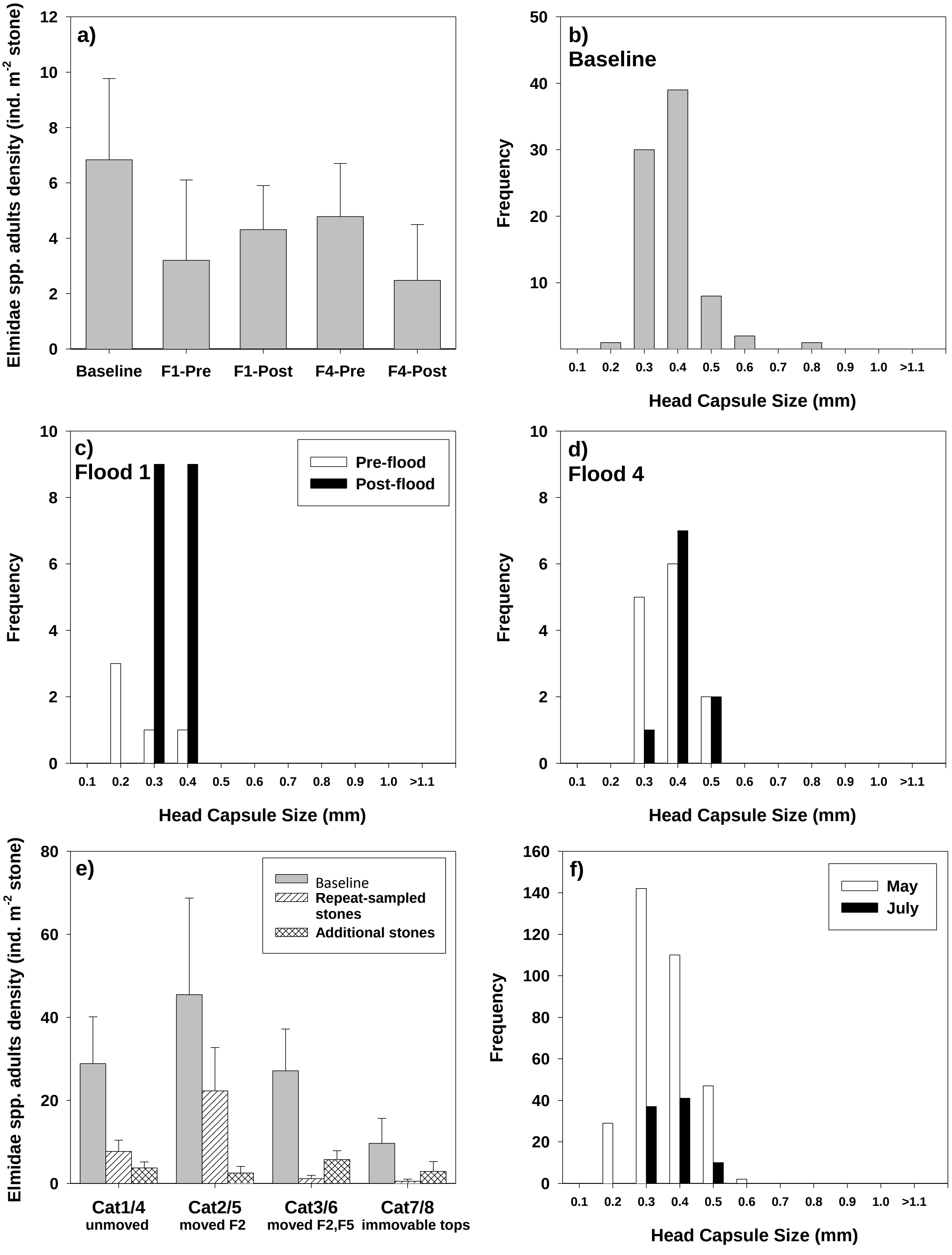

Figure 4.37 a) Mean density (+ standard error) and (b-d) population size frequency distribution of Elmidae spp. adults in the Molenaars River, on each of the five sampling occasions during the 2003 study, and (e) mean species density and (f) population size frequency distributions for the two sampling surveys in the Berg River in 2004. Density data show each of the stone movement categories separately for the Berg River study.

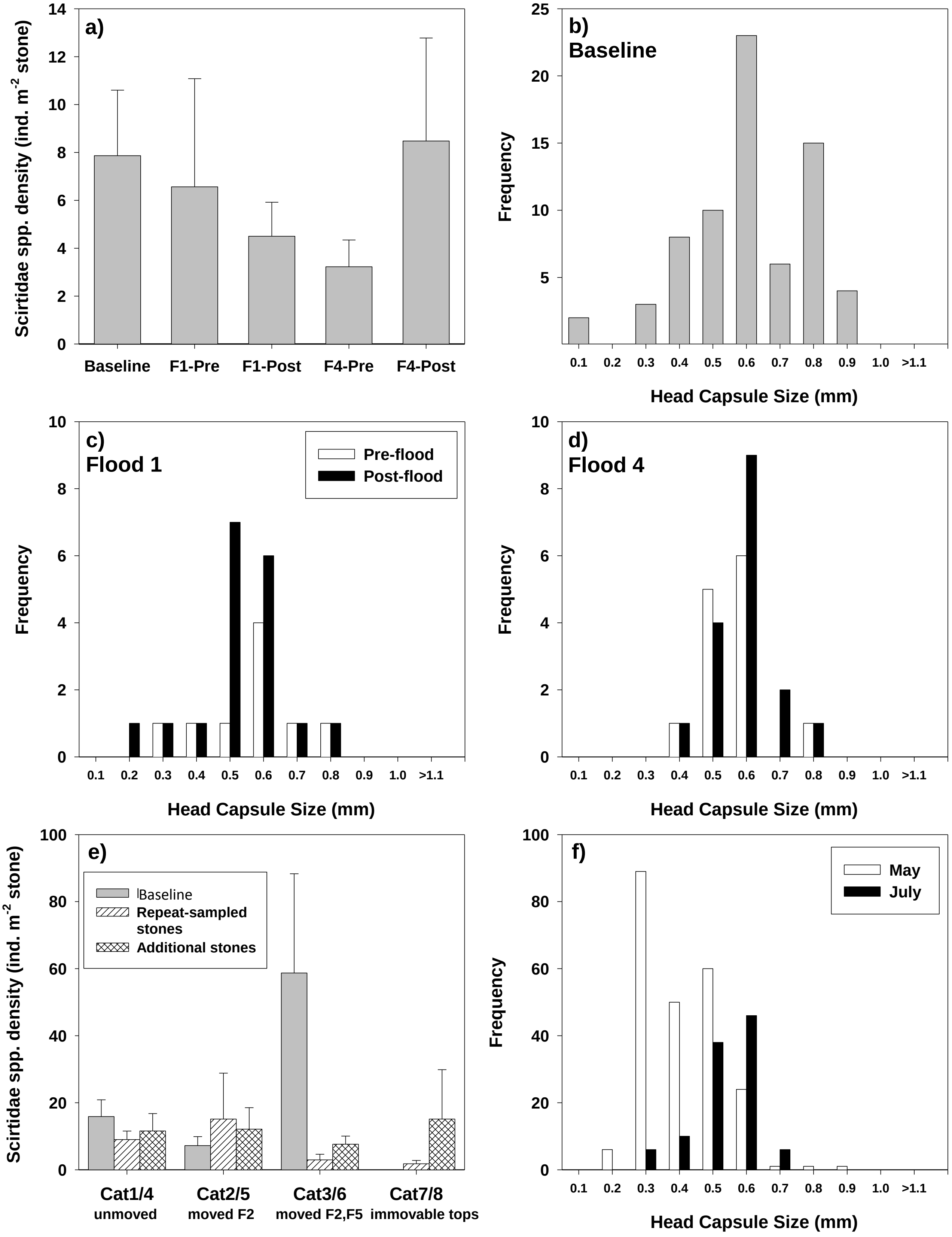

Figure 4.38 a) Mean density (+ standard error) and (b-d) population size frequency distribution of Scirtidae spp. in the Molenaars River, on each of the five sampling occasions during the 2003 study, and (e) mean species density and (f) population size frequency distributions for the two sampling surveys in the Berg River in 2004. Density data show each of the stone movement categories separately for the Berg River study.

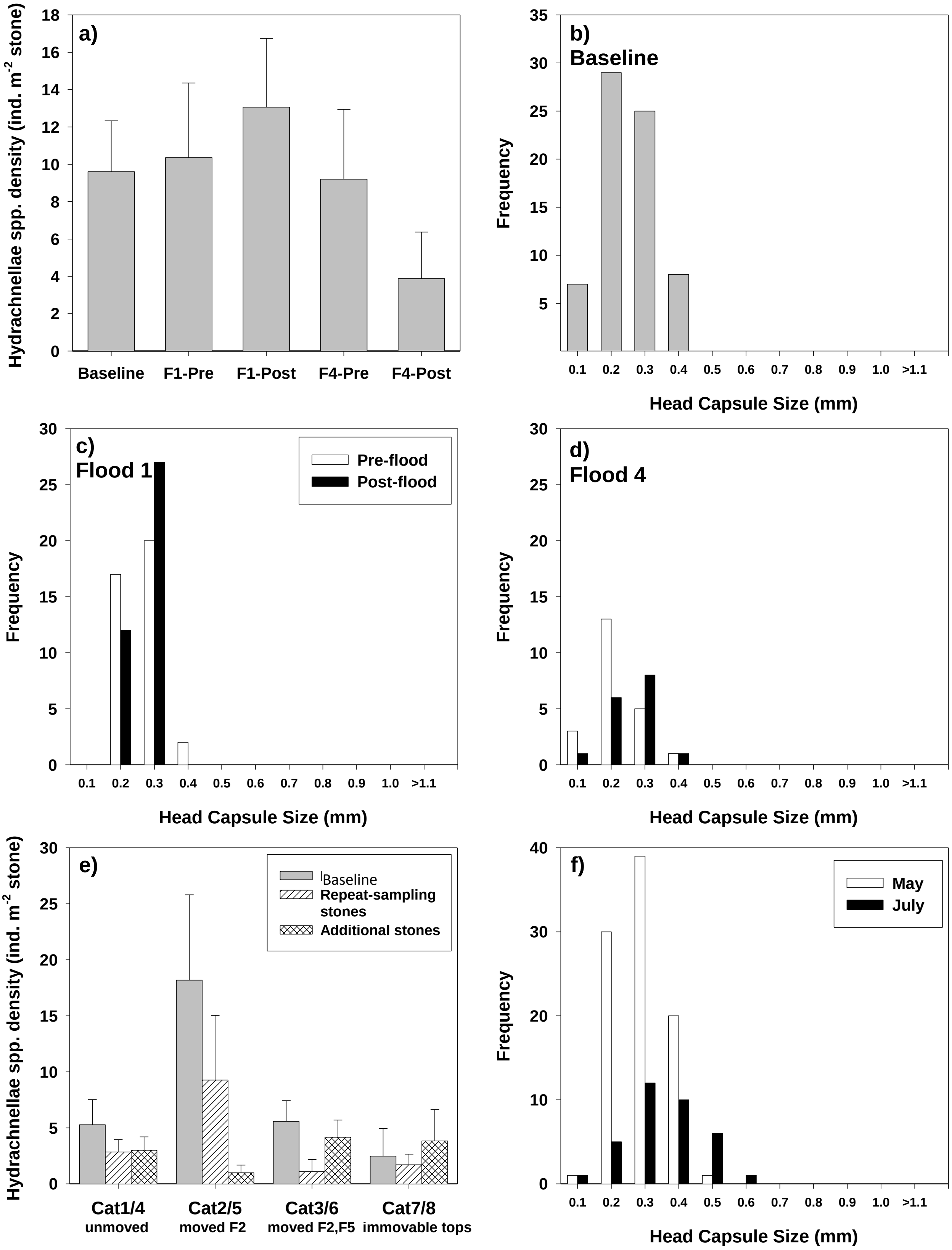

Figure 4.39 a) Mean density (+ standard error) and (b-d) population size frequency distribution of Hydrachnellae spp. in the Molenaars River, on each of the five sampling occasions during the 2003 study, and (e) mean species density and (f) population size frequency distributions for the two sampling surveys in the Berg River in 2004. Density data show each of the stone movement categories separately for the Berg River study.

Berg River the range was 12 000 - 31 000 Watts m^{-3} on unmoved, Repeat-sampled stones, but the range was lower, between 14 000 - 24 000 Watts m^{-3} on unmoved, Additional stones. As the applied power at the bed is discharge dependent, this increase in applied stream power ranges with increasing flood size is unsurprising.

In the case of the Berg River, all stream power values were high. Even though the range reported here was for the stones from which biological samples were taken, which were all situated within the main channel, there were only nine stones out of the 433 stones monitored over the flood where the applied power during Flood 5 was under 10 000 Watts m^{-3}. This suggests that, in terms of applied power, there were few patches within the stream bed that were associated with low hydraulic forces, which could have sheltered animals - applied stream power values during winter base flow conditions averaged around 100 Watts m^{-3}.

Relationships between post-flood invertebrate densities and stream power were hard to decipher for three reasons. In some instances inadequate recovery on repeat-sampled stones led to very low densities, for example of *Cheumatopsyche afra*. In the case of the Berg River samples, however, the control offered by the Additional stones data set avoided this problem. Secondly, patchiness in the distribution of most of the taxa resulted in zero densities on some stones that may or may not have been related to the magnitude of forces acting on the stones, thus obscuring possible relationships with applied stream power. Finally, the analysis could only test the relationship between <u>actual</u> post-flood invertebrate density and applied stream power, not the change in density of the different invertebrate taxa, since it is obviously impossible to measure both pre- and post-flood densities on individual stones. Given the probably very wide differences in starting densities, immediately before the flood, the analysis was limited therefore to examining whether the post flood distribution of invertebrates matched the distribution of forces on the stream bed, rather than the effect of different magnitudes of force on the degree of reduction in invertebrate density from a bed particle.

Whilst there were few significant relationships between the density of any taxon and the maximum applied stream power at that stone during the immediately preceding flood, most taxa demonstrated either non-significant negative relationships between density and applied power, or no relationship at all. Examples are provided in Figures 4.40 to 4.42. Correlation coefficients and regression equations are indicated on the plots only where these were found to be statistically significant.

Figure 4.40 shows the relationship between applied stream power and immediately-post flood densities for *Afroptilum* spp. and *Demoulinia* spp., on unmoved stones, in samples taken immediately following three floods. These were two small floods on the Molenaars River (Flood 1 and Flood 4) and Flood 5 on the Berg River. In the Molenaars River, both species were reduced by some 50 % in density over the two-month flood study period. In the Berg River, both species disappeared from stones that moved, whilst on <u>unmoved</u> stones *Afroptilum* enjoyed complete refugium and its densities remained unchanged on these stones.

Despite this substantial refugium, none of the relationships between *Afroptilum* spp. density and stream power was significant (Figure 4.40a). There is a suggestion in that densities respond weakly to the magnitude of applied stream power on a river stone, if one examines all three floods together, but clearly the relationship is not strong. *Demoulinia* was virtually absent from all stones in the Berg River after the floods (Figure 4.12e), and, expectedly, its densities showed no relationship to applied stream power.

For the Leptophlebiidae (Figure 4.41), different species were present at different densities in the two rivers. *Euthralus elegans* was the only one which remained present in high densities in the Molenaars River over

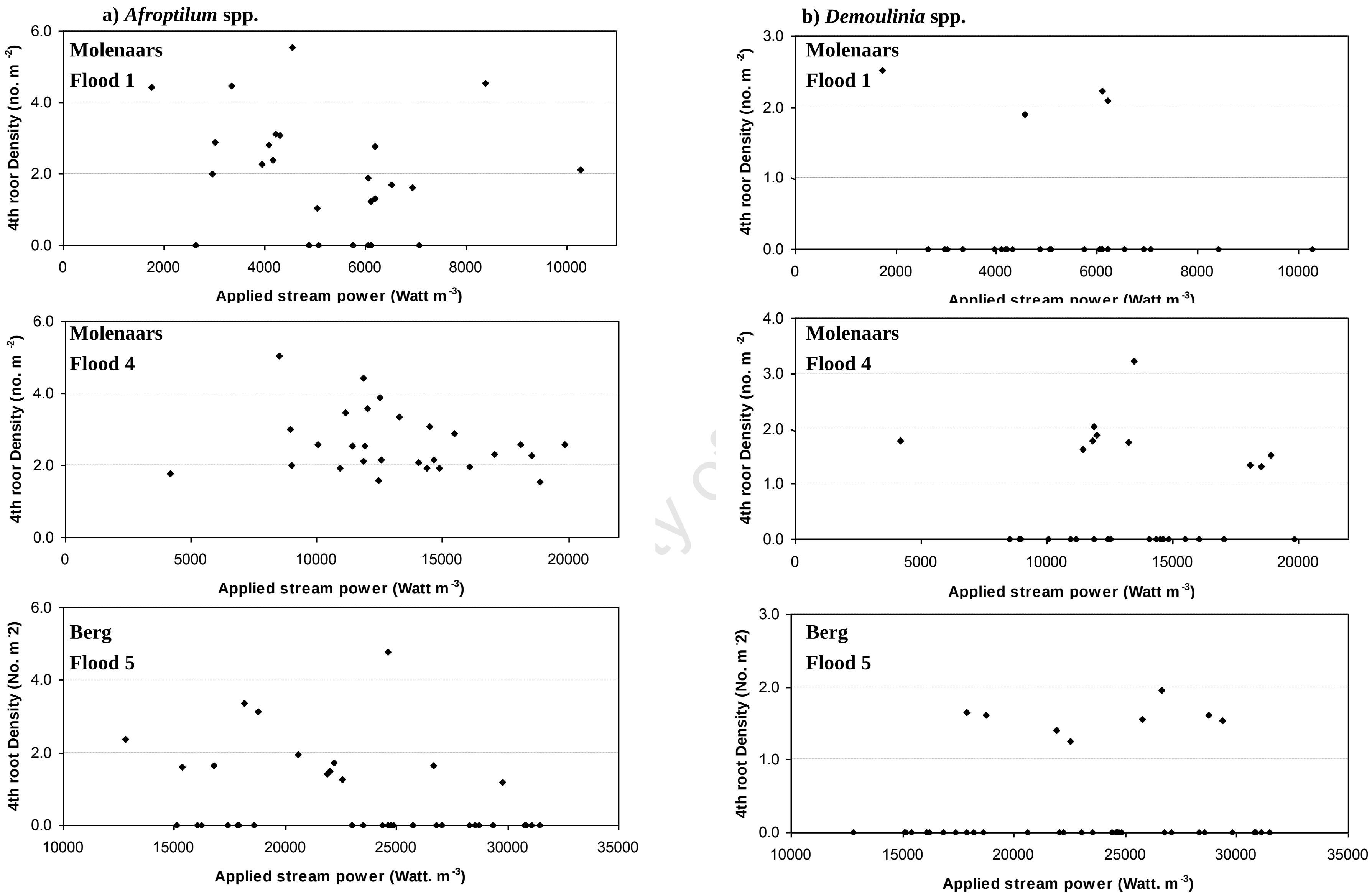

Figure 4.40 Relationship between the applied power at the bed, calculated for each stone sampled immediately after Flood 1, Flood 4 in the Molenaars River, and Flood 5 in the Berg River, against the post-flood density of invertebrates on the sampled stones, for a) *Afroptilum* spp. and b) *Demoulinia* spp. (all correlations non significant).

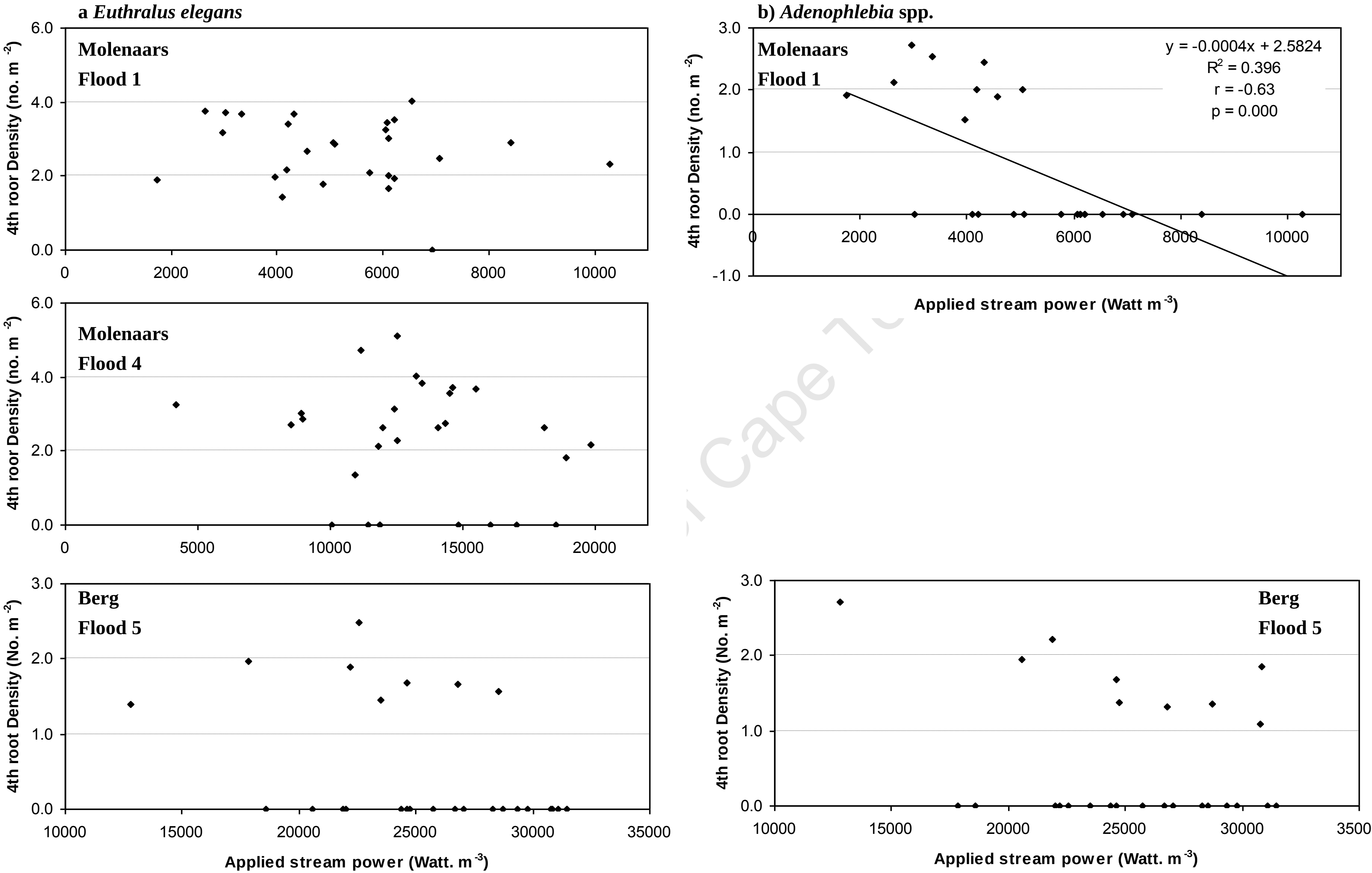

Figure 4.41 Relationship between the applied power at the bed, calculated for each stone sampled immediately after Flood 1, Flood 4 in the Molenaars River, and Flood 5 in the Berg River, against the post-flood density of invertebrates on the sampled stones, for a) *Euthralus elegans* and b) *Adenophlebia* spp. Only significant regression lines are shown.

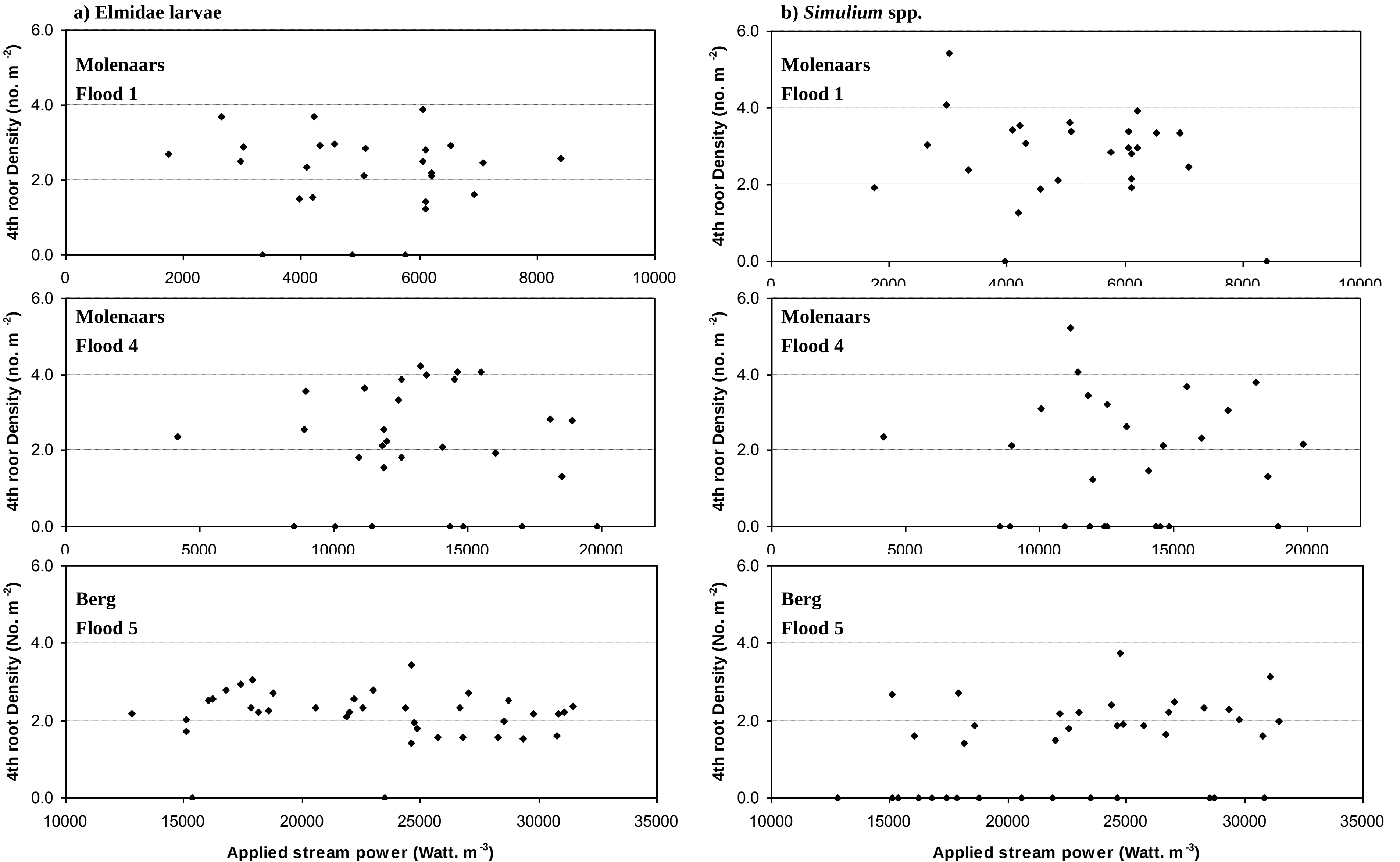

Figure 4.42 Relationship between the applied power at the bed, calculated for each stone sampled immediately after Flood 1, Flood 4 in the Molenaars River, and Flood 5 in the Berg River, against the post-flood density of invertebrates on the sampled stones, for a) Elmidae larvae and b) *Simulium* sp. (all correlations non significant).

the study period, whilst *Adenophlebia* spp. and *Aprionyx* spp. declined progressively over time, perhaps in relation to a late autumn emergence. *Adenophlebia* spp. was one of the few species that showed a significant negative correlation with applied stream power during Flood 1 in the Molenaars River (Figure 4.41b), whilst *E. elegans* showed no such relationship. In the Berg River, both species enjoyed a relative refugium on unmoved stones. The same trend as in the Molenaars was present - *E. elegans* showing no relationship to applied power; *Adenophlebia* spp. responding negatively, although this relationship was not significant in the Berg River.

Finally, two taxa that are frequently associated with the upper surfaces of river substrata, *Simulium* spp. and Elmidae larvae, are presented in Figure 4.42, in relation to changing applied power values. No correlations between densities and stream power were found.

4.7 DISCUSSION

Sampling design and confidence in the results

In the Molenaars River, invertebrate samples were collected from 50 stones at each of four pre- or post-flood sampling surveys, and 200 samples were collected at the initial Baseline survey, in order to assess flood-related impacts on invertebrate fauna. Due to the constraints of time and resources, however, only 30 stones, including samples from whole stones (23-27 samples) and tops of immovable boulders (3-7 samples), were processed for each survey, with 70 stones being processed of the 200 Baseline samples collected. Two randomly selected subsets of approximately 23 whole-stone samples from the Baseline data set were compared, as described in the preceding sections, to check that each subset produced the same representation of invertebrate community structure, and hence that this sample size was adequate for subsequent analysis of flood effects. The non-significant difference between the subsets of Baseline data samples (with the exception of two taxa) lends itself to the conclusion that this sample size was sufficient to use for further comparisons between pre- or post-flood assemblages or between these and the baseline data.

Invertebrate density data from all five collection times in the Molenaars River study showed a gradually declining population following the Baseline survey. Despite this, non parametric tests showed no significant differences in density between Pre-Flood 1 densities and Post Flood 4 densities in the majority of invertebrate taxa of the Molenaars River, taken individually, with a few exceptions. Indeed, multivariate analysis only discerned significant change in invertebrate community structure as a cumulative effect of four floods in the Molenaars River, suggesting that these small floods had little effect on invertebrate assemblages. An alternative, and quite probable, reason for the failure to discern statistically significant change could be the large variance associated with estimates of population density at each time interval over the study, which may have overshadowed changes resulting from small floods. To dismiss the non-significant declines in density and conclude that the series of Class I floods had no effect on invertebrate taxa would thus not be correct.

An important feature of the Molenaars River study was the sampling design - that of repeat-sampling marked stones. At the level of community structure, the biotope signature observed in the distribution of species assemblages on the repeat-sampled stones of the Pre-Flood 1 survey was very similar to that of the Baseline survey, as shown by the results of multivariate analysis. This robust analysis indicates that the relationships between species and their hydraulic environment became re-established very rapidly on repeat-sampled stones after initial sampling. In relation to univariate measures such as invertebrate density data, there were no significant differences between Baseline and Pre-Flood 1 densities of total invertebrate density and that of individual species for all but five taxa, also suggesting that recovery on repeat-sampled stones within the month-long period after initial sampling was adequate. These results suggest that such an approach to flood studies may have merit.

However, despite the similarity in the spread of samples from the Baseline and Pre-Flood 1 sampling, the actual pairs of invertebrate samples, i.e. taken from each individual stone sampled at Baseline and again at Pre-Flood 1, were only on average 67 % similar, based on multivariate analysis, after square root transformation of densities. The lack of statistical significance in the density reductions of individual taxa between the Baseline and Pre-Flood 1 surveys may also simply have been the result of high variances in density estimates, especially in patchily distributed taxa. The chances of a Type II error, i.e. of falsely concluding that there was no change in the invertebrate community cannot therefore be ruled out. If the results were to show absence of a full recovery, then the study approach would be flawed, in terms of quantifying flood effects.

Closer examination of the species data showed a combination of complete recovery by some species, as well as a failure by others to recover on sampled stones. Examples of the failure for species to recover on repeat-sampled stones were *Lestagella penicillata* and *Elporia* spp. Neither reached more than 46 % of the Baseline condition at any subsequent point after the Baseline survey. This was despite the fact that these taxa were associated with immature populations, which implies that there were unlikely to be losses to pupal or adult stages and despite evidence of recruitment of new instars over the winter period, at least in the latter species. Niemi *et al.* (1990) concluded that a complicating feature of pre-post disturbance studies is the fact that pre-disturbance densities are almost never re-attained because of natural temporal fluctuations and new gradients in resources. In the Molenaars River, exponential increases in *Lithogloea harrisoni* over the whole study period are a case in point (see also Johnson & Vaughn 1995).

These considerations throw some doubt over the results from the Molenaars River, suggesting on the one hand that the failure to find statistically significant changes in density should not be disregarded as evidence for flood effects, because of variability in density distribution, but on the other hand that the effects of floods might well be over-estimated on repeat-sampled stones, because of a failure of many taxa to recover after the initial sampling. This sampling effect can be considerably reduced by using the Pre-Flood 1 data as an alternative baseline condition against which to compare subsequent changes, and this indeed became the approach of this study.

Some concerns remain even after that, however. For example, the lack of change in *Elporia* spp. from Pre-Flood 1 (the alternative baseline condition) to Post-Flood 4 suggests a stable population throughout the winter. However, the size-frequency distribution for this species shows that it was recruiting new instars continuously, but no portion of the population developed to pupal stage over the study period. An alternative explanation for the stability in the *Elporia* spp. population density on the repeat-sampled stones, therefore, could be that larger individuals were relocating to non-sampled stones on the river bed, because of possibly poorer stone conditioning and food-resource availability on previously sampled stones. This topic is discussed in the next discussion section, on invertebrate recovery and resilience. A control sample of previously unsampled stones at each sampling occasion would have provided important comparative information in this regard, but was not collected.

With regard to the Berg River study, a control sample collected simultaneously with the repeat-sampled stones revealed that any sampling effect was not significant compared with the effects of the preceding floods on invertebrate assemblages or on the densities of individual taxa. There were some small exceptions to this: Chlorophyll a was lower on Repeat-sampled stones than on Additional stones, suggesting that the initial effects of stone scrubbing and marking may have been carried through to the post-flood period. Also, densities of some taxa that increased in densities over the study period, viz. *Pseudocloeon* spp. *Lithogloea harrisoni* and *Nadinitella crassi*, were also higher on Additional stones than on Repeat-sampled stones, although both data sets showed the same trend. Overall, therefore, the Repeat-sampled stones can be considered to provide a reliable measure of the magnitude of invertebrate response to flood disturbance, in the case where larger within-year floods occur, as the magnitude of the disturbance effect reduces the sampling effect to a relatively small error.

The Additional stones data set allowed for testing of the accuracy in predictive power of the hydraulic model, since the movement status of each of these stones was determined theoretically using the model, and the invertebrate responses, grouped into ‘moved’ and ‘unmoved’ categories were compared with those of the Repeat-sampled stones, whose movement status was obviously known through the actual recording of stone dislocation during floods. In all respects the two post-flood datasets showed the same pattern of change when compared with the Baseline condition, in each movement category. The similarity in flood responses of the invertebrates on Repeat-sampled and on Additional stones, in both movement categories, indicates that the hydraulic model has considerable potential for application in future disturbance studies, thus avoiding the error associated with the sampling effect of a repeat-sampled study design.

The Molenaars River study as a field experiment in post disturbance recovery

In many respects, the Molenaars River study emulates the sorts of artificial disturbance experiments of researchers over the past two decades (e.g. Robinson & Minshall 1986; Boulton *et al.* 1988; Lake *et al.* 1989; Lake & Schreiber 1991; Gawne & Lake 1996; Matthaei *et al.* 1996; Mathooko 1998) which have examined invertebrate assemblages on artificially disturbed substrata at increasing time since disturbance, by manually disturbing individual particles or patches of river bed. In this regard, the data collected in this study may provide useful information, specifically on the differences in recovery rates on previously denuded stones, which are relevant in flood studies. Such small-scale experimental disturbance studies are considered to simulate the effects of disturbance fairly adequately, especially since the dominant effect of floods is the overturning of individual stones (Downes *et al.* 1998a; Matthaei *et al.* 1999a). In this study then, the artificial disturbance of stones was effected during the Baseline sampling through the removal of all invertebrates from stones. The intensity of this artificial disturbance in the Molenaars River would be more akin to the acid-washing treatment of Boulton *et al.* (1988), than to the simple overturning of stream particles of other studies, or possibly might emulate an extreme scouring event in streams that carry high concentrations of suspensoids, because the sampling process included removal of animals, then cleaning and drying the stones sufficiently to mark them permanently.

Whilst Lake *et al.* (1989) found that 30 days was sufficient for full recovery of trapped organic material, invertebrate density and species density, Boulton *et al.* (1988) found that invertebrate species density but not total invertebrate density had returned to pre-disturbance levels in 32 days, on his acid-scoured stones. Similarly periphyton remained lower after 32 days than prior to the disturbance treatment. Robinson & Minshall (1986) concluded that a time period of over 27 days between disturbances may be adequate for equilibrium conditions to be established in invertebrate assemblages, but not periphyton. In this Molenaars River study, periphyton was not sampled, but organic debris did return to pre-disturbance levels within 30 days, whilst invertebrate taxa were variously more or less abundant after 30 days, with most taxa present in lower densities than at the pre-disturbance time. Community composition was on average 67% similar to the pre-disturbance condition. The recovery rates of the invertebrate taxa following initial disturbance were also variable. In this study therefore, recovery was slower than that measured in other studies, and affected by a range of factors, discussed below.

Factors affecting recovery rates are well described in the literature (e.g. Niemi *et al.* 1990), and include the presence of refugia, scale of disturbance and life-history traits of the recolonising population. In addition, the extent to which food resources are rapidly re-established may influence the effect of disturbance on invertebrates (Robinson & Minshall 1986; Death 2003). The scale of disturbance and the presence of refugia may in many ways be considered corollaries, since refugia are defined as habitats or factors that reduce the effects of disturbance (Sedell *et al.* 1990). In this artificial disturbance study, although the disturbance to both invertebrates and their potential food resources on each stone was extreme (total denudation), the scale of disturbance was small and its corollary, the availability of refugia was large - refugia were widely available in the form of all river-bed stones that were not sampled. Thus the key factor influencing recovery in this study would be the life history traits of the invertebrate taxa.

Mobility, or the propensity for dispersal, is logically one of the most important resilience attributes affecting the time to recovery after a disturbance (e.g. Lancaster & Hildrew 1993b; Townsend & Hildrew 1994; Winterbottom *et al.* 1997a). The Simuliidae and Chironomidae are well recorded early colonisers of disturbed substrata because of their mobility (Fisher *et al.* 1982; Biggs & Stokseth 1996), and these groups recovered well in the Molenaars River study after initial denudation of stones. On the other hand, the poor recovery by Elmidae larvae (Coleoptera), *Elporia* spp. (Diptera: Blephariceridae), *Lestagella penicillata* (Ephemeroptera: Telagonodidae) and both *Cheumatopsyche afra* and *Chimarra* spp., two net-spinning caddisflies, probably reflects the much poorer mobility of these animals than for example the baetid mayflies, chironomids and simuliids. Neither *Elporia* spp. nor Elmidae swims, but both groups move rather by crawling across the stream bed, and hence would take far longer to redistribute across the stream bed than more mobile, swimming and / or actively drifting taxa like the Baetidae and Simuliidae. *Lestagella penicillata* is a poor swimmer and tends to have a very clumped distribution over the stream bed (pers. obs.). Similarly, since *C. afra and Chimarra* spp. utilise built structures for capture of prey, they are less likely to range across the river than groups which browse or graze. The series of small floods during the Molenaars River study period may have served effectively to increase the mobility of these taxa, allowing some redistribution of these groups onto denuded stones, for example *C. afra and Chimarra* spp., whose densities on previously sampled stones increased with each time interval after the initial denudation and were greatest two months and four floods after the Baseline sampling survey. Winterbottom *et al.* (1997a) demonstrated that in some species mobility was increased as a function of discharge. In this light, a re-examination of the artificial disturbance study of Lake *et al.* (1989), where total recovery of invertebrate fauna occurred within 33 days of disturbance, might find that the small spates that occurred during the recovery period may have been a substantial contributing factor to the rapid recovery found in that study.

Townsend's (1989) definition of disturbance as being the 'opening up of space' for recolonisation, which may take place at a range of scales, is particularly applicable in these sorts of studies. Unoccupied space may be associated with the supply of resources, or critical to the capture of resources, and thus desirable for either competing species or for new recruits from within the stream bed. This is likely to be especially important where invertebrate densities are high, at which times recolonisation of substrata may take place in mere hours (Robinson & Minshall 1986).

In this regard, both the initial density of an organism, but also life-history characteristics that determine the availability of new recruits at the time of the disturbance, would be expected to influence recovery rates. The recolonising populations of a number of taxa, *inter alia Agapetus agilis*, *Simulium* spp., *Chimarra* spp. and *Lithogloea harrisoni* had a smaller mean or modal head size in the Pre-Flood 1 samples than in the Baseline survey, suggesting that the previously denuded stones may have been recolonised predominantly by young instars. For example, Simuliidae are year-round breeders in southern African rivers (de Moor 1994) and drift, particularly of younger instars, is substantial and constitutes an important mechanism for the dispersion and spatial differentiation of different developmental stages (de Moor *et al.* 1986). Similarly, the moderate and massive increases in Notonemouridae and *L. harrisoni* densities respectively, over the study period in the Molenaars River, and the frequency distribution of larval sizes, indicate the sequential hatching off eggs or migration of new instars from the hyporheos, which would provide a ready supply of individuals to colonise opened up space. On the other hand, new instars of *Cheumatopsyche* spp. tend to appear on the stream bed only later in the winter months (pers. obs.), and thus would not contribute to resilience of this group during early winter floods. Further, whilst the baetid mayflies *Demoulinia* spp. and *Afroptilum* spp. recovered rapidly on previously sampled stones prior to the onset of floods, they were susceptible to even the small floods during this study and, having no new recruitment, suffered large population losses over the season.

An additional factor in determining invertebrate resilience in this study was potentially the post-disturbance conditioning - in terms of algae and detritus, as well as the periphyton composition - of previously denuded stones. Recovery pathways of algal communities following disturbance by floods or drought include recolonisation by immigrant propagules, as well as *in-situ* recovery and growth of remnant residues of the algal mat (Biggs & Stokseth 1996; Peterson 1996; Stanley *et al.* 2004). In relation to floods, adnate forms of algae, mostly diatoms, are usually more resistant to scour and often provide a remnant layer for early recovery of populations (Grimm & Fisher 1989; Peterson 1996), except in the case of very large scouring floods. Non-attached, motile and loose flocculent assemblages have low resistance to floods, but under non-flood conditions drifting unattached diatoms resettle rapidly (Peterson 1996). Propagule attachment has been shown to be a function of velocity and is highest in slow-flowing (>0.3 m s^{-1}) habitats (Biggs & Stokseth 1996; Opsahl *et al.* 2003). An idealized sequence of recovery of algal assemblages post disturbance is suggested by Stevenson (1996) to be re-growth of adnate forms, mostly diatoms and basal cells of filamentous green algae that persist through the disturbance event, followed by fast-growing apically-attached forms that stand erect on substrata and, finally, stalked, filamentous or motile forms (Stevenson 1996).

Although algae are tolerant of desiccation (Peterson 1996; Stanley *et al.* 2004), mortality after sudden drying can occur within 0.5 to 4 hours (Stanley *et al.* 2004), depending on the degree of heat exposure. All ‘movable’ stones in this study were removed from the stream for marking, and remained in the sun for at least two hours. In addition, all organic material was burnt off the area of each stone that was to be marked with paint, by means of a gas torch, to ensure that the paint marker remained on the stone. Thus there were in all probability very few remnant algal cells on denuded stones during the Baseline survey, and recovery during the period that followed must have been largely a result of immigration from drifting propagules (Robson 2000). This feature, together with the prevailing autumn low-flow conditions, would have influenced the composition of algal assemblages on repeat-sampled stones. Thus the recovery process of stream periphyton during the Molenaars River study would probably have excluded *in-situ* recovery of adnate algal forms, but would rather have been characterised by a loose flocculent assemblage (Peterson 1996), with low representation of adnate and mucilaginous forms, which are slower to recover. Because different grazers are associated with exploitation of different algal life forms (Steinman 1996), this feature may well have influenced the resilience or recovery recorded for different grazer taxa, as is hypothesised in the case of scraper-grazer taxa, *Elporia* spp., Elmidae and *Agapetus agilis*.

Mention has already been made of the slow recovery of *Elporia* spp. and Elmidae. The cased-caddis *Agapetus agilis* would also fit this pattern of slow recovery, since despite signs of strong recruitment of new instars over the study period, this species also ended up with low densities on Repeat-sampled stones, relative to the Baseline condition. This was probably in part a reflection of its low mobility. However, as algal scraper-grazers (Schael 2005), these groups are thought to favour the utilisation of adnate or prostrate algal growth forms (Steinman 1996). For example, species within the genus *Agapetus* have been found to show a preference for, or significantly affect accrual rates of adnate diatoms, which were not affected or ingested by grazing mayflies (Poff & Ward 1992; Peterson *et al.* 2001). In this study, therefore, the failure of these groups to recover on Repeat-sampled stones may thus also reflect the fact that the species composition algal assemblages, or the dominant life forms, that were re-established on denuded stones did not correspond to the preferred food source of these particular grazer groups. Resource quality has been shown to influence invertebrate colonisation of substrata in other studies (e.g. Negishi & Richardson 2005).

In a slightly different vein, *Baetis* spp., by far the dominant taxon in the Molenaars River and present on almost every stone sampled, at all survey times, was the only baetid that had lower densities on repeat-sampled stones in the Pre-Flood 1 sampling than during the Baseline survey, and these were significantly so. In contrast, rapid recovery of the numerically subordinate *Demoulinia* spp., *Afroptilum* spp., *Pseudocloeon* spp. and *Demoreptus capensis* occurred from the Baseline to Pre-Flood 1 sampling times. The extent to which baetid species compete for resources in Western Cape rivers is a subject for mere speculation, but these taxa are all fairly similar with regard to mouthpart morphology. If *Baetis* spp. (mostly *B. harrisoni*, but probably comprising at least three species) were dominant competitors among the Baetidae mayflies, as suggested by their substantial numerical dominance within the invertebrate assemblages, then their apparent failure as a group to recover might simply reflect selection against the poorer resources probably associated with denuded stones - these stones in fact becoming space available for the lesser competitor species of Baetidae. In this instance, recovering periphyton assemblages may have been too sparse to support high densities of grazers, as was found in the study of Robinson & Minshall (1986). In the Molenaars River, periphyton biomass was not sampled, but in the Berg River study, one of the few differences between the post-flood repeat-sampled stones and the control set (Additional stones) was the significantly lower periphyton biomass on the former, suggesting slow post-sampling recovery.

Competition between hydropsychid caddisflies and Simuliidae is better documented. Hydropsychids have been shown to be superior competitors, either through aggression or because their collecting nets interfere with simuliid feeding (Hemphill & Cooper 1986; Hemphill 1988; Zhang *et al.* 1998) and mature hydropsychids are reported to constitute a significant predator regulating simuliid population size (de Moor 1992). Simuliidae also exhibit intraspecific competition (Wiley & Kohler 1981), although this more often leads to redistribution of individuals on a bed particle rather than displacement. In this study, both groups shared the fast run / riffle biotopes, and there was a significant positive correlation between *Simulium* spp. density and that of *Cheumatopsyche afra* in the Molenaars River during the Baseline survey, a finding similar to that of de Moor (1992) who showed substantial overlap in habitat utilisation between these groups. The rapid recovery by *Simulium* spp. on repeat-sampled stones one month after initial sampling is consistent with the other research findings indicating the high resilience and high mobility of this taxon (Hemphill & Cooper 1986; Hemphill 1988), and the increase in population density of this group is explained by the continuous recruitment of new instars over the study period. The pattern of recolonisation of stones by *Simulium* spp., with almost exactly the same distribution of densities over the stream bed in the Pre-Flood 1 period as in the Baseline sample, is a marvellous demonstration of its strong microhabitat preference. A second aspect of this pattern of recovery on denuded stones is also noteworthy: recolonisation of stones was more substantial on those stones that had previously supported *C. afra*, which species did not recover on denuded stones at all within the first month after Baseline sampling. The largest increases in *Simulium* spp. density were on those stones that had had the greatest densities of *C. afra* in the Baseline sample. This suggests that in the absence of *C. afra*, *Simulium* spp. was able to expand its population size on stones that provided the optimal hydraulic conditions for resource utilisation. Other studies (e.g. Johnson & Vaughn 1995) have also found a shift in relative densities of Hydropsychidae and Simuliidae after substratum disturbance in favour of the latter. These results are additional evidence for the suggestion that competitive displacement of Simuliidae by hydropsychid caddisflies in stream ecosystems is mediated by the effects of disturbance and the greater resilience of Simuliidae, thus allowing for long-term co-existence of these groups within the same microhabitat (Wiley & Kohler 1981; Hemphill & Cooper 1986; Hemphill 1988; Zhang *et al.* 1998; Robinson *et al.* 2004).

Invertebrate responses to floods

Molenaars River

In the Molenaars River study, the series of floods that occurred over the invertebrate study period were very small, with less than 3 % bed movement. The maximum average daily discharge for these floods was between 3 and 8 $m^3 s^{-1}$. To put this into perspective, base flow during this dry winter ranged between 1 and 4 $m^3 s^{-1}$, although this higher value was only measured at the end of winter. However, the fourth flood, in July, had an instantaneous peak discharge of about 29 $m^3 s^{-1}$, more than an order of magnitude higher than the winter base flows of that month.

The minor floods affected the recovery of invertebrates on denuded substrata in different ways. In some of the taxa which had shown little recovery in the month after the initial Baseline sampling, the increased velocities associated with the floods may have increased effective mobility and facilitated recolonisation (*sensu* Winterbottom *et al.* 1997a), as discussed previously. In other groups, a poor or only moderate level of recovery after initial Baseline sampling may have been associated with the life-history stages present in the river (e.g. absence of new recruits or sub-adult stages) and / or the poorer conditioning of the repeat-sampled stones, which may have been avoided by mobile taxa that are able to track resources efficiently (e.g. *Baetis* spp.).

Where recovery after initial sampling was rapid, the taxa exhibited various responses. Firstly, in some species only large instars appeared to be susceptible to floods, or were induced to emerge over the flood season, which may have been directly related to floods, or equally associated with other factors that determine life histories, such as resource availability, temperature etc. Other taxa showed various levels of susceptibility to increased flows. These species-specific responses to small flood flows are summarised in Table 4.15. Here the extent of recovery to the original densities after initial sampling, i.e. in the absence of floods, may be regarded as a measure of the resilience of a species, whilst, in the case of resilient species, the extent to which the series of floods was subsequently associated with reduced population densities is

Table 4.15 Summarised species-specific changes in density associated with small early winter floods in the Molenaars River, 2003. The resilience of different taxa is indicated categorically as 'high', 'medium' or 'low', based on the extent of recovery observed in the month after initial Baseline sampling, but prior to floods. Susceptibility to flow is also provided within categories of percentages, because of the low level of significance in the results, based on values in Table 4.10. These categories are as follows: ≈- no change (± 24 % increase or decrease), ↓ (25 - 69% decrease); ↓↓ (70 - 84 % decrease); ↓↓↓ (85 - 100+% decrease). Arrows facing upwards indicate increases in density.

Species / taxon	**Susceptibility to floods**	
	Description	**Change in density over flood period**
Species showing high resilience (good / full recovery after initial sampling, before floods)		
Afroptilum spp.	higher loss of small instars	↓
Demoreptus capensis	recruitment of new instars	≈
Pseudocloeon sp.		↓↓
Euthralus elegans		≈
Lithogloea harrisoni	recruitment of new instars	↑↑↑
Afronurus harrisoni	higher loss of large instars or flood-mediated emergence	↓↓
Afronurus sp.		↓↓
Notonemouridae	recruitment of new instars	↑↑↑
Simulium spp.	recruitment of new instars	↑↑↑
Orthocladinae		≈
Tanypodinae		↓↓
Scirtidae		≈
Hydrachnellae		↓
Species showing medium resilience (moderate recovery after initial sampling, before floods)		
Baetis spp.	may be associated with food quality	↓
Demoulinia spp.		↓
Lestagella penicillata		≈
Aprionyx spp.	Pre-flood autumn emergence - small numbers remaining depleted by floods	↓↓↓
Tanytarcini		≈
Chironomini		≈
Species showing low resilience (poor or no recovery after initial sampling, before floods)		
Cheumatopsyche afra		food-facilitated partial recovery
Chimarra sp.		flood-facilitated partial recovery
Agapetus agilis	recruitment of new instars	pattern not clear
Elporia spp.	recruitment of new instars	≈
Elmidae larvae		flood-facilitated partial recovery
Elmidae adults		↓

suggested as a possible measure of their susceptibility to flow. Where post Baseline sampling recovery was poor, floods appear either to facilitate recovery or to have no effect on recovery, and this is also indicated.

These data must be considered preliminary, since the patchiness of invertebrate distributions, especially when abundances were low, as well as the wide variance associated with estimates of population density, precluded statistically significant results in most instances. Nevertheless, they do represent a first indication of possible specie-level data for flood-related changes in density of invertebrates in Western Cape rivers.

Berg River

In the Berg River in 2004 there were two large intra-annual floods during the study period that moved between 25 and 43 % of the river bed, and three minor spates (DRIFT Class I and II) that moved less than 3 % of bed sediments. The larger floods represented a low and a high DRIFT Class IV flood, and moved all size categories of river bed materials, but to different degrees.

Many studies have used a level of 40 or 50 % of bed movement as a *threshold* for defining disturbance (e.g. Townsend *et al.* 1997b; Gjerløv *et al.* 2003). In comparison, therefore, the Class IV floods on the Berg River could be viewed as reasonably small disturbance events. On the other hand, most of the studies reported on are of rivers with relatively small bed material and hence generally low channel roughness compared to Western Cape rivers. A study of upper river reaches in the Western Cape (Jonker 2002) found median particle size to be between 200 and 300 mm, with the 70th percentile between 300 and 400 mm, and the 90th percentile ranging from 400 to 600mm. In contrast, Townsend *et al.* (1997b) considered large stones in their 27 rivers to be those with a diameter of >128 mm, with a maximum stone size, excluding bedrock, of some 250 mm diameter. Mean stone size in the study of Death & Winterbourne (1994) was 72-85 mm over their range of sites; and in the study of Lake *et al.* (1989) was 16 mm. The stone size range in the study of Matthaei *et al.* (1999a) was 2-64 mm. Only the studies of Downes *et al.* (1998a), with small, medium and large stone categories in the order of 40 - 100, 101-200 and >200 mm respectively, and Death (2003), where boulders were defined as particles >300mm, have remotely comparable substratum size categories. These results show that the study rivers for this thesis have substantially larger stones than most of the comparative studies on disturbance and thus greater bed stability: As a result, whilst the magnitude of forces acting on the bed, and potentially affecting invertebrates through increased shear stresses, might be similar to, or indeed considerably greater than, other studies, this might not result in equivalent bed movement. For example, in a New Zealand study of similar design to this present one (Matthaei *et al.* 1999a), a flood with return period of only six months was associated with some 68 - 72 % bed movement.

The floods in the Berg River were associated with a 58 % decrease in total invertebrate density, cumulative for the two-month period. Nearly all individual invertebrate taxa declined significantly, whilst three mayfly taxa increased in densities on stones over the study period - *Lithogloea harrisoni* and *Nadinitella crassi* (Telagonodidae) and *Pseudocloeon* spp. (Baetidae). Interestingly, the large floods on the Berg River appeared to exceed a disturbance threshold for other taxa that were not affected by the smaller floods on the Molenaars River, viz. *Simulium* spp., Notonemouridae and *Demoreptus capensis*. These taxa increased in densities over the two month study on the Molenaars River, but declined in the Berg River. In addition the Orthocladinae and Scirtidae, whose densities remained stable in the Molenaars River study, were reduced by the larger floods in the Berg River study.

Patchiness of stone movement

Perhaps one of the most profound consequences of small disturbances in streams, defined as within-year flood events, is the subdivision of the habitat into patches. Downes *et al.* (1998a) demonstrated how the spatial scale of disturbance is usually less than a whole reach or even a whole site, but rather the scale of individual stones. They argued that measuring biological response at a large scale, for example by combining samples taken from a whole site as has been the general practice in ecological studies over the past few decades, with the exception of artificial stream experiments, makes estimates of disturbance less accurate, because a large amount of information on variability is collapsed into an average response (Downes *et al.* 1998a). For example Thomson (2002) monitored invertebrate densities following four flood events spanning a year. His sampling strategy did not differentiate physically disturbed patches from those that were not, and thus could not interpret the significant differences in the reduction in invertebrate densities between the runs that were sampled on each occasion.

Matthaei & Townsend (2000) compared invertebrate densities in patches of stream bed that were differently affected by a flood, more than two months after the flood. Three patch types were defined according to the level of disturbance they sustained during the flood, as scour, fill and stable patches. Their results indicated that the disturbance history of the patch had a significant effect on species assemblage, with different species, or different developmental stages of the same species, preferring different patch types as defined above, although not consistently across different sites. Using the same approach, (Matthaei *et al.* 2003) found that periphyton assemblages sampled after flood events were also determined more by patch history (scour, fill or stable) than habitat parameters. Furthermore, temporal patterns of change were associated with patch type. In the immediate post-flood phase, periphyton densities were highest on stable patches, implicating a refugium function for these areas, especially since grazer densities were also higher in these patches than others. Two weeks later, densities had become highest in scour patches. Periphyton sampled three months after separate flood event was more than four times greater in fill patches than in scour or stable patches.

Englund (1991a) also demonstrated longer-term impacts of disturbance. In Swedish streams with patchy moss cover, the overturning of mossy stones created a more diverse habitat than in streams where this did not occur, created by the decaying moss on the undersides of overturned stones and because of upper stone surfaces having a cover of moss at various stages of recolonisation. He estimated recolonisation to be a process taking in excess of two years, whilst overturning of stones was estimated to occur naturally at a rate of less than 8% per annum. Different affinities of invertebrate taxa for moss cover resulted in a patchiness of associated invertebrate assemblage composition that remained 12 months after the initial disturbance. These studies emphasise the need to measure the patchiness of disturbance across the streambed.

In this light, the Berg River study allowed the effects of stone movement or non-movement during floods to be described in relation to the magnitude of the disturbance-response by invertebrates. The percentage decrease in total invertebrate density on stones that were unmoved over the whole study period, relative to those that moved in both large floods, was 40 % and 62 % respectively, a difference of some 20 %. This is an interesting result for two reasons.

Firstly, the difference in densities on moved vs. unmoved stones was much less than anticipated, or put differently, the fact that moved stones, sampled immediately after a flood, retained nearly 40 % of their Baseline densities is an unexpected result. It suggests that, despite flood-induced losses in density, which might represent mortality, many taxa were still present in reasonable densities on moved stones, presumably either because they never left that stone or because they colonised the stone in its new location during or at the end of the flood. This leads to consideration of how local persistence over the course of a flood is possible in the case of moved stones in the Berg River, since these stones could hardly be considered to constitute instream refugia.

Most studies of instream refugia, that is, excluding the hyporheic zone and floodplain areas, identify as most important either stable particles (which moved stones were not) or hydraulic refugia such as patches of stream where shear stress remains low during a flood (Lancaster 1990; Lancaster & Hildrew 1993a, b; Rempel *et al.* 1999; Lancaster 2000). Almost all studies that have demonstrated the use of hydraulic refugium during high flows have done so by means of artificially created refugia (Robertson *et al.* 1995; Winterbottom *et al.* 1997a; Winterbottom *et al.* 1997b; Lancaster 2000; Negishi & Richardson 2005), although Matthaei *et al.* (2003) were able to identify stable patches of stream bed that acted as refugia for periphyton and invertebrate grazers (see also Palmer *et al.* 1995). Nonetheless, these indicate that invertebrate densities in refugia were higher after floods than in non-refugium patches.

The availability of in-stream flow refugia is related to the heterogeneity of channel and bed morphology, where roughness elements create resistance to flow. Hydraulic dead zones have been posited as a novel approach to examining the refugium potential of stream reaches. This classifies streams on how they are expected to affect population changes in the biota through flooding. For example, Lancaster & Hildrew (1993b) identified different stream types according to a) the proportion of streambed occupied by hydraulic dead zones (non-flowing areas of transient storage within the water column e.g. turbulent eddies, channel margins, wakes around larger bed elements, reverse flows within pools) and b) changes in the frequency distributions of shear stress with increasing discharge. These stream types are illustrated in Figure 4.43.

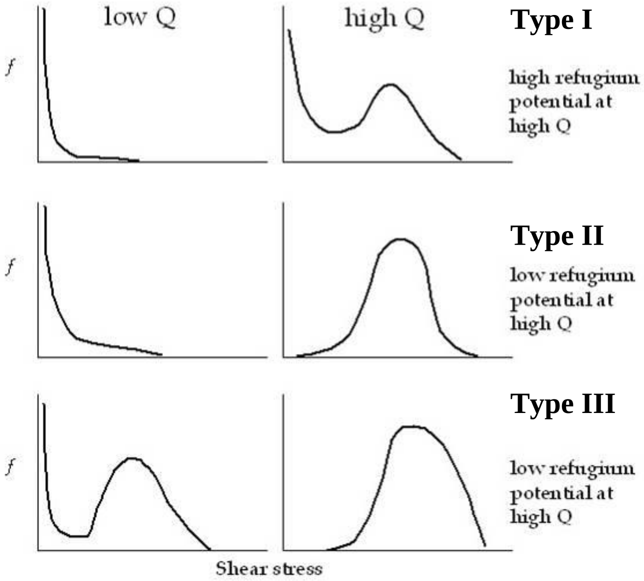

Figure 4.43 Characterisation of in-stream flow refugium potential in streams with different shear stress distributions at low and high flows (after Lancaster & Hildrew 1993b). *f* = frequency of occurrence.

Type I streams were characterised by a skewed unimodal distribution with a majority of low shear-stress spots at low flows, shifting to a bimodal distribution at higher flows. In this latter state, these streams have a greater proportion of areas with higher shear stress than during low flow conditions, but nevertheless retain a prevalence of areas with low shear stress, representing refugia that remain even at elevated flows. These streams were thus seen as being more retentive.

At the other end of the spectrum, Type III streams exhibited a bimodal distribution of shear stress at low flows, with high proportions of both low-stress and high-stress areas. This shifted to a unimodal, bell shaped distribution at high flows with few or no areas of low shear stress.

Type II streams showed the most extreme change in their refugium characteristics associated with flow changes: these were similar under low flow conditions to Type I streams, with a majority of low shear stress areas, but were not retentive at all under high flows, where there was a shift to mostly high shear stress across the stream bed, as with Type III streams.

In relation to this refugium-based description of different stream types, the Berg River may have fitted the Type III stream - that is, having a range of low and high shear stress points at low flows, but with all portions of the bed reflecting high shear stress (measured as stream power) at high discharges and hence exhibiting a low refugium potential. Indeed, none of the more than 400 points in the Berg River were characterised by low or unchanging applied stream power during flood flows, relative to the base flow condition. Even small stones that were located within the hydraulic shelter of large stones were nevertheless associated with elevated applied stream power. In addition, the moved stones were often simply overturned or moved less than 0.5 m from their original locations, thus remaining in unsheltered positions within the main channel.

The fact that invertebrate densities on moved, unsheltered stones were greater after floods than was expected indicates that, even in bed-moving spates, invertebrates that are eroded from the bed are not necessarily lost from the stream reach, even in the absence of obvious hydraulic shelter. Rather, displacement may result in some sort of scramble for cover, which may be more or less successful depending on the particular attributes, for example mobility, of different species. In addition, some taxa may simply remain on a moving stone, awaiting the return of low flow conditions to redistribute over the stream to preferred micro-habitats. An additional explanation, at least in the case of some taxa, could be that moved stones represent a less densely inhabited environment preferable for colonisation by young instars moving into the surface layers of the bed from the hyporheos, immediately after a flood. These species-specific responses are discussed below.

These results thus differ markedly from the small, experimentally-based, refugium studies that indicate higher densities in instream refugia during floods (e.g. Palmer *et al.* 1996; Winterbottom *et al.* 1997b; Negishi & Richardson 2005), since they provide no evidence of higher densities in some parts of the stream than in others. The current understanding of how invertebrates are affected by flood flows once they are dislodged from the substratum and entrained into the water column is poor, and difficult to research (Lancaster *et al.* 1996; Bond *et al.* 2000), although some behavioural studies indicate active responses to entrainment (Lancaster 1999). Speculatively, the high turbulence associated with the large roughness elements in the Berg River may create hydraulic dead zones (*sensu* Lancaster *et al.* 1996) that influence both invertebrate drifting distances as well as their re-entry into the bed. These are also considered to be instream refugia, but their transient nature makes them impossible to sample in empirical studies. Additional investigation of flood-related drift behaviour in these study rivers may help to explain reach-level resistance of invertebrates.

Secondly these results indicate a relative refugium afforded by stable stones. Whilst this was only some 20 % for total invertebrates, it was shown to be more or less important for different taxa. The fact that there were differences in densities on moved versus unmoved stones suggests that the density changes are in fact a consequence of invertebrate susceptibility to floods, rather than other mechanisms, for example related to life-history shifts that simply coincide with the winter period. Holomuzki & Biggs (2000) also found density decreases in a mayfly, caddisfly and snail on both stable and unstable (moved) substrata, and inter-specific differences in susceptibility to floods based on the behaviour of the species with regard to their occupancy of epibenthic areas of lower layers of the substratum. In the Berg River study, differential density changes on unmoved stones vs. those moved in both large floods are summarised in Table 4.16, for invertebrate taxa that occurred in sufficient densities to show patterns. This "susceptibility to flood disturbance" then is based on the percentage reductions recorded in this study, but is provided as categories rather than percentages because of the preliminary nature of the results. The summary is provided separately for Repeat-sampled stones (potentially overestimating the effect of floods because of the error associated with repeat sampling the same stone) and Additional (independent) stones, although the responses were highly similar in both cases.

Table 4.16 Summarised species-specific changes in density associated with a period of winter floods in the Berg River, 2004, showing the relative refugium possibly afforded by unmoved stones. Susceptibility to flow is based on percentage reduction in population density, and provided within categories of percentages. These categories are as follows: ≈- no change (± 24 % increase or decrease), ↓ (25 - 69% decrease); ↓↓ (70 - 84 % decrease); ↓↓↓ (85 - 100+% decrease). Arrows facing upwards indicate increases in density.

	UNMOVED STONES		**STONES MOVED IN BOTH LARGE FLOODS**	
	Repeat-sampled	**Additional**	**Repeat-sampled**	**Additional**
No relative refugium offered on unmoved stones				
Demoreptus capensis	↓↓↓	↓↓	↑↑↑	↑↑↑
Notonemouridae	↓	↓	≈	↑
Simulium spp.	↓	↓↓	≈	≈
Demoulinia spp.	↓↓↓	↓↓↓	↓↓↓	↓↓↓
Cheumatopsyche afra	↓↓↓	↓↓↓	Not present in Baseline	
Tanypodinae	↓↓↓	↓↓↓	↓↓↓	↓↓↓
Orthocladinae	↓	↓	↓	↓
Elmidae adults	↓↓	↓↓↓	↓↓↓	↓↓
Elmidae larvae	↓↓	↓↓	↓↓↓	↓↓
Low relative refugium offered on unmoved stones				
Cheumatopsyche maculata	↓↓↓	↓↓	↓↓↓	↓↓↓
Athripsodes bergensis	↓↓	↓↓↓	↓↓↓	↓↓↓
Afronurus sp.	↓↓↓	↓↓	↓↓↓	↓↓↓
Aprionyx spp.	↓↓	↓↓↓	↓↓↓	↓↓↓
Chironomini	≈	≈	≈	↓
Baetis spp.	↓	≈	↓	↓
Hydrachnellae	↓	↓	↓↓	↓
Euthralus elegans	↓↓	↓↓	↓↓↓	↓↓↓
Chimarra sp.	↓↓↓	↓	↓↓↓	↓↓↓
Afronurus harrisoni	↓	↓↓↓	↓↓	↓↓↓
Tanytarcini	↓↓	↓	↓↓↓	↓↓
Moderate to high relative refugium offered on unmoved stones				
Lestagella penicillata	↓	↓	↓↓↓	↓↓↓
Scirtidae	↓	↓	↓↓↓	↓↓↓
Afroptilum spp.	↑	↑	↓↓↓	↓↓↓
Lithogloea harrisoni	↑↑↑	↑↑↑	↓	≈

For many taxa there was little difference in the level of reduction on moved and unmoved stones, but possibly for different reasons. Species like *Demoulinia* spp., *Cheumatopsyche afra*, and Tanypodinae were mostly reduced to near-zero densities by the floods - apparently as a result of erosion of individuals by flood forces, rather than through pupation or emergence of subimagos, as indicated by the population size frequency data. Others such as the Chironomidae and Elmidae that may be more resistant to shear forces because of their smaller size may have simply held onto moving stones. Indeed, these taxa are well known to be difficult to dislodge from stones when sampling even by light brushing and often have to be removed with forceps.

In the case of taxa whose populations were augmented by new instars (e.g. *Baetis* spp., *Demoreptus capensis*, Notonemouridae and *Simulium* spp.) moved stones may have represented open space for recruits from the hyporheos, thus reducing the numerical differences in density between moved and unmoved stones. In this scenario the open space (moved stones) would presumably be initially denuded and then colonised by young instars. The only species with large enough sample sizes to test this was that for *Baetis* spp., where substantially smaller individuals were indeed found on moved stones than unmoved stones. This still does not indicate when such stones were colonised. Samples were taken as soon after a flood as possible, at still elevated discharge, but it is possible that animals had already begun to redistribute themselves over the stream bed. A final explanation for the higher than expected densities on moved stones could be that very mobile taxa (e.g. *Simulium* spp., *D. capensis*) may be able to counteract the effects of initial dislodgement by flood waters, by rapidly returning to the substratum rather than being washed away.

Invertebrate densities on the <u>top surfaces</u> of large immovable boulders did not decrease over the flood period, but changed in composition quite substantially, with *Baetis* spp. declining and being replaced in dominance by orthoclad chironomids. Other groups that increased in density on these surfaces were *Simulium* spp., *Lithogloea harrisoni*, *Lestagella penicillata*, *D. capensis* and other chironomids.

In relation to the type of refugium offered by unmoved stones - whether this was the top surface or the whole stones - the stones were generally subject to similar forces as those stones that moved during the floods, measured as stream power. This type of refugium - that of large stable particles (*sensu* Townsend *et al*. 1997b; Francoeur *et al*. 1998) - does not therefore constitute an hydraulic refuge, but rather one that may be associated either with more resistant taxa (e.g. *Lestagella penicillata*, *Lithogloea harrisoni*), with retreat- or case-building taxa (e.g. Tanytarcini, *Cheumatopsyche maculata*, *Chimarra* spp.), or with taxa that escape hydraulic forces by hiding within the sediments underneath these stones, and which would be exposed to the force of the flood if a particle were to move (e.g. Scirtidae, *Euthralus elegans*).

These results contrast with those of Bond & Downes (2000) who examined the effect of stone size and movement on two caddisfly species during floods. Before the floods the caddisflies preferentially colonised large stones (>20 cm top width), with an order of magnitude higher densities on these than on small (5-10 cm) rocks. The floods, including one of bankful discharge, were collectively associated with a 10 % reduction in density. After the floods, caddisfly densities were similar and low on both stone size classes, suggesting disproportionate dislodgement from large than small stones. In the present study *Cheumatopsyche maculata* also preferred larger rocks, but they afforded this species a small relative refugium during floods - notwithstanding which the population declined by 77 % over the flood season.

At a community level, stones that moved or remained unmoved appeared to differ very little, and were not distinguishable using multivariate analysis. This result needs to be interpreted in the light of the different starting conditions on moved and unmoved stones: from the Baseline survey, it became clear that stones that were destined to move were generally smaller, but, more importantly, had higher invertebrate densities than those that were destined to remain stationary over the flood period. The relatively greater reduction of densities on moved stones thus had the effect of cancelling out this initial starting difference, ensuring that the distribution of taxa after floods was generally more homogenous across different stone size classes. The failure of the multivariate analysis to discriminate between assemblages on moved and non-moved stones after the floods is probably due to this effect. It is noteworthy, however, that a number of taxa virtually only survived floods because of the relative refugium of unmoved stones - viz. Leptophlebiidae, most Trichoptera, and the mayfly *Afroptilum* spp. Similarly, *Lithogloea harrisoni*, which appears on the river bed at the start of winter, only increased in population density on unmoved stones.

Relationship between invertebrate density on unmoved stones and the distribution of forces over the stream bed

The concept that only a stone that moves represents some threshold constituting disturbance precludes, for example, the potentially destructive forces acting on large stable stones caused by extremely high shear velocities, but which are not competent to move the stone because of its weight. Such stones may provide refugium for organisms that can withstand extreme shear forces, but create conditions for the erosion of more mobile or fragile taxa, or their retreat into other habitats.

Bond & Downes (2000) provided evidence that hydraulic forces acting on even fixed stones may play as great a role in disturbance as stone movement, since densities of the species they examined were not different on fixed or movable stones. The results of this study agree, since stone movement was not necessary in the series of small non-bed moving spates in the Molenaars River to cause a reduction in invertebrate populations, and in the Berg River there was not a great deal of difference in densities of many taxa on moved and unmoved stones. This contrasts with Cobb *et al.* (1992) who found that insect densities started to decline with about 10 % displacement of stones.

Laboratory methods have been used to examine the responses of invertebrates to increasing flow forces (Lancaster 1999), but no field experiments have attempted to quantify this relationship. In this study, the attempt to find a relationship between invertebrate density and the distribution of applied stream power on unmoved river stones during a flood was not easy, and marred by a number of limitations. The chief one of these was that it is not possible to measure invertebrate density on a single stone both before and after a flood, as the measurement of the first will denude the stone of invertebrates, precluding further data collection. Thus the study could only examine the relationship between applied power and post-flood densities, not the change in density over the flood.

Even so, the results provide no substantial evidence that invertebrates change their distribution over the stream bed as a result of increasing flow forces, at least not in terms of relocating to stones with lower hydraulic stress during peak flood discharge. This may be in part explained by smaller scale, behavioural changes in distribution that could take place over the course of a flood, for example a retreat from exposed surfaces to the undersides of stones. Movement by individuals along a hydraulic gradient in response to increases in flow have been shown, for example, by Lancaster (1999) in her laboratory study. Also, underwater observations of *Elporia* spp. (Blephariceridae) movement in the Molenaars River during the ascending limb of one of the floods showed individuals on the uppermost surfaces to be progressively relocating downwards towards the bed, which might have been in response to the increased flows (pers. obs.). Such movement would mean that animals never experience or have to respond to the shear forces associated with maximum discharge, as would have been be measured or calculated at the top of the stone. Redistribution of animals immediately after a flood may also have confounded the effects of applied power on density distributions.

In conclusion, this preliminary field investigation of invertebrate responses to floods indicates:

- Increased hydraulic forces associated with even small spates, but where bed movement does not occur, effect a degree of reduction in invertebrate population densities, which cumulatively alter population dynamics over the winter season, although there are large differences in the extent to which species perceive small floods as disturbance.
- Large floods that include bed movement are associated with greater reductions in population and affect all stream invertebrates.
- Movement of the substratum is not a threshold for disturbance responses, since almost all taxa were reduced on unmoved as well as moved stones.
- The stability of stones over the course of a flood provides a relative refugium for many taxa, with lower population losses on these stones than on stones which move. However, there is a wide difference in the relative refugium afforded to different taxa, with unmoved stones offering no advantage to some species.
- Resistance to floods at a reach level is considerable, indicated by a 42 % survival of invertebrates after two floods that moved between 25 and 43 % of the river stones, including all stone size classes.
- Resistance to floods may be exaggerated by the immigration of new individuals of some species, in the form of new recruits of small instars from the hyporheos or upstream reaches, which would constitute a resilience response to floods in these taxa.
- No direct relationship was found between invertebrate densities on stones after a flood and the distribution of maximum forces acting on the stones during the flood, measured as unit applied stream power, possibly as a result of micro-scale movements of invertebrate within or on the same substratum particle.

These results add to the current understanding of how floods may affect ecological processes regarding species interactions, since they clearly demonstrate substantial differences in the disturbance response between different and potentially competing species. The preliminary species-specific results may aid in setting flow requirements in rivers, during Environmental Water Requirements studies, particularly where there is evidence of species interactions, or where pest species (Chironomidae, Simuliidae) may proliferate under regimes of little disturbance.

However, the observational data effectively represent only three replicates in what might be a very variable set of responses to floods, dependent on not only the specifics of the flood hydrograph (peak and duration), but the timing with the season, seasonal and inter-annual differences in initial population densities, and more. These need to be investigated to substantiate these results.

The successful outcome of the hydraulic model developed to describe incipient motion, in terms of its ability to indicate the movement status of previously unmarked river stones, raises the potential for simpler studies on flood responses to be undertaken in the future, free from the complicating error effects of repeat-sampled stones. This will enable large sample sizes to be processed, with more robust results.

Finally, in relation to quantifying disturbance, it seems apparent that, whilst increased flow forces provide one type of disturbance for stream biota, a qualitatively different level of disturbance may occur with the actual displacement of a bed particle. The spatial distribution of hydraulic forces acting on the substratum is likely to be different from the distribution of dislodged stones, so that both may need to be mapped and quantified independently in studies of disturbance.

5 QUANTIFYING HYDROLOGICAL DISTURBANCE IN THE MOLENAARS AND BERG RIVERS

5.1 INTRODUCTION

In Chapter 4 flood disturbance was measured in the Molenaars and Berg Rivers, both in terms of hydraulic forces acting on the stream bed as well as the response of the invertebrate community. These showed that small floods, without bed movement, were linked to variable responses, from minor reductions in population density of some species to increases in others. Larger floods, but those that still occurred at within-year return frequency, were associated with patchy bed movement and a reduction in almost all species' densities, but to dramatically varying degrees. In this chapter a threshold for flood disturbance that could be considered to be of evolutionary significance is proposed, based on the results of Chapter 4. Following this is an examination of the regime characteristics of flood events greater than this disturbance threshold, using the long-term flow record of the Molenaars and Berg Rivers, systems typical of Western Cape rivers.

Variability in stream flow has long been recognised as an ecologically relevant basis for describing or categorising rivers (e.g. Horwitz 1978), with the expectation that this can serve as a basis for predicting biological pattern and process. Variability in mean annual runoff has been described by Davies *et al.* (1995) as the overriding feature of southern African rivers.

Poff & Ward (1989) and Poff (1996) used 11 hydrological indices in their hydrological classification of USA rivers. They concluded that variables could be ranked according to their importance in defining a stream's "hydrological template" (*sensu* Southwood 1977, 1988). In their study the degree of intermittency was profoundly the major distinguisher between stream types. Flood frequency[1] and flood predictability[2], were next most important and provided the axes of their conceptual model of stream classification for perennial streams. Joubert and Hurly (1994) used the same hydrological indices in a classification of South African rivers. In their study, the rivers of the Western Cape were defined by a low overall predictability of flow (Colwell's index), with a relatively higher seasonal predictability of flow and a relatively low flood frequency, compared with other hydrological groupings.

Lytle (2001) differentiated between fine- and coarse-grained disturbances, which he defined respectively as those that affect only a portion of the population (in patches) or the whole population simultaneously. Coarse-grained disturbances are generally considered to be bankful and larger floods, with return periods in excess of a year, which are considered to move the bed substrata most efficiently (e.g. Poff & Ward 1989; Joubert & Hurly 1994; Poff 1996; Townsend *et al.* 1997b; Lytle & Poff 2004). Almost all studies using stream flow statistics to examine the role of the disturbance regime in structuring ecosystems have used coarse-grained disturbance levels as the basis for indices used for analysis of flood frequency.

Whilst even very small floods will have some ecological impact, as was shown in this study in Chapters 3 and 4, floods that occur at a frequency that is relevant to the life span of the species under investigation are likely to have evolutionary significance, for example in the evolution of species traits. A significant problem with the threshold for disturbance used in the stream flow analyses referred to above is that such

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[1] represented by the number of floods per annum and / or the median number of days between floods, where floods are determined as events of bankful or higher magnitude (1- to 2-year return interval floods)

[2] represented by both the maximum proportion of the total number of floods over the record occurring in any common 60-day period over all years in the record, and the maximum number of days in a year during which no flood occurred, common to all years

coarse-grained disturbances may not occur at a frequency relevant to the life-cycle attributes of the constituent species, particularly in the case of stream invertebrates. Over long time scales, organisms with an annual or shorter life cycle will have many generations that remain unaffected by floods that occur on average every 1.5 to 2 years or more. In relation to such organisms' life cycles, these are rare disturbances, even if they are severe, and should have little consequence for evolutionary adaptation. On the other hand, fine-grained disturbances, which are generally smaller in magnitude or severity, may be more in tune with species with short life cycles, but may be too weak a signal to elicit an evolutionary response. Successive fine-grained disturbances within a disturbance cycle (e.g. during the flood season), however, are common features of flow regimes. It is contended that the frequency and particularly the predictability in a disturbance cycle of fine-grained disturbances of sufficient magnitude, i.e. above a given threshold may act as a stronger determinant of species life cycle and behavioural adaptations, simply because a) they are of a magnitude that causes population reduction (i.e. they are disturbances) and b) they are simply more likely to affect the biota than larger but infrequent events.

The results of the previous chapters established a threshold for fine-grained (*sensu* Lytle 2001) flood disturbance in the Molenaars and Berg Rivers, based on the measurement of bed particle movement as a function of flood magnitude, and through measuring a concomitant response by invertebrate species and species assemblages. This is arguably a sounder basis for defining a disturbance threshold since it is based on a physical force rather than a hydrological statistic (see section 1.3, Chapter 1). Indeed, Clausen & Biggs (1997) indicated that in their streams the 3x median flow was associated with up to 40 % bed movement, which would then provide a physical basis for the use of this disturbance threshold.

The aim of this chapter is to describe the frequency and predictability of hydrological disturbance in the Molenaars and upper Berg Rivers as a characteristic of the natural flow regime, based on the ecologically-defined thresholds of disturbance that were quantified in Chapters 3 and 4. Medium- to long-term flow records are available for both rivers, from which a time series of flows equivalent to the threshold disturbance events was extracted.

Most studies of flow regimes have sought to classify like rivers into hydrological groups using flow variables or indices and / or to examine whether species assemblages within these groups confirm the biological relevance of the hydrological groupings. Also, the studies have examined variability of the total flow regime, i.e. of both low flow and high flow extreme events, and of general flow variability. In this study, hypotheses were formulated specifically about the nature of the flood regime itself, rather than other aspects of flow variability. The analysis has attempted to identify the ecological and evolutionary signals in this regime that might be responsible for adaptations displayed by the invertebrate fauna of these rivers, in relation chiefly to physical (e.g. mobility) and life-history attributes. As such, the focus of the analysis is on the within-year pattern of flood-flow variation, but includes an examination of the temporal stability of this within-year pattern.

Given their location in the winter rainfall limnological region of the Western Cape Province, the study rivers would be expected to show strong seasonal patterns in the occurrence and frequency of flooding (following Joubert & Hurley 1994 and Davies *et al.* 1995), with very few, or no, floods above the disturbance threshold expected to occur during the dry summer months, and with flood frequency (number per unit time) increasing to a maximum in the winter months (June – August). With regard to the concept that these systems are "predictably unpredictable" (*sensu* Davies *et al.* 1995), it is hypothesised that the low frequency of floods expected during the dry season carries a high predictability, whilst the timing and frequency of floods within the wet season should be unpredictable, or have a lower predictability. In other words, there is the expectation that despite a distinct wet season, there will be substantial variation in whether or not there are floods in any one month of the wet season. The number of floods would be expected to vary substantially from month to month during winter and, for each month, will vary year on year over the historical record. The analysis also attempted to identify the most predictable month or period of flooding, which could be an important driver of life-cycle patterns for many invertebrate species.

5.2 METHODS

5.2.1 Data sources

All hydrological analyses were based on the historical daily flow records for the gauges H1H018 on the Molenaars River and G1H004 on the Berg River, obtained from the Department of Water Affairs and Forestry hydrological database. The flow gauge for the Berg River, G1H004, was located some 4 km downstream of the flood study site. The gauge on the Molenaars River, H1H018, was located in the middle of the study reach, and some 5 km downstream of the flood study site. The Molenaars River data are considered to be accurate (King *et al*. 2004) and the full existing record from March 1969 until June 2007 was included in the analysis. The Berg River data are considered to be inaccurate for floods prior to 1980 (Howard 2004; see section 3.2.1*iv* of this thesis), and thus the record from April 1980 to April 2007 was used in the analysis. In the Berg River irrigation releases from an inter-basin transfer scheme are made during summer months and thus lowflows during these periods are not natural.

There were some missing data in both flow records, although these represented only 1.2 % of values for the Molenaars River and 2.5 % of values for the Berg River. The missing data were in the years common to both rivers, but the data were never missing from both flow records for the same period. All missing data were during winter months. A regression was performed on the daily discharge during the winter periods for the Berg and Molenaars Rivers. The strong linear relationship ($R^2 = 0.926$) allowed for extrapolation of the flows from one river to the other. Although a more correct patching of the record would have required disaggregation of monthly rainfall, this approach was considered to be an acceptable estimate given the high coefficient of determination of the regression equation and the small proportion of missing values. The patched data included 10 small floods ≤ 1:1 year return period for the Berg River data and one small flood for the Molenaars River data.

5.2.2 Statistical analyses

Analysis of the flow regime was conducted using Aquapak Version 1.05 2007 (Gordon *et al.* 2004), a general-purpose program that can be used for the processing of time-series data, and STATISTICA Version 7 2007 (Statsoft Inc. U.S.A.). Summary statistics were calculated to describe the temporal pattern of flow that comprises the overall flow regime, including the average daily discharge and Coefficient of Variation (CV) for annual daily average discharge values, where:

$$\text{CV}= \frac{\textit{Variance in daily flow}}{\textit{Mean daily flow}}$$

The use of daily flow in calculating the CV consistently returns higher values than it would using annual flow <u>volume</u> data, often used in hydrological studies to compare the CV of the mean annual runoff (e.g. Poff 1996), but is considered here to be more reflective of real variation at a more appropriate ecological scale. The CV can be used to describe either intra-annual variation in flow or inter-annual variation in flow. The former refers to the CV of daily discharge calculated for each individual year on record; the latter refers to overall variability in daily discharge over the full record. The choice of which time scale to use depends on the question being asked. The CV does not allow for consideration of the temporal sequence of flow or the predictability of changes in flow. From the perspective of this study, the important patterns in flow variability are those associated with the inter-annual variability of flows for different time periods, namely calendar months and seasons. In this context, an important question would be whether flow variation at different times of the year would influence species' survival and thus the evolution of life history or other adaptations, for example in summer, winter or transitional months. The CV of daily flows was therefore also calculated for each calendar month, based on the full data record, in order to describe the inter-annual variability of conditions pertaining at different times of the year, which may determine longer-term responses by stream biota.

5.2.3 Partial-duration flood series

The analysis of flood patterns was undertaken using Aquapak. A partial-duration series technique for analysing flood frequency is used to describe the characteristics of smaller, non-extreme flood events, as opposed to the annual maximum series, which tends to underestimate smaller return-period flood statistics (Gordon *et al.* 2004). The partial-duration flood-frequency analysis routine in Aquapak allows the extraction of a partial series of independent flood peaks above a selected threshold and fits a frequency distribution to the extracted series, thus providing flood-frequency estimates corresponding to a range of average recurrence intervals. This threshold for selecting floods can be arbitrarily set, depending on which flow levels are important to an analysis. For calculating recurrence intervals of one year or less, this threshold is commonly set such that on average 3N to 4N floods are included in the resultant time series, where N is equal to the number of years on record (Gordon *et al.* 2004). Such a step is automated in the Aquapak software.

For this study, the above rule of thumb was not adopted, because the threshold that would be set in this way was substantially higher than floods that have been observed to cause significant bed movement, and thus would underestimate the total number of these floods deemed over the record. Since the latter were the flows of interest, the threshold for defining or selecting floods was based rather on establishing a minimum value constituting a flood disturbance. This minimum value was taken from the flood-invertebrate response study described in Chapter 4. Not all flood events in the hydrological record would constitute a disturbance, according to the definitions in Chapter 1. For example, the first four floods measured in the Molenaars River in 2003 as part of the flood disturbance study (Table 5.1) were associated with less than 3 % of bed movement and elicited no measurable change in invertebrate assemblages (see Chapter 4), whilst the same applies for floods with a maximum average daily flow below 6 $m^3 s^{-1}$ in the Berg River (Table 5.2)[3].

On the other hand, floods that were associated with 26 – 40 % bed movement in the Berg River were associated with a significant decline in invertebrate densities for almost all taxa, and this threshold was thus considered to fall safely within the definition of a disturbance as an opening up of space for recolonisation by other individuals. The discharge associated with this level of bed movement – an average of 33 % - was therefore used to represent a threshold for disturbance. This level of bed movement

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[3] The flood peak flows reported upon in Chapter 4 were scaled to the average daily flow at the DWAF gauging weir on each river, so the threshold values for floods constituting disturbance in Tables 5.1 and 5.2 relate to flows at the respective weirs.

occurred at a discharge above 18 $m^3 s^{-1}$ in the Berg River and at a discharge above 30 $m^3 s^{-1}$ in the Molenaars River.

The partial-duration time-series analysis also requires stipulation of the minimum interval, as the number of days, between floods peaks and requires that the flow declines to some minimum threshold, defined as a percentage of the smaller peak, in order to identify independent events. For the Aquapak analysis, these parameters were manually set at three days for the interval between peaks and 65 % of the peak flow of the smaller flood, for the value which should be reached or exceeded between two independent flood events for both of them to be selected. Where contiguous flow peaks did not meet these criteria, only one event was deemed to have occurred, with the peak equal to the highest flow over that period. An example is provided in Figure 5.1, where independent flood events are marked with a dot.

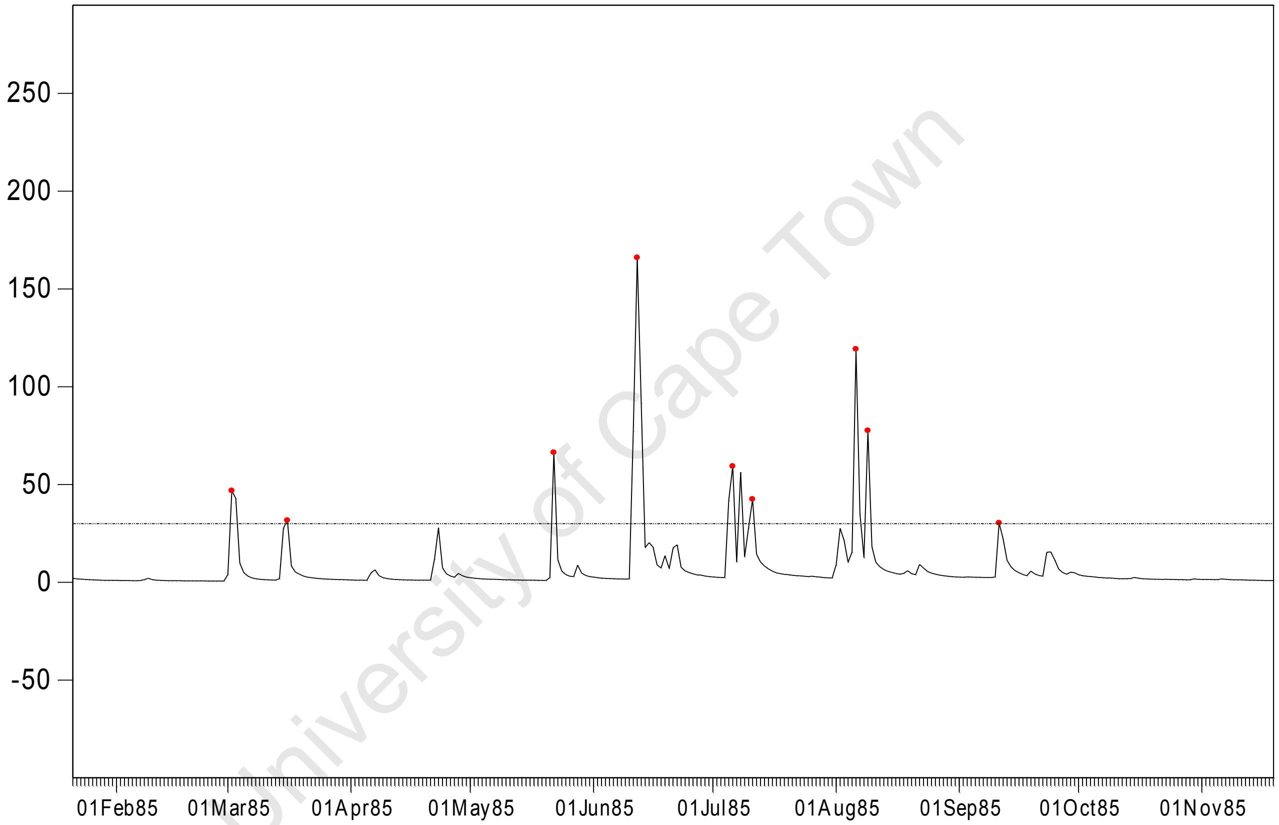

Figure 5.1 **Selection of independent flood events for the partial series analysis of floods, using Aquapak. Floods indicated with a dot were selected, according to the criteria described in the text. The dashed horizontal line represents the 30 $m^3 s^{-1}$ threshold chosen to represent disturbance for invertebrates in the Molenaars River (hydrological data sourced from DWAF database, gauge H1H018).**

5.2.4 Analysis of flood disturbance

The application of the above thresholds resulted in a flood series for each river, deemed to represent flood disturbances. The magnitudes of the individual events were not considered for this part of the analysis, given that they were all greater than the threshold deemed to cause population losses in invertebrates and algal standing stock. Rather, the frequency of disturbance has been demonstrated to be of more consequence in describing the disturbance regime (e.g. Poff & Ward 1989; Clausen & Biggs 1997, 2000).

The average flood frequency per calendar month and per year provides a measure of central tendency regarding the disturbance regime. However, perhaps more ecologically significant is the variation in flood disturbance, which was described graphically and statistically in terms of the frequency occurrence by month and according to year, to reflect on the within-season (monthly) and inter-annual temporal variability in the timing and frequency of floods.

5.2.5 Predictability of floods

Colwell (1974) defined three measures to describe fluctuations in physical or biological states over time, for cyclical data, based on information theory. The frequency of occurrence of each state, for example a particular quantity of discharge, for each time period is described in a state-by-time matrix. Predictability (P), which has a range of 0 – 1, describes the relative certainty of being able to identify the state of a system at a given time. Predictability is separated into a measure of constancy (C) and of contingency (M), where P = C+M. Constancy is a measure of temporal invariance – i.e. it is maximised where there is little variation in flow state over time. The contingency measure reflects how closely a shift in state corresponds to a particular time category, in other words describes the periodicity of, for example, flow states. A stream that has a fluctuating daily discharge could still have a high overall predictability if it had repetitive patterns of change in discharge. In this case the constancy component of predictability would be low, but the contingency component would account for high predictability.

Predictability values are sensitive to the actual categories of states, for example the number of flow categories state-by-time matrix. As a result, these should be in the based on physically meaningful thresholds, rather than being arbitrarily defined (Gordon *et al.* 2004). For example, Cobb *et al.* (1992) calculated predictability differences between sites based on the occurrence of a daily flow great enough to move at least 10% of the substratum, which was the threshold at which they recorded decreases in invertebrate abundances. These were better at differentiating between sites on the basis of their disturbance regime than arbitrarily defined flow classes. Wishart (1998) also calculated predictability indices based on flow categories linked to physical, flow-related disturbance. He identified three flow categories in his analysis – no flow, flow insufficient to move substrata and flow that moves sediment.

Poff (1996) showed that this index is also sensitive to temporal resolution in the hydrological time series i.e. the daily, monthly or seasonal window used for the analysis, and argued that inter-stream comparisons may only be valid where the same methods are used to calculate the index.

In this thesis study, what is of interest is not especially the predictability of the flow regime per se (e.g. in comparison to other rivers elsewhere), but rather the temporal variation in predictability of the disturbance regime, since this is posited to explain seasonal patterns in species' life cycles. For Colwell's indices, the data need to be assigned as relative frequency of occurrence to a set of states – in this case classes reflecting the magnitude of disturbance. Since all floods used for the analysis were large enough to cause population reductions, the flow states used for the predictability index were based on the number of floods per time interval (month), in other words, assuming that zero, one, two or more floods per month represented increasing degrees or magnitudes of disturbance. Thus the state classes were defined as 0, 1, 2, 3, 4, or 5 floods in a month.

A further index of predictability in the studies of Poff & Ward (1989), Joubert & Hurly (1994) and Poff (1996) considered the *predictability of flooding* (FLDPRED, dimensionless) to be represented by the maximum proportion of the total number of floods over the record occurring in any common 60-day window. In this study, the 60-day windows were calculated for six contiguous intervals, roughly equating to January / February, March / April, May / June and so on, but also at a daily time step, in order to identify the most predictable flood period, not tied to calendar month categories. The value of FLDPRED indicates how reliably – if at all – a flood disturbance period might be anticipated, and hence act as an evolutionary force in terms of species' life-cycle adaptations.

Finally, the probabilities of at least one flood, and of two or more floods, occurring in each calendar month were calculated from their percentage representation in the flow record.

5.3 RESULTS

5.3.1 General characteristics of the flow regime

The Molenaars River represented by H1H018 has a present-day Mean Annual Runoff (MAR) of 164 Mm^3 that is unaltered from the natural state and displays strong seasonality of low summer flows, with the occurrence of floods in a distinct wet winter season, a typical pattern for rivers in the winter rainfall region of the Western Cape. The mean and median annual discharge is 5.2 and 1.6 $m\ s^{-1}$ respectively, based on average daily flow values. Summer base flows are typically under 0.8 $m\ s^{-1}$, estimated as the 50th percentile of the flows recorded from December to March over the full record, increasing to winter base flows of some 5.0 $m\ s^{-1}$, the 50th percentile of the flows recorded from June to August over the full record. Winter floods are frequent, short-lived but intense (Figure 5.2).

The Berg River at G1H004 has a natural MAR of 136 Mm^3. This has been substantially altered under present day conditions, mainly through augmentation of summer releases from an interbasin transfer (50 Mm^3 per annum), but also through abstraction of water from one of the headwater tributaries and forestry impacts, resulting in a present day MAR of about 149 Mm^3. The elevated flow in the Berg River during summer compared to the natural situation is obvious when comparing the patterns of flow in the two rivers (Figures 5.2 and 5.3), which should be similar and both typical of Western Cape rivers. Despite its lower MAR and mean annual discharge of 4.7 $m^3\ s^{-1}$, median annual discharge in the Berg River is 2.7 $m\ s^{-1}$, nearly double that of the Molenaars River. This is the result of the altered summer flow regime. Summer base flows in the Berg River, also estimated as the 50th percentile of the flows recorded from December to March over the full record, are much higher than in the Molenaars River, about 3.2 $m\ s^{-1}$. Base flows in the Berg River also remain practically unchanged from summer to winter, where the 50th percentile of the daily flows from June to August is 3.8 $m\ s^{-1}$.

The annual coefficient of variation (CV) in daily flows in the Molenaars River ranged from 166 % to 341 %, with an average of 233 %. These values in the Berg River were a range of 114 % to 209 %, with an average of 155 %. Based on the whole record, the inter-annual variability was reflected by a CV of 268 % and 152 % for the Molenaars and Berg Rivers respectively.

For this study, the inter-annual variability of flow in each calendar month was of interest. The CV of all daily discharge data for each month is presented for the Molenaars and Berg Rivers respectively in Figures 5.4 and 5.5. These show the mean and standard deviation of the daily discharge for each calendar month, whilst the secondary axis indicates the CV. It is noteworthy that the most variable months are April, May and November, December, although the Berg River has a very low CV (*ca*. 50 %) for November through March during which time the IBT is operational.

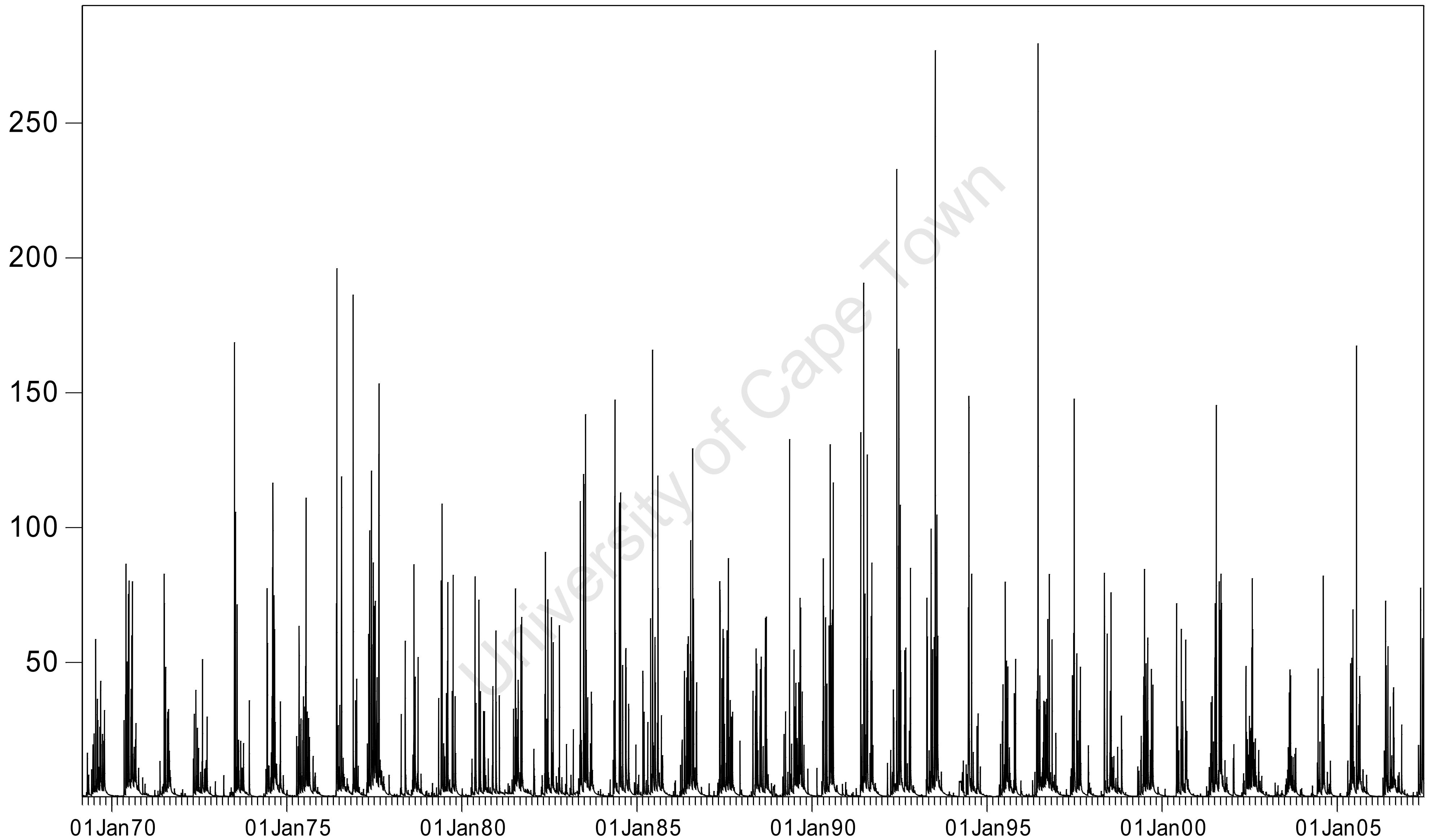

Figure 5.2. **Daily discharge time series for the Molenaars River (DWAF gauge H1H017), over the records from 1969 to 2007. Note the low summer base flows and infrequent floods (although flows were higher in the summer periods of 1981, 1983 and 1985).**

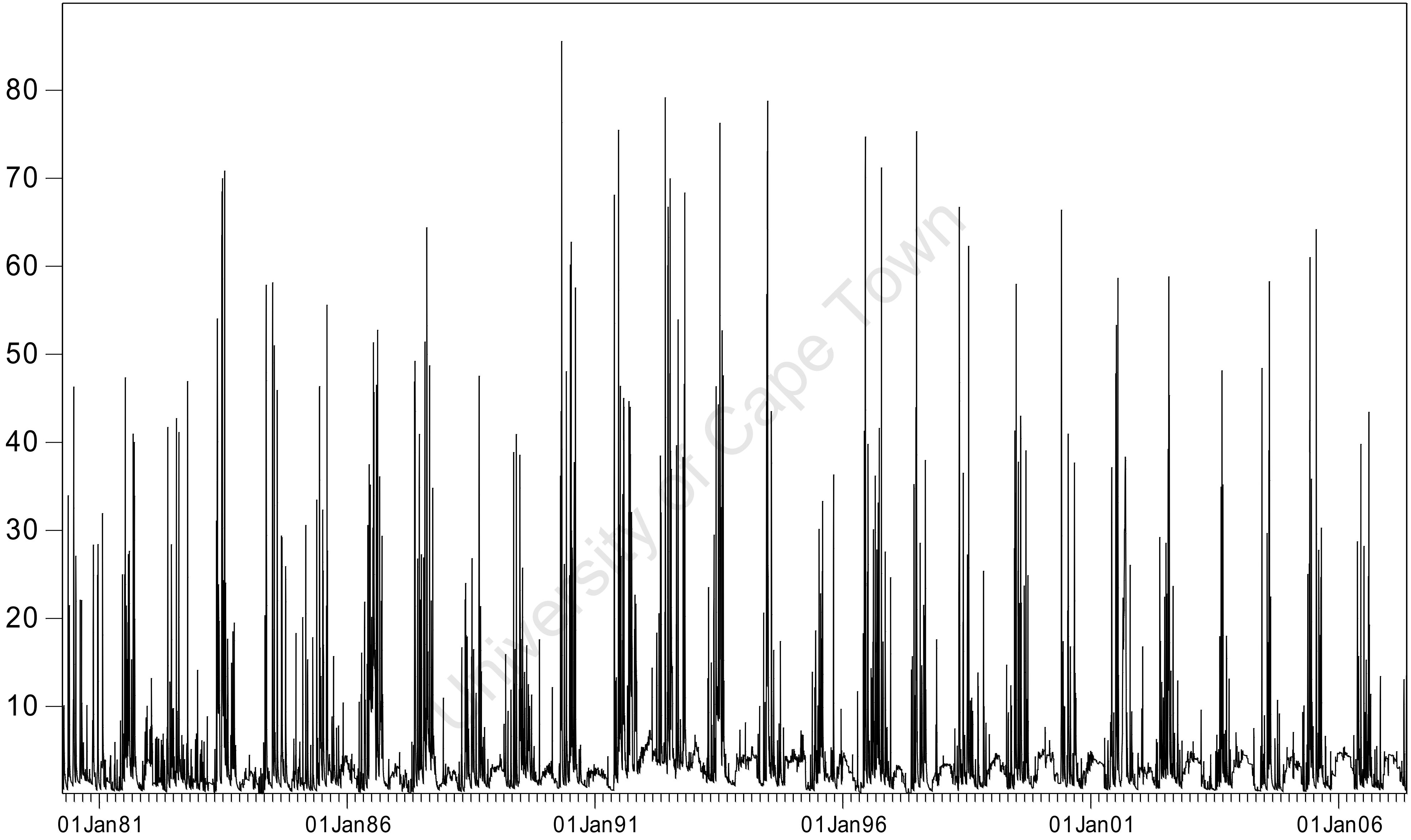

Figure 5.3. **Daily discharge time series for the Berg River (DWAF gauge G1H004), over the records from 1980 to 2006. Note the elevated summer flows as a result of interbasin transfer of water to supply irrigation needs.**

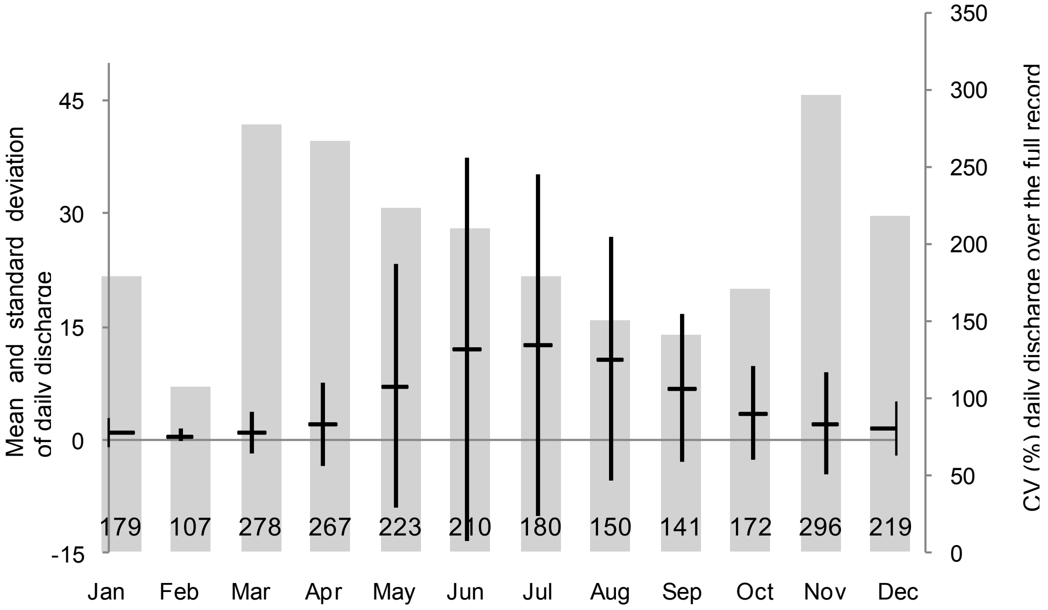

Figure 5.4. **Mean (cross bar) and standard deviation (vertical lines) daily flow over the full hydrological record for each calendar month, in the Molenaars River. The coefficient of variation of daily flow for each month is indicated in bars, and the actual percentage provided at the base of each bar.**

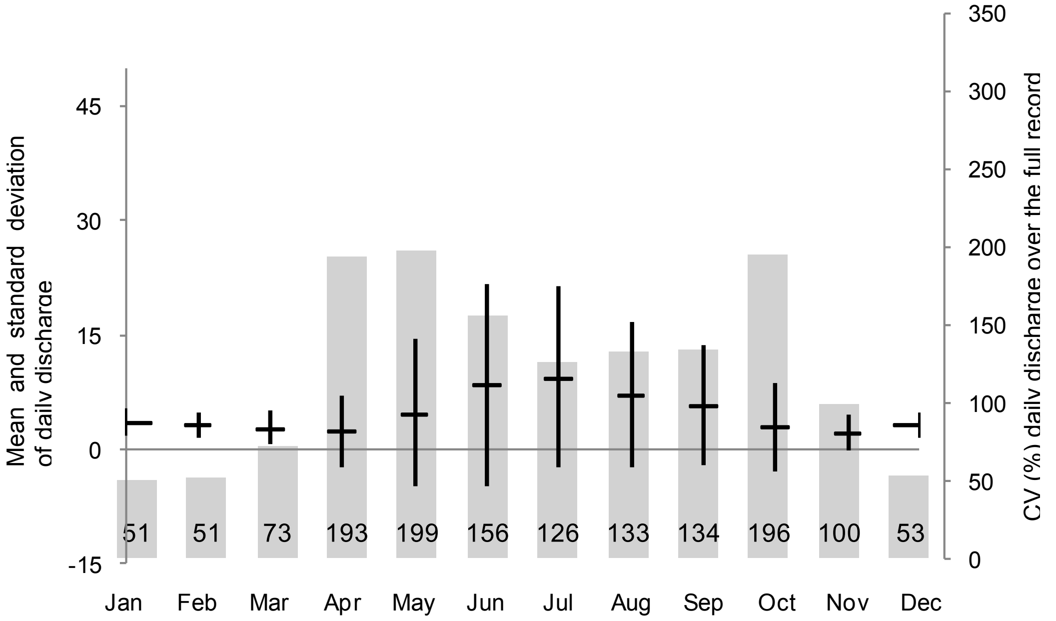

Figure 5.5. **Mean (cross bar) and standard deviation (vertical lines) daily flow over the full hydrological record for each calendar month, in the Berg River. The coefficient of variation of daily flow for each month is indicated in bars, and the actual percentage provided at the base of each bar.**

The CV, however, does not necessarily reflect variability as a result of floods that constitute disturbance per se. In order to examine the temporal aspect of flood disturbance itself, specific attention was focused on the patterns of flooding above the thresholds of 30 and 18 $m^3 s^{-1}$ in the Molenaars and Berg Rivers respectively.

5.3.2 Partial time series flood analysis

The different intra- and inter-annual return period floods derived from the partial series analysis of floods up to 2-year return period and the annual maximum series statistical analysis of floods with a 5- to 20-year return period for each river are presented in Table 5.1. It is noteworthy that the disturbance thresholds chosen for each river for this study, based on measurements of bedload movement and invertebrate response, are considerably smaller than the 0.24-year return period. That is, on average there will be more than four floods per year exceeding this threshold in both rivers.

Table 5.1. Results of the partial and annual maximum series analyses of flood return periods in the Molenaars and Berg Rivers.

Recurrence interval (yrs)	*Quantile: Molenaars River*	*Quantile: Berg River*
0.25	48.58	34.051
0.33	57.378	39.693
0.50	72.352	47.334
1.00	101.718	57.550
1.25	112.123	60.260
2.00	134.988	65.390
5	161.59	73.53
10	199.06	80.29
20	238.37	85.72
50	294.68	Record too short

5.3.3 Variability in flood frequency in the Molenaars and Berg Rivers

From the extracted flood sequences, the Molenaars River has an average (std. dev.) of 7.2 (2.6) floods per annum above the chosen threshold of 30 $m^3 s^{-1}$, with a coefficient of variation in flood frequency per annum of 37 %, whilst the Berg River has 8.3 (3.4) floods per annum above the threshold of 18 $m^3 s^{-1}$, with a slightly higher CV of 41 %. The Molenaars River data show a range between 3 and 15 flood disturbance events per annum whilst in the Berg River this is between 4 and 15 events (Figure 5.6).

The frequency of disturbance floods per calendar month for each year of the hydrological record is shown in a composite graph for the Molenaars River in Figure 5.7 and for the Berg River in Figure 5.8. The flood frequency is indicated on the vertical axis in each graph – up to five floods per month in the Molenaars River whilst in the Berg River there was a maximum frequency within any single month of four floods.

These composite graphs demonstrate graphically the substantial within-year variation in both the monthly *frequency*, as well as the *timing* of these floods, summarised in Tables 5.2 and 5.3. These show that the highest variability (CV) in monthly flood frequency occurs in the months that have an extremely low incidence of flooding, viz. November through March / April. The most consistent month in both rivers is July, which also experiences the highest mean flood frequency in both rivers. The frequency per unit time (in this case month) of floods over a disturbance threshold may be regarded as a measure of the disturbance intensity (cf. Townsend *et al.* 1987b). The inter-annual variation in monthly flood frequency provides the temporal dimension to this disturbance that determines the predictability of the disturbance regime.

5.3.4 Predictability of the disturbance regime

Colwell’s measures of predictability are based on the relative frequency at which the environment is in one of a number of specified states. In this study, the extent of disturbance is considered to be represented by flood frequency per unit time (month), and the frequency occurrence of these states is presented in Table 5.4 for the Molenaars River. The predictability, and its sub-measures constancy and contingency, of the disturbance regime defined in this way are presented in Table 5.5.

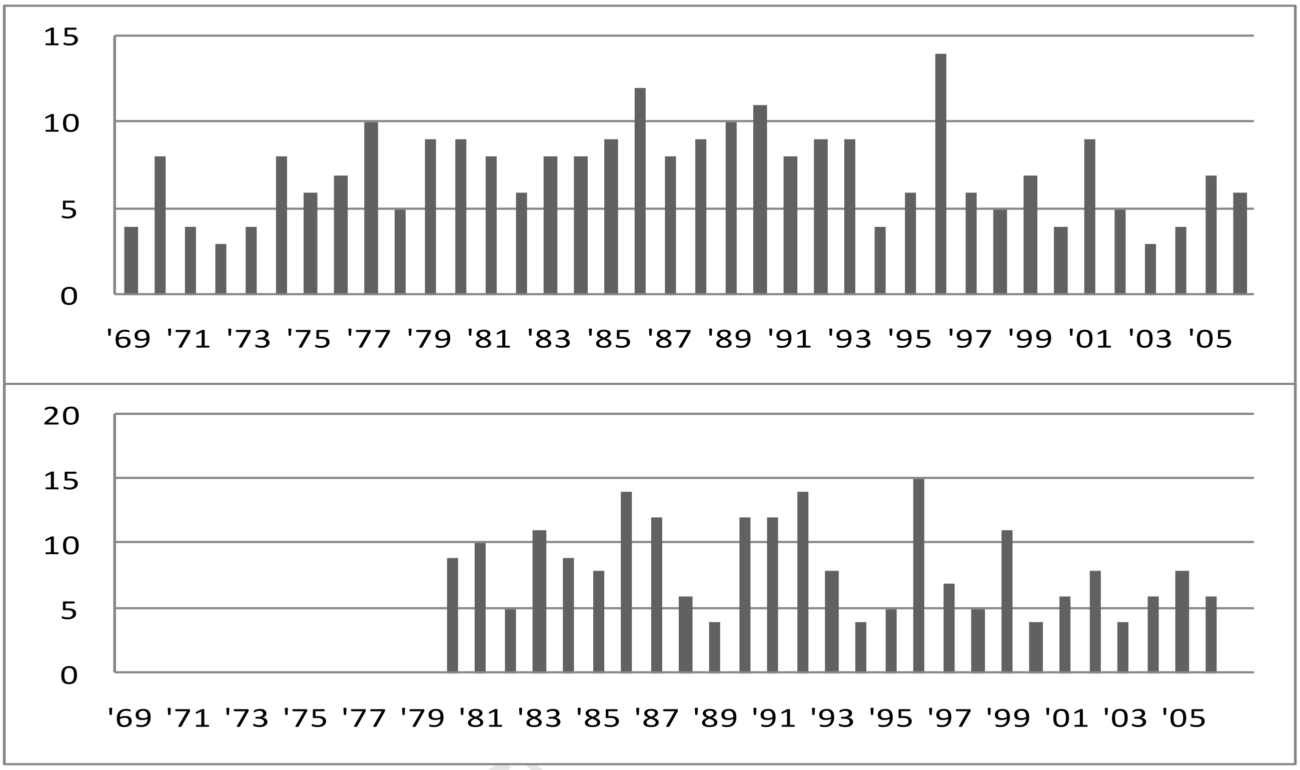

Figure 5.6. Inter-annual variation in the frequency occurrence of floods over the threshold (30 $m^3 s^{-1}$ in the Molenaars River and 18 $m^3 s^{-1}$ in the Berg River) for each year on record.

Table 5.2. Summary statistics for monthly disturbance flood frequency (FF) in the Molenaars River.

Statistic	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
Mean FF	0.03	0.00	0.08	0.18	0.92	1.41	1.74	1.42	0.84	0.29	0.11	0.13
Std. dev	0.16	0.00	0.35	0.51	0.81	1.09	0.98	0.92	1.03	0.61	0.31	0.47
CV (%)	616		461	282	87	78	56	65	122	211	295	361

Table 5.3. Summary statistics for monthly disturbance flood frequency (FF) in the Berg River.

Statistic	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
Mean FF	0.04	0.04	0.07	0.18	1.04	1.59	2.07	1.67	0.89	0.41	0.11	0.15
Std. dev	0.20	0.20	0.27	0.55	0.85	1.08	1.17	1.36	1.12	0.89	0.32	0.46
CV (%)	530	530	367	307	82	68	57	82	126	218	288	308

In Table 5.4, constancy is lowest when the row totals are equal, that is, when all states are equally possible. Contingency is lowest when the probability of occurrence of each state is independent of the month. The results indicate that the flood disturbance regime in the Molenaars River has a predictability of 62 % (0.62, Table 5.5), made up of contributions of both constancy and contingency, describing the dominance of a single state (zero or one flood), but also the fact that the states of one or more floods are strongly contingent on time of year, occurring generally in the wet season months. To answer how predictable the flood regime is <u>within</u> the "predictable" winter season (sensu Davies *et al.* (1995)'s description of

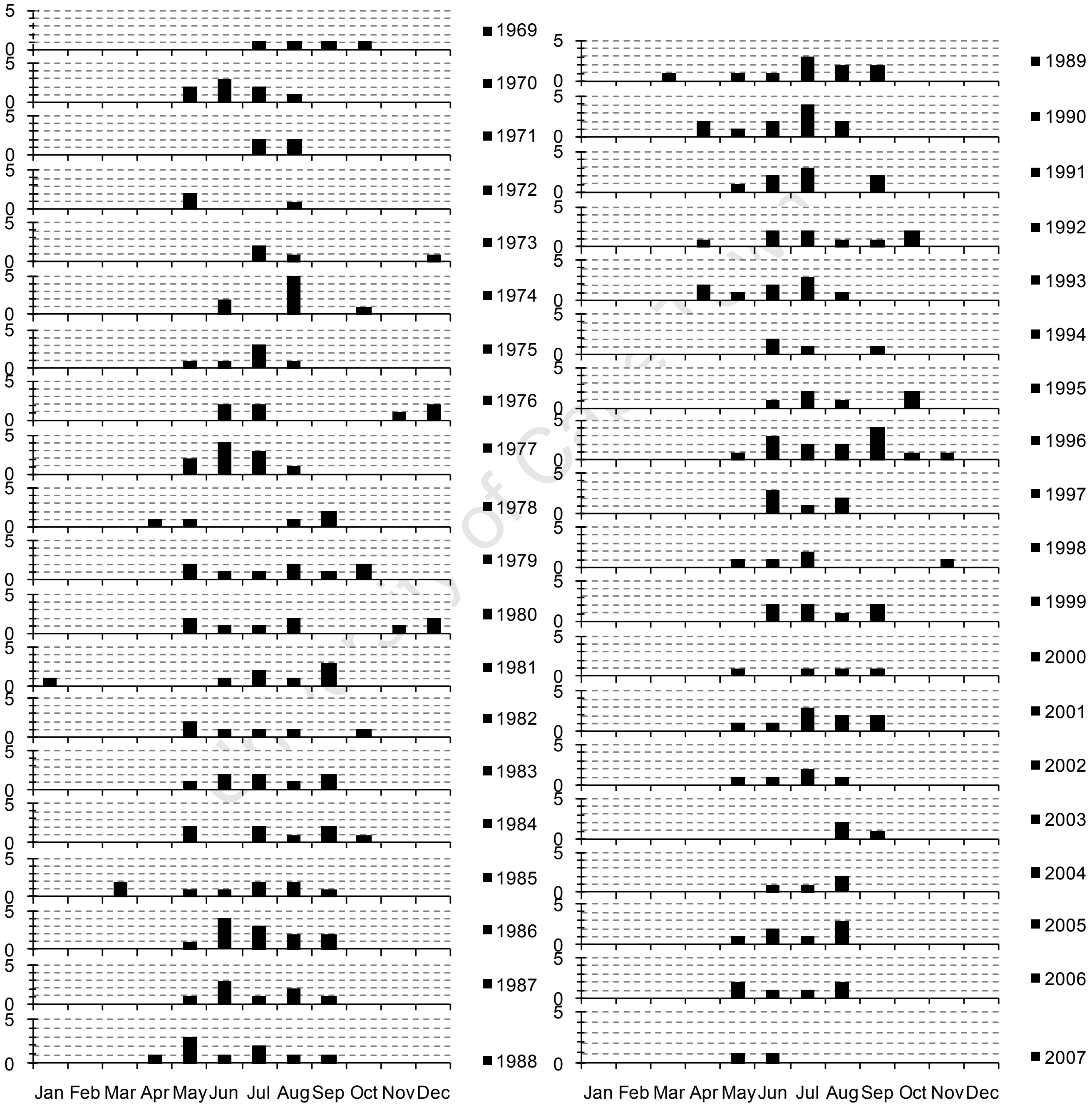

Figure 5.7. Frequency of floods over 30 m^3 s^{-1} in the Molenaars River for each calendar month and for the hydrological record from 1969 to 2007, demonstrating intra-annual and inter-annual variability in the flood regime. Note the record includes only January – June 2007. A maximum flood frequency of five floods per month was recorded in the Molenaars River only once (August 1974).

predictably unpredictable rivers), the indices were separated into a wet season: May – September (close to or greater than one flood per month on average – see Table 5.2) and a dry season (October – April). The disturbance regime is predictable during the latter (P=0.85), because of the high Constancy value (C = 0.82) resulting from the fact that the absence of floods is the dominant state. Within the winter wet season, Predictability is considerably lower (P = 0.29). Of importance is the fact that contingency is also very low, indicating that all states occur in all months during winter (Table 5.5).

The Berg River results (Tables 5.6 and 5.7) indicate a less predictable flood disturbance regime (P = 0.52) than the Molenaars River, but the contributions of constancy and contingency are similar to that in the latter. As with the Molenaars River, wet and dry season predictability values are considerably different, with flooding in the Berg River being even less predictable than in the Molenaars River (P of only 0.17),with both constancy and contingency at a minimum (Table 5.8).

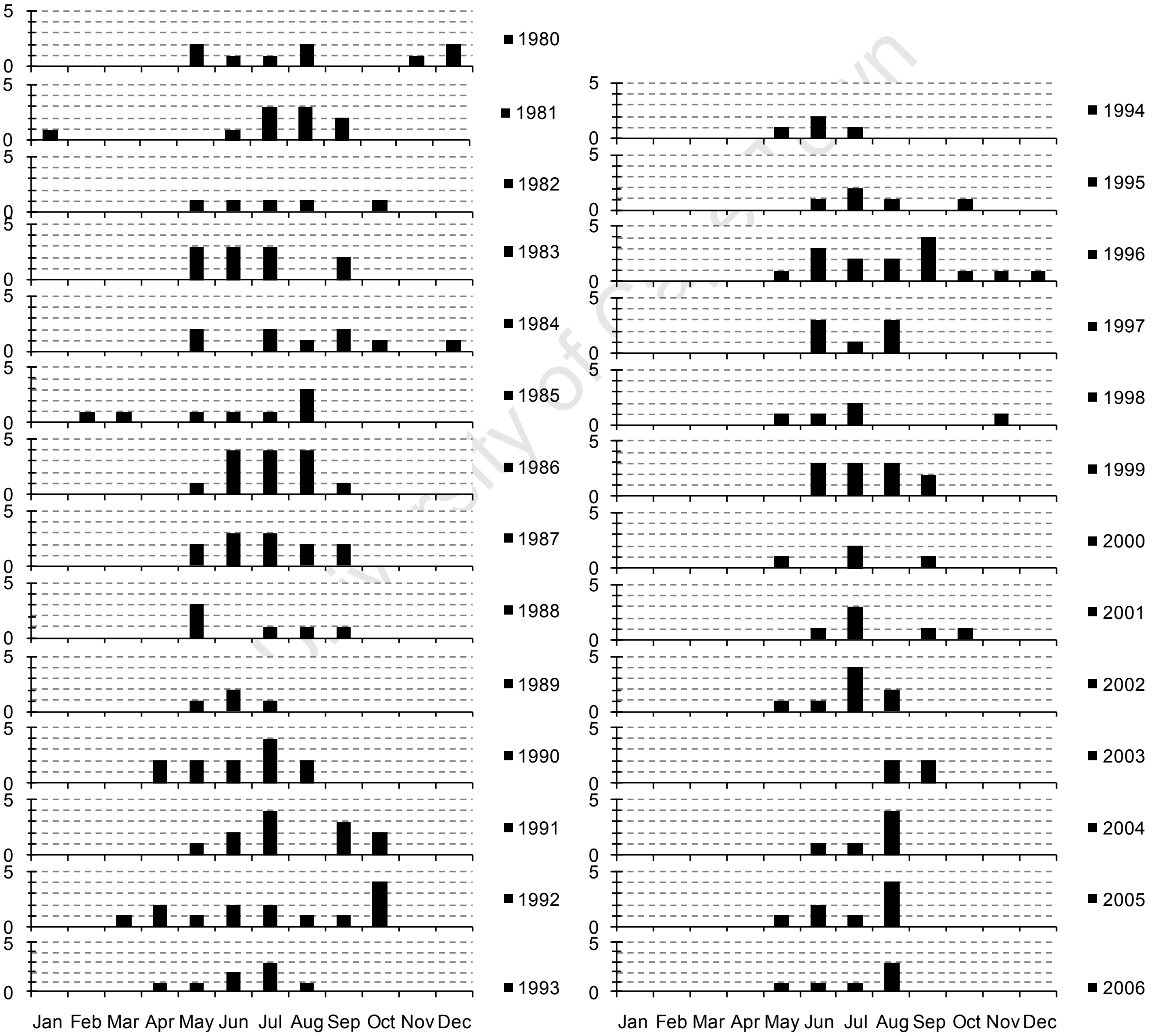

Figure 5.8. **Frequency of floods over 18 $m^3 s^{-1}$ in the Berg River for each calendar month and for the hydrological record from 1969 to 2007, demonstrating intra-annual and inter-annual variability in the flood regime. Note the record includes only January – June 2007. A maximum flood frequency of four floods per month was recorded in the Berg River.**

A further measure of flood predictability is the maximum proportion of all floods on record occurring within any common 60-day window, which was calculated for consecutive 60-day periods, at a time step of one day. The maximum proportion of floods in any window (FLDPRED) was 48 % for both the Molenaars and Berg Rivers. In the Molenaars River, this maximum of 48 % translates into a maximum of 3.4 floods per 60-day window (Figure 5.9a), whilst in the Berg River 48 % of the floods is equivalent to 4 floods per 60-day period as a maximum flood occurrence. In the Molenaars River the 60-day windows with the maximum flood occurrence (maximum proportion of floods) were represented by the periods 11 June to 9 August and 20 June to 18 August, which then may be seen as the two periods most likely to have floods (two peaks in Figure 5.9a). However, peak FLDPRED values between 0.46 – 0.48 were found for all 60-day periods starting between 5 and 20 June, and extending to between 3 and 18 August, equating

Table 5.4. **Number of times in the hydrological record (1969 – 2007) during which the Molenaars River experienced different levels of flood disturbance (flood frequencies in categories of 0 to 5 per month), for each calendar month. This was the basis for calculating Colwell's measures of predictability. The winter flood season months, based on average flood frequency, are shaded.**

Disturbance states	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
no floods	37	38	36	33	12	7	4	4	19	30	34	35
1 flood	1	0	1	3	17	15	11	18	9	1	4	1
2 floods	0	0	1	2	8	10	15	14	8	3	0	2
3 floods	0	0	0	0	1	4	7	1	1	0	0	0
4 floods	0	0	0	0	0	2	1	0	1	0	0	0
5 floods	0	0	0	0	0	0	0	1	0	0	0	0

Table 5.5. **Predictability and its elements, Constancy and Contingency, of the flood regime in the Molenaars River. These are described for the full hydrological cycle and for the wet and dry periods separately.**

	Predictability (P)	Constancy (C)	Contingency (M)
Overall predictability of disturbance regime	0.62	0.43	0.19
Wet season disturbance predictability	0.29	0.23	0.06
Dry season disturbance predictability	0.85	0.82	0.03

Table 5.6. **Number of times in the hydrological record (1980 – 2007) during which the Berg River experienced different levels of flood disturbance (flood frequencies in categories of 0 to 4 per month), for each calendar month. This was the basis for calculating Colwell's measures of predictability. The winter flood season months, based on average flood frequency, are shaded.**

Disturbance states	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
no floods	26	26	25	24	7	4	1	7	14	20	24	24
1 flood	1	1	2	1	14	10	10	6	5	5	3	2
2 floods	0	0	0	2	4	7	6	6	6	1	0	1
3 floods	0	0	0	0	2	5	6	5	1	0	0	0
4 floods	0	0	0	0	0	1	4	3	1	1	0	0

Table 5.7. Predictability and its elements, Constancy and Contingency, of the flood regime in the Berg River. These are described for the full hydrological cycle and for the wet and dry periods separately.

	Predictability (P)	Constancy (C)	Contingency (M)
Overall predictability of disturbance regime	0.52	0.31	0.21
Wet season disturbance predictability	0.17	0.07	0.09
Dry season disturbance predictability	0.77	0.74	0.03

to between 3.3 and 3.4 floods per 60-days over this time of the year (Figure 5.9a). The maximum proportion of floods - between 46 and 48 % - per 60-day period in the Berg River were found for all 60-day periods starting between 9 and 24 June, extending to between 7 and 22 August (Figure 5.9b).

The probability of occurrence of one or more, and of two or more floods in each calendar month may be reflected by the percentage representation of these frequencies in the flow record (Tables 5.8 and 5.9). There is a substantial increase in the chance of having at least one flood in a month from the month of May, but two or more (disturbance inducing) floods are readily experienced in the months of June – August.

Table 5.8. Percentage probability of flood frequencies of ≥ 1 and ≥ 2 floods in the Molenaars River for each calendar month

Flood freq.	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
.≥ 1 flood	2.6	0.0	5.3	13.2	68.4	81.6	89.5	89.5	50.0	11.8	10.5	7.9
≥ 2 floods	0.0	0.0	2.6	5.3	23.7	42.1	60.5	42.1	26.3	8.8	0.0	5.3

Table 5.9. Percentage probability of flood frequencies of ≥ 1 and ≥ 2 floods in the Berg River for each calendar month

Flood freq.	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
≥ 1 flood	3.7	3.7	7.4	11.1	74.1	85.2	96.3	74.1	48.1	25.9	11.1	11.1
≥ 2 floods	0.0	0.0	0.0	7.4	22.2	48.1	59.3	51.9	29.6	7.4	0.0	3.7

5.4 DISCUSSION

Many studies quoting coefficients of flow variation have based this measure on the inter-annual variability in the mean annual runoff (MAR), for example the comparisons made by Davies *et al.* (1995). Variability in daily discharge, however, probably represents a better estimate of the variability experienced by stream fauna. This may be expressed as intra-annual variability which explains a measure of the overall variability within the annual cycle of flow. Alternatively, the inter-annual variability relates to how "constant" this variability may be year after year. The Molenaars and Berg Rivers have <u>intra-annual</u> coefficients of variation (CV) of daily flow (i.e. mean of the annual daily flow CV) that are higher than the average values for perennial rivers in the Western Cape calculated by Joubert *et al.* (1994), but fall within

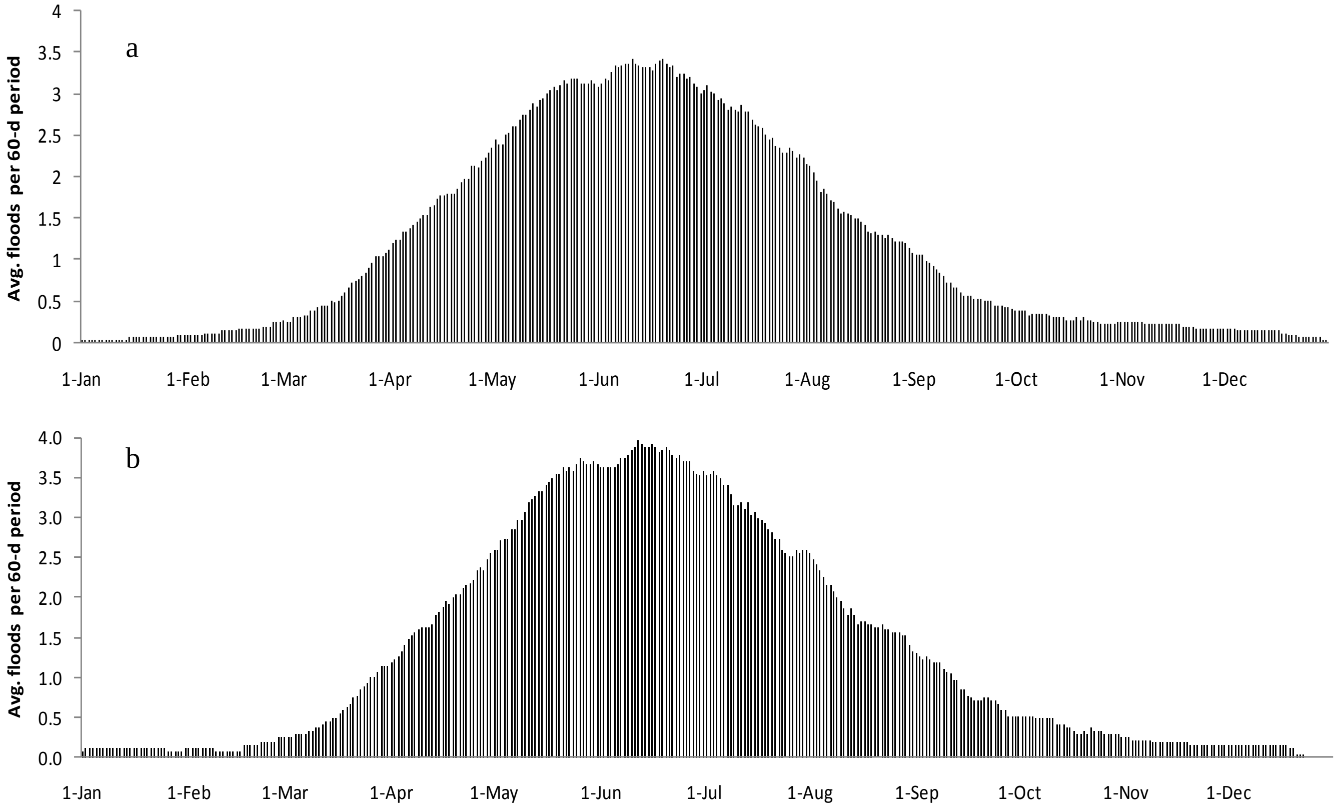

Figure 5.9. Average number of floods in the record occurring in consecutive 60-day periods (time step of 1 day) a) in the Molenaars River and b) in the Berg River. In both rivers, the maximum number of floods in a year occurring in a 60-day period was just under four, equal to 48 % of floods in the year, a measure of temporal predictability

the range of values given for "perennial runoff" to "perennial flashy" rivers described by Poff & Ward (1989) and Poff (1996).

This study did not aim to classify the Berg and Molenaars Rivers in terms of their flow regime, but rather to describe seasonal patterns of flow variability and of flow disturbance. Thus a comparison of how variable each month might be over long-term time frames (inter-annual variation), was made by examining the mean and CV of flows for each calendar month over the full record. These showed highest coefficients of variation in the Molenaars River in months that are characterised by low base flows combined with rare flood events, i.e. the months of March, April, November and December. In contrast, in the Berg River the high CV expected for these months was negated by the increased base flows resulting from the interbasin transfers during these periods. In the winter months the flows in the Berg River are affected by the abstraction of flow from the Wolwekloof tributary, and this may account to some degree for the lower variability in daily discharge in the Berg River compared to the Molenaars River.

An important caveat in the use of the CV as an indicator of, for example, the harshness of conditions in a river is that the variability in daily discharge does not necessarily reflect disturbance. For example, more than half the floods in the most variable months in the Molenaars River, which would contribute to the high CV, were lower than the threshold differentiating a disturbance event. Indeed, variability in flow can be a benign influence actually stimulating population growth, for example through the provision of increased nutrients to algal mats (Biggs and Stokseth 1996) or as an aid to dispersal and colonisation of new resources by invertebrates (e.g. Winterbottom *et al.* 1997a). Indeed this phenomenon was suggested by the Molenaars River flood study of 2003 and discussed in Chapter 4.

Poff & Ward (1989) used the combination of flood predictability and flood frequency as the major axes of a habitat template, where they argued that the position of a stream relative to these axes should provide an *a priori* basis for making predictions about the characteristics of the biota. Their conceptual model of stream classification is shown in Figure 5.10. Floods constituting disturbance events in the Molenaars and Berg Rivers show considerable intra-annual variability in their timing and inter-annual variability in the frequency (number per year) and periodicity (number per month) over the hydrological record. Flood frequencies are high (between 3 and 15 floods per annum, with an average of 7-8 in both rivers), and this frequency is higher in the winter months from May to September and very low at other times of the year.

In the stream classification of Poff & Ward (1989) flood frequency was calculated based on the frequency of floods greater than the 1:2 year return period flood as the threshold for disturbance. This is a circular argument, however, since the frequency of flood events based on a threshold that is itself defined by a frequency distribution must be a given: the 1:2 year flood is a statistical measure, defined as the 50th percentile of all the annual maximum floods in any record, which means that the frequency of floods greater than the 1:2 year will tend toward the same number given a long enough record in all rivers, regardless of the flashiness of the river. For example, there will be approximately 50 such event in 100 years of record, which makes this an inappropriate basis for comparison between rivers.

This study has chosen a more empirical, and considerably lower, threshold for disturbance than that of Poff & Ward (1989), based on actual measurement of bed movement in each of the study rivers, which removes the problem of the threshold for disturbance being linked to a return period frequency, instead reflecting a measured disturbance response. Although Clausen & Biggs (1997, 2000) also used a hydrological statistic to define their disturbance threshold - 3 x the median annual discharge – this is not based on a frequency analysis of flood events per se. These authors indicated that this flow statistic was associated with bed movement in many New Zealand streams, and demonstrated that the frequency of these disturbances was highly correlated with species composition across a range of streams. This value (3x median) is considerably lower than the disturbance threshold used for the Berg and Molenaars Rivers, which is understandable because the flow threshold for bed disturbance in New Zealand's gravel bed rivers would naturally be lower than the flow threshold for bed movement in cobble / boulder beds that are characteristic of the rivers used for this study.

The hypothesis investigated in this chapter was that the disturbance regime will be predictable by season, but that within the wet season disturbance will be unpredictable. The results indicate that the Molenaars and Berg Rivers may indeed be characterised as having a period of very low levels of disturbance, from October / November to April, albeit where the regime is characterised by minor fluctuation in discharge, mainly below the threshold for disturbance. This is reflected in the high daily flow CV in some summer months. From a flood perspective, conditions remain constant, reflected in the Constancy component of Colwell's Predictability index which is between 74 and 82 %. During this time, in the Molenaars River, there is between 13 (April) or 12 (October) and zero (February) percentage chance of one disturbance taking place per month, whilst the Berg River has a slightly higher chance of a flood in October, at 26 percent. Alongside this lowflow period is a period characterised by high frequencies of disturbance, from May to September, with one or more floods on average per month. This pattern of a season of disturbance alternating with a season of mild flow conditions has a fairly high predictability (52 – 62 %), based on Colwell's measures. Here both constancy and contingency contribute to the overall predictability, indicating both the relatively invariable summer disturbance regime and the somewhat repetitive pattern of increased floods during the wet season.

However, within the disturbance season, the timing of floods is not predictable: in winter, the extent of flooding, as represented by the number of disturbances per month, has a low predictability (less than 30 %). Contingency, or the measure of how repetitive the pattern is, is particularly low. Further, no more than 48 % of floods over the available record occur within the same two-month window (the FLDPRED index), demonstrating variability in the *timing* of floods within the winter months. Poff (1996) found that this index ranged from 44 to 96 % across 806 small rivers in the USA, indicating that on a comparative basis, flooding in the Molenaars and Berg Rivers is unpredictable.

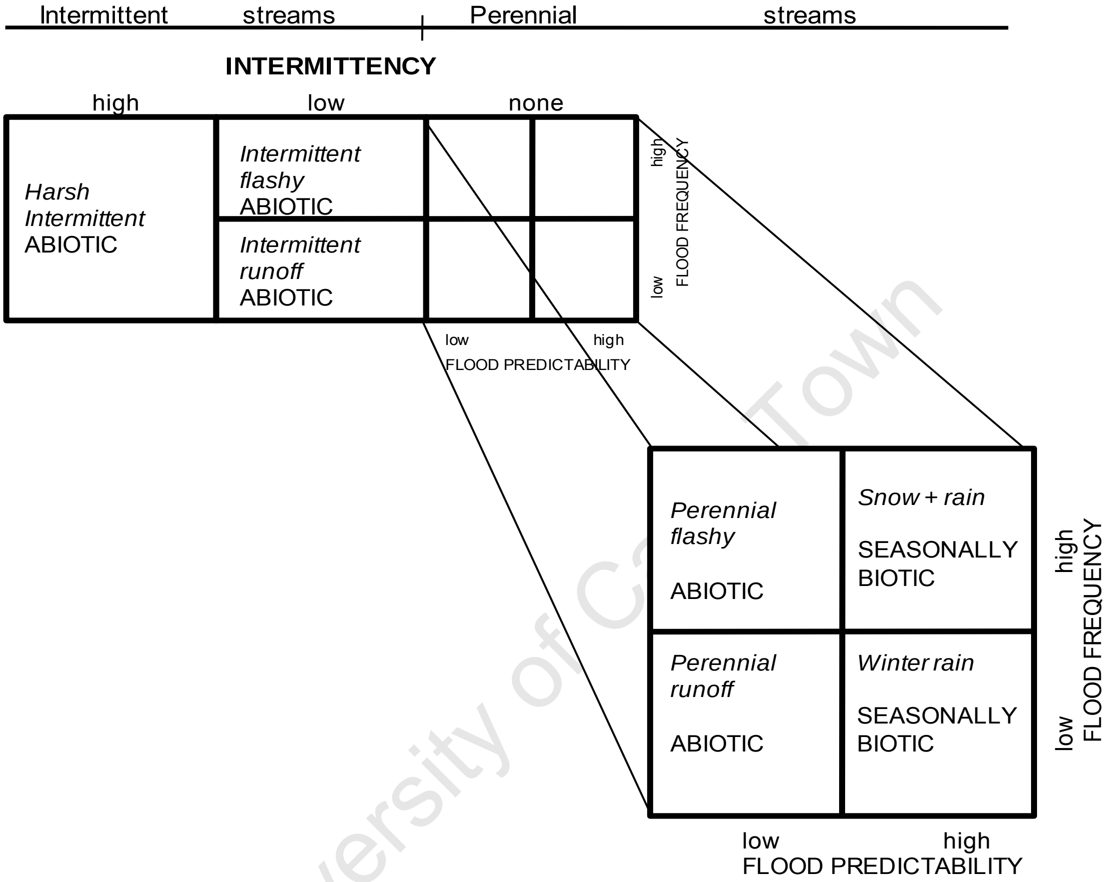

Figure 5.10. **Conceptual model of stream classification based on four temporal components of the discharge regime, as proposed by Poff & Ward (1989). The degree of intermittency is the first variable in the classification. For streams with low intermittency and for perennial streams, flood frequency determines the next level of the classification. For perennial streams, flood predictability provides a further axis in the classification. The position of a stream in relation to these variables will determine the degree of abiotic vs. biotic control of stream communities.**

In conclusion, the characterisation of rivers in the Western Cape as "predictably unpredictable" is borne out by the analysis of the flow regimes of the Molenaars and Berg Rivers. Strong seasonality of flood disturbance is a feature of the regimes. Winter disturbance is unpredictable on a month to month basis. In contrast, the summer period is characterised by a high predictability of disturbance, because floods are almost non-existent. Variation in daily discharge during summer is considerable, but this variation is for the most part below the disturbance threshold.

5.4.1 Implications for community structure

Adaptation to disturbance regimes occurs as a response to the imposition of mortality-inducing conditions, and may include morphological, behavioural and life history traits. The variability of the flow regime means that the adaptations of stream biota will carry both costs and benefits. For example life histories linked to autumn emergence may avoid mortality as a result of floods in winter, but would not benefit from the access to resources that might be afforded by an extended period of benign conditions in years that floods occur later in the disturbance season. The responses to this variability form the basis for natural selection filtering out those species with successful traits from a potential pool of stream biota, and thus determining the observed community composition (Lytle & Poff 2004). Lytle & Poff (2004) argued that the mode of adaptation to the flow regime will depend on the different components of the regime: they regarded the magnitude and frequency of disturbance as a driver of morphological adaptations aimed at increasing resistance or resilience to floods (e.g. drag-reducing body shapes) and thereby maintaining population persistence, whilst the predictability or unpredictability of individual floods should result in behavioural adaptations, for example speed of orientation within the water column, often including reactions to correlated environmental cues. Predictability in the timing of disturbance, should allow for the evolution of life-history adaptations that temper the importance of flood frequency (Lytle & Poff 2004). Life history adaptations to the flood disturbance regime include metamorphosis synchronised with the average timing (onset) of the flood season (Lytle 2002), and life cycles that maximise growth and reproduction during stable and mild periods (Gasith & Resh 1999).

For an adaptation to become represented in the biota of a stream, its benefits should outweigh the costs, averaged over many generations. For example, where the timing of disturbance is highly predictable, an optimal life history response would be one that synchronises life history events, such as age at reproduction, with the timing of floods. No optimal life history pattern is possible, however, regarding the timing of maturation age and emergence where floods are highly unpredictable. On the other hand, life history adaptations may include bet-hedging, such as asynchronous hatching of a portion of eggs laid at a given time (Zwick 1996). The latter may be important adaptations to low predictability in the timing of disturbance, or to high variability in the intra-annual frequency of fine grained disturbances.

The success of adaptations to the disturbance regime may be measured in community persistence over time. Few trajectory studies of persistence as described by Hildrew & Giller (1994), i.e. that measure persistence at one or a few sites, sampled repeatedly over a period of years, are reported in the literature. Most indicate that in the medium term, communities tend to be generally persistent, albeit unstable with regard to absolute invertebrate abundance. In these studies, increased temporal variability, and reduced habitat stability are associated with lower persistence. However, few of these studies examine persistence in relation to the long-term, regime, characteristics of their study rivers. This is the subject of the following chapter.

6 TEMPORAL DYNAMICS IN INVERTEBRATE ASSEMBLAGES

6.1 INTRODUCTION

Short term disturbance studies, namely those covering weeks to a period of months following disturbance have concluded that most invertebrates are highly susceptible to floods, but recovery after a flood is relatively rapid, in the order of days to months, for organic matter, algae and invertebrates (e.g. Robinson & Minshall 1986; Niemi *et al.* 1990; Stevenson 1990; Death 2003). These findings were discussed in Chapters 3 and 4 in the light of the flood response study. Here, factors that govern the extent of population reductions were considered to be the intensity or scale of the disturbance across the stream bed, and its corollary, the extent of instream refugia, as well as the resistance of different species to the effects of floods. Factors that determine recovery include the resilience traits of individual species, such as mobility, and the proximity of colonisers located in various refugia. Given the range in both resistance and resilience capabilities of invertebrate species, and given the physical differences between streams in the types and amount of refugia that are offered, it would seem that floods, at least those that occur on intra-annual time scales, may be considered to add considerably to the spatial and temporal patchiness of stream ecosystems, through the continual reorganisation of their biota.

In addition to this, floods occur within the context of a temporally changing set of environmental conditions, for example seasonal shifts in ambient conditions and / or resources. End-points of stream recovery can seldom be expected to be the same as the starting point, the pre-flood state, and this makes understanding the effects of disturbance even more complex. Any attempt to find a generalised pattern of post-flood dynamics in stream biota must therefore allow for this variety in ambient conditions (e.g. Robinson & Minshall 1986), something which few studies have been able to achieve. The examination and explanation of biological pattern and process requires firstly the contextualisation of how the regime-type attributes of floods interact with the broader cyclical patterns in stream environments and how these together determine the nature of intra-annual variability. Regime-type attributes include a) average frequencies of floods; b) the duration of periods between floods; and c) seasonality or predictability of flooding within annual cycles. For example, a flood may have a very different impact on biotic assemblages depending on antecedent water temperatures and flow conditions, which themselves influence resource availability and community composition. Clearly an important question is the degree to which floods on their own determine the nature of intra-annual variability in biological assemblages, versus other possible explanatory variables.

A further consideration is the degree of inter-annual variability in the disturbance regime, and its effect on the persistence, or lack thereof, and the stability of populations or communities. The stability and persistence of a population over inter-generational time is a phenomenon gaining exposure in disturbance studies and in long-term monitoring studies (e.g. Bradt *et al.* 1999; Bradley & Ormerod 2001; Scarsbrook 2002; Metzeling *et al.* 2002; Woodward *et al.* 2002). Townsend *et al.* (1987) defined persistence as the extent to which the species complement of a community remains unchanged over a time period encompassing at least one complete population turnover. Stability of natural populations or communities refers to the extent to which there is constancy in the relative densities of species over time periods encompassing at least one turnover of generations (Connell & Sousa 1983). The relative density of a species refers to its ranked abundance relative to other species in a community (Meffe & Minckley 1987).

These two measures, stability and persistence, refer to different properties of communities. Measuring the persistence of a community demonstrates the extent to which its species are buffered against extinction in the face of disturbance. Persistence of a population, according to Connell & Sousa (1983), indicates that a population or species either did not become locally extinct over a given period, or if it did, that it was able to recolonise the area within one generation.

Stability on the other hand is predicted to vary more substantially on inter-annual time scales where environmental variability, such as that caused by floods, is high.

In this study, two key questions relating to seasonality and inter-annual variability are:

- how does the interaction between seasonality in ambient conditions and resources and flood occurrence or frequency affect community assembly patterns in rivers at the within-year scale, and to what extent are floods the driver of this
- how much does inter-annual variability in the disturbance regime, which defines predictability, affect population or community persistence and stability.

The strength of the impact of floods at both of these scales may determine life history responses, which are the outcome of both intra- and inter-annual variation in conditions.

6.1.1 Seasonality and inter-annual variability in Mediterranean rivers

The characterisation of rivers in the Western Cape as "predictably unpredictable" (sensu Davies *et al.* 1995) is borne out by the analysis of the flow regimes of the Molenaars and Berg Rivers presented in Chapter 5. These rivers have strongly seasonal flooding. The period from October / November through to April is characterised by very low flood frequencies. Fluctuation in discharge is mainly below the threshold for disturbance defined in Chapter 5 of this study, and base flows maintain strong perenniality. After this summer lowflow period is one characterised by high flood frequencies, from May to September, with one or more floods on average per month. This pattern of seasonal disturbance has a fairly high predictability. However, in winter the occurrence of floods is unpredictable on a month to month basis, and inter-annual variability is high. Undoubtedly such a regime must influence the nature of invertebrate communities, particularly the temporal dynamics in their composition.

The thermal regime is considered to be one of the most important water quality parameters affecting the distribution and abundance of riverine plants and animals and in Western Cape rivers also displays a very strong seasonal pattern, albeit without the variability during the winter period that characterises the flow regime. Temperature may thus provide alternative or complementary explanations of community patterns. As with the hydrological analysis, however, time series data are the most useful for detecting the influence of this parameter. For example, increases in invertebrate abundance are more likely to be related to cumulative degree days, rather than to spot temperatures recorded once a month.

Rivers in Mediterranean regions, such as that in the Western Cape, all show strong seasonality in physical conditions. Gasith and Resh (1999) proposed a number of hypotheses regarding the consequences of the flooding and drought regimes on biota of Mediterranean streams, inter alia:

- faunal abundances will be lowest during the wet winter season, when flooding occurs;
- during spring, after floods, invertebrate benthic biota will increase in abundance from individuals colonising from nearby habitats or refugia;

- invertebrate species composition and abundance will shift rapidly between late summer and winter, but more gradually from the end of winter to summer;
- change in species composition from winter to summer will occur earlier in years where floods are reduced in number or decline in frequency earlier; and
- species adaptations to the wet and dry periods will result in constant levels of species richness, despite changes in composition, although diversity will be lowest in late summer, when evenness is low as a result of dominance by species that can tolerate the conditions associated with summer drought.

Many studies have demonstrated strong seasonal patterns in invertebrate assemblage composition and virtually all of these show consistently repeating wet and dry season communities, that are only rarely overridden by the effects of floods or droughts (e.g. Bunn *et al.* 1986; Meffe & Minckley 1987; McElravy *et al.* 1989; Feminella & Resh 1990; Boulton & Lake 1992; Boulton *et al.* 1992; Cobb *et al.* 1992; Jacobsen & Encalada 1998; Robinson & Minshall 1998; Wright & Symes 1999; Pardo 2000, although for exceptions see Brewin *et al.* 2000 and Thomson 2002).

Most such studies have been based on data collected bi-annually or aggregated into wet versus dry periods (e.g. Bunn *et al.* 1986; McElravy *et al.* 1989; Jacobsen & Encalada 1998; Beche *et al.* 2006). However, if wet or dry periods coincide with other periodic changes in the ambient conditions, then the cause of the observed biological pattern is difficult to separate out (Suren & Jowett 2006). Viewed in this way, flood disturbance effects are simply one of a number of forces driving biological pattern that show intra-annual cycles, such as higher or lower base flows and temperature. Clearly an important question is the degree to which floods *per se* determine the nature of intra-annual variability in biological assemblages, versus other possible explanatory variables. In this regard, a necessary approach is to examine the effect of floods through the course of an annual hydrological cycle, with a sampling strategy that is adequate in temporal scale (time interval) to attempt to separate out seasonality from disturbance impact (e.g. Boulton & Lake 1992; Flecker & Feiferek 1994; Miller & Gollada 1996; Silva Filho & Maltchik 2000; Suren & Jowett 2006).

6.1.2 Aims of this chapter

In this chapter, longitudinal gradients in invertebrate community composition within the foothill reach of the Molenaars River are compared with community changes occurring over seasonal and inter-annual time scales. It is anticipated that temporal patterns should be far stronger than inter-site differences, both in physico-chemical variables and in the biota. Given the strength of the temporal signal in the data, links between biotic patterns and a number of flow variables are explored, including descriptors of flood disturbance (e.g. time since last disturbance, number of disturbances during the prior period). The predictions regarding seasonal dynamics in invertebrate assemblages, community structure and overall abundance as a result of flooding patterns, as proposed by Gasith & Resh (1999) are tested.

In addition, temporal patterns in invertebrate community structure are examined against those of other abiotic variables, besides flow, that show marked seasonal fluctuation, in order to compare the contribution of these variables to the observed biotic pattern with that of flow.

The Molenaars and Berg Rivers are strongly perennial and the low-flow period is not considered to be as harsh as in those systems that experience extreme drying. As such, the window period for growth and reproduction should be wider, and extend to the period between floods, that is, from October to April. In relation to the hypotheses proposed by Gasith & Resh (1999), the comparatively mild late summer stress in the Molenaars River should result in a situation where habitat quality does not preclude sensitive taxa; thus diversity should not become reduced as a result of dominance by tolerant species, but should rather remain constant throughout the year. Given the low levels of summer temperature stress in these rivers compared to less perennial systems, it is hypothesised that temporal changes in invertebrate assemblages or densities should track most strongly changes in flow, and specifically will be driven by variations in the onset, intensity and duration of the flood season.

Following this, measures of persistence and stability in invertebrate assemblages are derived for each calendar month and are examined in the light of differences in the predictability of flooding over the year. Although most studies of community persistence and stability take the form of a comparison of a number of rivers with contrasting disturbance regimes, this study has a different focus, examining year-on-year persistence and community stability for <u>different seasons, or calendar months</u>. Here the specific hypothesis to be tested is that the invertebrate community in summer should be more stable and more persistent year-on-year than that in winter, because of the low levels of disturbance in the former and the frequent flood events that characterise the latter. Also, year-on-year measures of persistence and stability for the winter months should be influenced by interannual differences in flood intensity and frequency, with greater persistence in years with similar flood patterns. Alternatively, if invertebrates are adapted to the flow regime, even to the lack of predictability in flooding, then their assemblages should be predictable and persistent, because at local or reach level they have evolved resistance to disturbance. In that case, persistence should be high year-on-year for all seasons, but stability may still vary as a result of short term climatic differences within each winter flood season.

6.2 METHODS

6.2.1 Sampling programme and data sets

The two data sets from the Molenaars River used for this analysis of temporal pattern are described in Chapter 2. Briefly, they comprise quantitative monthly invertebrate data from the Molenaars River at the eight study sites, from August 1991 to February 1993, and a set of semi-quantitative data collected monthly from 1994 to 1997, from four of the study sites. A range of physico-chemical parameters and periphyton were sampled to help in explaining the patterns in the invertebrate data. In rocky, open-canopied rivers with clear water, algae attached to rock surfaces (periphyton) are often the dominant food source for invertebrate fauna. Periphyton may vary temporally in availability, as a result of flow disturbance and temperature, but may increase rapidly in the face of low invertebrate grazer densities where these themselves are regulated by floods and / or temperature (Biggs & Close 1989). As such, an indication of periphyton availability was considered to be an important informant in investigating invertebrate dynamics.

Periphyton biomass, as well as substratum composition, velocity, depth, temperature and chemical data were collected alongside the biotic data during the 1991-1993 sampling, but not for the second data set. Discharge and water quality data were available from the nearby DWAF flow gauging weir, and used to represent conditions over the study reach as a whole, and for both sampling periods. This was considered to be an adequate data set for the whole study reach, given the low level of longitudinal change, or inter-site differences, displayed in the site-specific data (Ractliffe & Brown 1994).

The first data set was collected over 18 months from late August 1991 until early February 1993, during which time samples were collected from nine sites along the Molenaars River, on 17 occasions: storm events in winter 1992 delayed sampling with the result that on average a little longer than a monthly period elapsed between one sampling occasion and the next. Notwithstanding, the period between sampling occasions was never more than 6 weeks.

The second data set comprised semi-quantitative invertebrate data, originally collected as part of a wider biological monitoring programme covering nine sites along the Molenaars River. Sampling was undertaken on a monthly to six-weekly basis, depending on weather conditions, from January 1994 to July 1997. Four of the sites monitored in this way corresponded to those sampled between 1991 and 1993. This data set comprised a total of 41 sampling events at each of these four sites.

6.2.2 Historical data sets

The long-term average daily discharge record at DWAF flow gauge station H1H018 was obtained from the DWAF database. A wide range of chemical data is collected from this gauge, at varying intervals. The most relevant physico-chemical variables in the assessment of possible drivers of periphyton biomass and invertebrate assemblage patterns were considered to be temperature and the plant nutrients: ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), nitrite nitrogen (NO_2^--N), soluble reactive phosphorus (PO_4^{3-}-P) and, to a lesser extent silica (Si). These, along with pH and conductivity, were obtained from the DWAF database.

6.2.2.i Simulation of water temperature data

The temperature data available from the DWAF record were sparse, with large gaps in the record. Because of the requirement for time series temperature data, a generic multiple linear regression model (Rivers-Moore *et al.* 2005), based on average air temperature data and including a flow rate factor, was used to simulate daily maximum water temperature data for the period 1970 – 1998. The regression equation for maximum water temperature (WT_{max}) used from those evaluated by Rivers-Moore *et al.* (2005) was a generic one, in the absence of site-specific data:

$$WT_{max} = \beta + A1.AT_{mean} - A2.Flow^{-1}$$

where β, A1 and A2 are coefficients (4.004, 0.8995 and 0.4827 respectively), AT_{mean} is the mean daily air temperature and $Flow^{-1}$ is the inverse of the average daily discharge.

Maximum and minimum air temperature data were obtained from the Cape Town Weather Office, for Weather Station No. 0021825 4 - Paarl (33.7500°S 18.9670°E). This station was chosen because it had the most intact record of those in relatively close proximity. Many weather stations record only maximum and minimum temperatures, as in this case. Although mean temperature is not simply the average of these values, in the absence of an actual daily mean temperature measurement, AT_{mean} was calculated as the mean of the daily maximum and minimum values, which was considered to be an acceptable surrogate.

The absolute temperature values calculated in this process were deemed to be less important than the provision of relative measures, particularly in the comparison of inter-annual differences in temperature regime. Nevertheless, the accuracy of the simulated time series was evaluated through comparison with the actual DWAF river temperature measurements taken over this period. A complicating factor was that the DWAF temperature data were collected at various times of the day, and thus do not necessarily represent the maximum, minimum or mean temperature on the day of collection, to some extent negating the use of these data, particularly given the range in daily temperature at various times of the year. To overcome this partially, the comparison of simulated versus actual data was restricted to those measurements in the DWAF data-set that were obtained after 13:00 hours and before 16:00 hours. Figure 6.1 shows the results of this comparison. The simulated data were generally slightly higher than the spot DWAF temperatures – this might be error in the model or the fact that the DWAF data may not reflect true maxima.

On average, the difference in temperature was 1.5 degrees (Statistica comparison of means). Correlation analysis showed, however, a strong, significant correlation between these variables (r = 0.71). As a result of this analysis, the simulated temperature data set was included in the analysis of temporal patterns that follows.

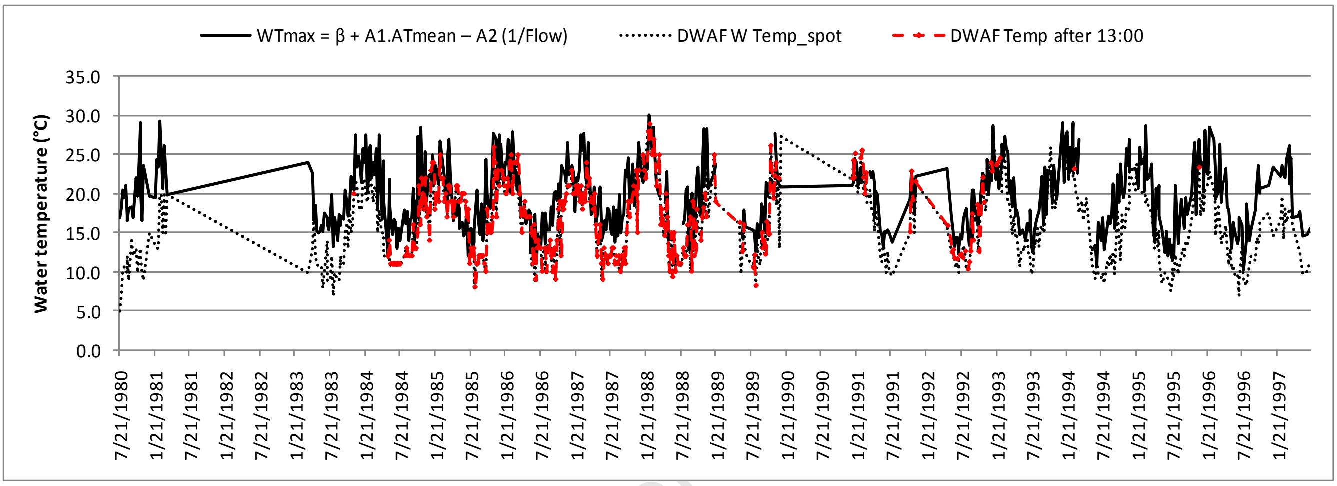

Figure 6.1. **Comparison of simulated maximum water temperature in the Molenaars River and measured spot data from DWAF Gauge H1H018. The full complement of spot temperature readings is indicated by the dotted lines, whilst those collected after 13:00 hours are shown in red.**

6.2.3 Field sampling methods

6.2.3.i Abiotic variables

The abiotic variables that were measured at each of the sampling sites during the 1991-1993 sampling period are described below.

a <u>Physical and chemical properties of the water</u>

Current speed and water depth were recorded simultaneously using a Price AA current meter and a graded, top-setting wading rod (Scientific Instruments Inc.). At least three replicate samples were taken at each site, from the riffle area where invertebrate and periphyton samples were collected. The concentration of Total Suspended Solids (TSS) in the water column was measured by filtering *in situ* a known (approximately 1000 ml) volume of water through pre-weighed Watman GF/F (0.45 µm pore-size) filters, pre-combusted at 450 °C for three hours. The filter papers with filtrate were dried for 48-72 hours at 60 °C, re-weighed to determine TSS, and further combusted for four hours at 450 °C to determine the ash-free dry weight of organic matter. This also allowed for the inorganic fraction to be calculated. Weighing was done on a Mettler AE 100 laboratory balance accurate to 0.1 mg.

To determine the Total Dissolved Solids, the filtered river water was evaporated at approximately 60 °C to prevent the loss of volatile organics, in pre-weighed Pyrex glass beakers weighed on a Sartorius precision laboratory balance accurate to 1 mg.

Water temperature was measured using a spirit thermometer, accurate to 0.5 °C. pH was recorded with a Crison Portable 506 field pH meter, accurate to 0.01 pH units, and conductivity was measured with a Crison Conductimeter Portable 523 field meter, accurate to 0.01 mS m^{-1}, with built-in temperature compensation of 25°C. Conductivity and TDS are well correlated in most rivers, because of the charged nature of most dissolved particles. Although both were measured, conductivity was used as an indication of the concentration of dissolved substances, since long-term records for the Molenaars River also exist for this variable. Some data gaps in the conductivity measurements were patched using TDS data, based on the significant correlation between the two (Pearsons $r = 0.57$, $p < 0.05$) and using a regression equation derived from these data.

Because processing of nutrient samples was costly, only 12 sets of samples from the 17 sampling trips were processed to provide data on concentrations of the nutrients most commonly implicated in excessive plant growth resulting from nutrient enrichment of water bodies (Dallas *et al*. 1998): ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), nitrite nitrogen (NO_2^--N) and soluble reactive phosphorus (PO_4^{3-}-P). All filtered water for the analyses, except that for analysis of ammonium, was bottled in polythene scintillation vials that had been pre-cleaned in 5% ExtranR solution (phosphate-free), and rinsed first in deionised and then double-distilled water. Samples for analysis of ammonium were stored in HCl-washed glass vials within polythene containers. All water samples were stored on ice in the field and frozen immediately on return to the laboratory. Samples were analysed by the Council for Scientific and Industrial Research laboratory, using a TECHNICON AutoAnalyser lII Segmented Flow System. The principles of the methods employed are outlined in Mostert (1983) and Windt (1993).

6.2.3.ii Periphyton

The 1991-1993 data included a measure of the periphyton biomass at each site. The periphyton, or more correctly the biofilm layer, covering the stones on the river bed was measured each month by brushing off the periphyton from a known area of the upper surface of three randomly selected river cobbles, using a toothbrush. Samples were filtered through pre-weighed GF/F (0.45 µm pore-size) filter paper, dried at 60 °C to a constant weight and weighed. The organic (ash-free dry weight) periphyton biomass was measured using the same process as that for TSS. Some of the samples collected at the end of December 1991 were damaged and thus this month is represented by a single sample at each site. Seasonal and inter-annual changes in periphyton density were explored in relation to flow and temperature, and also in the light of patterns in invertebrate abundance of the study period.

6.2.3.iii Invertebrate samples

a Data set from 1991 – 1993

Three randomly selected replicate benthic samples were collected from riffle biotope at each site. The decision to restrict the number of both periphyton and invertebrate replicate samples to three was entirely pragmatic, and was made recognising that insufficient replication may increase heterogeneity or “noise” and potentially affect the interpretation of resulting patterns in the data (see Hurlbert 1984). However, this was mitigated by sampling only within riffles, as well as the fact that invertebrate densities in the

Molenaars River are very high, compared to most Western Cape rivers, and thus three replicates represent many thousands of individuals.

A box sampler described by King (1981) was used to collect invertebrate samples (area of 0.1 m^2, front-face of 250 µm and sides of 80 µm mesh, ending on the downstream end of the box in an 80 µm mesh funnel with detachable jar). It was placed on the stream bed, as flush with the stream bottom as possible, and the cobbles and gravel lifted systematically from the area within the box and brushed gently to loosen the animals clinging to their surfaces. The stones were then placed outside of the box. After all movable cobbles had been brushed clean, the gravel and sand inside the box sample area were agitated, down to a depth of 15 cm, to release any further invertebrates in the substratum. Samples were preserved in 4% buffered formalin and transferred into 70% alcohol upon return to the laboratory.

Samples were strained through a 250 µm sieve, to reduce processing time, with the fraction of the sample between 80 and 250 µm not analysed. Whilst this may have implications for estimating the densities of very young instars, identification of animals within this fraction, even to genus level, is not possible. The remaining sample was sorted, and invertebrates were identified and counted. For very large samples, sub-sampling was necessary: animals that were easily visible with the naked eye were picked from the sample initially, followed by sub sampling using a standard plankton splitter.

b Data set from 1994 – 1997

A single composite sample was collected at each of the sites on each sampling occasion, incorporating the full range of available biotopes at the site, including the marginal vegetation. Samples were collected using a 1 mm mesh net held downstream as the substratum was agitated by kicking. A strict time allocation of fifteen minutes' kicking time was observed for the collection of all samples.

The live sample was emptied into a trough and visually inspected to record the presence of invertebrate families. For each recorded taxon, abundance was rated on a scale of one to four, where 1 = a single sighting; 2 = an estimated abundance of between 1 and 10 individuals; 3 = between 10 and 100 individuals; and 4 = between 100 and 1000 individuals.

c Invertebrate identification and classification

For the comparison of spatial and temporal patterns, the taxonomic level to which invertebrates were identified was initially mainly to Family level, but in some cases to sub-Family or tribe level (e.g. Chironomidae), or to a higher level where groups were very sparsely represented in the samples and did not warrant the extra effort (e.g. Hemiptera). However, three of the eight sites were selected for the life-history study (Chapter 7) and the data from these sites were re-identified to the more refined taxonomic level of genus or species for that analysis. Invertebrate identifications were performed using Nikon SMZ 1500 and SMZ 1B dissecting microscopes, an Olympus compound microscope and numerous field guides and taxonomic keys (de Moor 2002; Barber-James & Lugo-Ortiz 2003; de Moor & Scott 2003; Mansell 2003; Samways & Wilmott 2003).

For the longer-term data set (1994 – 1997), visual identification of taxa in the field was undertaken at the level of Family for the most part, with the exception of the baetid mayflies and leptocerid caddisflies, for which genera or morpho-species were recorded, based on obvious differences observable in the live material, and a knowledge of the major species present in the river.

In order to explore the relationship between invertebrates likely to be feeding on algae and the seasonal variation in periphyton biomass, the functional feeding group (FFG) affiliation of each taxon (*sensu* Merritt & Cummins 1984) was identified. In the South African context FFG classifications have been proposed by *inter alia* King *et al.* (1988) and Palmer (1991). Schael (2005) prepared a tentative revision of the basic FFGs for her Western Cape rivers study, by combining feeding mode and food preference, with 20 FFGs defined. She also provided a list of the FFG affiliation of the species encountered in her study, based on best available ecological information.

The species-level FFG information provided by Schael (2005) was the basis for the designation of algal consumers in this present study, where most of the data were identified only to Family-level. The various taxa were therefore assigned to a FFG based on the dominant FFG affinity of species in the taxon, and on observational evidence of their position (top or bottom) on stones. All Families where scraper or grazer feeding groups were dominant (based on Schael 2005) were included in the list of algal consumers, unless these were considered to be associated with gravels under the surface layer of the stream bed (e.g. Scirtidae). This list of algal consumers thus included Elmidae, Hydraenidae (Coleoptera), Hydroptilidae and Glossosomatidae (Trichoptera), Heptageniidae and Telagonodidae (Ephemeroptera) and the chironomid subfamily Orthocladinae and tribe Tanytarcini. The Baetidae (Ephemeroptera) were also included on the list, even though they are generally considered to be collector-gatherer feeders. This was because most of the Baetidae in riffle biotopes in the Molenaars River are found on the upper surfaces of rocks where periphyton is abundant, rather than under the cobbles and gravels below the surface layer which are characteristically occupied by the Leptophlebiidae, and where detritus dominates. Whilst this is a very coarse estimate of "algal consumers", it does provide a basis for an assessment of whether algal biomass is a factor influencing temporal patterns of change in invertebrate community composition.

6.2.4 Analytical methods

6.2.4.i Overview

Initial analysis of the data was aimed at identifying spatio-temporal patterns in the abiotic and biotic data from the 1991-1993 period and from 1994-1997. The study area comprised the 13 km foothill zone of the Molenaars River, with less of a range in gradients than, for example a reach including mountain stream or lowland river, it was expected that the strength of the temporal signal in the data should be far stronger than any longitudinal trend. However, since some of the sites were shown to be more enriched by nutrients than others, with higher phosphate levels and at times increased periphyton biomass, a subset of the more natural sites was used for the investigation of relationships between temporal change in invertebrate assemblages and community indices or the dynamics of specific taxa on one hand, and periphyton and physical parameters on the other hand. The analysis was done using a combination of uni- and multi-variate statistical methods.

6.2.4.ii Univariate analyses

Longitudinal trends and inter-site differences in the physico-chemical and biotic characteristics in the Molenaars River were explored both graphically and using non-parametric statistics. In most cases, data did not conform to the assumptions of parametric methods, even after transformation, thus requiring the use of Kruskal-Wallis ANOVA by ranks. Where Kruskal-Wallis tests were significant, pair-wise differences between groups were explored using Dunn's Pairwise multiple comparison procedure.

Pearson correlation as well as regression analysis was used to assess relationships between variables. The former measures the strength of the linear relationship between two variables, and is compared with the null hypothesis that no linear relationship exists between them (correlation coefficient, r, is zero). The coefficient of determination (R^2) is a measure of how much the dependent (y) variable may be explained in terms of the independent (x) variable. Significance testing in a correlation analysis requires that the assumptions of independence between variables as well as normality in distribution are met. In order to stabilise the variances, 4th-root transformation (invertebrate data) and log transformation (periphyton) of data was performed. Other physical data were not transformed, unless explicitly indicated.

Patterns in invertebrate data were explored using simple abundance measures: total invertebrate density (numbers per m^2), taxon density (total number of taxa per sample, converted to number of taxa per m^2), rarefied taxon richness (number of taxa per 100 individuals), Shannon Wiener diversity and Pielou's evenness. The latter three measures were generated from the routines provided in the PRIMER package (see next section). Rarefied taxon richness relates the number of taxa in a sample to the log of the total number of individuals in that sample, to account for the observed phenomenon of naturally increasing species richness found in samples with large numbers of individuals. In this study, the rarefied taxon richness refers to the number of taxa in a sample of 100 individuals. The Shannon-Wiener diversity index (H´) was calculated for each site each month, where

$$H' = -\Sigma_i \, p_i(\ln p_i)$$

and p_i is the proportion of the total abundance contributed by the ith taxon. This diversity index combines a measure of taxon richness with the equitability of the distribution of each taxon across the assemblage, and was used specifically to investigate inter-annual changes in diversity (e.g. from one summer to the next). Similarly, Pielou's evenness is a measure of equitability which indicates how evenly the individuals are distributed among the different species. Pielou's evenness was calculated as

$$J' = H' \text{ (observed) } / H'_{\text{max}}$$

where H'_{max} = the maximum possible diversity that would be achieved if all species were equally abundant.

In addition, differences over time in the density of particular groups that at times dominated the assemblages, as well as some considered to be sensitive to alterations in the natural environment, were examined.

6.2.4.iii Multivariate analyses

The software package PRIMER (Plymouth Routines in Multivariate Ecological Research) Version 6, described in Section 3.2.3 of Chapter 3, was used to investigate the relationships between sites and within sites over time, based on the composition of their invertebrate fauna.

Initially, the analyses of the 1991-1993 data were performed using all individual replicates collected from each site. The multivariate patterns resulting from this approach were not dissimilar from those produced using the averages of the three replicates at each site, but the naturally patchy micro-distribution of riverine invertebrates tended to make graphical representation of the data unclear. For this reason, whilst testing for significant differences between sites, the ANOSIM routine was performed using the replicate data, but for the CLUSTER analysis and ordination, averaged data from each site were used, for clarity of presentation.

The 1994-1997 data set spanned a 2.5-year period, from August 1994 to January 1997. Data from all four sites were pooled for investigation of temporal patterns, using the PRIMER routines. Because the abundance estimates for each of these samples was on a scale of 1 to 4, combining the data into a single class was done by using the average of each value, so that where a taxon was collected at only one of the four sites, its pooled average abundance class would be 0.25.

6.2.4.iv Analysis of persistence and stability

Persistence is examined by comparing the invertebrate assemblage at two times, over an interval of a year, which is regarded as sufficient for most river taxa to encompass a population turnover. The 1994 – 1997 data set for the Molenaars River allowed for year-on-year measures of persistence to be calculated for calendar months across a two or a three year cycle. Persistence between 1994 and 1995was examined for the calendar month of January to July, where only two years of data were available. For the months of August to December, persistence was calculated for the years 1994 and 1995, as well as between 1995 and 1996 and between 1994 and 1996, since three years of data were available. Inter-annual differences in persistence were determined for months where the sampling data were sufficiently extensive and these were related to changes in flood flows in these years.

One of the measures used to explore persistence was a Persistence Index (Meffe & Minckley 1987), defined as 1-Turnover (T), where:

$$T = \frac{(C+E)}{S1+ S2}$$

and where C = the number of taxa that have colonised at Time 2; E = the number of taxa that have become extinct at Time 2; and S_1 and S_2 are the number of taxa present in the samples at Times 1 and 2 respectively. This index provides the same measure as the Bray-Curtis similarity index based on presence absence data, obtained from the PRIMER routines.

A second measure of persistence used for this study was Jaccard's measure of persistence, defined in Townsend *et al.* (1987) as:

$$JACC = \frac{j}{a + b - j}$$

where j = the number of taxa in common at both times; a = the number of taxa present at Time 1 and b = the number of taxa present at Time 2.

Because the measurement of persistence is based on presence / absence data, it was calculated for the year-on-year monthly samples for both the 1991 – 1993 and 1994 – 1997 sampling periods.

Stability measurements were based on calculating Spearman's rank correlation coefficient for pairs of samples for the year-on-year monthly samples for the 1991 – 1993 sampling period. This only allowed stability to be measured for the months that overlapped, namely August to February, covering winter, spring and summer seasons. The Spearman's rank correlation coefficient provides a measure of the strength of association between pairs of variables without assigning a dependent or independent variable. The variable correlated in this case is the abundance rank of each taxon in the paired samples. The Bray Curtis dissimilarity measure used in the PRIMER routines is also a measure of stability used in the recent literature (e.g. Scarsbrook 2002; Beche & Resh 2007).

6.3 RESULTS

6.3.1 Patterns in abiotic conditions in the Molenaars River

6.3.1.i Spatial pattern: between site differences

The assessment of spatial, or longitudinal, pattern in the Molenaars River was based on 17-months of data collected from Sites 1 to 8 between 1991 and 1993. Many of the variables were represented by single, unreplicated data for each site for each sampling occasion. Inter-site comparisons were thus based on the data set for each site, taken over the full sampling period. No significant differences between sites were found in temperature and conductivity (Kruskal-Wallis one-way ANOVA, $p > 0.05$), although the range of values for each of these was lower for Site 1 than for any of the other sites. Site 6, downstream of a trout farm had a significantly higher pH than Site 1 (Kruskal-Wallis $H = 17.70$, $p = 0.013$, Dunn’s multiple comparison $p = 0.046$). Total Inorganic Nitrogen was not significantly different between sites, but a significant difference was found between sites based on their Phosphate concentrations (Kruskal-Wallis $H = 15.31$, $p = 0.032$). Although there were no pairwise differences (Dunn’s multiple comparisons, all $p > 0.05$), Site 8 had the lowest and Site 6 the highest mean PO_4-P concentration. Both the percentage sand cover within the substratum and the TSS were also significantly higher at Site 6 than at other sites (Kruskal-Wallis $H = 18.28$, $p = 0.011$ and $H = 42.98$, $p << 0.001$ respectively).

Three to six replicate velocity measurements were taken randomly from riffle biotope at each site, on most sampling occasions, although data from some months were excluded because of faulty instrumentation. The data did not conform to the assumptions of equal variances for parametric testing (Levene’s test, $p < 0.001$), and thus 2-way ANOVA (sites and months) was not possible. A Kruskal-Wallis non parametric ANOVA was performed on the replicate data from each site, separately for each of the sampling occasions, to test for inter-site differences in velocity. This gave a non-significant result for all months except for the July 1992 data (Kruskall-Wallis $H= 13.37$, $p = 0.037$). Dunn’s post-hoc multiple comparisons of the July 1992 data showed that Site 8 was generally characterised by faster current speeds than the other sites, but in this month only.

6.3.1.ii Temporal patterns in the data

DWAF historical data were analysed to show long-term trends in abiotic conditions whilst monthly measurements taken from the sampling sites were pooled and subjected to Kruskal-Wallis ANOVA to explore temporal patterns in physical and chemical conditions over the study period.

6.3.1.ii.a.1 Water depth and velocity patterns

Changes in water depth and velocity would be expected to follow those of discharge, which in the Molenaars River is highly seasonal, given the winter rainfall, summer drought climate of the Western Cape Province (Figure 6.2). No long-term data are available for water depth and velocities in the Molenaars River, and this analysis is restricted to the site data collected from 1991 - 1993. Average water depth and velocity from each site in each month were used as spatial replicates in the examination of temporal change, because there were no significant differences between sites as explained in the preceding section. This was with one exception: the July 1992 data for Site 8 were removed from the analysis of temporal changes in velocity because of the significantly higher velocity at this site compared to other sites in this month (see results above).

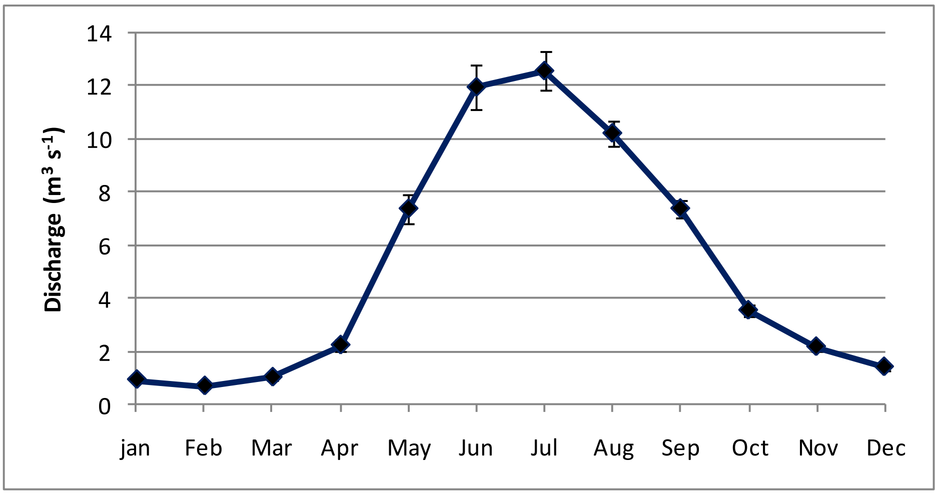

Figure 6.2. **Monthly average discharge in the Molenaars River, taken from DWAF flow gauge H1018, based on the record from 1978 to 2008. On average February has the lowest discharge and July the highest (data from the Department of Water Affairs & Forestry). Standard error bars shown.**

The results of this temporal analysis showed significant differences with time (Kruskal-Wallis H=99.38, p << 0.001), and post-hoc multiple comparisons largely corresponded to expected patterns. For example, velocities in June and August 1992 were significantly higher than those measured in January and March 1992 and from December 1992 through February 1993 (Figure 6.3), and there was a significant increase in velocities from March to April 1991. Velocities in December 1991 were marginally higher than expected, given the relatively low discharge in the river at that time, and not significantly different from the autumn and winter velocities. This may be a consequence of the fairly low replication of the measurements. Average water depth in the Molenaars River followed much the same pattern as that of velocity, as would be expected.

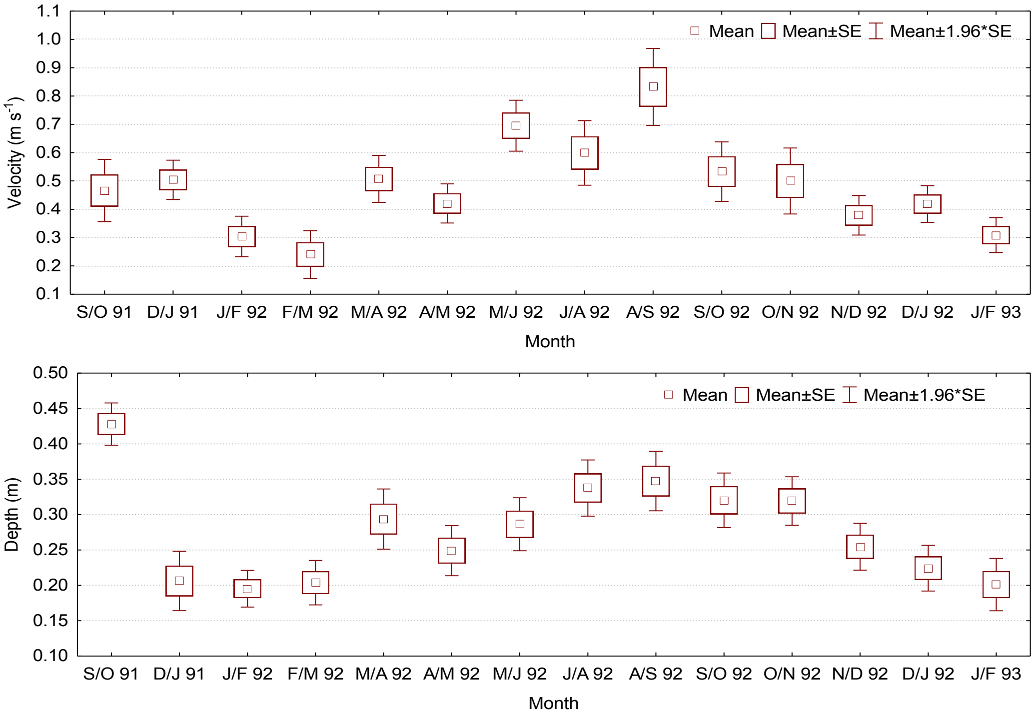

Figure 6.3. **Mean monthly water velocity and water depth in the Molenaars River, taken from random flow measurements at each site between September 1991 and February 1993. Site data are pooled because inter-site differences were not significant. Note the gap in data between September / October (S/O) 1991 and December / January (D/J) 1991, where data were not collected.**

6.3.1.ii.a.2 Water temperature

The simulated long-term maximum daily water temperature followed a very similar but inverse pattern to the flow regime (Figure 6.4). A two-way ANOVA returned significant differences in temperatures between calendar months, with significant differences also found between years (Table 6.1). Monthly temperatures showed a smooth pattern of decline and increase, from a peak in February to a July low (Figure 6.5), mirroring the pattern of average discharge (Figure 6.2). A post-hoc Turkeys test revealed non-significant differences ($p > 0.05$) in temperature between the mid-summer months of January and February, which were the only two consecutive months whose temperatures were not significantly different. Non-significant differences in temperature (all $p > 0.05$) were also found between March and December (early and late summer), between May and September (late autumn and early spring), and between June and August (early and late winter). April, October, November and July were different from all other months.

Table 6.1. ANOVA results for analysis of differences between months and over years in daily maximum water temperature in the Molenaars River. The analysis is based on simulated data

	Sum of Squares	Degrees of freedom	Mean square	F-statistic	p value
Year	777	23	33.8	5.4	0.0000
Month	96694	10	9669.4	1543.8	0.0000
Year x month	7201	263	27.4	4.4	0.0000
Error	55125	8801	6.3		

The relationship between temperature and discharge is apparent from the temporal sequences in Figure 6.4, but the simulated temperature series contains a flow term, and thus the data sets are not totally independent. In order to test the relationship between the two variables statistically, independent sample sets are required. Hourly temperature logging has been undertaken in the Molenaars River since October 2007 and a sequence of these data was used to examine how well mean daily flow and mean daily temperature were correlated. The results (Figure 6.6) show a strong, significant negative correlation between these variables (Pearsons correlation coefficient $r = -0.75$, $p << 0.001$), indicating that temperature, even measured independently of flow, tends to co-vary with discharge, and these two potential drivers of invertebrate pattern are very closely linked.

Significant inter-annual differences in simulated temperature were found in this study (Table 6.1). The years 1972, 1973, 1984-1986, 1993 and 1997, were warmer (Figure 6.7) and significantly different from many of the cooler years (Tukeys post-hoc test, $p < 0.05$). In relation to study period 1991 - 1993, the years 1991 and 1992 were not significantly different from each other, but both were significantly cooler than 1993.

The time series for discharge and simulated daily maximum temperatures over the period 1991-1993 are highlighted in Figure 6.8. Spot temperature data collected by DWAF at H1H018 are also shown. Most of these were collected in the early morning, however, and do not reflect maximum temperature.

Temperatures from April through July in 1991 were generally higher than over the same period in 1992, with means in 1991 between 1-2.5 °C higher than the corresponding months in 1992 (Figure 6.9). After the largest flood of the year, 31 July 1991, temperatures remained low until after the September floods in

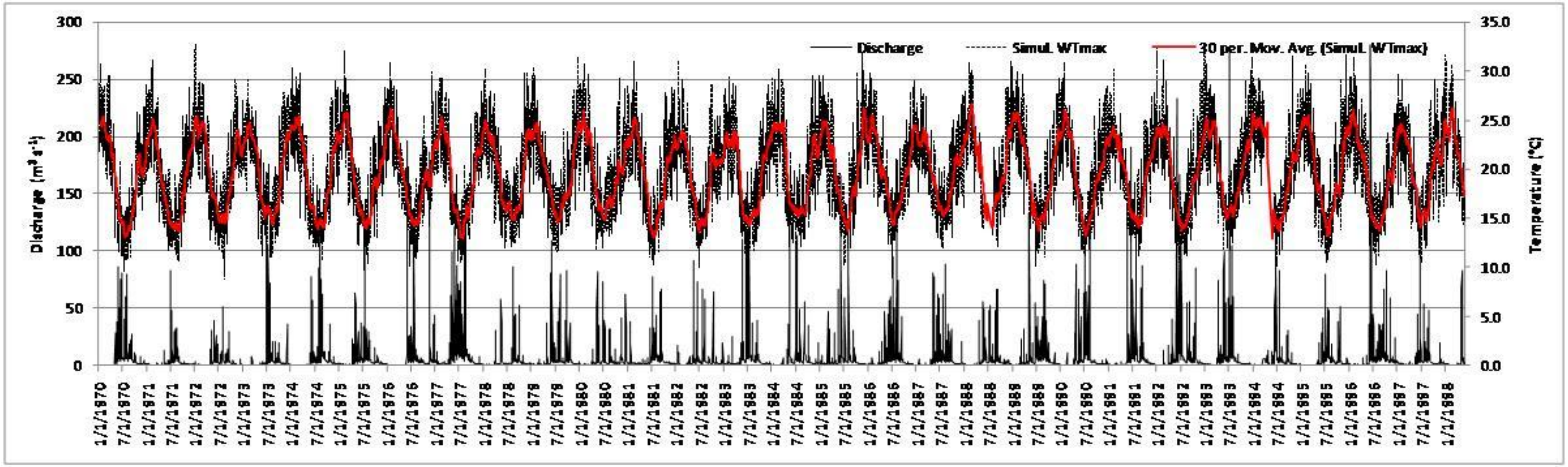

Figure 6.4. Time series of simulated daily maximum water temperature (WT_{max}) with daily flow data.

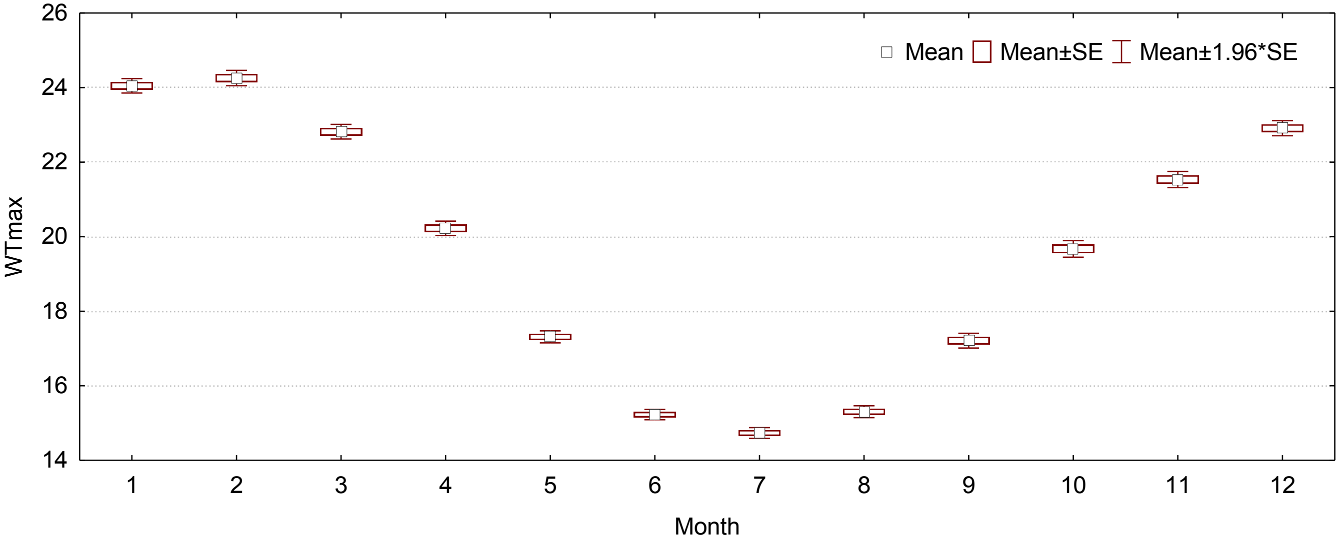

Figure 6.5. Summary daily maximum water temperature (WTmax, in ° C) in the Molenaars River, based on simulated data from 1970 to 1998. Calendar months are numbered in sequence.

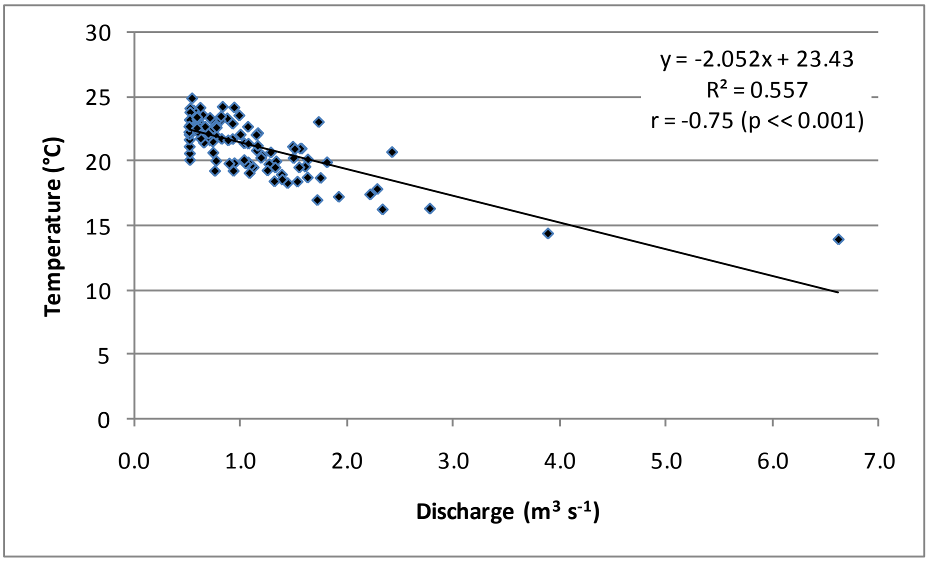

Figure 6.6. **Correlation between average daily discharge and average daily water temperature in the Molenaars River, based on logged temperature data over the period October 2007 to April 2008 (data courtesy of Justine Ewart-Smith, Freshwater Research Unit, University of Cape Town).**

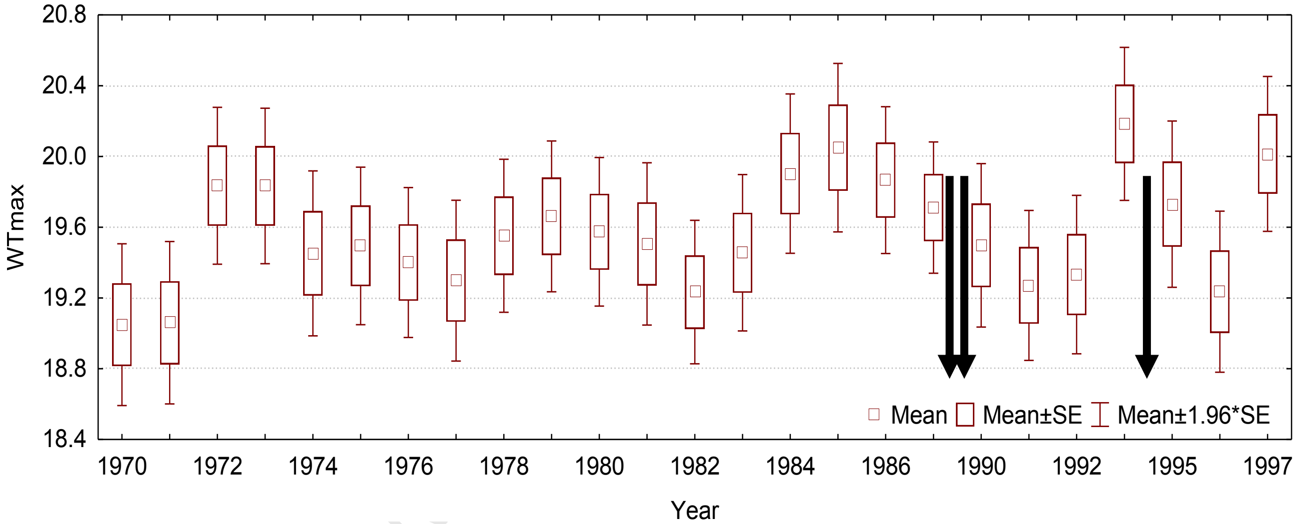

Figure 6.7. **Summary of annual statistics for simulated daily temperature maxima (WTmax, in ° C) in the Molenaars River, based on simulated data, for the years 1970 – 1997. Note the years 1988, 1989 and 1994 are not included in the analysis (indicated by arrows), because of the extent of missing data in these years.**

1991, with a relatively sharp increase only in late September (note the shape of the 30-day running mean for temperature in Figure 6.8). The opposite occurred in 1992: here autumn (March – May) was cooler, and the lowest temperatures were experienced in June and July 1992, with temperatures increasing from August 1992, a month earlier than in 1991 (Figure 6.9).

In relation to the second period for which invertebrate data were collected, 1994-1997, Figure 6.10 shows discharge and simulated daily maximum temperatures in the Molenaars River. Most spot readings taken by DWAF at H1H018 again do not correspond to maximum temperature, being collected in the morning hours. However, recent hourly data indicate that in winter temperatures vary diurnally by about 1.5 °C, which suggests that the simulated maximum temperatures may be overestimated for the winter period by approximately 1 °C. The simulated winter temperatures were higher than during the 1991-1993 period,

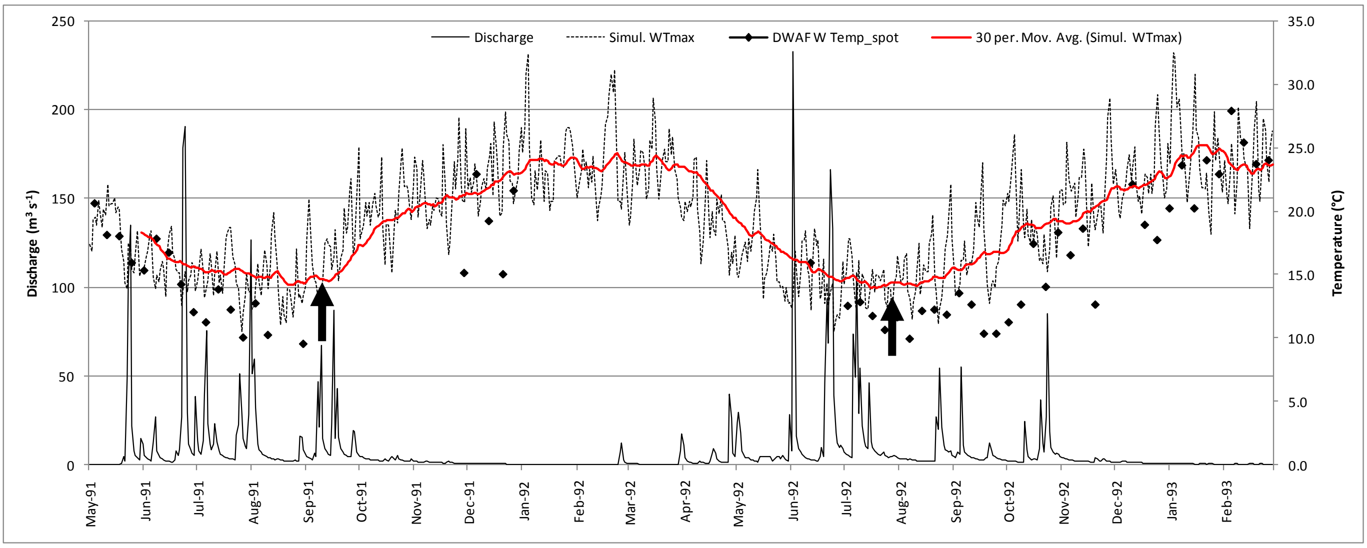

Figure 6.8. **Summary of simulated maximum temperature data (WTmax, in ° C) and daily average flow from the DWAF record at H1H018 over the 1991-1993 study period. The red line shows a 30-day running mean for the simulated temperature data set. Arrows indicate the onset of a seasonal increase in temperature, which differs in timing by about a month between the two years.**

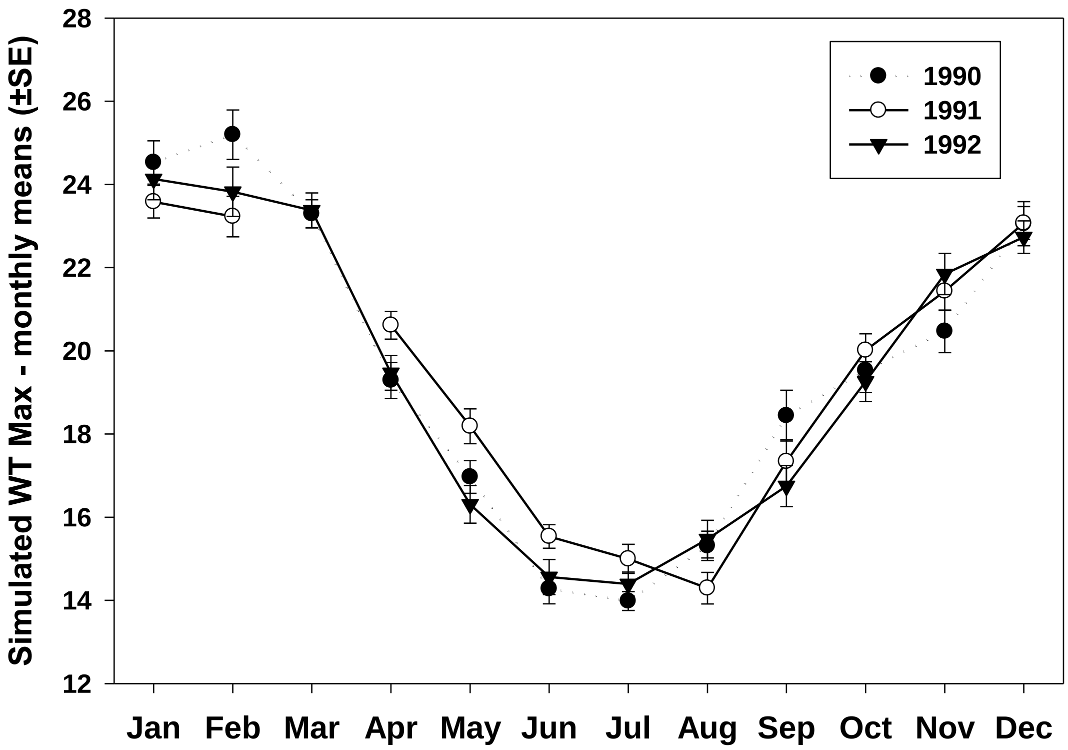

Figure 6.9. **Comparison of monthly mean values of simulated maximum daily temperature (WTmax, in ° C), for the years 1990 to 1993.**

evident by comparing the trend lines in Figures 6.8 and 6.10. Simulated mean monthly temperatures were warmer in July in 1994 than the other years, whilst the months from August through November in 1996 were considerably cooler than the previous two years (Figure 6.11)

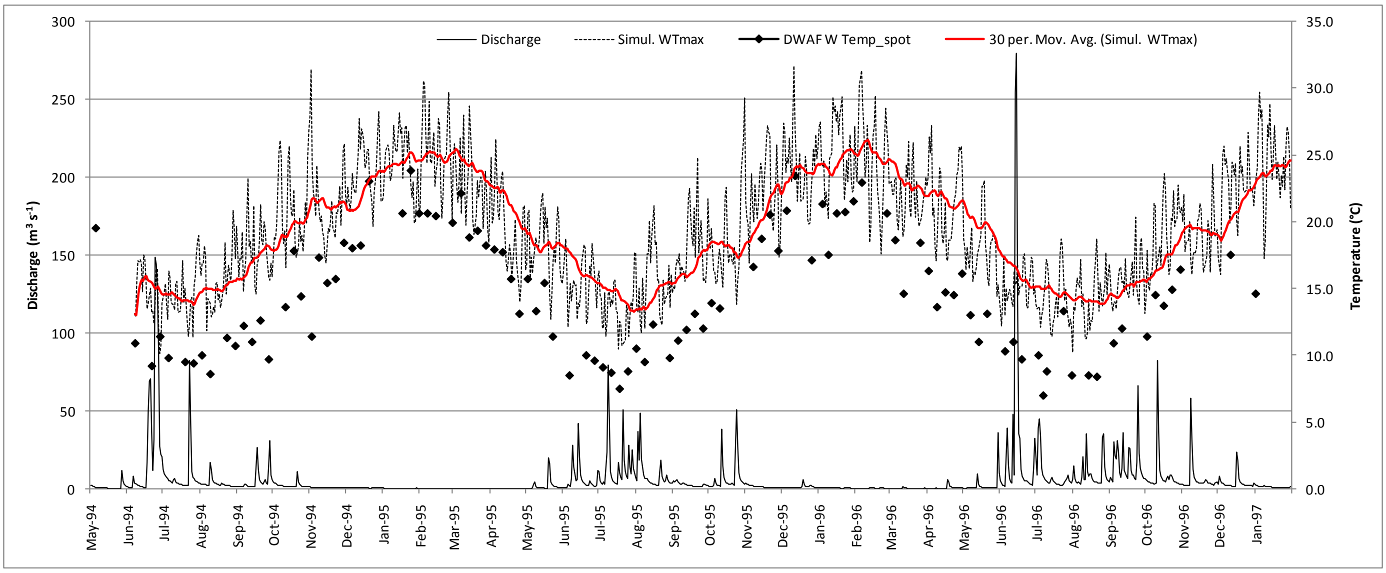

Figure 6.10. **Summary of simulated maximum temperature data (WTmax, in ° C) and daily average flow from the DWAF record at H1H018 over the 1994-1997 study period. The red line shows a 30-day running mean for the simulated temperature data set.**

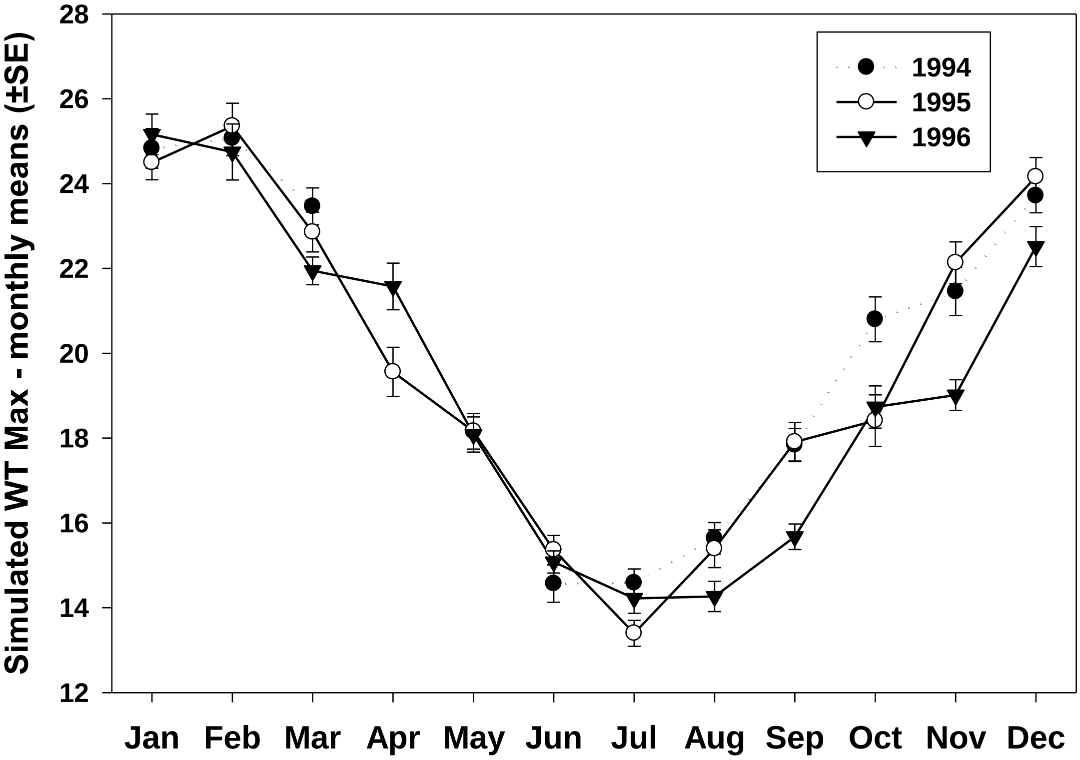

Figure 6.11. **Comparison of monthly mean values of simulated maximum daily temperature (WTmax, in ° C), for the years 1994 to 1997.**

6.3.1.ii.a.3 Chemical variables

Water chemistry data were analysed for monthly variation in concentration, and the time series was also explored over the period 1978 to 2007 to identify possible longer-term temporal patterns. Summary monthly data based on the full data set from the DWAF record are presented alongside summarised data from the field-collections made during the study period itself, excluding the data from Site 6 where significant differences were found between this site and the others (refer to section 6.3.1(i)).

The Molenaars River, typical of drainage systems in the Cape Fold mountain region, has pure, acidic water, as shown by the low pH and conductivity values in Figures 6.12 and 6.13. Both variables differed significantly between months (Kruskal-Wallis H (11, N=1227) = 141.7, p < 0.0001 and H (11, N=1218) = 39.9, p < 0.0001 respectively. pH shows a smooth pattern of seasonal pattern of change, with June and July minima and January / February maxima (Figure 6.12). Although January is also associated with maximum conductivity values (Figure 6.13), the data show a prolonged period from February through to May when conductivities remain very similar, before declining to July lows.

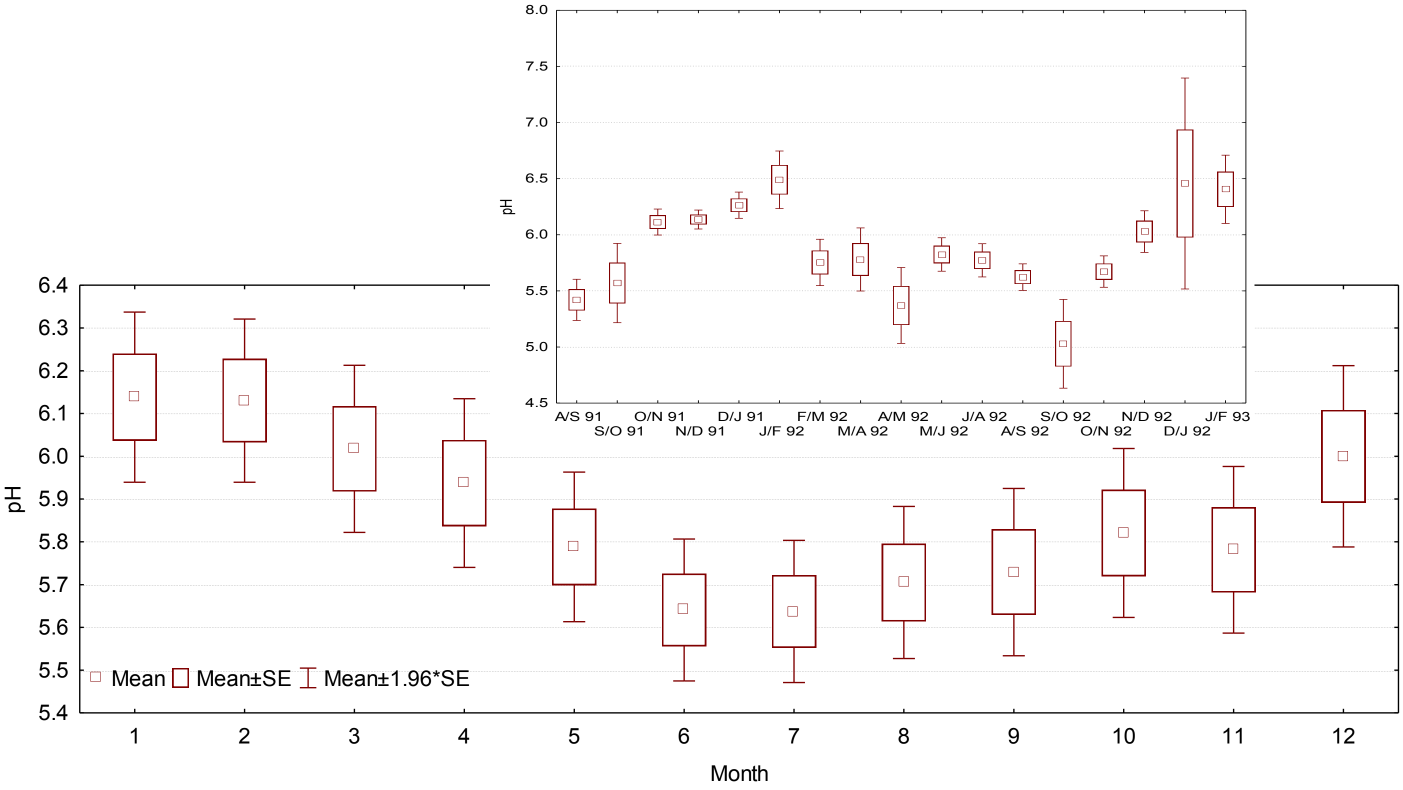

Figure 6.12. Summary of long-term pH data for each calendar month, from the DWAF record at H1H018, data collected weekly or monthly between 1978 and 2008, but not continuous. Inlay: summarised monthly data collected during the 1991-1993 sampling period for this study.

Although the pH values recorded from 1991-1993 at the study sites conformed to this longer-term pattern (inset in Figure 6.12), summer maxima of around 6.5 in both 1991/92 and 1992/93 were much higher than the long term averages for these months. The long-term data (Figure 6.12) show an abrupt increase in pH values in early 1990, sustained at more neutral pH levels for the remainder of the period of record, which may explain why the long term average is lower than values recorded in the 1990s. Statistical testing of the difference in pH between the pre- and post-1990 periods showed a significant increase from a mean pH of 5.2 to one of 6.5 (Mann-Whitney U Two-sample test, p < 0.001).

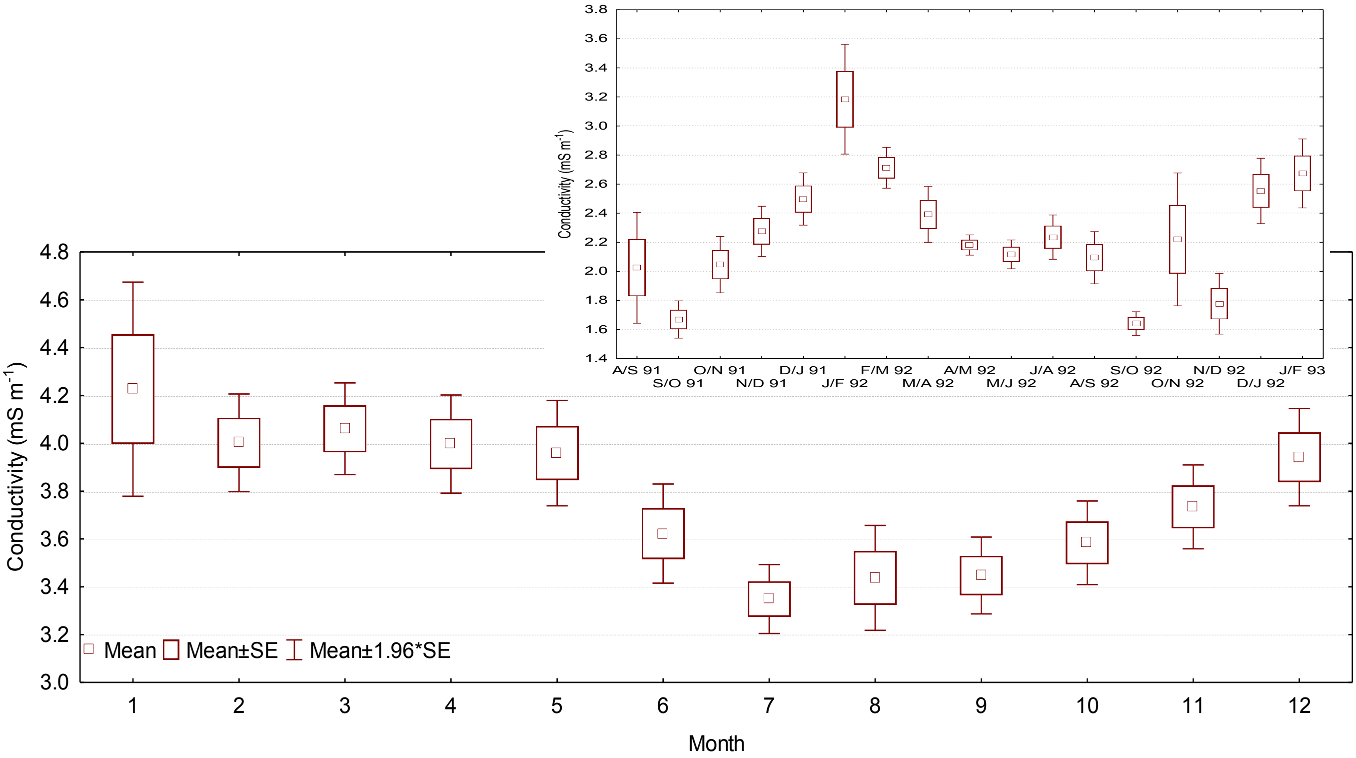

Figure 6.13. Summary of long-term conductivity data for each calendar month, from the DWAF record at H1H018, data collected weekly or monthly between 1978 and 2008, but not continuous. Inlay: summarised monthly data collected during the 1991-1993 sampling period for this study.

Conductivity values over the period 1978 – 2007 also show an increase, between 1983 and 1997, but this is less marked and the river can be characterised as low in dissolved salts, with values rarely exceeding 8 mS m^{-1} (Figure 6.14). This period of increased conductivity corresponds to the period during which both the road tunnel at the western end of du Toitskloof (immediately upstream of Site 1) and the bridges and road through du Toitskloof, were constructed.

The concentration of total inorganic nitrogen (the sum of ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N) and nitrite nitrogen (NO_2^--N) in the Molenaars River also showed significant differences between months (Kruskal-Wallis H (11, N=1170)= 73.3, $p < 0.0001$), but with a late summer (March) maximum and a mid-winter (July) minimum (Figure 6.15). The data also show a depression in these nitrogen compounds in February. Interestingly, the data collected during the 1991-1993 field study were characterised by highest values in the Jan / Feb '92 and Dec / Jan '93 periods, with lower values in Mar / Apr '92.

Orthophosphate was the only compound analysed that showed no seasonal pattern (Figure 6.16), with no significant differences between months (Kruskal Wallis test, p=0.3). Indeed, the time series plots for the nutrient data between 1978 and 2007 (Figure 6.17) show a trend of increasing concentrations particularly in orthophosphate levels, but also in total inorganic nitrogen. Silicon data from the same record are also indicated in Figure 6.17 as a comparison – these show very well defined seasonal patterns (March maximum, July minimum, as with inorganic nitrogen), but there is no trend in the data over the longer-term, 30-year time-period

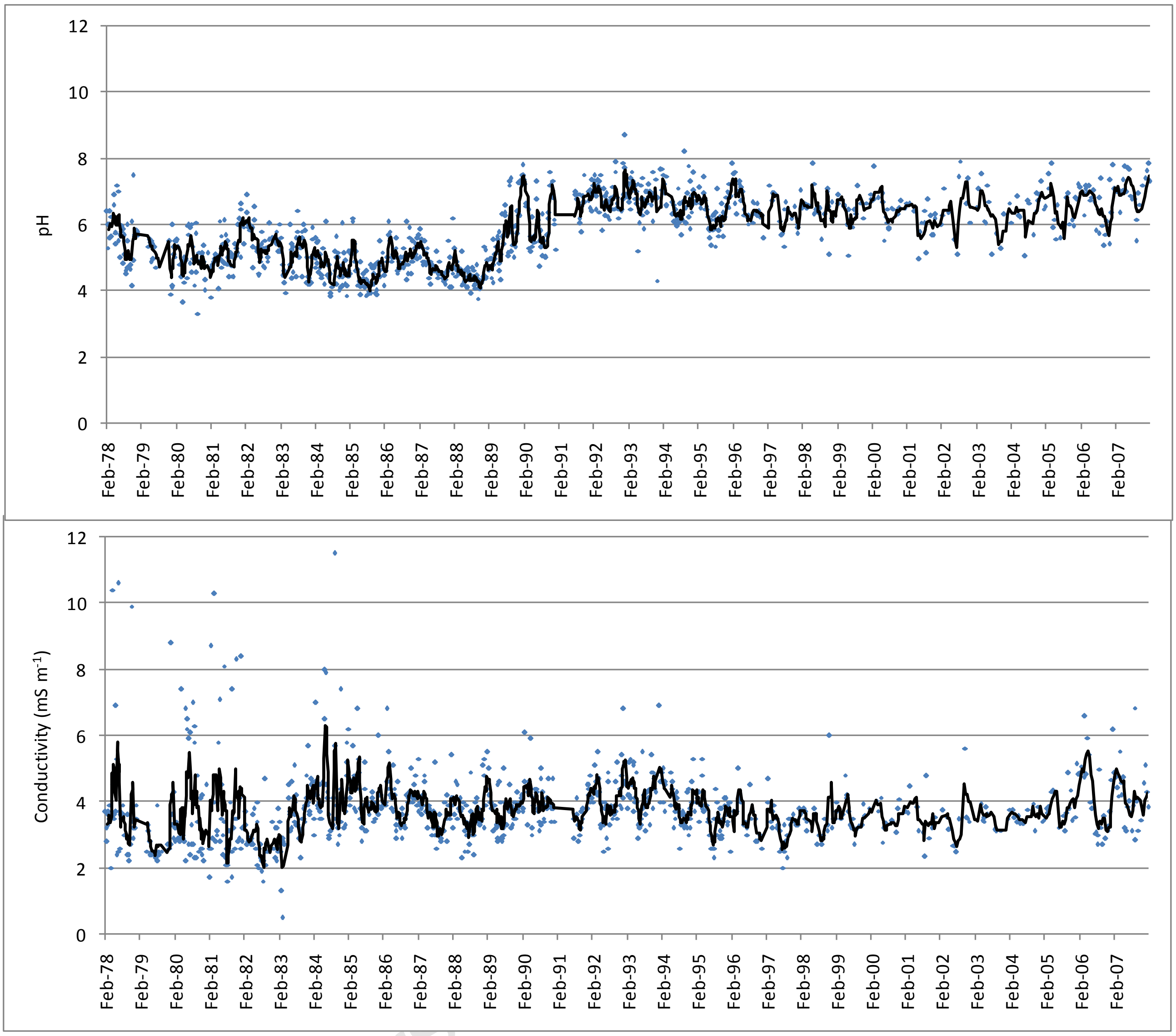

Figure 6.14. Temporal trends in pH and conductivity in the Molenaars River, based on data from DWAF gauging stationH1H018. The line represents the 5-sample moving average in each case.

Four time intervals were identified from examining these trends, based on dates from which the data suggested periodic jumps in phosphate levels: pre 1983, from 1983 – 1989; from 1990 – 1998; and from 1999 – 2007. Since the data were not normally distributed, even after transformation, Kruskal-Wallis analysis of variance was performed, with significant difference between the four time periods for both variables (Kruskal-Wallis H (3, N=696) = 385.7, $p < 0.0001$ and H (3, N=1017) = 136.4, $p < 0.0001$ for orthophosphate and total inorganic nitrogen respectively). Orthophosphate concentrations showed a steady increase over this period, whilst in the last decade total inorganic nitrogen declined from the high levels recorded in the 1990s (Figure 6.18).

Average values of total inorganic nitrogen under 0.5 mg l^{-1} are generally consistent with oligotrophic conditions (DWAF 1996), but where these are combined with phosphates concentrations between 0.015 and 0.047, this suggests mesotrophic conditions (DWAF 2002). The data in Figures 6.17 and 6.18 indicate oligotrophic conditions in the Molenaars River prior to 1983, but with increasing levels of mesotrophy, especially after 1990.

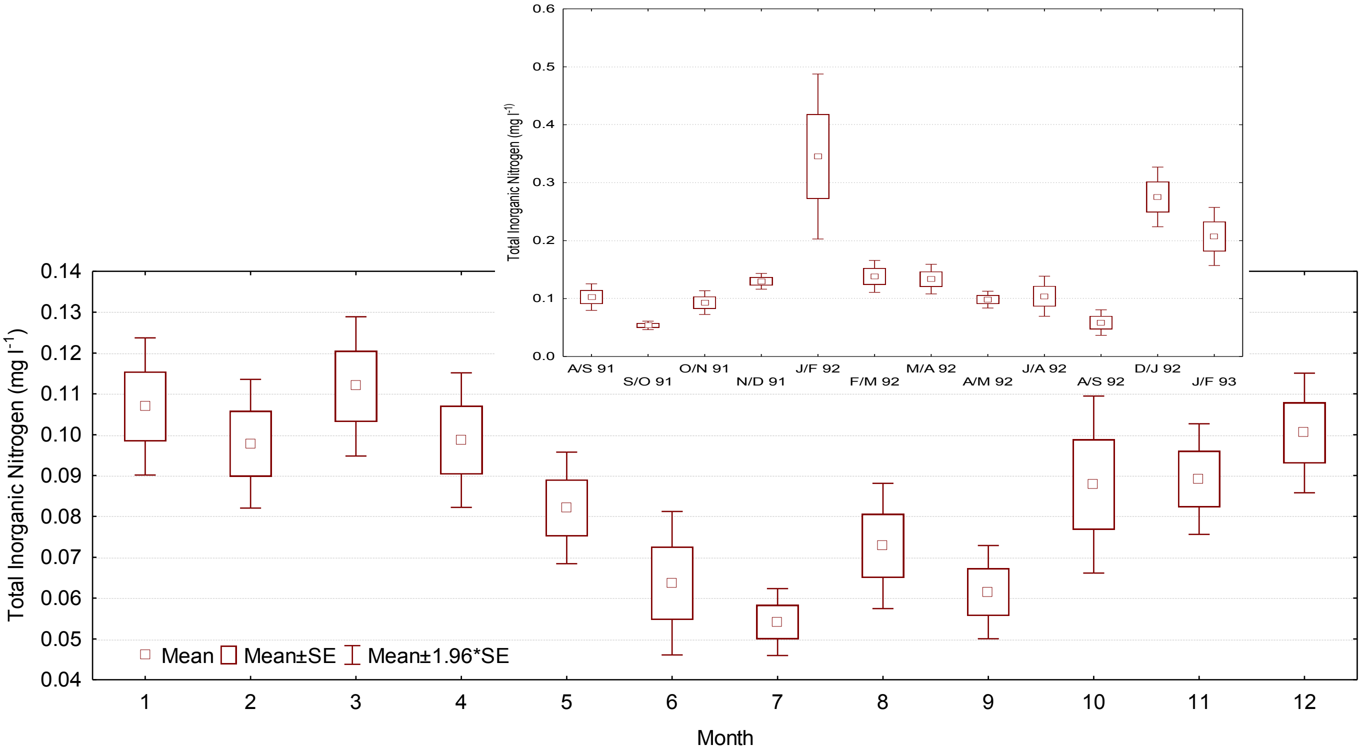

Figure 6.15. **Summary of long-term Total Inorganic Nitrogen (nitrate/nitrite/ammonia) data for each calendar month, from the DWAF record at H1H018, data collected weekly or monthly between 1978 and 2008, but not continuous. Inlay: summarised monthly data collected during the 1991-1993 sampling period for this study. Note that only some months are represented in the inlay data.**

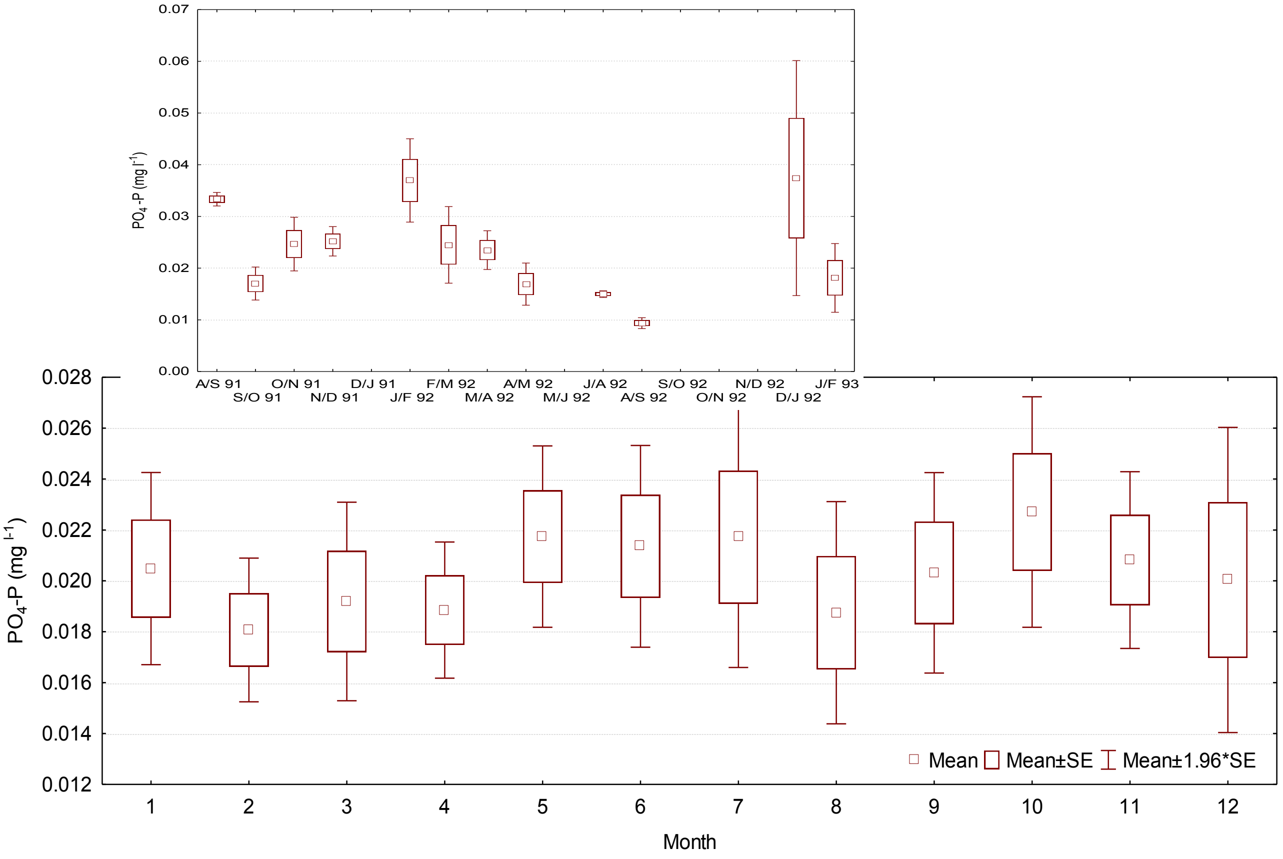

Figure 6.16. **Summary of long-term phosphate data for each calendar month, from the DWAF record at H1H018, data collected weekly or monthly between 1978 and 2008, but not continuous. Inlay: summarised monthly data collected during the 1991-1993 sampling period for this study. Note that only some months are represented in the inlay data.**

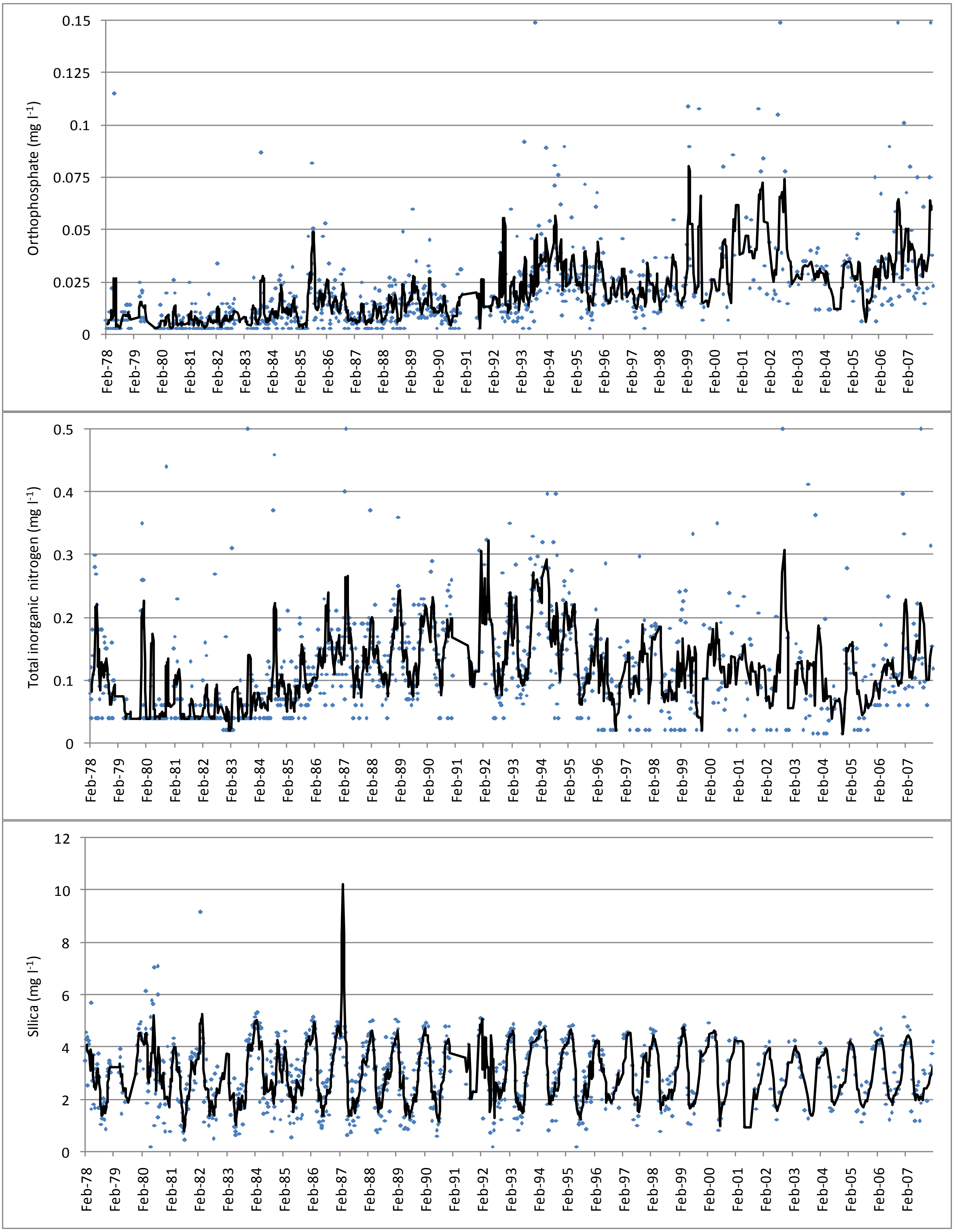

Figure 6.17. Temporal trends in Orthophosphate (PO_4^- -P). Total inorganic nitrogen (NO_3^- NO_2^- and NH_4^+ - N) and Silica (Si^+) in the Molenaars River, based on data from DWAF gauging stationH1H018. The line represents the 5-sample moving average in each case.

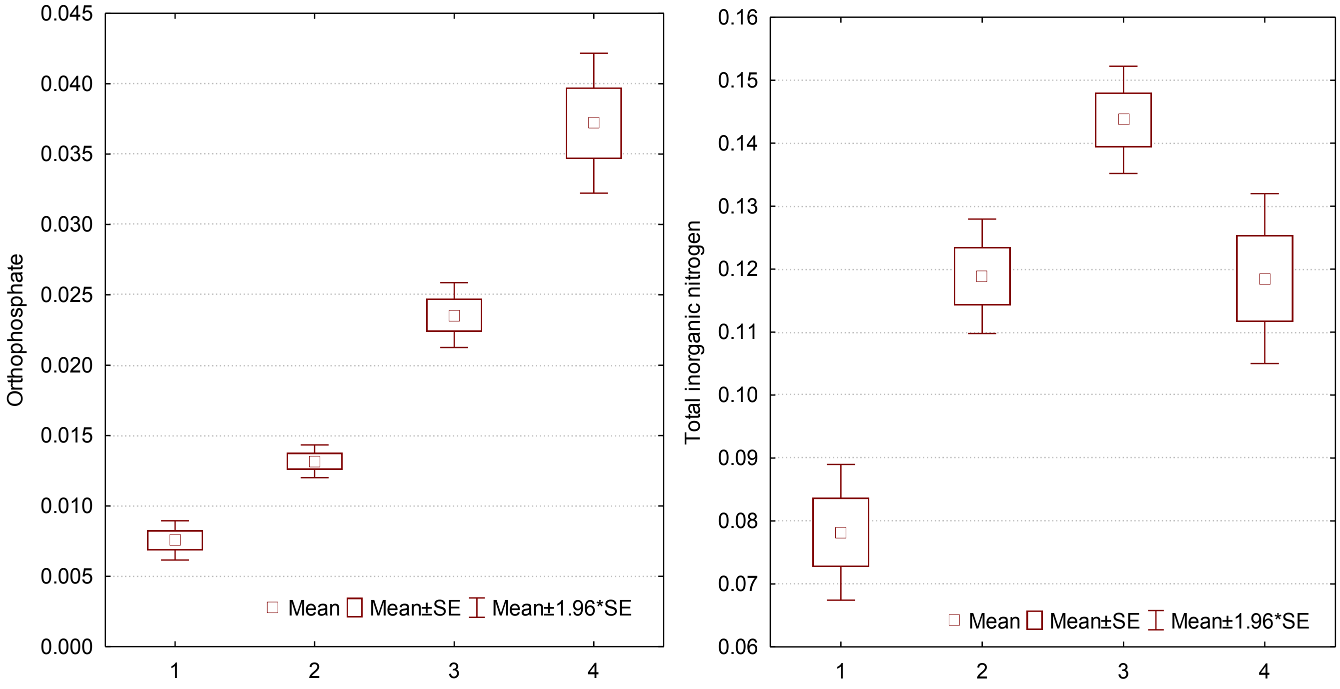

Figure 6.18. Box and whisker plots summarising nutrient concentrations (in mg l^{-1}) in the Molenaars River for four time periods: 1 = prior to 1983; 2 = 1983 – 1989; 3 = 1990 – 1998; and 4 = 1999 – 2007. Orthophosphate concentrations showed a steady increase over this period, whilst in the last decade total inorganic nitrogen has declined from the high levels recorded in the 1990s. Note that period 3 covers the field study undertaken for this thesis.

6.3.2 Periphyton biomass

The periphyton biomass data did not fulfil the assumptions of normality and equal variances, and thus Kruskal Wallis non-parametric one-way analysis of variance was used to examine differences between sites, conducted separately for each of the 17 monthly data sets. Significant between-site differences were found in seven of these data sets. These differences were largely due to significantly higher periphyton biomass values at Site 3 for most of the months sampled (Dunn's pairwise tests, $p < 0.05$). Although other differences between sites were not significant, overall mean periphyton density was higher not only at Site 3 but also at Site 6 and to a lesser extent Site 4, than at the other sites (Figure 6.19). Sites 3 and 6 were downstream of a trout hatchery and trout farm respectively, and the distance between sites 3 and 4 was less than 1 km.

Sites 3, 4 and 6 were thus removed from the data set for the analysis of temporal patterns in periphyton biomass, because of their generally elevated biomass, and the fact that the seasonal pattern of change at these sites obscured the temporal patterns evident at the other sites.

Although the monthly mean values for periphyton are associated with high variability (Figure 6.20), they illustrate a winter / spring period (A/S91 and A/S92, S/O92) where biomass was elevated, declining to low biomass in early-summer (N/D91, D/J91 and N/D92), after which biomass increased again in late summer in 1992 (J/F92 and F/M92). The low values in S/O91 are probably as a result of a spring flood of 87 $m^3 s^{-1}$ occurring just prior to sampling, with comparatively high discharge (5.5 $m^3 s^{-1}$) continuing through the sampling period. No marked increase in periphyton occurred in January-February 1993. In 1992 there was a second trough of lower autumn values (M/A92), which was the only autumn sampled for this study, as sampling was completed before autumn 1993.

Almost overriding this pattern, however, was a substantial difference in the periphyton densities for the same period in consecutive years. The red arrows in Figure 6.21 indicate the same period from late winter through spring in 1991 and 1992. These are characterised by much higher periphyton values in 1992: late September 1992 in particular had more than double the average periphyton biomass than the same period in 1991.

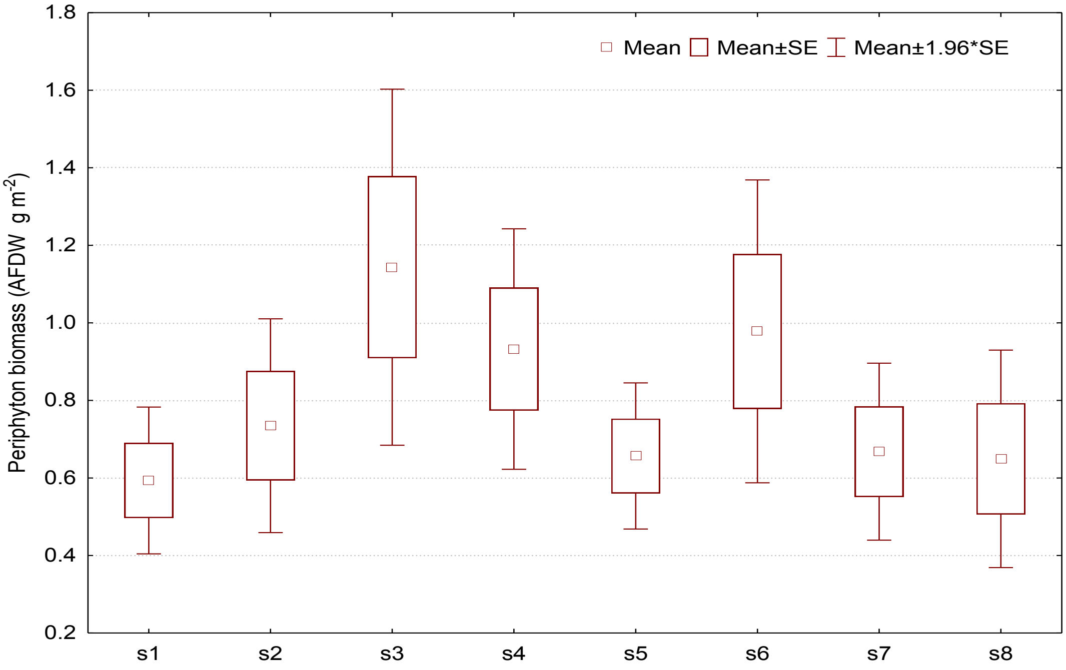

Figure 6.19. Mean and standard error for periphyton biomass at the eight sampling sites in the Molenaars River, based on the average biomass at each site over the full sampling programme.

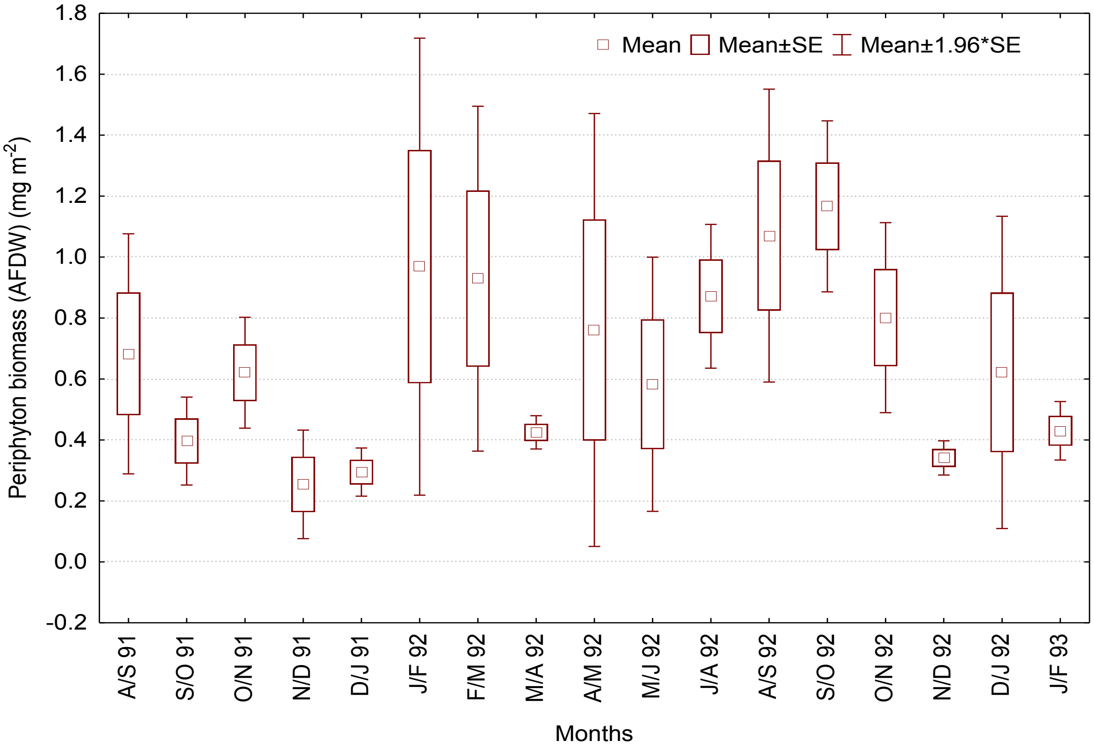

Figure 6.20. Periphyton biomass in the Molenaars River combined for Sites 1,2,5,7 and 8.

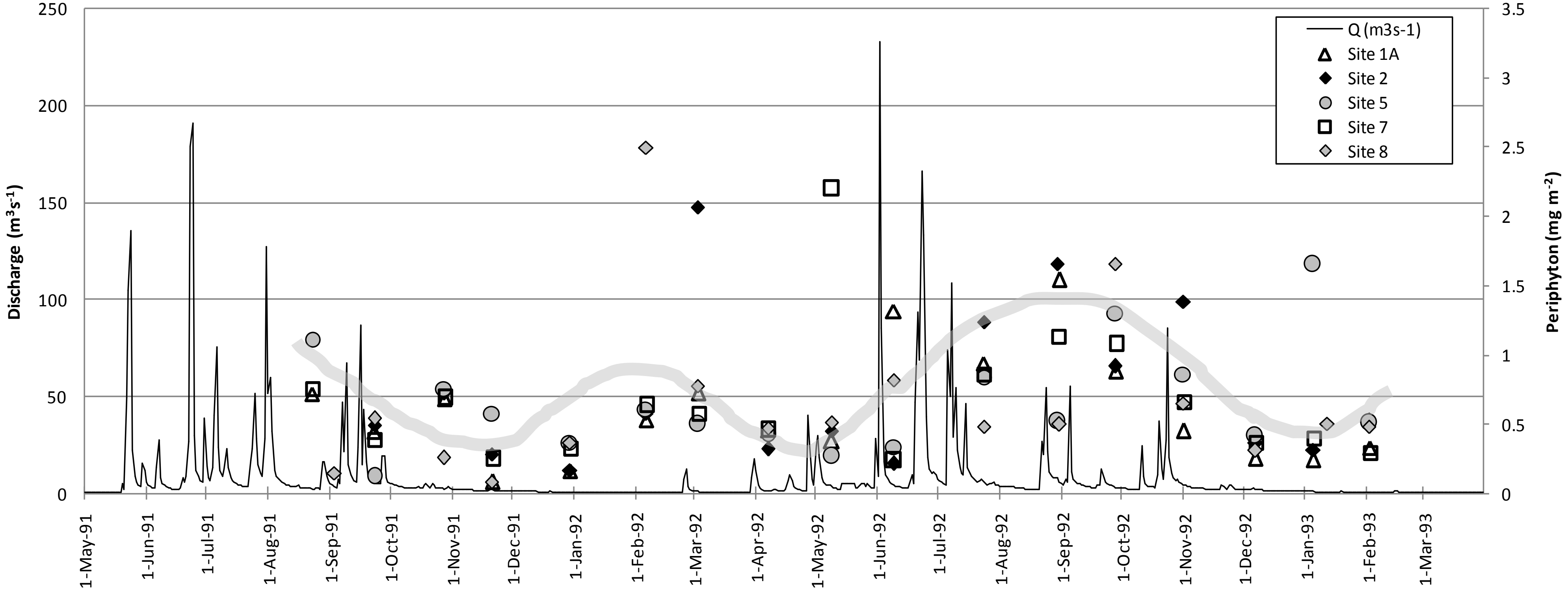

Figure 6.21. Changes in average periphyton density over time at each of the sampling Sites 1,2,5,7 and 8 in relation to the flow patterns over the 1991-1993 study period.

No correlation could be found between periphyton density and flow, measured as average daily discharge, or floods, measured as the number of days since the last flood with peak daily flow greater than 30 $m^3 s^-$, or flood frequency, as the number of such floods in the preceding 30- or 60-day period. There were also no significant correlations between periphyton density and temperature, measured as the maximum daily temperature, or as the average temperature since the last flood, or as the cumulative degree-days since the last flood or cumulative degree-days in the preceding 30-day period. The pattern of periphyton density, did not, therefore, follow simple seasonal patterns, either in relation to temperature or to flow. The data suggest low periphyton availability in late spring / early summer and again in autumn. However, periphyton density as measured in this study only represents what is present on the stream bed, not what might be being consumed by invertebrate grazers. For example, high invertebrate densities may alter periphyton biomass, but not necessarily its productivity. Changes in periphyton biomass are thus compared with fluctuations in densities of grazing invertebrates in the following sections, in order to draw some conclusions regarding the interplay of flow and temperature on both periphyton and invertebrate dynamics and to make preliminary remarks on the possible importance of periphyton to invertebrates in the Molenaars River.

6.3.3 Invertebrate assemblages

6.3.3.i Invertebrate patterns over the 1991 – 1993 sampling period

a Community structure

Cluster analysis and multi-dimensional scaling (MDS) were undertaken on both the full set of replicate samples, identified to family level, and on a second data set where the replicates were averaged for each site-time. Only the plots using averaged densities per site–time are presented here, for clarity of representation, although the patterns were highly similar in both cases. The data suggest strong seasonal shifts in invertebrate assemblage composition. Cluster analysis split samples into a broadly summer group and winter group, at 52% similarity (Figure 6.22a). The spring samples in 1991 grouped with the winter groups, whilst spring samples in 1992 grouped with the summer group, as did the autumn samples of 1992.

Within these main groups were number of further distinct clusters. These mostly represented reasonably distinct groupings of samples from each calendar month, circled in Figure 6.22b. In particular, the samples from each month during spring and autumn formed discrete clusters. In Figure 6.22b the groups are arranged in a circular pattern, indicating sequential change not only according to seasons but per calendar month. The 1991 winter and spring samples fell outside of this tight circle, being more widely dispersed. The S/O91 samples were closely linked with those of the previous month, and widely separated from the O/N and N/D 1992 groups.

The mid-summer samples also tended to be less distinct by month, and formed a series of overlapping groups in Figure 6.22b. Also, although the M/J92 samples formed a discrete group, those of the following two winter months J/A and A/S92 were interspersed with each other.

Another feature of the plots is that, whilst differences between sites mostly did not override the pattern of sequential monthly change, there were some anomalies as a result of inter-site differences. Site 5 in J/A92 and Site 5 and 6 in A/S92 were more similar to the A/S91 samples than the other 1992 winter sites. These sites were affected by a landslide in the valley at that time, which resulted in massive quantities of sediment smothering the river bed at Site 5, until these were scoured in the late winter floods. The samples

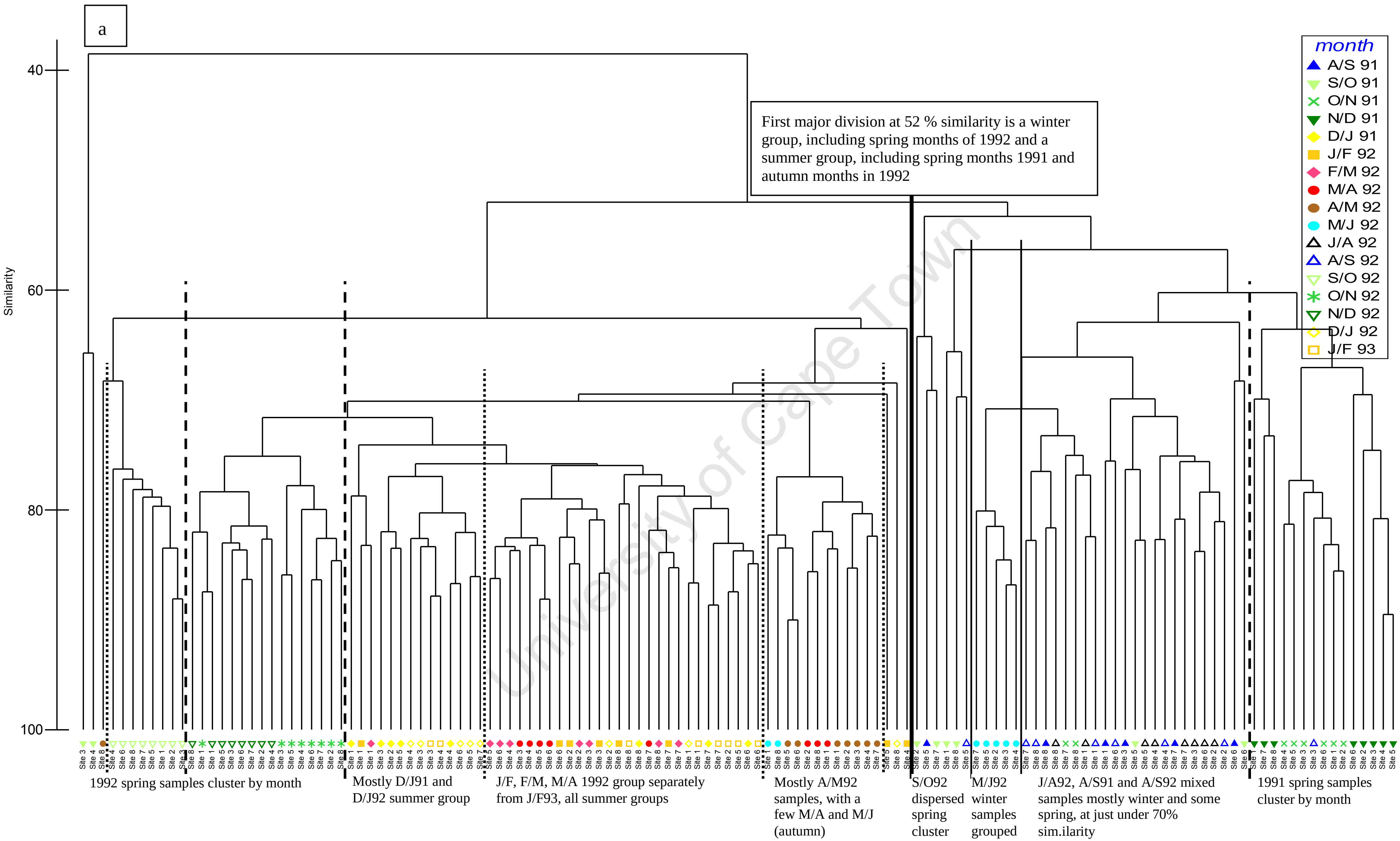

Figure 6.22a. **Cluster analysis plot of the relationship between invertebrate assemblages at eight sites in the Molenaars River, and over time from monthly samples in August / September 1991 (A/S 91) through to January / February 1993 (J/F 93).**

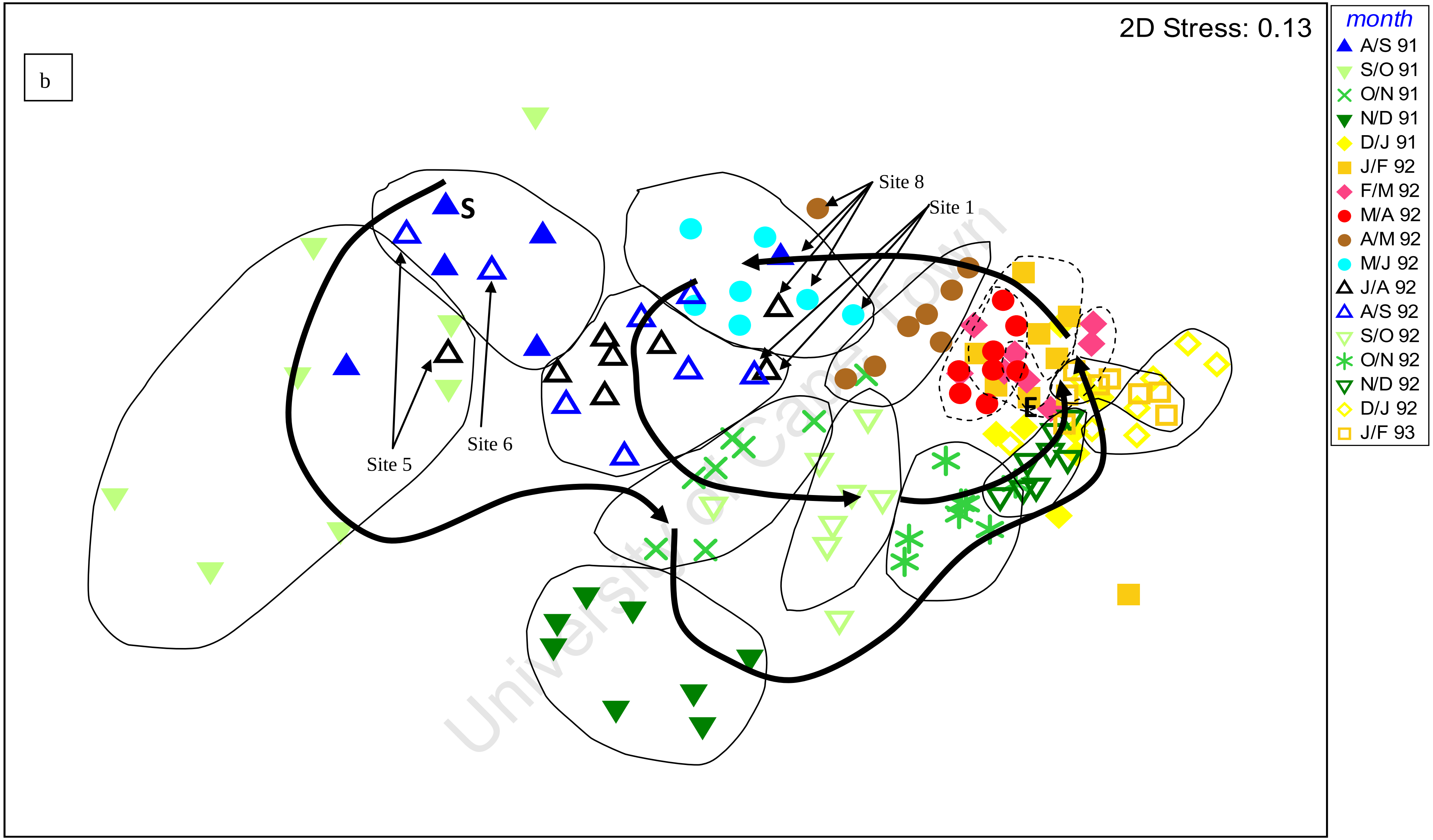

Figure 6.22b. MDS plot of the relationship between invertebrate assemblages at eight sites in the Molenaars River, and over time from monthly samples in August / September 1991 (A/S 91) through to January / February 1993 (J/F 93). Distinctive monthly groups of samples are circled; overlapping months in summer 1992 are circled with dashed lines. Black arrows indicate the temporal sequence from start (S) to end (E) of sampling. Arrows indicate samples discussed in the text.

at these sites on these occasions were virtually devoid of any animals, as a result of this, albeit temporary, loss of favourable habitat.

Differences in assemblage composition at the sites and over months were explored with a two-way crossed multivariate analysis of similarities (ANOSIM routine in PRIMER) using the replicate density data for each site. Differences between individual months far outweighed differences between sites (Table 6.2), with a Global Rho-value for month differences of 0.740, compared with 0.404 for site differences. An R-value of 1 denotes that all the replicates within one group are more similar to each other than they are to any of the replicates of another group, and thus that the groups are perfectly discriminated.

The pairwise tests for each of the pairs of sites that were different were significant in all cases, except for Site 3 versus Sites 2 and 4. However, the R-statistic was small in most pairwise comparisons of sites, suggesting inconsequential differences in their invertebrate assemblages. R-values for the five pairs of sites exhibiting high R-value (most different from each other) are indicated in Table 6.2. These largely concerned Sites 3, 4 and 5, which were significantly different overall from Sites 1 and 8.

Table 6.2. Results of a 2-way ANOSIM testing for significant differences in invertebrate assemblages between sites and sampling times (months) in the Molenaars River between 1991 and 1993.

Groups	R Statistic	p-Value
GLOBAL TEST - differences between sites	0.404	0.001
Site 8 vs. Site 3	0.702	0.001
Site 1 vs. Site 5	0.691	0.001
Site 8 vs. Site 5	0.671	0.001
Site 8 vs. Site 4	0.622	0.001
Site 1 vs. Site 4	0.611	0.001
GLOBAL TEST - differences between months	0.740	0.001

The pairwise tests for between-month differences involved a number of permutations, as a result of having 17 months to compare. Significant differences were found between all pairs of samples, with the exception of F/M92 versus M/A92. That a summer assemblage differs significantly from a winter one is hardly surprising, but of greater interest is the change in assemblage from month to month. These results demonstrated significant shifts in community assemblage, not only seasonally, but on a month by month basis, with the exception of F/M92 versus M/A92. The strength of this month-to-month difference is shown by the R-value in Figure 6.23. Working consecutively through the figure, the first major shift in community assemblage occurred between the late September sampling in 1991 (S/O 91) and the late October sampling (O/N 91), as denoted by a high R-value, and again a large shift between late November (N/D 91) and late December (D/J 91). This strong month-to-month discrimination became less pronounced during the summer months, with a both very small and statistically non-significant difference between February / March 1992 and late March / April 1992 (F/M92, M/A92 in Figure 6.23). A large change was then apparent between late April / early May and May/June samples (R-value= 0.736), this interval being characterised by the largest flood over the sampling period (see Figure 6.21). Indeed, this flood had a recurrence interval of just under 20 years. It is noteworthy, however, that the Apr/May 92 sampling was carried out after two floods of 30 – 40 m^3 s^{-1} (a threshold for disturbance, as defined in Chapter 5), but the larger shift in assemblage structure came only with this larger June flood.

The two winter months following the M/J92 sampling were increasingly less distinct, mirroring the overlapping positions of these samples in Figure 6.20b. However, the transition from winter to spring in 1992 was once again characterised by a high level of discrimination, on a monthly basis. This time, however, the largest R-value was between late August (A/S 92) and late September (S/O 92), a month earlier than in 1991. This period also had a large shift between late November (N/D 92) and late December (D/J 92), as with the 1991 year.

Thus key shifts in invertebrate assemblages, where consecutive months are associated with high levels of discrimination in the fauna, appear to be a winter-spring "jump", marked shifts in the early to mid-summer period, but relative stability in late summer and early autumn, and a further marked change at some threshold associated with winter conditions.

Correlation between community shifts and environmental variables is discussed in the next section.

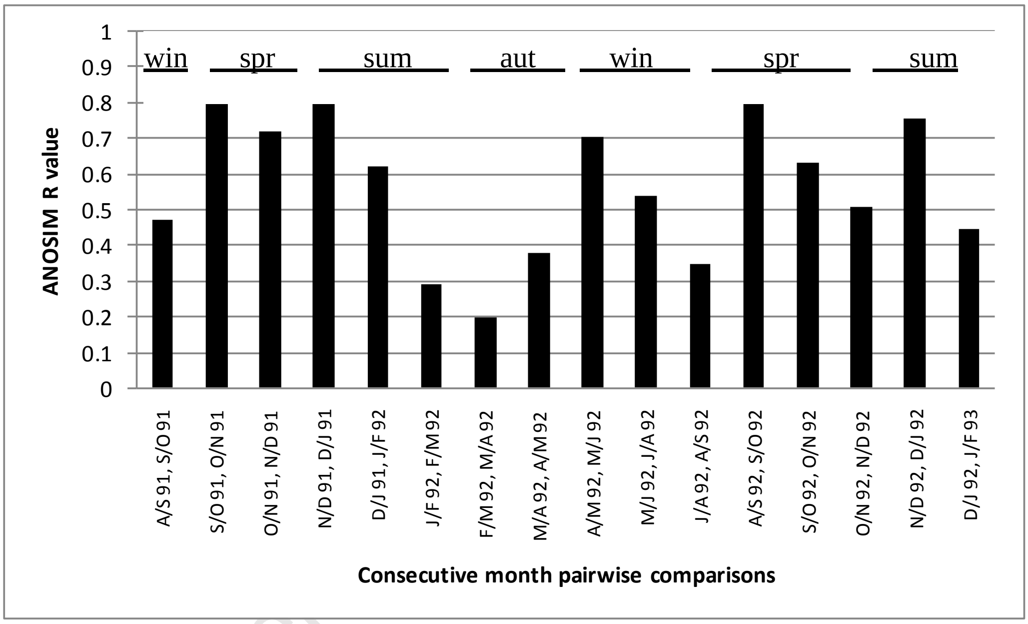

Figure 6.23. ANOSIM pairwise test R-values between consecutive calendar months in the Molenaars River, from August / September 1991 (A/S 91) through to January / February 1993 (J/F 93). The Global Rho value for differences between times was 0.753, indicating a high level of temporal change in invertebrate assemblages composition.

b Univariate community measures

A range of univariate measures was examined, in relation to their month-to-month dynamics. These were total invertebrate density (N), taxon density (S, the number of taxa recorded in each sample, expressed per m^2), rarefied taxon richness (ES, rarefied taxon richness), Shannon Wiener diversity (H'), and Pielou's evenness (J'). However, because of the site difference evident in the PRIMER analysis, only selected sites were used for this reach averaging. To select these sites, Kruskal Wallis non-parametric one-way analysis of variance was used to identify differences between sites, conducted separately for each of the 17 monthly data sets, for each of the afore-mentioned univariate measures.

Few significant between-site differences were found (Kruskal-Wallis $p > 0.05$) for all of these variables, and in almost all cases where differences were found, pairwise comparisons were either not significant (Dunn's pairwise test, all $p < 0.05$) or in some instances indicated differences between Site 1 and Sites 3, 5 and 6, and between Site 8 and Sites 3, 4, 5 and 6. This was a similar result to the PRIMER differences between sites (see Table 6.2) and considered to be linked to the slightly enriched conditions affecting the middle portion of the study reach. These findings justified the use of Sites 1, 2, 7 and 8 for the calculation of reach averages, for the exploration of the dynamics the univariate community measures.

Monthly fluctuations in all variables are evident from Figure 6.24, as well as large differences in density and species richness in the spring months of 1991 versus 1992 (colour coded green in Figure 6.24). Spring and summer months were associated with higher invertebrate densities, compared with the other seasons. However, diversity tended to be highest in late summer, autumn and winter, probably because of the combination of moderate to high species richness and high evenness in these months. Spring, and especially the spring of 1992 where numbers were considerably elevated, showed the lowest diversity, evenness, taxon density and rarefied taxon richness. This is consistent with the multivariate results which showed massive dominance by baetids and chironomids in spring, but growing numbers of less abundant taxa during the summer and autumn months.

c Relationships between invertebrate assemblages and abiotic measures

Given the seasonality of both temperature and flooding, possible relationships between these variables and the community dynamics were explored.

For the temperature effect, cumulative temperature degree days for the 14-day and for the 30-day period prior to each sampling event were highly correlated (Pearsons $r = 0.961$, $p < 0.05$), and thus the latter was chosen to explore the temporal influence of temperature on invertebrate assemblage structure. With regard to flow disturbance, three measures were examined: the number of days of flood flow above the disturbance threshold (defined in Chapter 4 as 30 $m^3 s^{-1}$) in the preceding 30-day and in the preceding 60-day periods, the peak flow in the 30-day period prior to sampling, and the number of days of low flow since the last flood > 30 $m^3 s^{-1}$. These represent a flood frequency term, a flood magnitude term and a post-flood recovery term respectively.

There was a high correlation between the number of floods measured over a period of 30 versus 60 days (Pearsons $r = 0.926$, $p < 0.05$). Both this and the peak flow and the number of days since the last flood were also strongly correlated with each other and with the temperature variable i.e. cumulative degree days over 30-d period prior to sampling (Table 6.3), although the relationships were not linear in the case of the number of floods versus the number of days since flooding. The significant correlations indicate the close relationship between floods and temperature. The regression equation used to model temperature did contain a flow term, which might contribute to the correlation between these flood and temperature measures, but for high flows this term was relatively insignificant compared to the other terms in the regression.

These temperature and flood variables, which were considered to be potential drivers of invertebrate assemblages, were used in the PRIMER routine BIOENV to identify the environmental variables most strongly correlated with the pattern of community change. In addition, the relationships between each of these variables and the univariate indices data were explored graphically and statistically.

The total invertebrate densities were lowest during the wet season, during the period of frequent flooding, and increased after the cessation of floods (Figure 6.25a and d). There were four occasions when substantial (M/J92) or unexpected (S/O91, N/D91 and A/S92) reductions in total densities were observed.

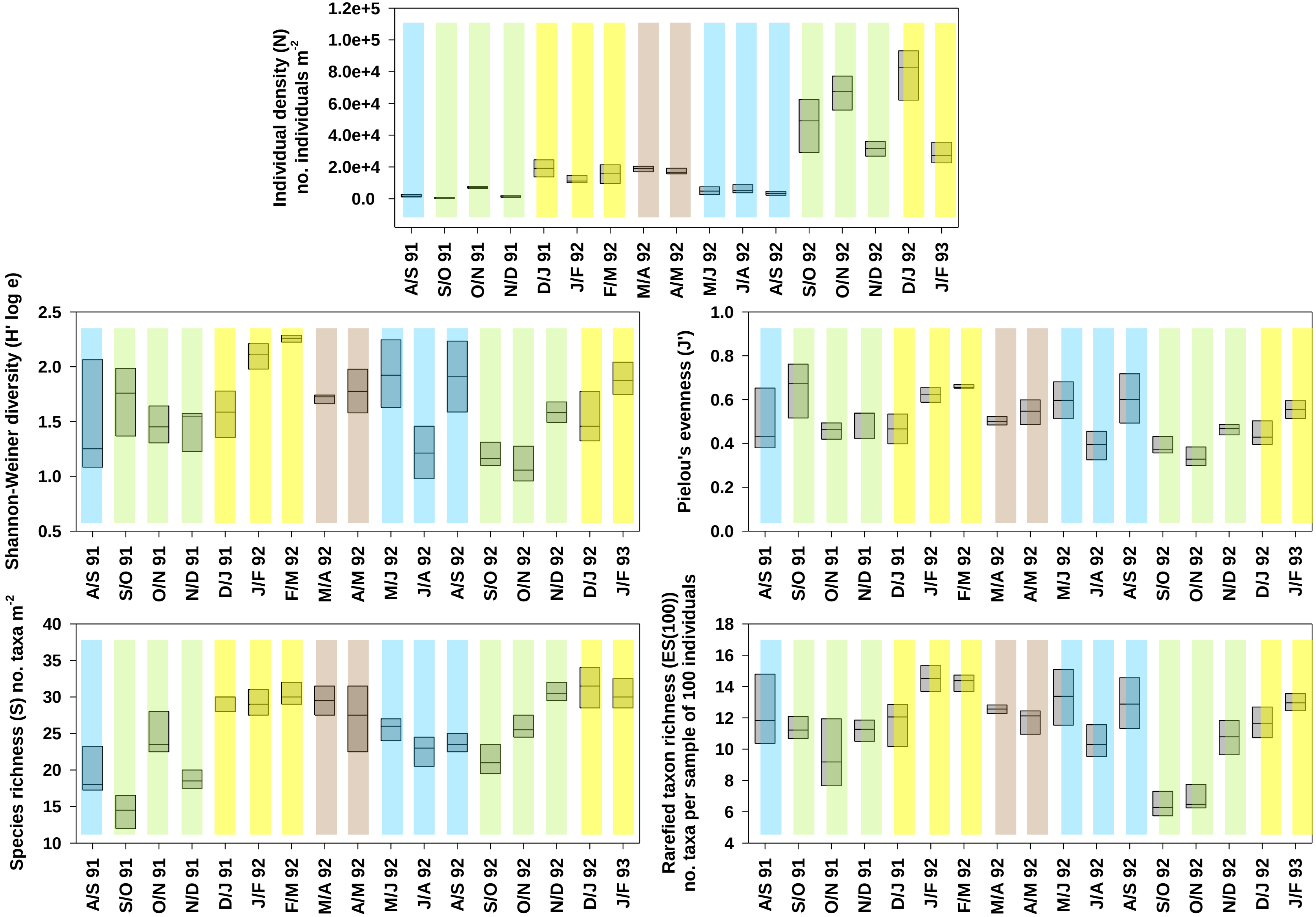

Figure 6.24. Seasonal patterns in total invertebrate density (N), Shannon Wiener diversity (H') and Pielou's evenness (J'), taxon density (S, the number of taxa recorded in each sample, expressed per m^2) and rarefied taxon richness (ES, the number of taxa in a sample of 100 individuals). Box = 2 x Std. Error; division in box = median value. Seasonal affiliation indicated by blue (winter), green (spring), yellow (summer) and brown (autumn). Data pooled from Sites 1,2,7 and 8.

Table 6.3. Results of Pearson's correlation analysis between temperature and three disturbance measures: cumulative temperature in the 30 days preceding sampling, number of floods in the previous 60-day period, peak flow in the previous 30-day period and number of days since the last flood > 30 $m^3 s^{-1}$. n = the number of data points used in the correlation, r = the correlation coefficient and p = the level of significance.

Variables tested	n	r	p
No. days since last flood > 30 $m^3 s^{-1}$ vs. cumulative temperature for 30 d prior to sampling	17	0.840	<< 0.0001
No. floods in 30-d period prior to sampling vs. cumulative temperature for 30 d prior to sampling	17	-0.795	<< 0.0001
Peak flow in 30 d period prior to sampling vs. cumulative temperature for 30 d prior to sampling	17	-0.852	<< 0.0001
No. days since last flood > 30 $m^3 s^{-1}$ vs. no. floods in 60-d period prior to sampling	17	-0.820	<< 0.0001
No. days since last flood > 30 $m^3 s^{-1}$ vs. Peak flow in prior 30 d period prior to sampling	17	-0.824	<< 0.0001
Peak flow in prior 30 d period prior to sampling vs. no. floods in 60-d period prior to sampling	17	0.704	<< 0.0001

immediately prior to sampling, and this explains these deviations in the overall trends. The M/J92 decline in densities was not unexpected, but was large, and followed from a 230 $m^3 s^{-1}$ flood four days earlier. The sampling dates S/O91 and A/S92 were also associated with floods in the week preceding sampling, albeit smaller than the June 1992 flood. The fact that the densities in these later winter / spring months were even lower than those caused by the 230 $m^3 s^{-1}$ flood may indicate that the magnitude of a flood beyond some threshold may not cause ever-increasing degrees of population reductions, or equally could be associated with the timing of the flood: densities prior to the S/O91 and A/S92 sampling periods were already very low, and immediate post-flood recovery may have been thus inhibited. The unexpectedly low invertebrate densities in N/D91, compared with the trend of increasing numbers during spring, are not explained by any of the flood or temperature measures.

What is also apparent in Figure 6.25 is that there were very large inter-annual differences not only in summer densities, but also the timing of when densities increased from winter lows to spring / summer highs: D/J in 1991 versus S/O in 1992. Inter-annual differences in the frequency of flood flows (days of discharge greater than 30 $m^3 s^{-1}$) were fairly marked, with S/O91 having four times the number of flood-days than S/O92 (Figure 6.25a). The months of A/S and S/O 91 were also characterised by higher peak flows than the corresponding period in 1992 (Figure 6.25c).

Figure 6.25b demonstrates the change in invertebrate density as against cumulative temperature in the 30 days preceding sampling. The large inter-annual variation in density was not matched by similar inter-annual differences in temperature, except for slightly higher values in A/S and S/O in 1992 compared with the previous year. Similarly, the number of days since flooding did not appear to match the inter-annual differences in total invertebrate density (Figure 6.25d).

On the other hand, taxon density (Figure 6.26) was far less subject to inter-annual variation, and showed a very close fit with the cumulative temperature, from visual inspection of the plot (Figure 6.26b), with the exception of the months S/O91 and N/D91, already discussed.

Correlation analysis revealed significant relationships between the temperature and flood frequency (Fld60) variables and both invertebrate density ($\sqrt{\sqrt{N}}$) and taxon density (S) (Figure 6.27), but not with

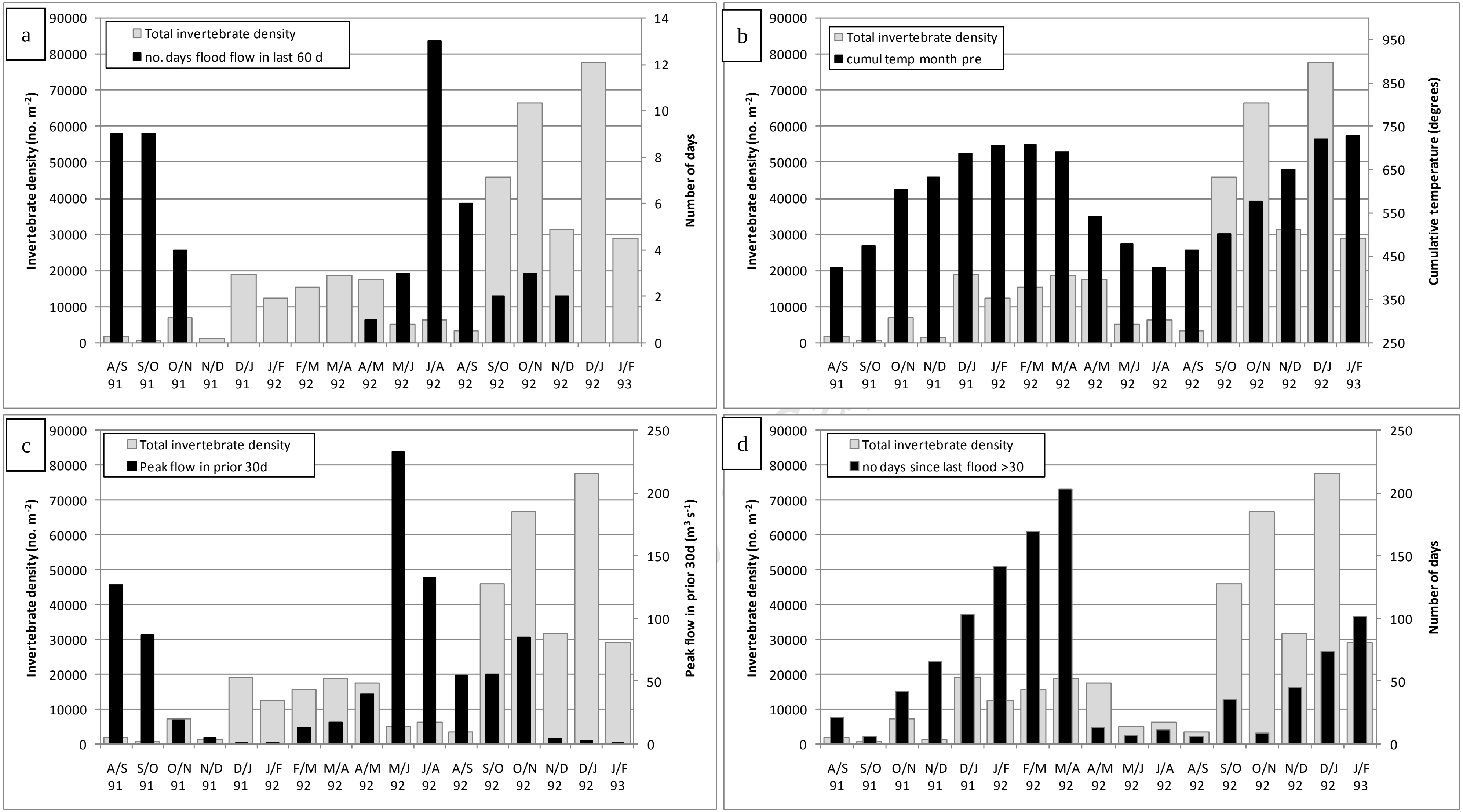

Figure 6.25. Total invertebrate densities in the Molenaars River, based on averages from Sites 1, 2, 7 and 8 over the 1991-1993 study period, alongside (a) number of days of flooding (over 30 m^3 s^{-1}) in the 60- day period prior to the date of sampling; (b) cumulative temperature in the 30-day period prior to sampling; (c) peak flow in the 30 days prior to sampling; and (d) the number of days since flood flows > 30 m^3 s^{-1}.

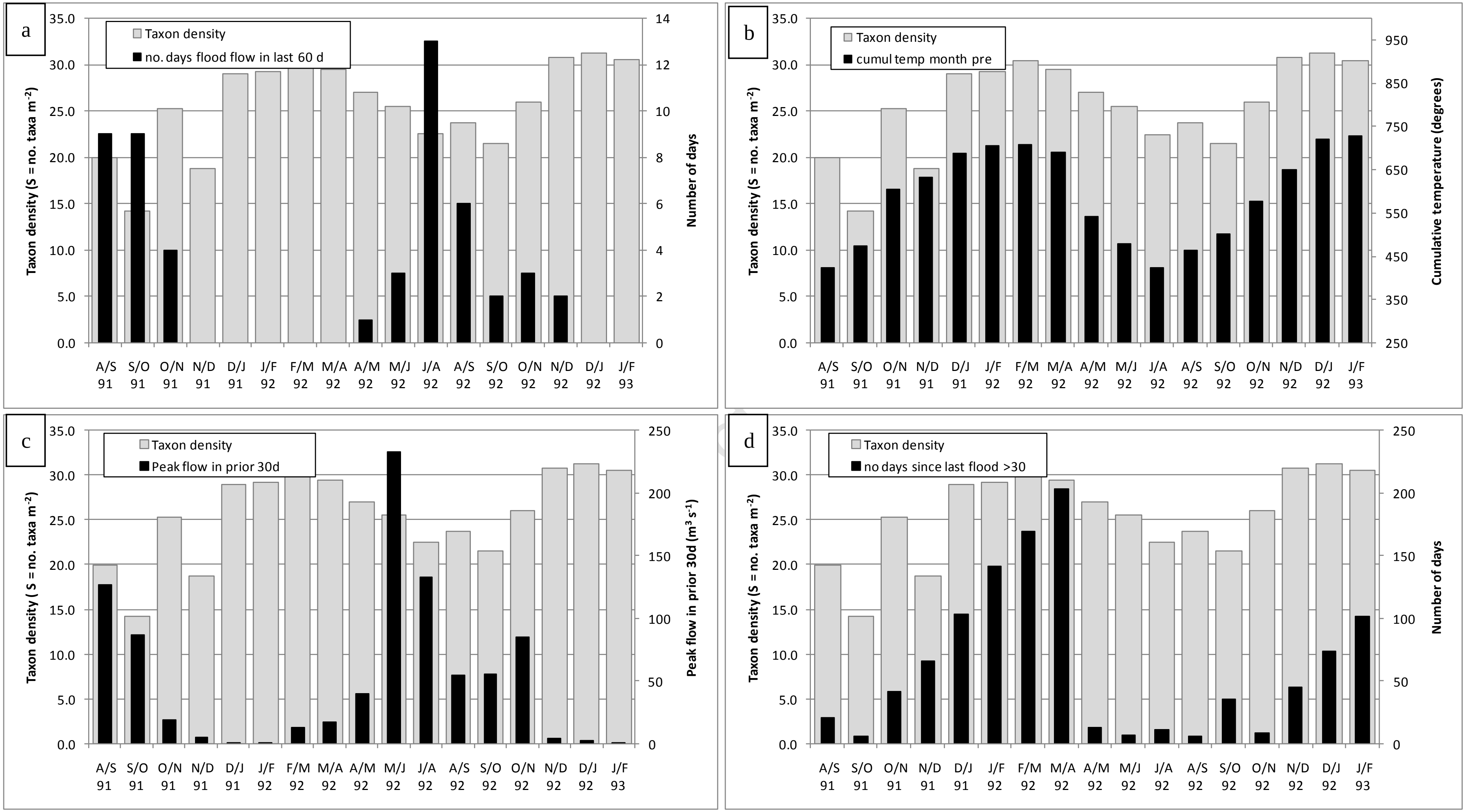

Figure 6.26. Taxon density (number of taxa per m^2) in the Molenaars River, based on averages from Sites 1, 2, 7 and 8 over the 1991-1993 study period, alongside (a) number of days of flooding (over 30 m^3 s^{-1}) in the 60-day period prior to the date of sampling; (b) cumulative temperature in the 30-day period prior to sampling; (c) peak flow in the 30 days prior to sampling; and (d) the number of days since flood flows > 30 m^3 s^{-1}.

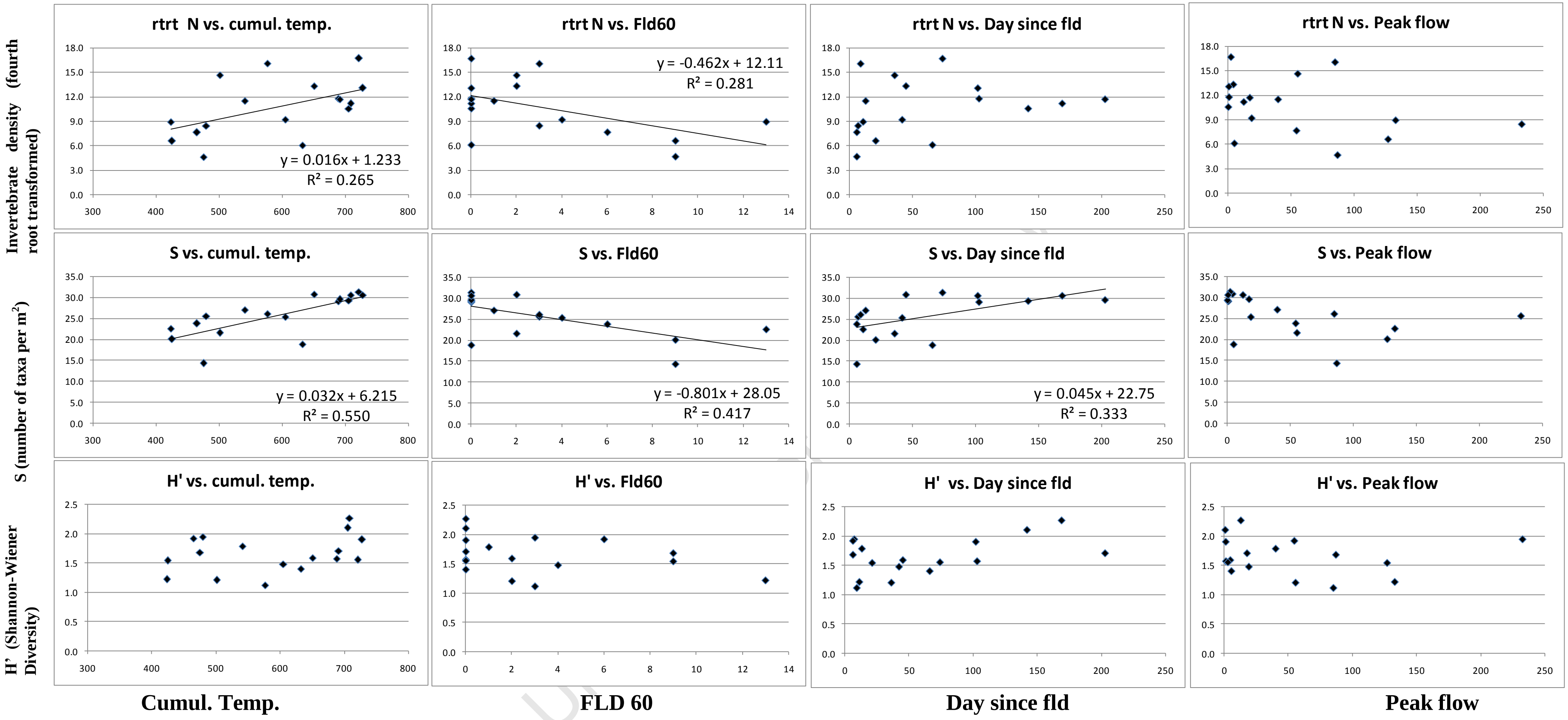

Figure 6.27. Relationship between three community indices and four drivers of temporal patterns in invertebrate communities. Community indices: rtrt N = total invertebrate density (numbers per m^2, data 4th root transformed); S = richness (number of taxa per m^2); H' = Shannon Wiener diversity. Abiotic drivers: cumul. temp. = cumulative temperature in the 30-day period prior to sampling; Fld60 = number of days of flooding (over 30 $m^3 s^{-1}$) in the 60-day period prior to sampling; Days since fld = number of days since flood flows > 30 $m^3 s^{-1}$; and Peak flow = peak flow ($m^3 s^{-1}$) in the 30 days prior to sampling). Regression equations are shown for those variables that returned significant linear correlation (all $p \leq 0.05$). Data from Sites 1, 2, 7 and 8.

peak flow in the 60 days preceding sampling (flood magnitude). No relationship was observed between invertebrate density and the number of days since flooding (post-flood recovery term), but there was a significant relationship between the latter and taxon richness. There was no relationship between either the temperature or the flow terms and Shannon Wiener diversity, rarefied taxon richness or Pielou's evenness.

The variable used for BIOENV analysis included the temperature and flood variables described above, as well as the temperature and discharge on the day of sampling, the average phosphate and total inorganic nitrogen concentrations in the preceding month, and the mean periphyton density at the time of sampling. The results show that the cumulative temperature in the 30 days preceding sampling had the strongest individual correlation with the community patterns, and that additional variables added little to the explanatory power (Table 6.4). However, fairly good correlations were also achieved between the community patterns and FLD60 and FLD30, which themselves were highly correlated.

Table 6.4 BIOENV results to examine which environmental variables best explain the MDS and cluster patterns based on invertebrate assemblages in the Molenaars River, from August 1991 to February 1993, as shown in Figure 6.20. The correlation coefficient (ρ_w) for the flood and temperature variables which have the highest correlation values on their own are presented, as well as the combinations of variables that resulted in the highest ρ_w values overall (in bold text). Temp. = cumulative temperature for 30 d prior to sampling; FLD30, FLD 60 = number of days with flood flows > 30 $m^3 s^{-1}$ in the prior 30 d or 60 d period; Q = discharge on the day of sampling.

Combinations of 2, 3 and 4 variables	Correlation coefficient ρ_w
Temp.	0.556
FLD60	0.489
FLD30	0.400
Temp., FLD60	**0.557**
Temp., Q	**0.557**
Temp., FLD60, FLD30	**0.557**

The fact that month-to-month changes in invertebrate assemblages were significant and at times substantial, as indicated by the high ANOSIM values in Figure 6.23, combined with the high value of the best BIOENV correlation, $\rho_w = 0.577$, means that these variables can be considered to be strong drivers of intra-annual change in invertebrate assemblages, at the monthly scale. The relationships between community change (the ANOSIM value) and the flood and temperature variables are presented graphically in Figure 6.28.

SIMPER analysis was undertaken to determine which of the invertebrate taxa were most responsible for patterns of change, comparing sequential months over the study period. The SIMPER routine computes dissimilarity (1- similarity) between each pair of samples in the two groups of samples being compared, and then calculates an average, along with the separate contributions to dissimilarity from each taxon. The average dissimilarity and taxa in order of their percentage contribution to dissimilarity between the monthly samples are presented in Table 6.5. Although the analysis of dissimilarity was undertaken using root-root transformed data, the average density for each taxon in each of the monthly comparisons is presented as densities per m^2, for clarity. The Dissimilarity / Standard deviation ratio in Table 6.5 indicates the consistency with which each taxon discriminates between monthly groups (see section 3.4.2iv of Chapter 3 on PRIMER methods).

These results show a complicated relationship between temperature, flood effects and community structure. From A/S91 to the following month S/O91, the significant ANOSIM value of 0.47 indicates a moderately large change in invertebrate assemblage. During the preceding month the cumulative temperature was 475 degree-days (Figure 6.28a). This period was associated with four floods (Figure 6.28b), the peak flow of which was some 90 m^3 s^{-1}, where sampling was undertaken only six days after the flood event (Figure 6.28c).

The invertebrate taxa contributing most to the dissimilarity between A/S91 and S/O91 (Table 6.5) were all reduced in density from the one month to the next, with the exception of one minor group, the hemipterans.

In contrast, the comparison of S/O91 and O/N91 samples returned a very high ANOSIM value, indicating strong differentiation between the months. The absence of floods in the preceding period meant that the O/N91 samples were collected 42 days after the last flood event (>30 m^3 s^{-1}). The highest discharge for the month was 18 m^3 s^{-1} and the cumulative temperature increased by over 100 to 605 degree-days over that of the period A/S91 to S/O91.

The chief drivers of biological pattern in this period were the Orthocladinae, Tanytarcini, Baetidae and Simuliidae (Table 6.5). All of these increased dramatically in density. The order of magnitude increase in chironomid pupae from

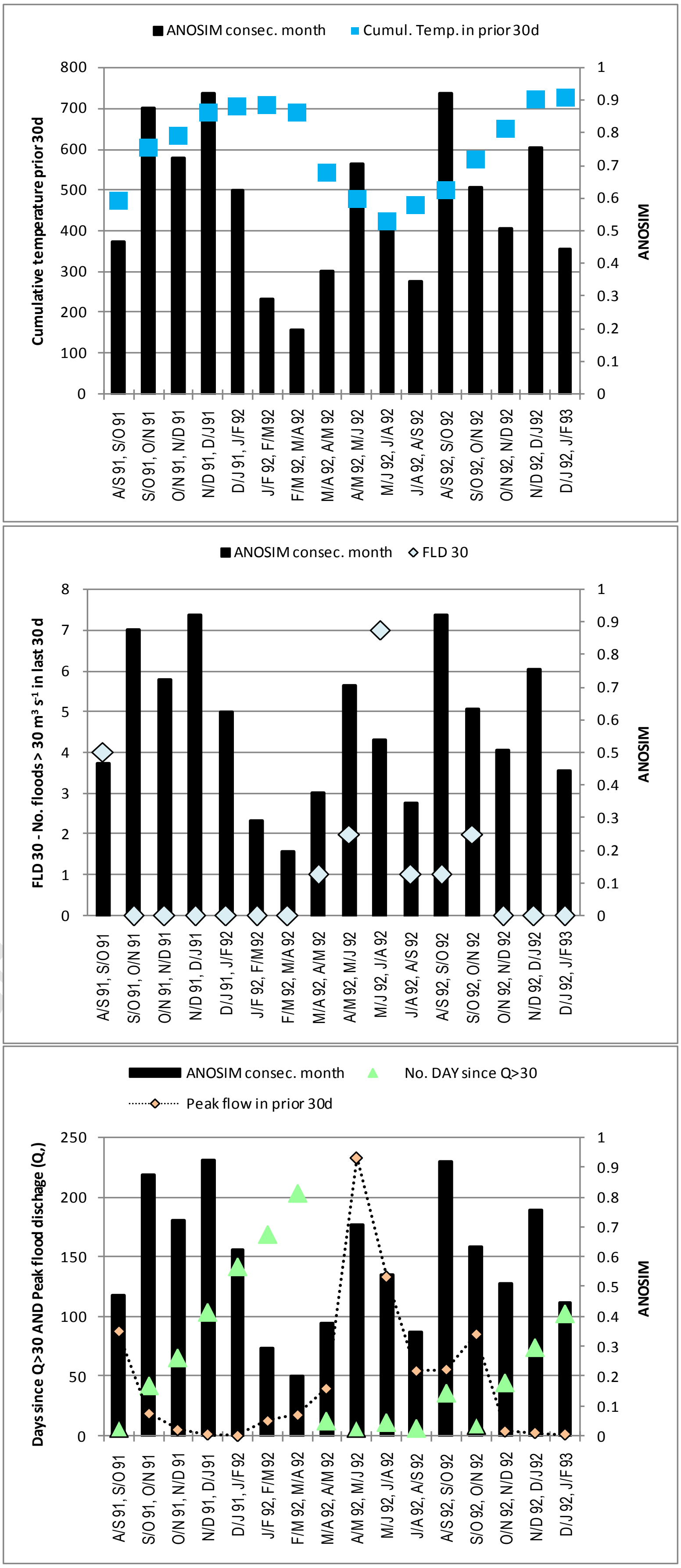

Figure 6.28. Graphical relationship between the ANOSIM value (Rho, between 0 and 1) and temperature and flood variables over the 1991 – 1993 study period.

S/O91 to O/N91 along with increases in all chironomid sub-families suggests an increased rate of turnover of generations associated with the temperature increase, and in the absence of floods.

Although the period between O/N91 and N/D91 was very similar from an environmental perspective to that between S/O91 and O/N91 – no floods, a similar, slightly increased temperature budget, and with lower but still reasonably elevated base flows, there was still a substantial difference in community structure, with an ANOSIM Rho value of 0.72 (Figure 6.28). This change was, unexpectedly, chiefly associated with a decline in the Orthocladinae so abundant in O/N91 (Table 6.5). The same temperature and flood pattern was repeated from N/D91 to D/J91, albeit with a higher cumulative temperature. Despite this, this period was nevertheless characterised by the largest shift in community structure over the whole sampling period (ANOSIM Rho = 0.92), associated with blooms of baetids and chironomids, for example, baetids increasing from 1250 to some 14 000 m^{-2}.

The summer period, from D/J91 through M/A92, was associated with both stable cumulative monthly temperatures, an absence of floods and low base flows (Figure 6.28), and was characterised by a reduction in the difference between monthly samples, becoming insignificant in the F/M92 to M/A92 period. Interestingly, although there were no floods above the disturbance threshold, there were small spates of 12 and 17 $m^3 s^{-1}$ just prior to the F/M92 and M/A92 sampling periods respectively.

Figure 6.29 shows the monthly density of a number of selected taxa, and in most cases their abundances, whether high or low, were relatively stable over this period. A greater range of taxa also contributed to the dissimilarity between monthly samples than during the spring months, as shown by the SIMPER results (Table 6.5).

The period from M/A92 to A/M92 was characterised by a higher differentiation of the invertebrate assemblage than during summer, and was associated with both a decline in the cumulative temperature (from 691 to 541 °C) and a flood of 40 $m^3 s^{-1}$, the latter some two weeks prior to sampling in A/M92 (Figure 6.28). In contrast to the spring period, this community shift, although significant, was not very substantial (ANOSIM Rho value = 0.38) and was associated with decreases in baetids, simuliids and some chironomid taxa, the reverse of the spring pattern. Other groups also declining in densities included the *Caenis* spp. (Caenidae), *Cheumatopsyche* spp. (Hydropsychidae), whilst *Chimarra* sp. (Philopotamidae), *Elporia* spp. (Blephariceridae) and hydraenid and scirtid beetles all increased substantially.

A second major reduction in Baetidae and Orthocladinae, as well as substantial reduction in densities of almost all taxa with the exception of the Notonemouridae, accompanied the shift in community from A/M92 to M/J92 (Table 6.5). This change followed two floods in the intervening 30 days, including, just seven days prior to sampling, a flood with a peak daily discharge of 233 $m^3 s^{-1}$ (a 1:20 year return period). Indeed, overall invertebrate density decreased by 70% on the A/M92 densities. Only three taxa were reduced to zero, however, and all of these occurred at densities of fewer than three individuals m^{-2} in A/M92. The ANOSIM Rho value (0.71) was similar to the values recorded during the highly variable spring months (Figure 6.28). The cumulative temperature for the 30 day period prior to sampling was similar, although somewhat less than during the M/A92 – A/M92 period.

The second winter month – the shift from M/J92 to J/A92 – was less pronounced (ANOSIM Rho value of 0.54). This was despite seven floods (above the 30 $m^3 s^{-1}$ threshold) in the 30 days prior to sampling, the largest with a peak daily discharge of 1130 $m^3 s^{-1}$ and the last occurring 11 days prior to sampling. These

Table 6.5. Condensed SIMPER results of taxa contributing to the differentiation of monthly groupings, based on cluster analysis of Molenaars River samples from 1991 to 1993. The assemblages from each site for each month are shown in Figure 6.20. The taxa contributing to 40 % of between-month dissimilarity are shown. Densities are in no. m^{-2} for each group, in the order in which it is listed. Diss = dissimilarity. The Diss / SD ratio indicates the consistency with which taxa discriminate between groups across all samples.

Pairs of months selected for comparison (% dissimilarity between months)	**Taxa contributing most to the first 40% of the total dissimilarity value**	**Average density in each month compared (# m^{-2})**		**Avg. Diss between group elements**	**Diss / SD ratio**	**Cumul. % contrib. to Diss**
		1st listed month	**2nd listed month**			
A/S91 vs. S/O91 (40.5)	*Elporia* spp.	100	3.8	2.34	1.29	5.76
	Elmidae	75.6	35.4	2.26	1.48	11.33
	Cheumatopsyche spp.	12.2	2.9	2.19	1.46	16.73
	Telagonodidae	68.3	23.8	2.10	1.20	21.91
	Scirtidae	32.2	4.6	1.92	1.35	26.64
	Orthocladinae	635.0	242.5	1.90	1.52	31.34
	Hemiptera	0.0	3.8	1.87	1.58	35.96
	Simulium spp.	32.8	10.4	1.86	1.18	40.56
S/O91 vs. O/N91 (48.6)	Orthocladinae	242.5	3868.3	5.37	3.23	11.05
	Baetidae	35.4	1328.8	4.56	4.30	20.43
	Chironomid pupae	6.7	187.1	3.49	1.92	27.60
	Simulium spp.	10.4	248.3	3.17	1.93	34.12
	Tanytarcini	3.3	92.5	2.89	2.19	40.07
O/N91 vs. N/D91 (36.3)	Orthocladinae	3868.3	98.3	5.70	4.24	15.72
	Chironomid pupae	187.1	9.2	2.68	2.38	23.11
	Telagonodidae	15.4	1.3	1.77	1.99	27.99
	Simulium spp.	248.3	261.3	1.46	1.52	32.02
	Cheumatopsyche spp.	15.8	10.0	1.35	1.37	35.73
	Agapetus agilis	7.5	1.7	1.32	1.54	39.38
	Simuliid pupae	3.8	19.2	1.29	1.22	42.92
N/D91 vs. D/J91 (49.7)	Tanytarcini	20.8	6138.8	4.51	1.92	9.08
	Baetidae	1251.3	13972.7	3.93	3.99	17.01
	Cheumatopsyche spp.	10.0	958.3	3.41	3.02	23.88
	mites	3.3	281.3	2.43	2.74	28.77
	Athericidae	0.8	183.1	2.35	2.65	33.51
	Simulium spp.	261.3	2207.1	2.33	1.55	38.21
	Adult beetles	13.3	576.0	2.31	2.73	42.86
D/J91 vs. J/F92 (27.2)	Baetidae	13 972.7	3381.3	1.82	2.59	6.70
	Tanytarcini	6138.8	3501.7	1.73	1.34	13.08
	Telagonodidae	12.9	235.4	1.16	1.84	17.37
	Leptophlebiidae	296.0	1235.0	1.12	1.42	21.47
	Orthocladinae	1285.8	3172.5	1.10	1.37	25.51
	Simulium spp.	2207.1	1354.6	1.07	1.24	29.46
	Cheumatopsyche spp.	958.3	333.3	1.03	1.69	33.24
	Ecnomidae	6.0	57.1	0.85	1.22	36.35
	Tanypodinae	420.8	910.8	0.81	1.37	39.32
	Agapetus agilis	21.3	0.4	0.79	1.32	42.21

Table 6.5 cont. Condensed SIMPER results of taxa contributing to the differentiation of monthly groupings, based on cluster analysis of Molenaars River samples from 1991 to 1993. The assemblages from each site for each month are shown in Figure 6.20. The taxa contributing to 40 % of between-month dissimilarity are shown. Densities are in no. m^{-2} for each group, in the order in which it is listed. Diss = dissimilarity. The Diss / SD ratio indicates the consistency with which taxa discriminate between groups across all samples.

Pairs of months selected for comparison (% dissimilarity between months)	**Taxa contributing most to the first 40% of the total dissimilarity value**	**Average density in each month compared (# m^{-2})**		**Avg. Diss between group elements**	**Diss / SD ratio**	**Cumul. % contrib. to Diss**
		1st listed month	**2nd listed month**			
J/F92 vs. F/M92 (23.5)	Tanytarcini	3501.7	1284.6	1.42	1.32	6.05
	Simulium spp.	1354.6	1927.1	0.97	1.28	10.17
	Leptophlebiidae	1235.0	1615.0	0.97	1.18	14.28
	Caenis spp.	18.3	52.5	0.90	1.35	18.10
	Ecnomidae	57.1	0.4	0.89	1.18	21.90
	Chironomini	135.8	15.8	0.80	1.73	25.32
	Afronurus spp.	77.1	53.8	0.75	1.24	28.54
	Cheumatopsyche spp.	333.3	520.4	0.75	1.45	31.71
	Orthocladinae	3172.5	3223.8	0.73	1.41	34.80
	Elmidae	271.7	483.8	0.70	0.87	37.76
	Notonemouridae	20.4	35.0	0.69	1.34	40.70
F/M92 vs. M/A92 (21.1)	Baetidae	4150.8	9965.4	1.17	1.75	5.54
	Tanytarcini	1284.6	350.4	1.05	1.23	10.51
	Caenis spp.	52.5	137.1	1.01	1.42	15.32
	Elporia spp.	0.0	31.3	0.97	1.80	19.93
	Leptophlebiidae	1615.0	957.5	0.85	1.33	23.95
	Tanypodinae	507.9	138.8	0.75	1.77	27.51
	Turbellaria	21.3	8.3	0.70	1.37	30.84
	Deuterophlebiidae	19.3	2.1	0.67	1.18	34.04
	Leptoceridae	20.0	33.8	0.65	1.27	37.15
	Chimarra sp.	30.0	34.2	0.65	1.22	40.24
M/A92 vs. A/M92 (24.4)	Hydraenidae	0.0	195.0	1.74	1.93	7.10
	Scirtidae	300.0	614.6	1.26	1.37	12.28
	Collembola	0.0	30.8	1.07	1.55	16.65
	Caenidae	137.1	0.8	0.96	0.99	20.56
	Elporia spp.	31.3	285.0	0.96	1.05	24.47
	Baetidae	9965.4	5955.2	0.94	1.52	28.34
	Simulium spp.	969.6	325.2	0.85	2.00	31.84
	Chimarra sp.	34.2	130.8	0.80	1.24	35.12
	Cheumatopsyche spp.	527.1	167.7	0.80	1.53	38.38
	Tanytarcini	350.4	85.8	0.76	1.38	41.48
A/M92 vs. M/J92 (28.6)	Baetidae	5955.2	1205.2	2.37	1.90	8.29
	Orthocladinae	4988.3	861.4	2.28	2.45	16.26
	Scirtidae	614.6	57.1	1.43	1.35	21.25
	Hydraenidae	195.0	25.7	1.41	1.33	26.16
	Chironomid pupae	37.1	2.4	1.38	2.04	30.98
	Elmidae	491.9	54.8	1.37	1.78	35.77
	Simulium spp.	325.2	159.0	1.10	2.01	39.63
	mites	123.8	8.6	1.08	2.35	43.39

Table 6.5 cont. Condensed SIMPER results of taxa contributing to the differentiation of monthly groupings, based on cluster analysis of Molenaars River samples from 1991 to 1993. The assemblages from each site for each month are shown in Figure 6.20. The taxa contributing to 40 % of between-month dissimilarity are shown. Densities are in no. m^{-2} for each group, in the order in which it is listed. Diss = dissimilarity. The Diss / SD ratio indicates the consistency with which taxa discriminate between groups across all samples.

Pairs of months selected for comparison (% dissimilarity between months)	**Taxa contributing most to the first 40% of the total dissimilarity value**	**Average density in each month compared (# m^{-2})**		**Avg. Diss between group elements**	**Diss / SD ratio**	**Cumul. % contrib. to Diss**
		1st listed month	**2nd listed month**			
M/J92 vs. J/A92 (31.3)	Orthocladinae	861.4	3518.8	2.24	1.76	7.15
	Afronurus spp.	31.0	2.9	1.79	2.11	12.86
	Baetidae	1205.2	213.3	1.71	1.24	18.32
	Chimarra sp.	63.8	5.0	1.53	1.43	23.20
	Chironomid pupae	2.4	29.6	1.48	1.56	27.93
	Chironomini	29.5	4.6	1.45	1.49	32.57
	Telagonodidae	446.5	289.0	1.33	2.07	36.83
	Hemiptera	0.0	6.7	1.31	2.37	41.03
J/A92 vs. A/S92 (29.5)	Orthocladinae	3518.8	1326.3	2.31	1.40	7.82
	Scirtidae	92.1	35.8	1.61	1.34	13.29
	Elporia spp.	77.9	54.2	1.58	1.41	18.64
	Collembola	28.8	3.3	1.55	1.27	23.90
	Chironomid pupae	29.6	88.8	1.33	1.37	28.40
	Elmidae	83.3	34.6	1.20	1.17	32.46
	Hemiptera	6.7	1.7	1.13	1.34	36.30
	Adult beetles	10.0	7.9	1.09	1.13	39.99
	Chironomini	4.6	5.0	1.07	1.17	43.61
A/S92 vs. S/O92 (38.3)	Orthocladinae	1326.3	21937.0	5.65	2.69	14.31
	Baetidae	225.0	10672.5	5.44	3.09	28.09
	Simulium spp.	109.2	3317.6	3.62	2.12	37.26
	Chironomid pupae	88.8	1088.0	2.43	2.37	43.42
S/O92 vs. O/N92 (29.0)	Baetidae	10672.5	36354.2	2.71	1.71	9.35
	Chironomid pupae	1088.0	39.6	2.15	3.53	16.79
	Orthocladinae	21937.0	7185.0	2.01	1.58	23.74
	Simuliid pupae	1.3	58.3	1.37	1.69	28.46
	Simulium spp.	3317.6	6139.2	1.37	1.32	33.18
	Chironomini	4.2	77.5	1.36	2.10	37.87
	Elporia spp.	35.5	18.8	1.00	1.20	41.32
O/N92 vs. N/D92 (24.9)	Tanytarcini	181.3	1574.2	1.52	2.43	6.09
	Baetidae	36354.2	15757.1	1.44	1.80	11.88
	Cheumatopsyche spp.	30.8	452.5	1.34	1.43	17.25
	Chimarra sp.	5.0	76.7	1.22	1.90	22.16
	Gyrinidae	0.0	32.1	1.13	2.00	26.70
	Agapetus agilis	22.5	52.9	0.94	1.34	30.47
	Orthocladinae	7185.0	3634.2	0.92	1.68	34.16
	Tanypodinae	94.2	391.7	0.90	1.44	37.78
	Elmidae	217.9	338.3	0.80	1.06	41.00

disturbances were associated with a decrease in density of most taxa, but from an already low base (Table 6.5). In contrast, orthoclads increased substantially over this period as did the density of their pupae.

Table 6.5 cont. Condensed SIMPER results of taxa contributing to the differentiation of monthly groupings, based on cluster analysis of Molenaars River samples from 1991 to 1993. The assemblages from each site for each month are shown in Figure 6.20. The taxa contributing to 40 % of between-month dissimilarity are shown. Densities are in no. m^{-2} for each group, in the order in which it is listed. Diss = dissimilarity. The Diss / SD ratio indicates the consistency with which taxa discriminate between groups across all samples.

Pairs of months selected for comparison (% dissimilarity between months)	Taxa contributing most to the first 40% of the total dissimilarity value	Average density in each month compared (# m^{-2})		Avg. Diss between group elements	Diss / SD ratio	Cumul. % contrib. to Diss
		1st listed month	2nd listed month			
N/D92 vs. D/J92 (25.8)	Hydra	3.3	516.3	1.50	2.27	5.71
	Baetidae	15757.1	41375.4	1.41	2.16	5.46
	Leptophlebiidae	148.8	2123.8	1.40	1.80	10.88
	Afronurus spp.	6.3	279.6	1.36	1.98	16.16
	Cheumatopsyche spp.	452.5	2719.2	1.34	1.86	21.35
	Tanytarcini	1574.2	7507.9	1.25	1.39	26.20
	Telagonodidae	5.8	310.4	1.24	1.79	31.02
	Simuliid pupae	61.3	2.9	1.07	2.46	35.18
	mites	209.2	1580.0	0.94	1.48	38.83
	Elmidae	338.3	1113.8	0.76	1.15	41.78
	Caenis spp.	28.8	42.9	0.71	1.07	44.55
	Athericidae	30.8	174.2	0.71	1.48	47.29
	Chironomini	53.8	310.8	0.70	2.20	49.99
	Agapetus agilis	52.9	7.3	0.69	1.26	52.68
D/J92 vs. J/F93 (21.7)	Baetidae	41375.4	12925.4	1.57	2.31	7.23
	Tanytarcini	7507.9	4263.8	1.23	1.48	12.91
	Simuliid pupae	2.9	40.8	0.88	2.14	16.98
	Simulium spp.	4622.1	3230.0	0.82	1.33	20.74
	Leptophlebiidae	2123.8	2676.7	0.80	1.28	24.43
	Orthocladinae	6194.2	4073.3	0.78	1.40	28.05
	Elmidae	1113.8	515.4	0.76	1.28	31.55
	Chimarra sp.	210.4	110.0	0.69	1.37	34.71
	Turbellaria	22.1	30.4	0.64	1.55	37.65
	Cheumatopsyche spp.	2719.2	1216.7	0.63	1.40	40.55
	Caenis spp.	42.9	10.8	0.62	1.17	43.43
	Trichoptera pupae	17.9	59.2	0.61	1.38	46.25
	Telagonodidae	310.4	154.2	0.59	1.31	48.95
	Scirtidae	149.2	37.5	0.54	1.26	51.43

Cumulative temperature for the 30 day period preceding sampling was 423 degree-days, the lowest of the sampling period (Figure 6.28). The A/S92 samples were fairly similar to those of the previous month, similar to the low levels of month-to-month variability displayed during the summer period. The sampling, again, was conducted six days after the only flood of the 30-day period, one of 55 m^3 s^{-1}. Chironomid numbers did not increase, despite only a small rise in cumulative temperature compared to the prior month. Most other taxa, in particular the mayflies, maintained stable densities over the J/A92 to A/S92 period.

However, the following month was a different matter: the ANOSIM Rho value for A/S92 vs. S/O92 was the highest of any paired months, driven by an explosion in Baetidae, Orthocladinae and Simuliidae densities and increased presence of chironomid pupae (Table 6.5). This was despite very similar conditions in respect of floods (one flood, peak discharge 55 $m^3 s^{-1}$) but with slightly increased cumulative temperature (576 degree-days in the period before the S/O92 sampling versus 501 degree-days the previous month), and importantly, the sample was collected 30 days after the last flood exceeding the threshold value of 30 $m^3 s^{-1}$. This community shift was similar to the one recorded in 1991, but a month later viz. S/O91 vs. O/N91, which was 42 days after the last flood above the threshold, and was characterised by the same suite of invertebrates exhibiting a large increase in densities, although in 1991, the cumulative temperature was much higher – 605 degree-days - consistent with its taking place a calendar month later.

The remaining spring and early summer months had a similar pattern, with fluctuating densities of baetids and chironomids being largely the driving factors behind large shifts in community structure each month, amidst increasing temperatures and the cessation of floods. By the middle of summer, from D/J92 to J/F93, monthly change in community structure was still moderately high, but more stable than the spring.

Thus the major shifts in invertebrate assemblages depicted by the ANOSIM values were associated with high variability in invertebrate assemblages particularly in the absence of floods (a large number of days since the last event) and in the face of increasing temperatures, and largely the result of rapid chironomid, baetid and simuliid population growth. The period of high variability extended from early spring until mid-summer (September to December), although it differed somewhat between the two years of this study. Conversely, the absence of floods above the disturbance threshold, combined with high but stable temperatures, was associated with increased stability, in late summer. The community shifts in the March to May period were more subtle and less variable, and arose from smaller magnitude increases or decreases in population size of a wider range of invertebrate taxa. It is unclear as to what the different contribution is from floods versus temperature in this period. Winter floods clearly increased the stability of populations on a month-to-month basis, but only after a fairly abrupt community shift associated with the onset of floods and with declining temperatures. Baetids and simuliids did not reach high numbers in winter, although chironomid densities were occasionally high. It is possible that the very large flood exaggerated the monthly variability during winter (the A/M92 to M/J92 period).

The difference between each month’s assemblage was to a large degree one of a change in the abundance of taxa, rather than their presence or absence. However, this was not always the case. correlation with the flow terms, reflecting its winter affiliation. More detailed analysis of these patterns, at

Figure 6.29 shows the monthly densities of a number of taxa, selected to show just this variation in intra-annual change at a population level. A few taxa appear to be restricted to summer months, such as *Caenis* spp. (Caenidae), but the vast majority or the taxa occurred year-round, although with substantially elevated densities in spring (e.g. Chironomidae), early summer (e.g. Athericidae), mid to late summer (e.g. *Afronurus* spp. (Heptageniidae). A number of taxa are restricted to the winter months, such as *Elporia* spp. (Blephariceridae), Hydraenidae.

Figure 6.30 to 6.33 present the results of correlation and regression between the major invertebrate taxa and these four temporal drivers. Cumulative temperature was most strongly related to most of the invertebrate groupings, although the Fld60 variable, representing flood frequency, was most strongly correlated with total numbers, the Baetidae, Philopotamidae and grazer density. The number of days since the last flood was also an important variable for many groups, especially the Leptophlebiidae, Heptageniidae, Leptoceridae and Hydropsychidae. These all have low winter densities, with increases in either spring or early summer. Few groups were correlated with the peak flow in the preceding month – the flood magnitude term.

A number of taxa exhibited no clear relationship to any of the variables, viz. scirtid beetles, Notonemouridae, Glossosomatidae, Telagonodidae and Chironomidae. These groups had highest densities in later summer and autumn, or a strong year-round presence in the river. On the other hand, the Blephariceridae was the only taxon to show a negative correlation with temperature, and positive correlation with the flow terms, reflecting its winter affiliation. More detailed analysis of these patterns, at

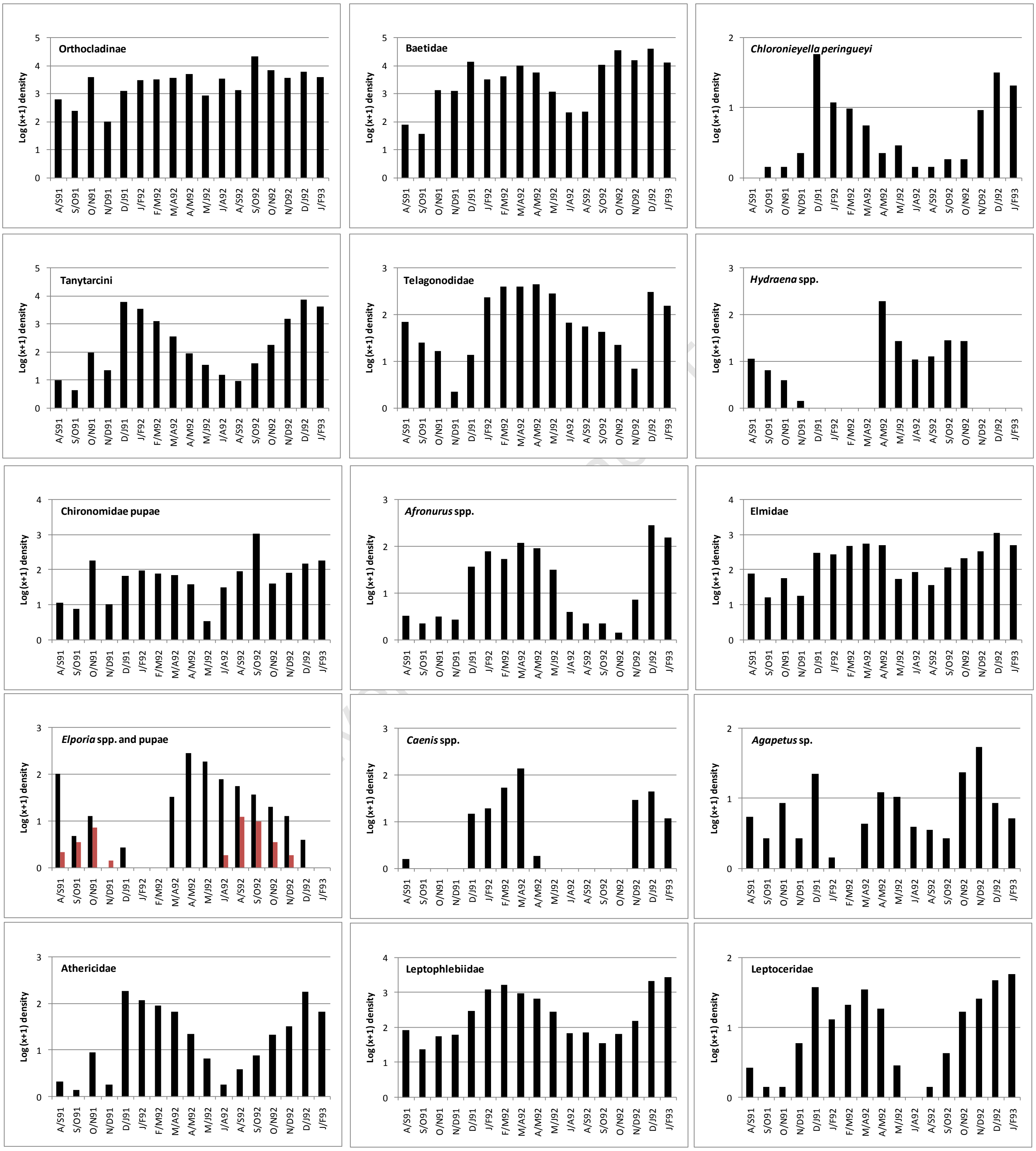

Figure 6.29. Monthly changes in density (logarithmic scale) of selected taxa in the Molenaars River from August 1991 to February 1993, as an illustration of community dynamics.

Figure 6.30. Relationship between densities of the major Ephemeroptera groups in the Molenaars River from 1991-1993 and four abiotic drivers. Density data are 4th root transformed. Cumul. temp = cumulative temperature in the 30-day period prior to sampling; Fld60 = number of days of flooding (over 30 m^3 s^{-1}) in the 60-day period prior to sampling; Days since fld = number of days since flood flows > 30 m^3 s^{-1}; and Peak flow = peak flow (m^3 s^{-1}) in the 30 days prior to sampling). Regression equations are shown for those variables that returned significant linear correlation (all p ≤ 0.05). Data from Sites 1, 7 and 8.

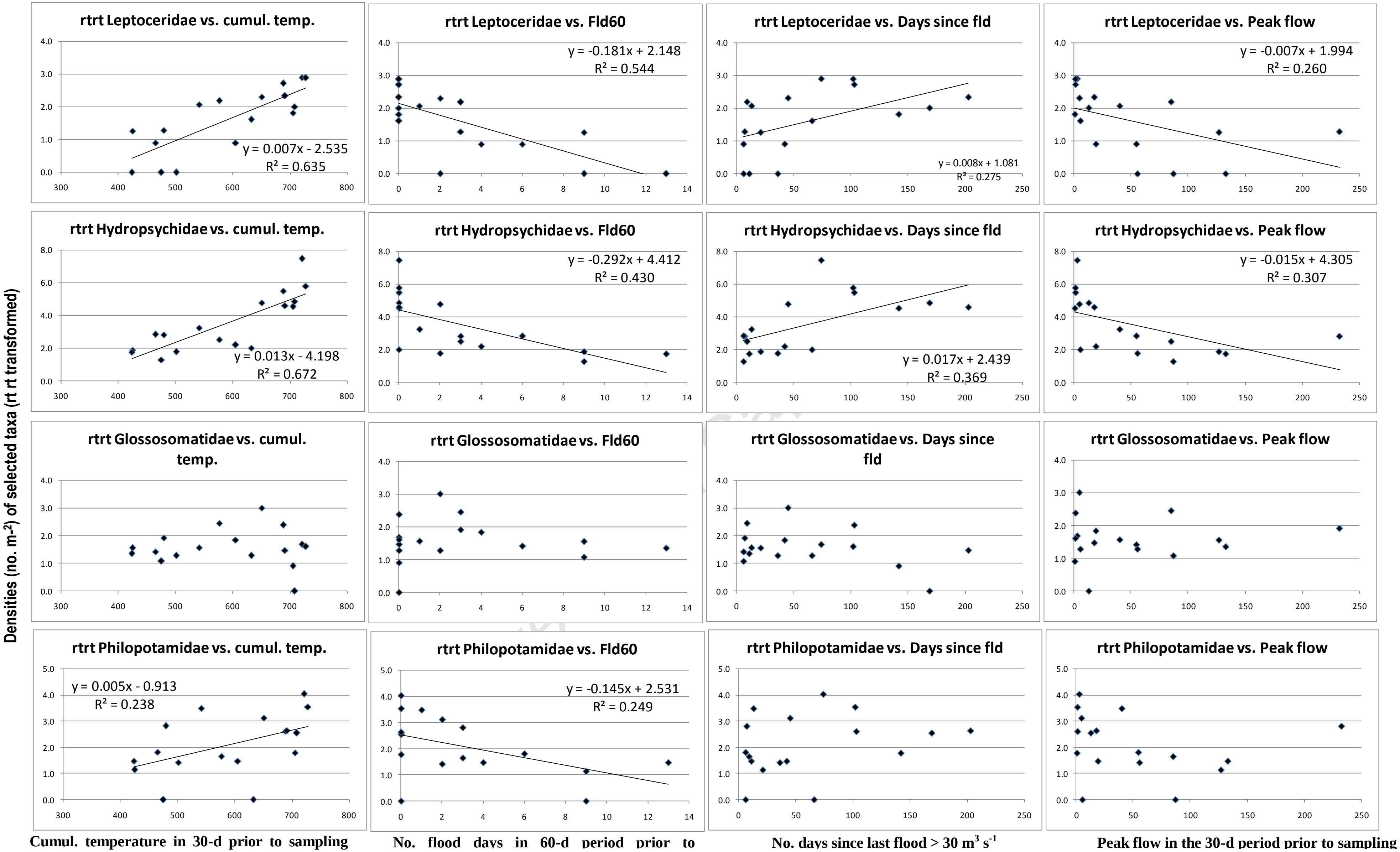

Figure 6.31. Relationship between densities of the major Trichoptera groups in the Molenaars River from 1991-1993 and four abiotic drivers. Density data are 4th root transformed. Cumul. temp = cumulative temperature in the 30-day period prior to sampling; Fld60 = number of days of flooding (over 30 $m^3 s^{-1}$) in the 60-day period prior to sampling; Days since fld = number of days since flood flows > 30 $m^3 s^{-1}$; and Peak flow = peak flow ($m^3 s^{-1}$) in the 30 days prior to sampling). Regression equations are shown for those variables that returned significant linear correlation (all $p \leq 0.05$). Data from Sites 1, 7 and 8.

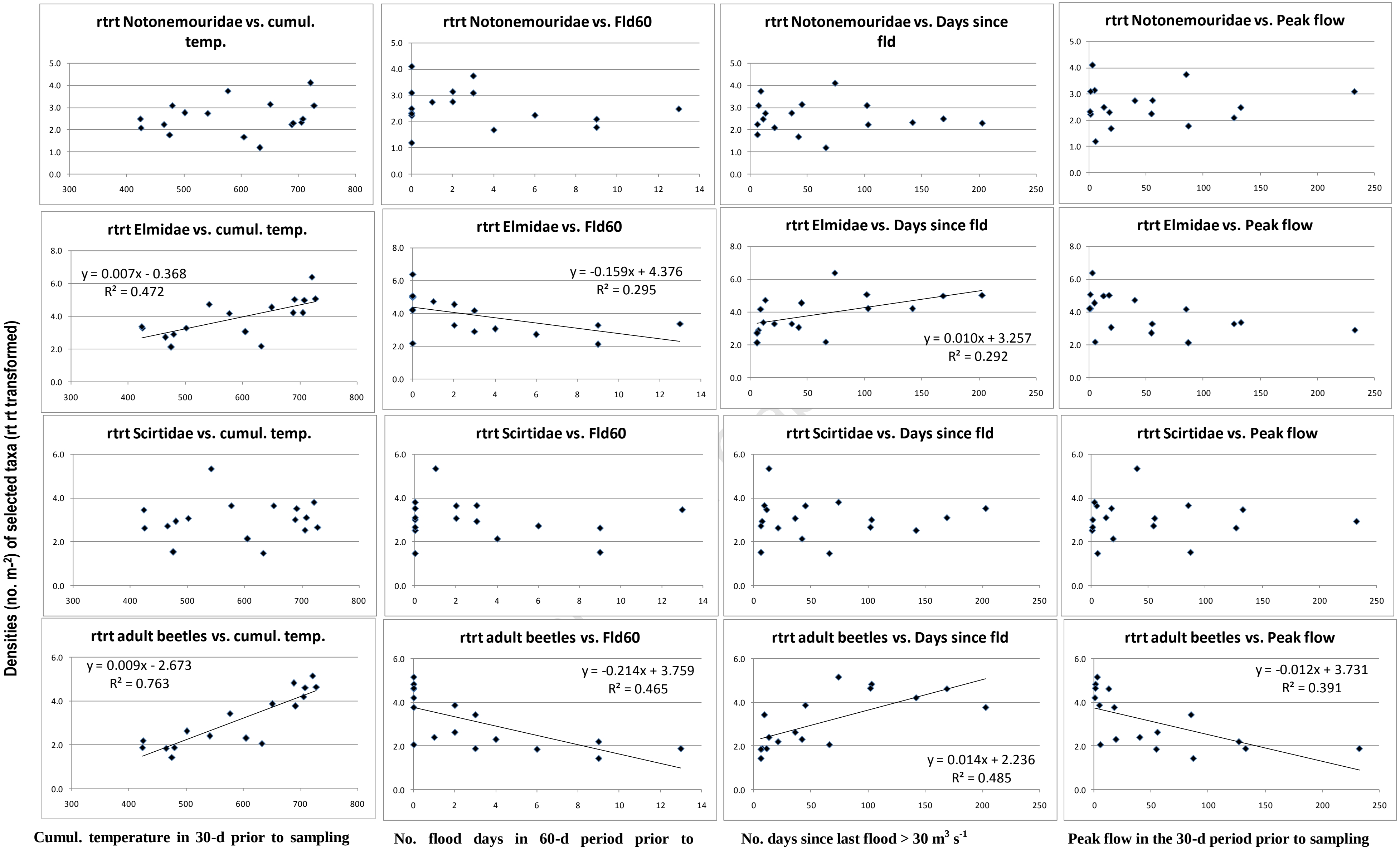

Figure 6.32. Relationship between densities of the Notonemouridae (Plecoptera) and the major Coleopteran groups in the Molenaars River from 1991-1993 and four abiotic drivers. Density data = 4th root transformed. Cumul. temp = cumulative temperature in the 30-day period prior to sampling; Fld60 = number of days of flooding (over 30 $m^3 s^{-1}$) in the 60-day period prior to sampling; Days since fld = number of days since flood flows > 30 $m^3 s^{-1}$; and Peak flow = peak flow ($m^3 s^{-1}$) in the 30 days prior to sampling). Regression equations are shown for those variables that returned significant linear correlation (all $p \leq 0.05$). Data from Sites 1, 7 and 8.

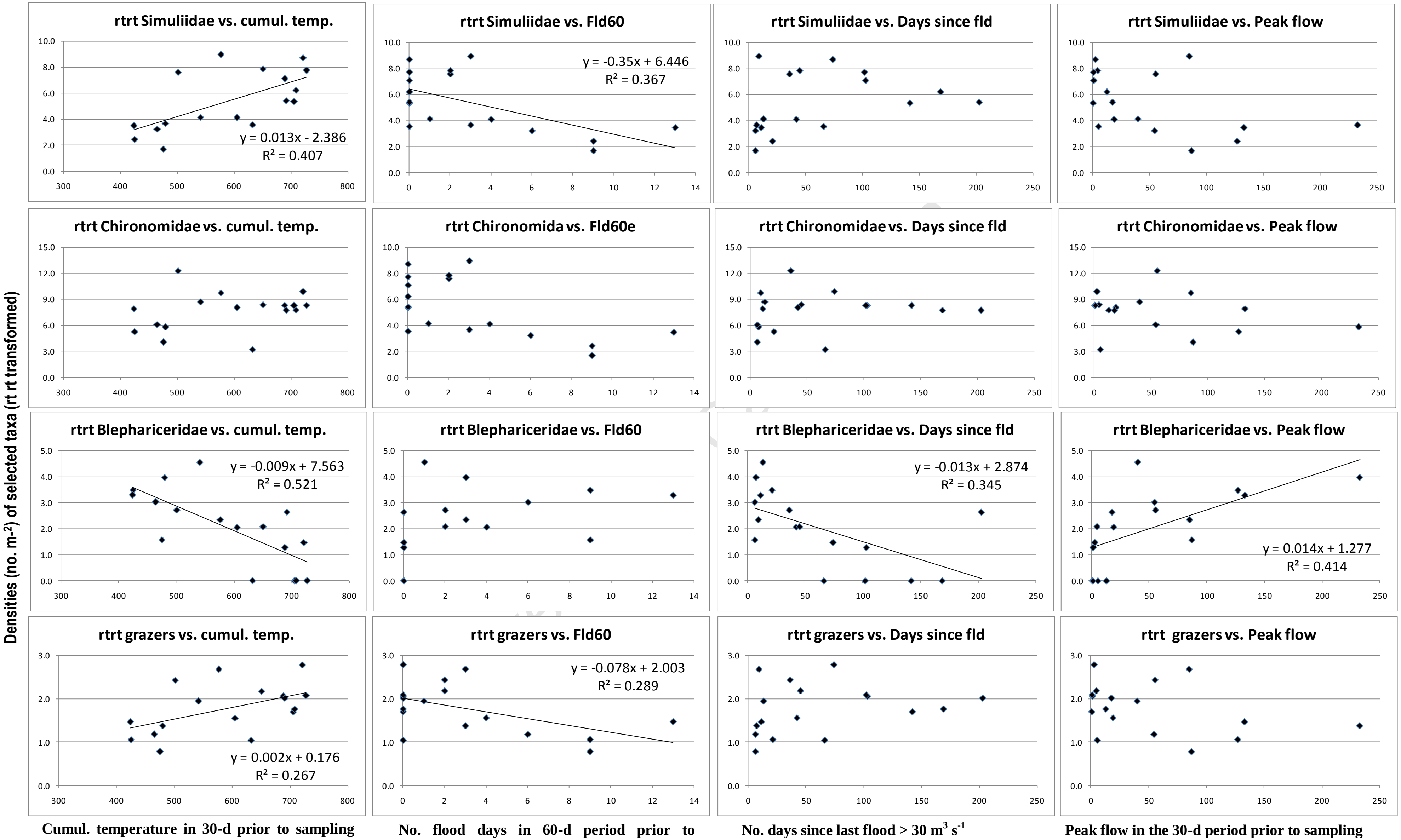

Figure 6.33. Relationship between densities of the major Diptera groups in the Molenaars River from 1991-1993 and four abiotic drivers. Density data are 4th root transformed. Cumul. temp = cumulative temperature in the 30-day period prior to sampling; Fld60 = number of days of flooding (over 30 $m^3 s^{-1}$) in the 60-day period prior to sampling; Days since fld = number of days since flood flows > 30 $m^3 s^{-1}$; and Peak flow = peak flow ($m^3 s^{-1}$) in the 30 days prior to sampling). Regression equations are shown for those variables that returned significant linear correlation (all $p \leq 0.05$). Data from Sites 1, 7 and 8.

a species level and including an assessment of change n population structures, is presented in Chapter 7.

Grazer density was correlated with flood frequency and cumulative temperature, but no correlation was found between grazer density and periphyton density. As shown in section 6.3.2, periphyton was not significantly correlated with any of the temperature or flood measures either.

Invertebrate patterns over the 1994 – 1997 sampling period

The second data set used in this study covered the period 1994-1997, and comprised data from sites corresponding to four of those sampled during the 1991-1993 period. The longer temporal period covered by this data set allowed for some evaluation of the *inter-annual* variability in community structure, already demonstrated by the 1991 and 1992 differences in spring assemblages.

ANOSIM showed no significant differences between the four sites (Global R = 0.069, p = 0.13), and thus these were combined for presentation of temporal patterns over the 2.5-year period. In a one-way analysis for difference between months, significant ANOSIM differences were found between months (Global R = 0.523, p = 0.001), but most pairwise differences were not significant at the 1% threshold for statistical significance used in these multiple comparisons (see methods section 3.2.3ii on significance thresholds to prevent falsely rejecting the null hypothesis). Despite this, the MDS plot shows some interesting trends.

The month-to-month shifts in invertebrate assemblages demonstrated by the 1991-1993 data (refer to Figure 6.22) are mirrored by this second data set (Figure 6.34). The samples again split firstly into a broad winter-spring group and a spring-summer-autumn group (Figure 6.34). Again, a cyclical pattern in the MDS plot is clearly evident. Four seasonal groupings were differentiated by cluster analysis: a winter group (June/July and July/August); two groups comprising mainly spring samples, one associated with the winter group and another with the summer group; and a summer – autumn group.

However, despite the repeated seasonal grouping of samples, as with the 1991-1993 data, invertebrate assemblages were clearly characterised by strong inter-annual differences, evident in the wide distances between samples from the same month in consecutive years. For example, the N/D month in some years clustered with the summer samples, whilst in others with the winter samples. Also, the shift from A/M to M/J in 1995 was substantially different from that in 1996. The former, from A/M95 (sample collected 9 May 1995) to M/J95 (sample collected on 7th June 1995) was characterised by a substantial change in assemblage structure, with the two samples grouping with the summer / autumn and the winter clusters respectively (arrow 1 in Figure 6.34). Interestingly, the only flood between the two sampling dates was a small flood of 20 $m^3 s^{-1}$ in mid-May 1995, under the 30 $m^3 s^{-1}$ threshold for disturbance. In contrast, the period A/M96 (sample collected on 20th April 1996, i.e. some two weeks earlier in the year than in 1995) to M/J95 (sample collected 20 May 1996, also some two weeks earlier than the M/J sample for 1995) was not characterised by a change in assemblage, with both samples clustering with the summer / autumn cluster (arrow 2 in Figure 6.34). In this 1996 year, there was only one small fresh of 9 $m^3 s^{-1}$ between the two sampling dates.

The A/S samples were taken on 30 August, 12 September and 1 September in 1994, 1995 and 1996 respectively. The samples from the first two of these years are closely grouped, but are different from the 1996 sample (arrow 3 in Figure 6.34). There were no floods over 30 $m^3 s^{-1}$ in the 5-week period prior to the A/S94 and A/S95 sampling, and only three (1994) or four (1995) floods during the entire winter prior to these sampling dates (top of Figure 6.34 and Figure 6.35). In contrast the 1996 period was characterised

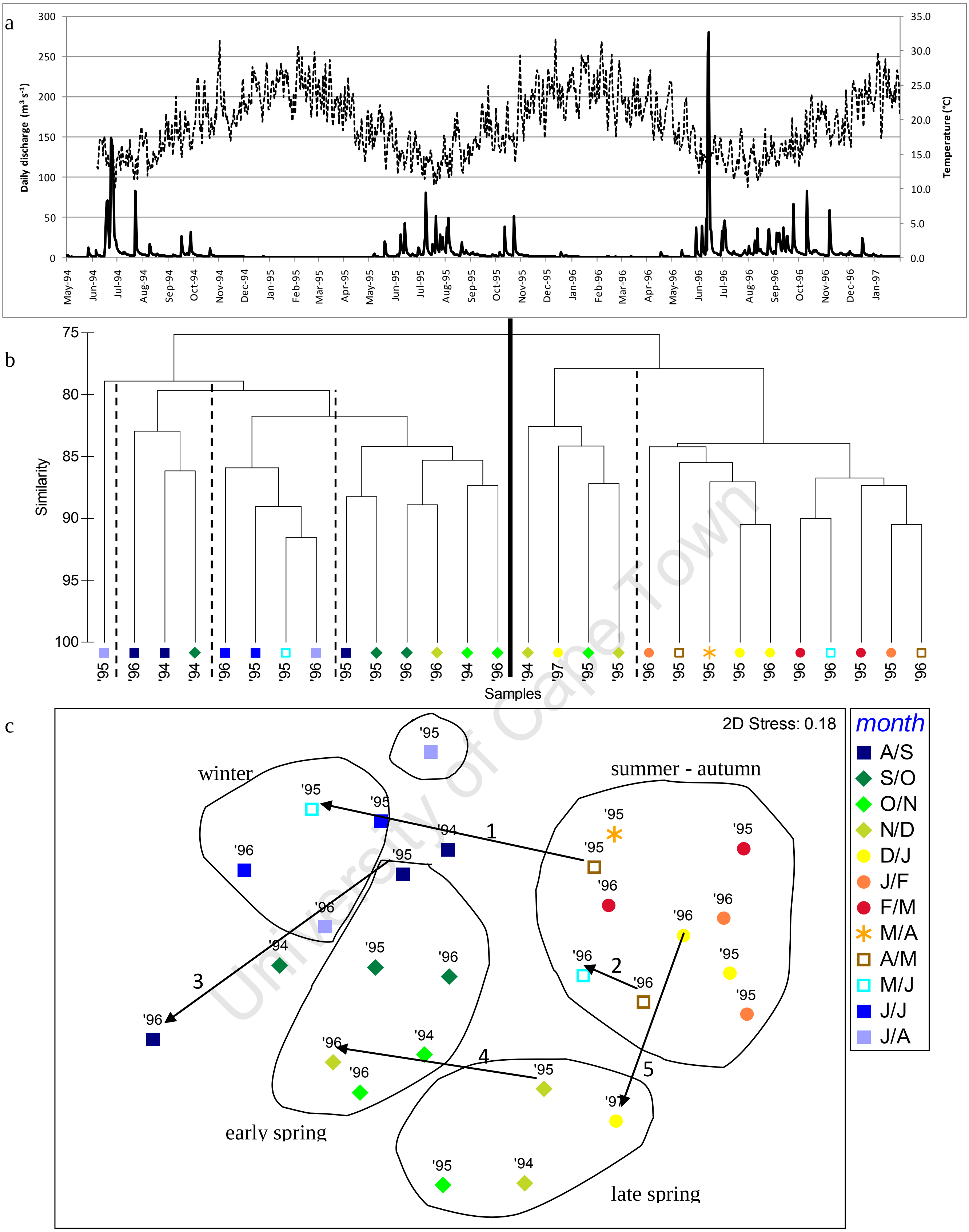

Figure 6.34. **a) Flow (solid line) and simulated maximum temperature (dashed line) in the Molenaars River over 2 ½ years, from August 1994 through to January 1997. b) Cluster analysis and c) MDS plot of the temporal shifts in invertebrate assemblages from monthly samples collected over the same period. Seasonal affiliation is indicated by symbol: filled square – winter; diamond – spring,; circle – summer; star – late summer (one sample only); hollow square – autumn. Calendar month is indicated by colour and year designated above each sample. Five groupings defined at a cut-off of 80% similarity generally corresponding to seasons, with one slightly out-lying winter sample. Numbered arrows are discussed in the text.**

by eight floods during the winter period prior to A/S96 sampling, the last of which was a mere four days before the samples were collected. This may account for the difference between this sample and those from the same dates in the previous years.

The hydrological data presented in Figure 6.34 and 6.35 show that 1996 was a substantially wetter year than the two years preceding it. Fourteen floods in total in 1996 were double the long-term average of 7.2 floods > 30 $m^3 s^{-1}$ in the Molenaars River. The floods did not begin earlier in the season, but were more numerous and extended into November 1996. Along with this, temperatures were 1-2 °C cooler in 1996 between September and November (refer to Figure 6.10 and Figure 6.11). The N/D96 and D/J97 samples were thus more characteristic of the early spring and late spring periods respectively, than the equivalent samples of the previous two years which were characteristic of late spring and summer respectively (arrows 4 and 5 in Figure 6.34)

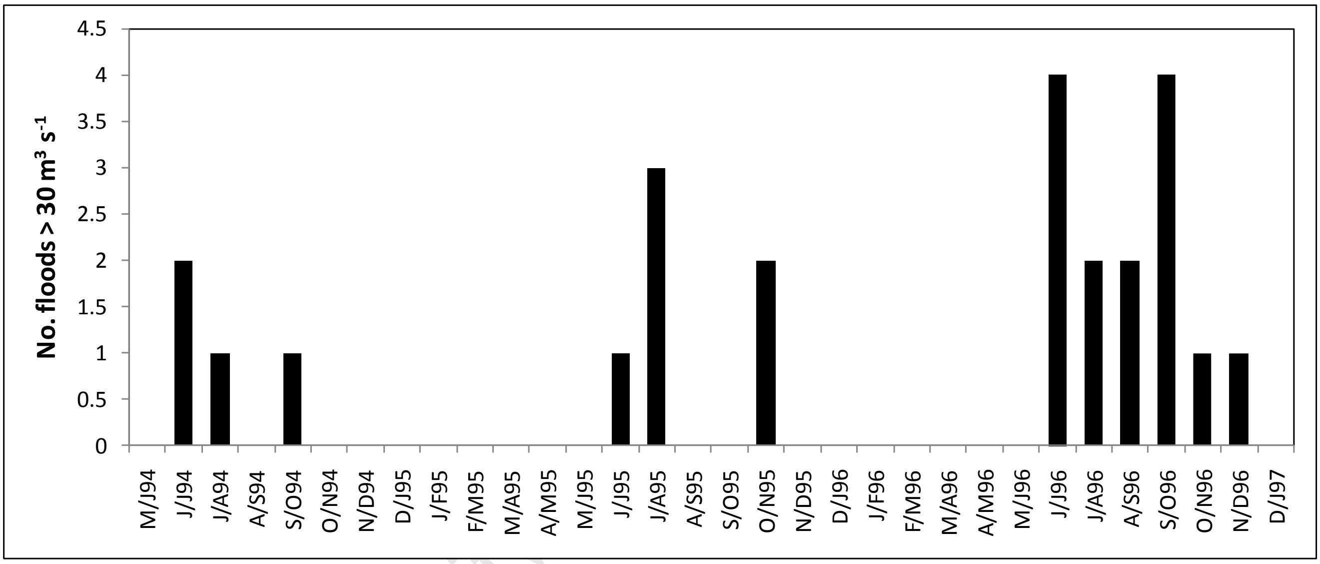

Figure 6.35. Number of floods above the 30 $m^3 s^{-1}$ threshold in the Molenaars River for the period of approximately one month between successive sampling occasions, i.e. from the date of one sample to the date of the following one. Sampling began in A/S94 (30 August 1994) and the data indicate that there were no floods in the 30-d period before this. On the other hand, there was one flood between the A/S94 sample and the S/O94 sample, which was collected on 6 October 1994. The long term average is 7.2 floods per annum over the 30 $m^3 s^{-1}$ threshold; hence these data represent two dry and one wet year.

The community shifts in Figure 6.34 reflect changes in both the taxon richness (Figure 6.36) and a coarse measure of relative abundances of the taxa associated with each month's sample (Figure 6.37), although these abundances were evaluated simply on a scale of 1-4, based on examination of the samples in the field (see section 6.2.4 iii). SIMPER results indicated that most invertebrate families were present year-round, and simply differed seasonally in their densities, as with the 1991-1993 data. Some of the species identified in the field were sparsely represented, and no clear pattern of occurrence, either by season or between years, could be distinguished. However, some species or genera identified in the field demonstrated clear seasonal patterns of occurrence in the river, being absent at particular times of the year. The shifts in relative densities over the 2.5-year period for selected species are shown in Figure 6.37. *Baetis harrisoni*, *Pseudocloeon vinosum*, and *Baetis* sp. 3 (Ephemeroptera: Baetidae) showed various patterns in occurrence, from year round to a winter-spring peak and summer low, to a summer – autumn peak respectively, as indicated in Figure 6.37. At the coarse level at which abundances were estimated for this data set, no differences in abundance were evident that may have been associated with wetter or drier years. The Glossosomatidae (Trichoptera), on the other hand (all *Agapetus* spp.) appeared to increase in density from winter through spring, but in 1996 were less abundant and patchily represented in the samples from mid-winter onward.

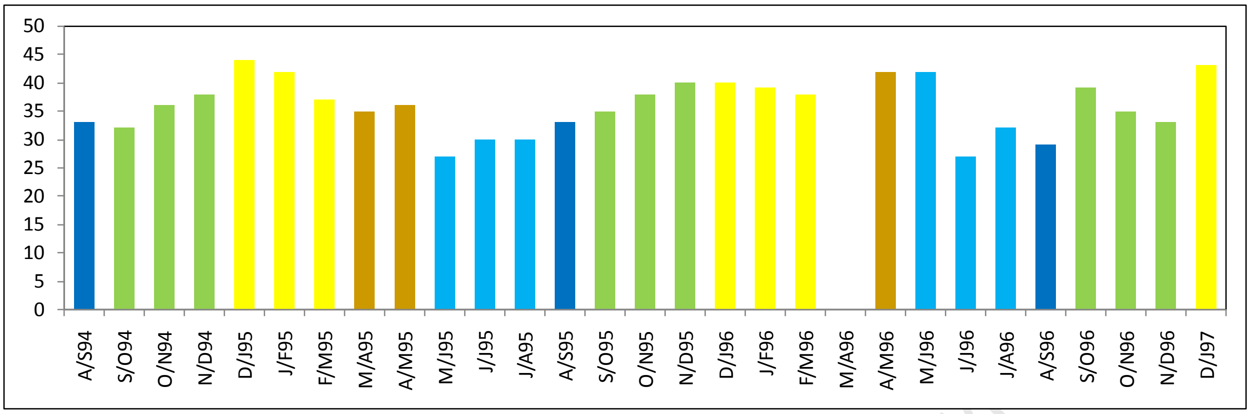

Figure 6.36. Total number of taxa in the Molenaars River for each sampling date from August 1994 to January 1997. The A/S sampling date in each year is shown in dark blue. The A/S sampling date in each year is shown in dark blue to help with locating the start of each new year of sampling. Seasons are colour-coded: blue – winter; green – spring; yellow – summer; brown – autumn. No sample was collected in M/A96.

The Blephariceridae (Diptera) comprised a single genus, *Elporia*, and showed a definite expansion in the period over which it was present in the 1996 winter, relative to the preceding two (Figure 6.37), which would appear to be related to the fact that 1996 represented a wetter than average year, whilst the previous two years were drier than average. This genus was also the only taxon that showed a significant negative correlation with cumulative temperature and a positive correlation with peak flow in the 30-day period preceding sampling in the 1991-1993 data set analysis (refer to Figure 6.33). Similarly, the Telogonodidae appear to recruit strongly in early autumn, and reach population density peaks at the end of winter, with numbers tailing off during spring and summer.

The Elmidae (Coleoptera) and to a lesser degree *Afronurus* spp. (Heptageniidae) showed a seasonal pattern of spring growth in densities, highest abundances in summer, and declining numbers through autumn to winter and early spring lows. These groups are both algal feeders, and their increases in density in late spring is in contrast with the spring blooms in chironomids and baetids forcefully demonstrated in the 1991-1993 data Finally, the Scirtidae, a coleopteran group whose densities were not correlated to either flow or temperature variables in the 1991-3 data set (Figure 6.32) appeared in all seasons, with generally higher densities in the summer and autumn of 1996, following the dry winters of 1994 and 1995 (Figure 6.37).

Overall however, seasonal variation in the number of taxa was well demonstrated for the period 1994 – 1997 as with the first data set from 1991-1993 with winter lows and late spring – summer peak taxon density. Interestingly, in 1996, the number of taxa in April / May and May / June remained high and drop off from its summer level, as it did in 1995, and this was despite four floods >30 $m^3 s^{-1}$ occurring prior to the May / June sampling in 1996, whereas there were no floods greater than this threshold in the autumn of 1995 – indeed that year was characterised as a drier than average year, with a late start to flooding.

Baetis harrisoni

Elporia spp.

Pseudocloeon vinosum

Helodidae

Baetis sp 3

Elmidae

Afronurus spp.

Agapetus agilis

Telagonididae

Athripsodes bergensis sp.A

Leptophlebiidae

Oecetis sp.

Figure 6.37. Relative abundance[4] of selected taxa, for each sampling date from August 1994 to January 1997. The y-axis represents the average of the abundance classes from all four sampling sites. Abundance classes were based on a scale of 4, where 1 = a single observation, 2 = 2-10 individuals, 3 = 11 – 100 individuals; and 4 = 101 – 1000 individuals. Colour-coding as per Figure 6.36.

6.3.3.ii Year-on-year persistence and stability

Whilst intra-annual variability, in terms of the pattern of change from month to month and over seasons, has thus far been the focus of this chapter, both the 1991-1993 and 1994-1997 data sets showed considerable inter-annual variability in both the number and timing of floods and of invertebrate community structure.

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[4] abundance was rated on a scale of one to four, where 1 = a single sighting; 2 = an estimated abundance of between 1 and 10 individuals; 3 = between 10 and 100 individuals; and 4 = between 100 and 1000 individuals. These graphs show the average of the abundance classes from all four sampling sites on each date

In this study, the specific hypothesis to be tested is that invertebrate assemblages during the summer lowflow period, characterised by low levels of disturbance, should show greater year-on-year persistence than those assemblages subject to frequent flood events, where these measures should be influenced by interannual differences in flood intensity and frequency. Alternatively, if invertebrates are adapted to the flow regime, even to the lack of predictability in the timing or magnitude of floods, then their assemblages should be predictable and persistent, because at local or reach level they have evolved resistance to disturbance. In that case, persistence should be high year-on-year for all seasons, but stability may still vary as a result of short term climatic differences within each winter flood season.

Both Jaccard's persistence index and that using Bray Curtis presence absence data were calculated for each of the sampling sites, and for the calendar months Aug/Sep to Jan/Feb, which reflects the months for which two years' worth of data were collected. A one-way ANOVA showed that there were indeed differences in persistence between various months (ANOVA F=4.99, p = 0.0001). Post-hoc Tukeys test showed that the months of A/S, S/O, O/N and N/D were significantly different from D/J and J/F. These are indicated by letters denoting months with similar persistence in Figure 6.38. These results demonstrate a small, but significantly higher level of persistence in the summer months than in the late winter or spring months. Stability in the spring months, especially S/O, was substantially lower than the winter or summer months, and also lower than the persistence measure. The Spearman's rank correlation measure of stability gave a similar result (data not shown).

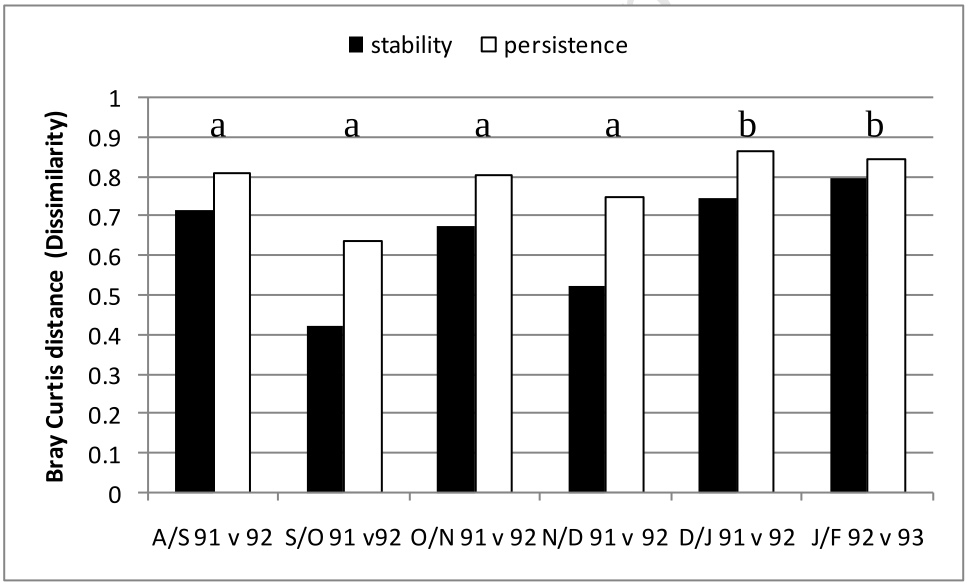

Figure 6.38. **Persistence and stability for year-on-year comparisons of calendar months for which data were available: the months of A/S to J/F between 1991 and 1992. Months with significantly different persistence levels are indicated by similar letters, a or b.**

Differences in year-on-year persistence by month over the 1994-1997 period were somewhat less demonstrable (Figure 6.39). The comparison of 1994 with 1995 showed the same levels of persistence year on year for the winter spring and summer months. Only the 1995 vs. 1996 comparison showed lower persistence levels, associated with the autumn and winter periods, where flood differences were pronounced.

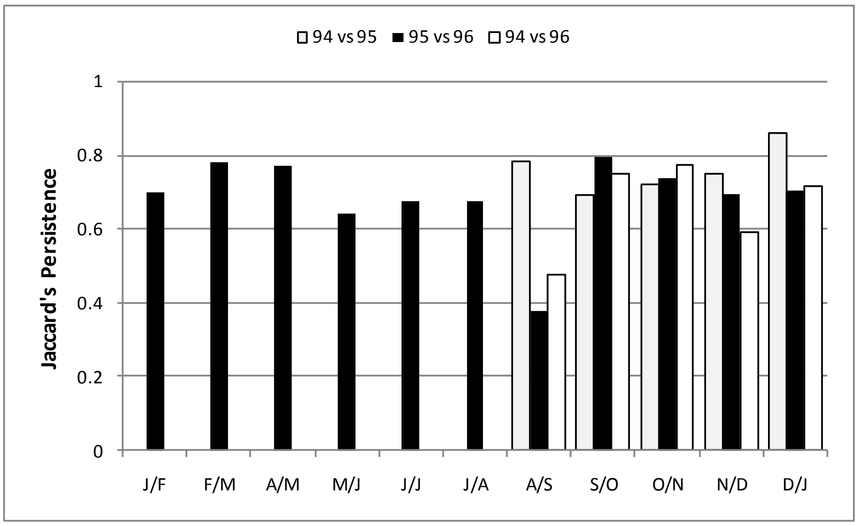

Figure 6.39. Jaccard's persistence index for year-on-year comparisons of calendar months for the 1994-1997 data.

6.4 DISCUSSION

This study has demonstrated strong, cyclical patterns of intra-annual variation in invertebrate community structure in the Molenaars River that are a) characterised by significant changes in community composition at monthly time scales and b) strongly correlated with temperature and the occurrence of floods in the immediate past.

This demonstration of the temporal scale of community change has been possible, firstly because of the time step – a month – at which the investigation was conducted. Most studies of temporal change on an annual or greater scale have identified two periods, be these winter / summer seasons or wet / dry phases, either through only sampling at two intervals, or by aggregating data into these (e.g. King 1981; Bunn *et al.* 1986; McElravy *et al.* 1989; Jacobsen & Encalada 1998; Beche *et al.* 2006; Beche & Resh 2007). It is argued that this finer scale investigation has the advantage of exposing smaller scale shifts in communities that help to differentiate between seasonal variables and flow, or flood effects, on invertebrate communities. A similar argument has been made by Suren & Jowett (2006).

The second important aspect of this study, in relation to demonstrating and attempting to explain intra-annual pattern is the fact that it has examined these patterns over more than one year. A general pattern of intra-annual change can only be demonstrated after examination of how variable such a pattern might be on time scales of more than one year. Shortcomings in this study are acknowledged, however, in the extent of inter-annual replication, and especially the lack of quantitative data for the longer-term examination of pattern, but do not preclude some insights being drawn regarding the stability of intra-annual patterns of assembly in invertebrate communities.

The Molenaars River is very characteristic of streams in Mediterranean climates, experiencing hot dry summers when flow is low, and cool winters during which most of the annual precipitation is concentrated within a few months. Gasith & Resh (1999) predicted that generalised invertebrate community patterns should result from this hydrological regime, viz. low densities at the end of winter, as a result of reductions in population sizes by floods; constant levels of species richness, because adaptations to the flow regime should allow for species to exploit their environment throughout the year; a gradual increase in densities from winter lows to summer maxima, but a decline in diversity measures in summer due to the dominance by taxa capable of exploiting harsh late summer conditions; and a rapid changeover from summer to winter communities, linked to the onset of winter floods.

Low densities during winter, or the immediate post-winter period compared with the summer period, have been documented by *inter alia* Bunn *et al*. (1986), Boulton *et al.* 1(992), McElravy *et al.* (1989), Feminella & Resh (1990), Beche *et al.* (2006) and Suren & Jowett (2006). Where these studies have examined inter-annual patterns, both wetter than average years and drought years were associated with lower post-flood season densities than were average or dry years (e.g. McElravy *et al.* 1989; Beche & Resh 2007). The obvious implication is that increased levels of disturbance influence densities in the post-disturbance period.

Seasonal patterns in overall invertebrate densities were also observed in the present study, with lowest density in the winter months. However, densities were not necessarily lowest at the end of the flood season as predicted by Gasith & Resh (1999), particularly in the second year of the study. Densities in the winter-spring period did not follow a uniform trend of progressive decreases to a post-flood low, as predicted by Gasith & Resh (1999), but fluctuated on a monthly basis during the winter and especially spring, and these fluctuations appeared to be driven by a number of interacting factors.

Firstly, there was a strong negative correlation between invertebrate densities and the frequency of floods in the immediate period preceding sampling, suggesting that this may be an important factor in larval population dynamics. Community patterns were significantly correlated with flood frequency in the 60-day period preceding sampling, as were the densities of a number of individual taxa, a finding shared by Suren & Jowett (2006) and McElravy *et al.* (1989). The absence of floods, however, was not always associated with increases in density, whilst density reductions occurred in some months even where there were no floods in the preceding 30 or 60 days, for example in autumn. Even comparing months only within the winter period, similar changes in density were observed in cases with only one and with seven floods in the preceding 30 or 60 days. The variance in this relationship was more pronounced during the spring months, associated with the dramatic fluctuations in chironomid and baetid densities.

Secondly, a large reduction in densities of individual taxa and total invertebrates was observed after the large (1:20 year return period) flood between the April/May and May/June sampling in 1992. This was associated with a marked change in community structure, in agreement with the rapid changeover from summer to winter communities predicted by Gasith & Resh (1999). In the winter of 1995, however, a similarly large shift in community occurred from A/M to M/J, but no floods above the threshold for disturbance had yet occurred in the autumn period preceding this. The following year, the samples collected in A/M and M/J were very similar, despite a very similar temperature and autumn flows as the previous year, and the autumn-winter shift predicted by Gasith & Resh (1999) occurred a month later, again coinciding with a 1:20 year flood. Whilst the shifts in invertebrate assemblages may easily be explained by the large floods in 1992 and 1996, the shifts in 1995 appear to be unrelated to either temperature or flow cues.

Thirdly, the lowest densities recorded during the winter months were clearly those where the sampling was conducted within seven days of the previous flood. For example, the July / August 1992 samples were collected following a period with seven flood days, including a large flood, but had substantially greater densities than the samples from August / September, which were associated with only one flood of half the size, but which occurred six days prior to sampling. The July / August sample, however, was collected two weeks after floods subsided and contained chironomid densities of some 3500 m^{-2}, three times higher than those of the following August / September month. Flecker & Feifareck (1994) measured orders-of-magnitude fluctuations in invertebrate densities between successive months in their study of streams in the Andes. The most dramatic of these were the increase in density that accompanied the onset of flow after a dry summer period and the decrease in density following the first winter storm. That study also found no trend of declining densities over the wet period, and the month-to-month fluctuations were also driven by fluxes in chironomid densities, and were related to the number of days since flooding.

Under these circumstances, invertebrate assemblages could be anticipated to show a strong relationship with the number of days after a flood. This was not the case, taking into account the full annual cycle, however: there was no linear relationship between either invertebrate densities as a whole or densities of any but a few individual taxa with the number of days since the last flood, and even the significant relationships were weak. In some cases, a linear relationship appeared to hold up to about 50 days post flood, but fall away thereafter. In the studies of Grimm & Fischer (1989) and Giller *et al.* (1991), densities showed a post-flood increase up to some threshold time, where after this relationship did not hold, suggesting that other forces, not directly related to floods, are more likely to influence species populations and invertebrate assemblages in longer periods. Indeed, McElravy *et al.* (1989) found no relationship between invertebrate densities and this variable, but in their study there was always a minimum of 20 days, and often far more, that elapsed between the end of a storm and the collection of samples.

Grimm & Fisher (1989), working in a desert stream subject to aseasonal flash flooding, found macroinvertebrates whose densities had been reduced by some 80 % by large floods took 13 – 52 days to reach pre-flood levels. In their study, successional change in invertebrate assemblage structure was inhibited in cases where post- flood sequences of recovery were short because of the onset of the next flood, but that for sequences longer than 40 days without new floods, there were complex natural patterns of succession in invertebrate assemblages that were not related to flood disturbance. These were considered to be rather mediated by biotic interactions.

In this present study, the time between the last disturbance flood and the sample collection was very important in respect of community structure in the late winter – spring periods in both 1991 and 1992. Where 30 or more days had passed since a flood, at this time of the year, the community change was extremely marked, dominated by massive population outbursts of chironomids, baetids and simuliids, relative to the preceding month's sample, but also characterised by increases in many other, less numerically dominant taxa such as Leptophlebiidae, Hydropsychidae and many beetle families. This set of circumstance happened to prevail in the period between late September and late October in 1991, but occurred a month earlier, between late August and late September, in 1992. The difference in timing of these population increases between the two years was simply as a result of the floodless period during late winter of 1992 rather than in spring as was the case in 1991. Extended periods of benign conditions like these in the midst of winter are fairly common, as demonstrated in the presentation of inter-annual variation in flood frequencies for each calendar month for the Molenaars and Berg Rivers in Chapter 5. The relatively low predictability by month of floods in the Molenaars River has a corollary: periods of 30 days or more when conditions are relatively benign will be a feature of the winter flood season. This may have implications for life history adaptations, as will be further examined in Chapter 7.

Floods are often referred to as "reset" mechanisms, returning a community to a pioneer state, and preventing the attainment of equilibrium conditions (*sensu* Connell's (1978) Intermediate Disturbance

Hypothesis (IDH) and Huston's Dynamic Equilibrium Model of community structure (Huston 1979, 1994). However, disturbance is rarely cataclysmic. Ledger *et al.* (2006) refer to the importance of the "biological legacy" following a disturbance, namely the types and quantities of organic matter and biota that persist through a disturbance event, and make the point that there are few examples where such remnant matter or biota are not fairly substantial. The nature of persisting biota will exert a strong influence on the process of recovery, since the endpoint in any trajectory of change is highly contingent on the antecedent conditions. Disturbance can leave behind either minor or major legacies, to continue the analogy of Ledger *et al.* (2006). For small floods the remnant fauna may be similar to that prior to the disturbance, so that whilst these may be strong enough to alter relative densities, subtly change the availability of resources or influence competitive outcomes, they seldom override the seasonal pattern of community change, since their effect on populations is not total. Given the temporally changing set of environmental conditions faced by these remnant populations, it may be more appropriate to refer to "post-flood trajectories of community assembly" to describe what happens after a flood, rather than to use the term recovery, since the latter implies that factors other than the disturbance effect remain static, or that an end-point identical to the starting point can be reached. In contrast where ambient conditions are also changing after floods, these will equally affect the pattern of invertebrate community assembly, and determine the rate at which species densities and resources accumulate to levels at which biotic interactions may assume importance in ecosystem structuring.

Gasith & Resh (1999) predicted that after the wet season floods in Mediterranean systems, invertebrate biota will gradually increase in abundance from individuals colonising from nearby habitats or refugia. This study had a different outcome. Even the degree of community shift associated with the 1:20-year flood at the onset of winter was far exceeded in magnitude by the dramatic increases in abundance and the dynamic shifts in invertebrate assemblage during spring months: these do not resemble a recovery to a pre-flood state, but rather suggest a chain of events that are set in motion through the combined effects of floods, flood cessation and temperature cycles. The spring period was associated with the highest month-to-month variability in invertebrate community structure of all, where densities were as large as those in summer, but strongly dominated by baetids or chironomids. The large fluctuations between months could have been partly the result of the sampling coinciding with hatching of eggs or emergence of adults of these two groups, as a result of increased generation turnover, as will be examined in the next chapter.

The predictions regarding low diversity during the summer (Gasith & Resh 1999) are not borne out by this study. This is not surprising, since the Molenaars River does not experience the same summer drought conditions as many Mediterranean rivers, given its strong perenniality. Temperature does reach a maximum during summer, and high temperatures combined with increases in algal biomass may cause periodic stress in terms of water quality, but seemingly not to the point of eliminating sensitive taxa. Rather, diversity was highest in late summer and in winter, driven by increased representation of beetle, trichopteran and mayfly groups and reduced dominance of baetids and chironomids, and lowest in spring, which could be a consequence of the chironomid and baetid dominance then. In line with the predictions of Gasith & Resh (1999), species richness remained reasonably constant year round, although it was slightly lower in winter. This may have been influenced by the much smaller sample densities in these months.

The relationship between invertebrate density and temperature was marginally stronger than the flood measures. Temperature, as cumulative degree days, explained a greater proportion of the overall pattern in community change over the study period than did floods, notwithstanding the examples discussed above for the independent influence of flood disturbance. Boulton *et al*. (1992) also showed very strong seasonality in invertebrate assemblages correlated not only with discharge, but with temperature and algal standing crop.

Most of the taxa implicated in the changes to community structure during the late winter and spring months were those assigned to the category of algal consumer / grazer. The results of this study suggest that invertebrate grazers are more strongly affected by flood flows and post-winter increases in temperature than by the availability of periphyton resources, since no relationship with the latter could be found. Discarding the influence of periphyton on patterns of invertebrate assembly, however, may be simplistic. Firstly, periphyton density as measured in this study only represents what is present on the stream bed, not what might be being consumed by invertebrate grazers, and non-significant correlations might mask a real contribution by periphyton resources that enable the massive outbreaks of chironomids and baetids in spring and early summer. Figure 6.40 shows the seasonal and interannual patterns in periphyton and grazers, alongside changes in flow and temperature. Periphyton densities increase during winter when invertebrate grazer densities are low, most clearly seen during 1992. Indeed, Wellnitz & Rader (2003) report that whilst flood flows can reduce algal densities, constantly elevated base flows result in substantially greater accumulation of biomass than under conditions where flow velocities are slow. Hence, whilst grazers are kept in low densities by frequent floods, periphyton biomass is likely to increase rapidly during the intervals between floods in winter and spring, aided by elevated base flow, thus providing a large resource for those taxa resilient or resistant enough to floods to utilise it.

Algal abundances are quick to respond after floods, and may reach peak densities within a few days (Stevenson 1990). Thus a series of increases and losses may have occurred within the period between each monthly sampling in this study, making interpretation of the data ambiguous. Temporal sampling on a monthly schedule may not provide an adequate basis to evaluate densities during a period characterised by frequent floods. This weakness is particularly true given the potential importance of periphyton as a factor influencing invertebrate densities in spring and early summer. Power (1992), working in a Californian stream, revealed that seasonal blooms of attached filamentous algae are a natural feature even of largely unenriched systems, and are associated with seasonal changes in macroinvertebrate communities, particularly reduction in their densities as a result of floods. In these rocky, open-canopied rivers, algae are quickest to recover from the scouring action of winter high flows, through vegetative regrowth by basal holdfasts (Power 1992). Power (1992) provided preliminary evidence to show that, following winter flooding, filamentous algae enjoy a window of time with favourable growth conditions in a community with only one “functionally significant” trophic level: producers unchecked by herbivory. This was followed by sequences of more complex trophic relationships as invertebrate numbers built up.

The periphyton AFDW data for the 1991-1993 period in the Molenaars River, notwithstanding poor replication, show a complex pattern of change over time, in relation to flow, temperature and grazer densities (Figure 6.40). Periphyton densities were reduced by floods although not in proportion to flood magnitude. However, in late winter / early spring, along with a turnabout in temperatures from July minima, periphyton density increased, whilst corresponding grazer densities remained relatively low. This is in line with the notion of a window of opportunity during which algae are able to increase without a top-down herbivore control. Initial post-flood increases in periphyton are usually associated with diatom dominance (Biggs 1996), which comprises a major food source for invertebrates. In 1991, but more

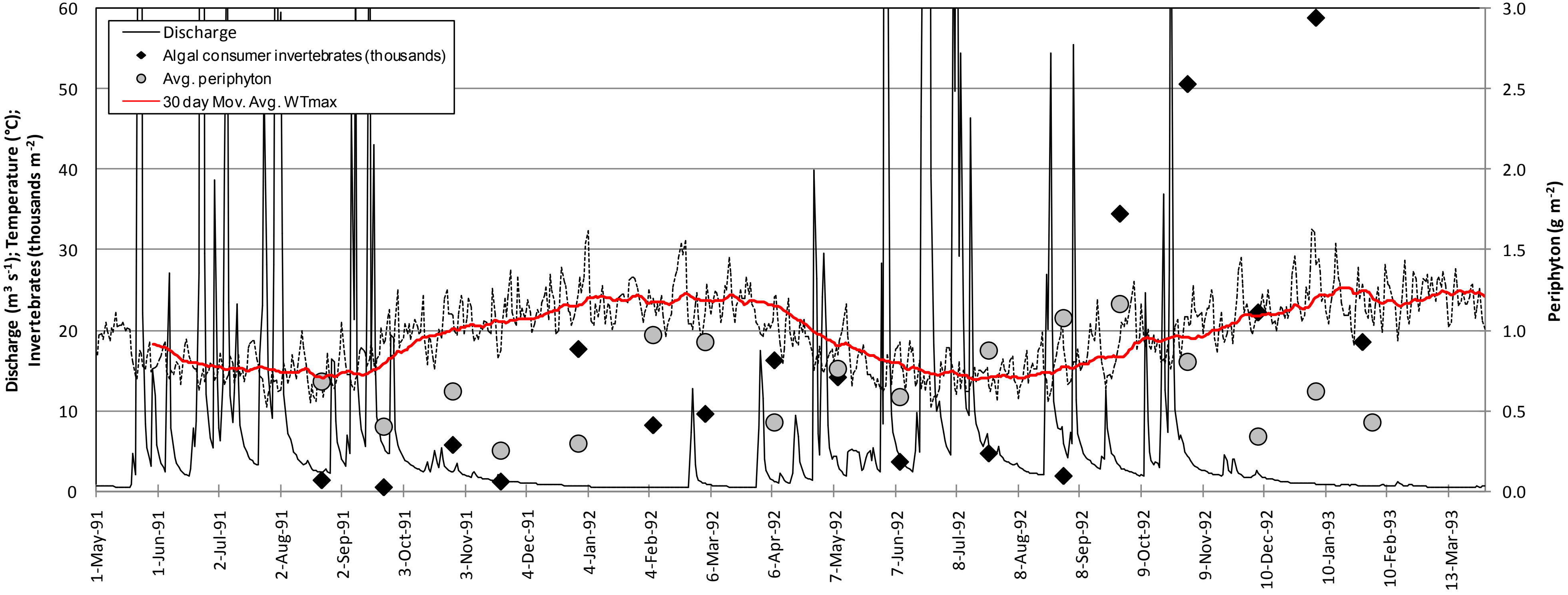

Figure 6.40. **Patterns in invertebrate grazer numbers and periphyton density (AFDW), with changes in temperature and the flow regime in the Molenaars River from 1991 to 1993. Data are based on averages from Sites 1, 7 and 8.**

demonstrably in 1992, the late winter - spring increases in periphyton was followed by the blooms of grazers, chironomids and baetids as already described, from October onward, resulting in early summer declines in periphyton biomass. By late summer periphyton biomass once again increased: this is speculated to be an increase driven by Cyanophyta, blue-green algae, which are typical of the late summer flora in the Molenaars River (J. Ewart-Smith, Freshwater Research Unit, University of Cape Town, unpublished data 2008), and which are unpalatable to invertebrates

The pattern of intra-annual change in community structure was repeated in annual cycles through the study period, from 1991 to 1993 and 1994 to 1997. The exact timing of population reductions and population growth was variable though. This may have reflected short-term impacts of floods on densities. Similarly, temperature patterns varied subtly from year to year, particularly during spring. However, other factors not incorporated into this study may influence life-history events such as recruitment or emergence, and these could explain changes in density that are unrelated to either the temperature or flow regime, despite the seeming synchrony between these latter variables and invertebrate densities. Without data that, for example provide insights into growth rates or mortality, surveys like this one will be limited in the degree to which they can ascribe causes to observed biological patterns.

The differences in timing of density changes within each year were reflected in only moderate levels of stability, measured year-on-year, chiefly during the winter and spring season, with higher stability in community structure in summer (no data were available for autumn). The higher stability in the winter period than in spring appeared to be the result of low numbers and relatively small fluctuations in population sizes of individual taxa. The fact that invertebrate assemblages after each of the 1:20 year return period floods – before the M/J sampling in 1992 and before the J/J sampling in 1996 – were not outliers in the MDS similarity plots (Figures 6.22 and 6.34) speaks to a high resistance in the invertebrate community over the course of these events. Also, year-on year, the winter months, June July and August were characterised by greater constancy in their community composition than were the spring or autumn months, suggesting that invertebrate assemblages during the major flood period are in fact fairly predictable. This points to other processes that might offset the effects of disturbance, such as continuous recruitment among some taxa.

Persistence levels followed much the same pattern, but were higher all round. In the 1994 – 1997 period, persistence was lowest when the driest year was compared with wettest year. This is similar to the findings of McElravy *et al.* (1989) and Beche & Resh (2007), although their studies incorporated a far wider range of climatic variability. This present study was conducted in a series of “normal” years, and presumably more pronounced differences in stability could be expected to occur in the Molenaars River over a more expanded time slice. Persistence in the Molenaars River appears to be the consequence of a fairly continuous presence of most of the taxa comprising the invertebrate fauna of the river, although a few groups are restricted to winter or summer months, whilst stability is provided by fairly consistent year-on-year patterns of monthly change in their individual densities. Lower stability in spring months appears to be associated with year-on-year differences in the densities of the chironomids, baetids and simuliids, whose numbers may fluctuate as a result of generation turnover occurring on time scales shorter than this study was able to measure.

This study shows, therefore, that invertebrate assemblages are highly variable over intra-annual time scales in the order of months or less. During the winter months, when floods are more regular and / or frequent, constancy in community structure from month to month is higher than in spring, where temperature increases interact with variable frequencies of floods, resulting in greater fluctuation in densities. The summer period, where floods are absent and temperatures are relatively constant, has highest month-to-month constancy.

Despite this intra-annual variability in invertebrate assemblages, a general seasonal pattern of change in community assemblage was repeated year on year, driven by the combination of temperature, and the onset and cessation of floods. Flood frequency and magnitude, and the time interval between floods show inter-annual variability that is fairly well correlated with in these community dynamics. This study was conducted over a period constituting fairly average climatic conditions, and larger inter-annual fluctuations in seasonal patterns of assembly are expected to be demonstrated with an increased time slice.

Examination of the life history characteristics of the invertebrate biota in the Molenaars River may help to explain the large fluctuations in species densities from month to month exhibited in this study river. This is the subject of the next chapter.

7 INVERTEBRATE LIFE-HISTORY ADAPTATIONS TO FLOODS

7.1 INTRODUCTION

In disturbance studies the terms resistance and resilience can be used to describe adaptations by species to increase their survivorship. Resistance traits describe adaptations to withstand the physical stresses of disturbance and resilience traits describe adaptations to ensure that some proportion of the population escape these stresses and are available to recolonise river habitats (Townsend & Hildrew 1994; Lancaster & Belyea 1997). A generally-held view is that invertebrate species display low resistance but high resilience to disturbance (e.g. Niemi *et al.* 1990). Factors affecting recovery at a local or landscape level include the scale of disturbance and the presence of physical refugia which are generally provided by the heterogeneity of the physical environment (Hildrew & Giller 1994). From a biological perspective, recovery is influenced by the resilience characteristics of the organisms, in particular their mobility, and their life-history traits which in part determine the availability of new recruits at the time of the disturbance.

Where disturbance is highly predictable, the evolution of mechanisms that avoid exposure to mortality-inducing floods would be expected (Lytle & Poff 2004), particularly where resistance to floods is low. These mechanisms include metamorphosis to a life stage that is outside of the stream or located in the hyporheos, with metamorphosis timed to coincide with the start of the flood season (Lytle 2002). Life cycles may also be geared to maximise growth and reproduction during periods of stable flow (Gasith & Resh 1999). Short life cycles and rapid development from larvae to adult allow species to take advantage of the sometimes brief periods during which conditions are favourable (Lytle & Poff 2004).

Synchrony in life histories to the average onset of the disturbance period is advantageous where disturbance is predictable and severity is certain, but can come with costs, such as lost opportunity to feed and grow if floods are late or small, or increased mortality if floods are early (Lytle 2002; Lytle & Poff 2004). Even within regions with strongly seasonal flood regimes, high inter-annual variability in flood frequency or magnitude may preclude the evolution of adaptive responses that favour avoidance of disturbance periods. Instead, bet-hedging adaptations such as asynchronous hatching of portions of eggs over an extended time may be more useful (Huryn & Wallace 2000). If vulnerable life stages are exposed to disturbance then allocation of resources to the development of resistance traits, such as armouring and anchors, would be of increased value in maximising survival.

High levels of inter-annual variability in flooding patterns, where disturbance may not regularly affect stream populations at certain times, may also constitute a noisy evolutionary signal. Here mortality-inducing floods may be too variable in timing to act as a dominant evolutionary force because of the increased advantages of risk taking, in years when floods do not occur. Other factors may then become as or more important as drivers of species attributes and in determining the development trajectory of species, for example, competition for resources and predation (e.g. Peckarsky *et al.* 2001). Similarly, the reduced fecundity resulting from rapid development rates and small size on emergence is only advantageous, on the basis of a balance of fitness cost, where the risk of mortality is high (Huryn & Wallace 2000). Development at higher temperatures also constitutes a cost in terms of allocation of resources to increased respiration rather than growth and fecundity (Rowe & Ludwig 1991). In the Molenaars and Berg Rivers, considerable inter-annual variation in the timing, frequency and magnitude of bed-moving floods is a characteristic of the long-term flow regime. The examination of responses to individual flood events in

Chapter 4 suggests that for most taxa, reductions in populations are the immediate consequence of frequently-occurring, intra-annual, floods that are capable of initiating bed movement. Where floods are clustered into a winter season, therefore, it would be expected that progressive reduction of the invertebrate standing stock would take place. The predictions of Gasith & Resh (1999) of lowest densities at the end of the flood season are a logical corollary of this expected incremental decline in population density.

The flood study (Chapter 4) compared pre-flood population densities with those after a series of three to five floods, using post-flood samples collected within four days of the last flood event. These showed density decreases of 34% in the Molenaars River after a series of non-bed moving spates and 56% in the Berg River following five floods, two of which moved some 40% of the stream bed. However, temporal patterns in invertebrate assemblages in the Molenaars River over longer time scales i.e. months (Chapter 6) show that density reduction as a result of a flood appears to be of a more transient nature than a whole winter season: month-to-month changes in invertebrate density in the Molenaars River showed reductions during the flood season only when samples were collected very shortly after a flood, i.e. within seven to ten days. Moreover, increases in overall invertebrate density occurred in winter and spring months when samples were collected after a period of some 30 days without any flooding. Whilst these increases were not displayed by all of the taxa, being generally displayed more obviously by the simuliids, chironomids and baetids, most invertebrate species in the Molenaars River do at the very least show year-round presence. The observation of either only short-term impacts of floods, and / or the continuous representation of species throughout the winter months, suggests higher levels of resistance to floods than previously registered, or a resilience that stems from active use of refugia and rapid recolonisation of the stream bed after disturbance. These refugia must exist despite the fact that even intra-annual floods above the disturbance threshold, i.e. occurring on average seven times each year, move upward of 40% of the substratum. Besides physical, or in-stream, refugia used by surface-dwelling populations, Lancaster & Beylea (1997) also described changes in habitude (i.e. where different life stages use different microhabitat types, such as hyporheic eggs) and temporal refugia through complex life cycles (e.g. different phases occupy different habitat types), that increases resilience in the face of disturbance.

An examination of the life-history patterns of these invertebrates may not only provide the key to understanding how resistance and / or resilience attributes account for the relatively constant presence of invertebrate species in the Molenaars River, and their high year-on-year persistence, but should also explain the large fluctuations in species densities from month to month exhibited in this river.

7.1.1 Aims of this study

The focus of this chapter is an exploration of the sorts of life-history patterns, at least in relation to larval development, that have evolved in various invertebrate species inhabiting the Molenaars River. Most life-history patterns are the consequence of evolutionary trade-offs between the risks of mortality and the benefits associated with being in a place where resources maximise growth and reproductive output. In the case of the Molenaars River (and other Western Cape rivers with similar hydrological patterns), these trade-offs could result in a number of life-history 'types', all of which may be equally beneficial. Whilst the particularities of the flood regime, in combination with the temperature, which reaches seasonal peak values during the low-flow period would be expected to play an influential role in life-history adaptation, the data provided here cannot do more than provide a preliminary sketch of differences in broad development periods and some indication of cohort patterns in the selected species.

The data used to characterise the various life-history types were taken from the 1991-1993 Molenaars River samples, providing a basis to compare population size structure alongside density changes for a number of key taxa.

7.2 METHODS

Three of the eight sites used for analysis of family-level patterns in Chapter 6 were selected for the life-history study. The samples from Sites 1, 7 and 8, which were shown in Chapter 6 to have no significant differences in densities of the range of taxa examined, were pooled to provide a reach-level picture of changes in population size distribution of a number of species. Not all samples from each month were available for this analysis, because of some errors during the curation of the samples between the time of the initial family-level analysis of densities and the later species-level identification and head-capsule measurement.

In some cases, identification below genus was impossible, especially for all life stages, and data were therefore pooled at a genus level for some taxa. In the case of *Baetis* species, the early instar data were pooled, but split into different species for larger size classes.

Invertebrate identifications and measurements of head-capsule width were performed using Nikon SMZ 1500 and SMZ 1B dissecting microscopes, an Olympus compound microscope equipped with a measuring graticle, as well as numerous field guides and taxonomic keys (de Moor 2002; Barber-James & Lugo-Ortiz 2003; de Moor & Scott 2003; Mansell 2003; Samways & Wilmott 2003). No information on development stage was obtainable from this data set, for example the designation of first or second instars, but rather size was used as a proxy for relative age. The presence of pupae was recorded for holometabolous species, and existing literature on emergence periods and adult flight was used to assist with interpretation of the results. The study did not extend to include a range of data often collected in focussed life-history studies, such as growth rates and biomass or secondary production estimates.

Density estimates for each species for each month were obtained by multiplying the family-level average densities by the proportion of head counts belonging to each of the species comprising that family. This was because whilst the average densities would have been calculated as the average of all replicate samples from the sites, not all these samples were available for head-capsule width measurement, owing to problems with the curation of some of the samples between the initial processing and the life-history analysis. It was therefore considered to be more consistent with the analysis in Chapter 6 to use the original data estimates of density, than to multiply up the subsample used for size measurement. In a small number of instances (Plecoptera and Chironomidae), no samples for one or more of the months were adequately curated, preventing any population size determinations for those months, and resulting in data gaps.

A final caveat was that the 1991-1993 samples were collected from riffle biotopes only, and then only three replicates per site. The pooling of sites to some extent increased the spatial replication, but only in riffles. Other biotopes that might be preferred by different life stages were therefore not sampled, providing an incomplete picture of the inhabitation of the river bed by larval stages during the year.

Despite the qualifications above, size frequency and density data were compiled for 19 of the most abundant species or genera in the Molenaars River. Life-history patterns were described based on visual interpretation of the size frequency data. Cohorts – groups of animals hatching at the same time – were identified by visual inspection, where peaks were evident and were separated by troughs in the size-frequency data. Voltinism – the number of times per year that the generational cycle from egg to adult was completed, was defined from Lake *et al.* (1985) as follows: univoltine = one generation per year; bivoltine = two generations per year; multivoltine = more than two generations per year; semivoltine = one generation every two years.

7.3 RESULTS

The size frequency data demonstrate clear differences in life-history responses to floods in the Molenaars River (Figures 7.1 to 7.19).

Only a small number of taxa were restricted to the summer period or had life-history patterns where developmental stages were clearly timed so as to avoid the flood period altogether. The other two life-history patterns hypothesised to occur, namely a winter seasonal development, with avoidance of summer in-stream conditions, and a mix of asynchronous development and / or multivoltine life cycles, were prevalent in the Molenaars River. In addition, a fourth life-history type, namely a slow seasonal life history with synchronised development throughout the year, was also displayed by many taxa, contrary to expectations.

7.3.1 Summer life histories with restricted exposure to winter floods

Of all the taxa examined, only two displayed life histories that were clearly geared toward avoiding winter floods - the leptophlebiid (Ephemeroptera) species, *Adenophlebia peringueyella* and *Aprionyx* spp. (probably one species, *A. peterseni*). In the 2003 autumn pre-flood survey (Chapter 3), both species were found to favour slackwaters significantly above riffles or runs. The patterns of change in population size distribution shown in Figures 7.1 and 7.2, therefore, are somewhat incomplete, since the 1991-1993 data were collected from riffles only[5]. These show the appearance of young nymphs in the spring, with two or three cohorts apparent at the time of their first appearance in November / December. The shape of the size-frequency distribution, especially that of *Aprionyx* spp. suggests that these cohorts were possibly recruited outside of riffle areas where hatching could have occurred initially. Fairly rapid development, and the achievement of mature size classes by a substantial proportion of the population occurred in the case of *Aprionyx* by February / March (Figure 7.2) and by April / May in *Adenophlebia* (Figure 7.1). Recruitment of new cohorts in the riffles appeared to continue until January / February. Whilst densities were high in summer, most animals seemed to disappear in later summer and autumn. The 2003 autumn survey (Chapter 3) showed that these species inhabit mostly the more sheltered habitats during autumn, and field observations indicated emergence from slackwaters and river margins in summer and autumn. The sudden decline in *Aprionyx* densities in February / March, particularly of larger instars, suggests their relocation to slower-flowing habitats from which emergence is undertaken by crawling up emergent boulders (pers. obs.). A similar phenomenon was evident in the case of *Adenophlebia peringueyella*, but somewhat later in the season, with numbers declining substantially in the April / May period.

An additional feature, however, is that a small number of individuals appeared to persist through the winter, which suggests that nymph growth in these life cycles is not wholly restricted to summer in the Molenaars River. Low densities of mid-sized nymphs were recorded during winter in both species, but

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[5] The 1991-1993 samples were collected from riffles only using a box-sampler. The 2003 samples were collected from individual stones on the surface of the river bed, thus representing the surface hydraulic forces associated with riffles, runs, slackwaters. These indicated a preference in these species for slackwaters in autumn, and their virtual absence from samples after floods.

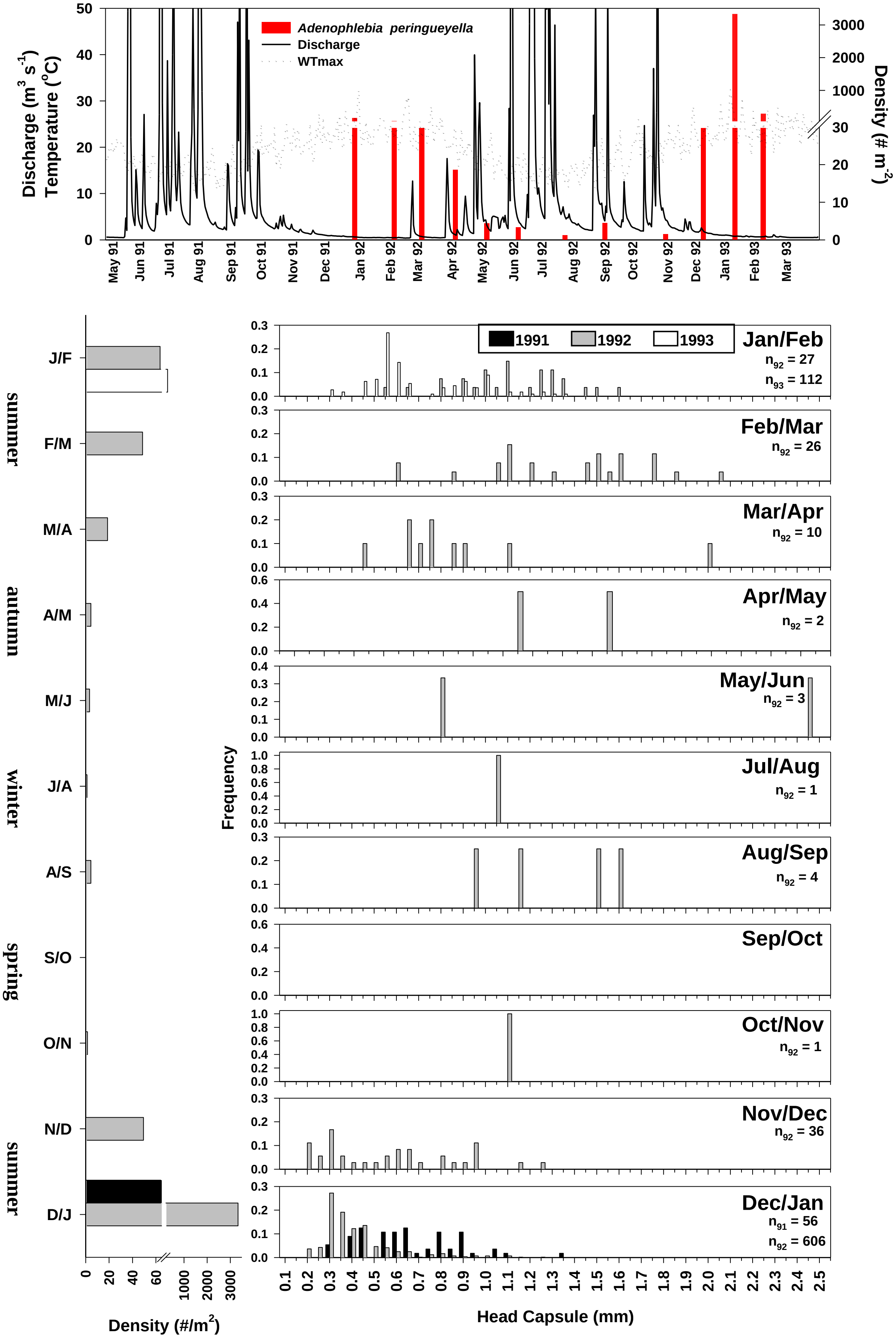


Figure 7.1 *Adenophlebia peringueyella* densities (side panel and top, red bars) and head-capsule size frequency by sampling period for each year. The number of head capsules used to create each frequency-distribution is indicated for each month / year.

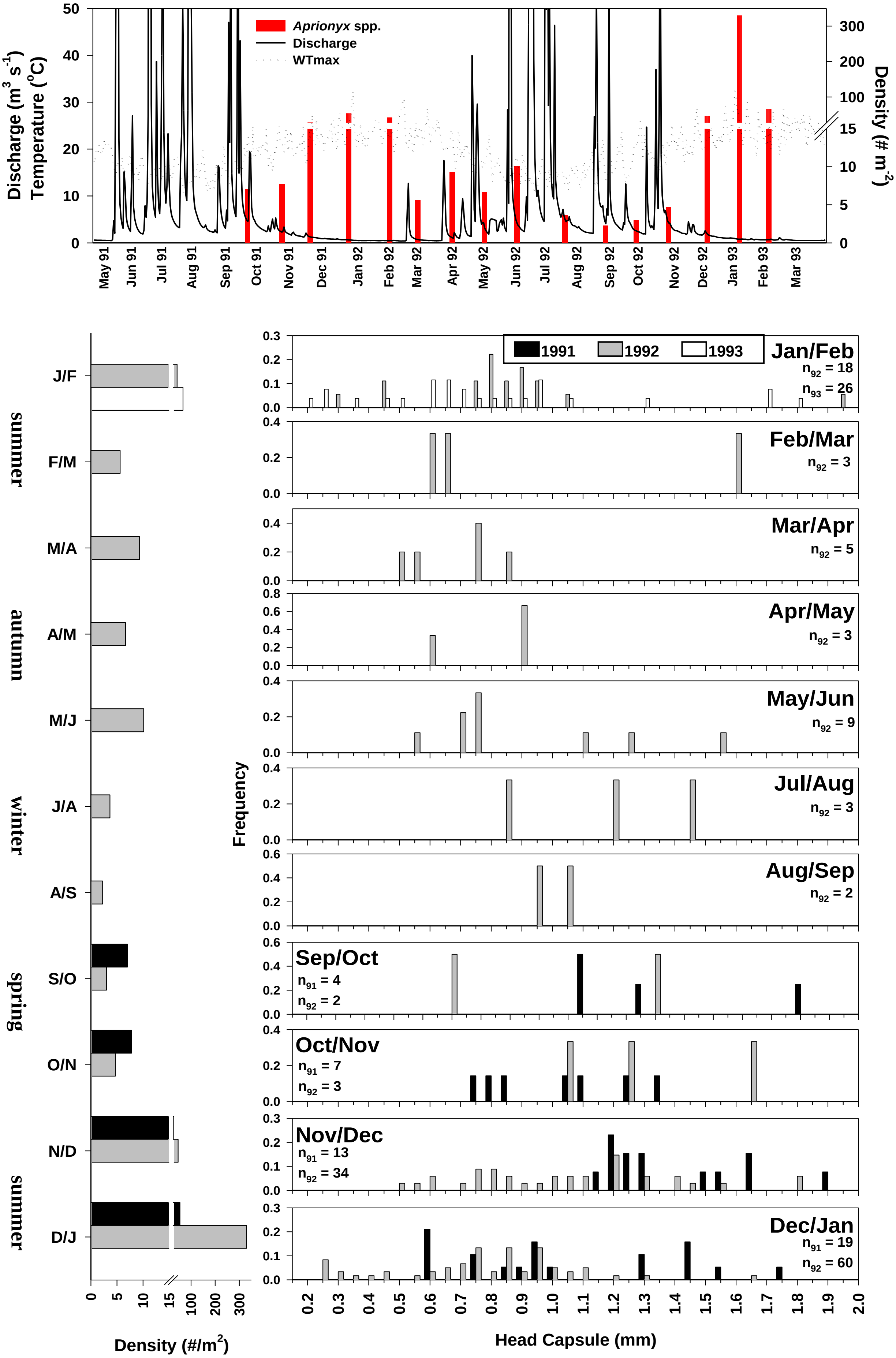


Figure 7.2 *Aprionyx peterseni* densities (side panel and top, red bars) and head-capsule size frequency by sampling period for each year. The number of head capsules used to create each frequency distribution is indicated for each month / year.

especially *Aprionyx* spp. These matured in early spring. This phenomenon of portions of a cohort or population developing at different rates is known as cohort splitting (Lancaster *et al.* 2008). In the case of *Aprionyx* spp. the early spring months saw an overlap of mature and new instar nymphs, whilst for *Adenophlebia peringueyella* winter densities were very low, and there was a hiatus in the presence of this species in the river over the spring period.

The cased caddisfly, *Cheumatopsyche afra* (Trichoptera: Hydropsychidae) was present in the Molenaars River in moderate to high densities year-round. Pupation was largely restricted to summer months, although a few pupae were observed in mid winter (Figure 7.3). This species may thus be considered to be at least partially included in the group of summer-based life cycles where exposure to winter floods is reduced. Recruitment of new instars was apparent from October / November, although the presence of a somewhat larger cohort of half-grown larvae in the October / November 1991 data set suggested that recruitment could have occurred a month earlier than this. Simultaneously, two cohorts of larger instars from the previous generation were completing their development in that period with pupation of that generation presumably ending by December. Larval development of the spring recruits appeared to be very rapid, with the first spring cohort reaching what is presumed to be final instar stage (maximum size) by December / January, followed by pupation, a generation time of approximately four to five months.

New recruits formed a substantial component of the population from November to February, which was also the period of highest population density. Four or five cohorts were visible each month over this period, based on interpretation of the peaks in the size-frequency data, along with pupae being collected every month from October to April. Recruitment continued until March / April, but pupae were absent in the river from this time, although a small number of pupae were collected in the mid- to late-winter sampling, suggesting that at least some individuals reached maturity during this winter period. The remaining larvae grew slowly over winter until spring, when the pupation took place from October to early December, suggesting either cohort splitting or the development of a second, winter generation from eggs hatching in late summer.

Chimarra spp. (Trichoptera: Philopotamidae) followed a similar pattern (Figure 7.4), with spring recruitment occurring at the same time as the maturation and pupation of the overwintering population. Growth of new cohorts appeared to be initially rapid, in at least three cohorts, but with continued recruitment until autumn. The pattern of development suggests that of the spring (S/O and O/N) recruits may reaching final instar stage between late January and April, but with a considerable proportion of the population remaining in the river during winter, growing very slowly and suffering losses during floods, but ultimately reaching maturity in the late winter – spring period of the following year.

7.3.2 Winter-resistant and / or summer intolerant life histories

The species with larval development most strongly restricted to the winter months was *Elporia uniradius* (Diptera: Blephariceridae) (Figure 7.5), which was absent from surficial sediments in the Molenaars River for at least three months during the height of summer. A second and third species, *Elporia capra* and *E. capensis*, were difficult to differentiate, and were not included in this life-history analysis. They were present during the same period, but numerically *E. uniradius* was substantially dominant. Larval development occurred over a period of only eight months, with recruitment of young (small) individuals dominating in the March / April and April / May samples, and pupae present in the river some three months after the initial appearance of this species in the river. Peak densities coincided with peak recruitment and in the mid-winter months, with no significant decline in densities following the 1:20-year

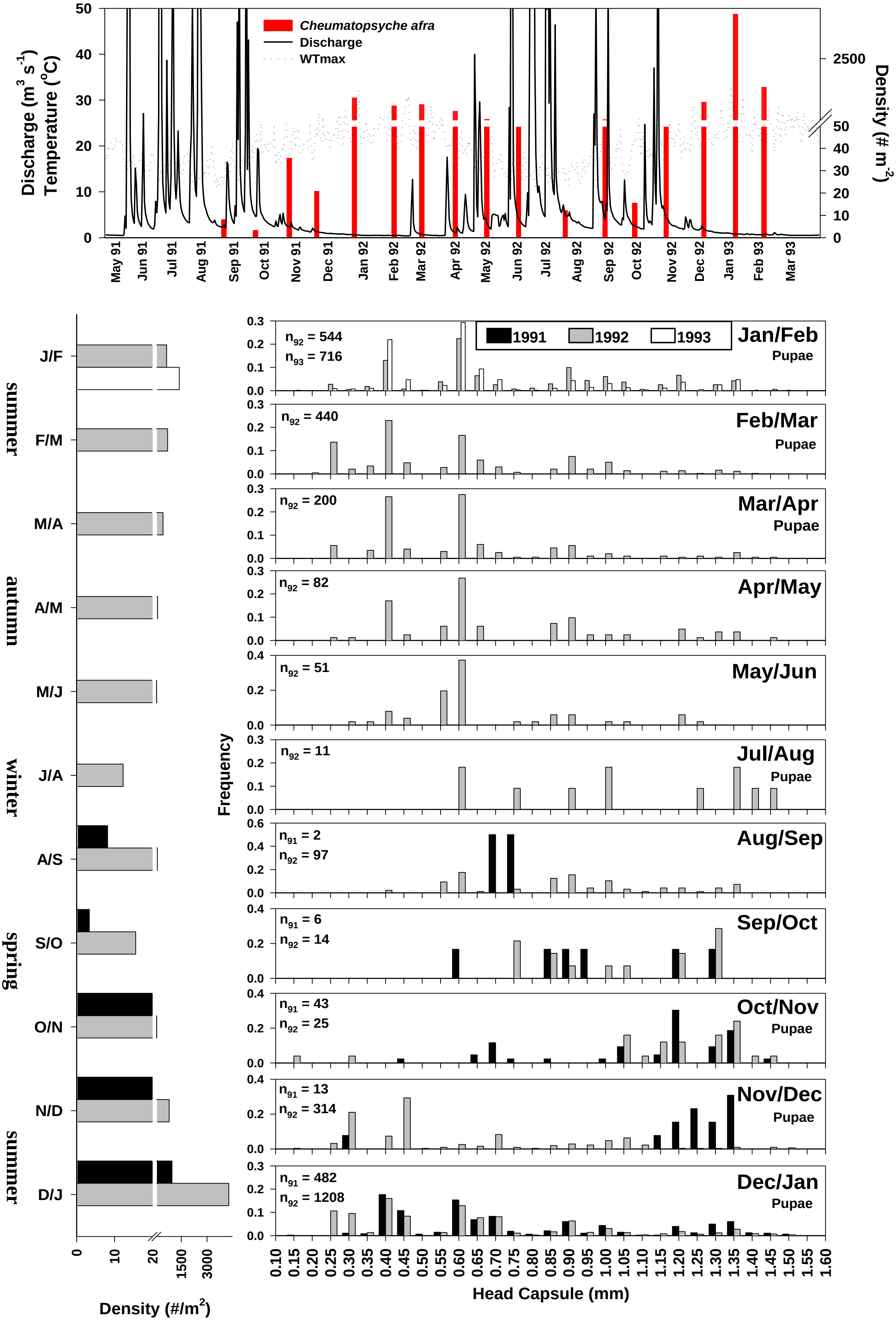


Figure 7.3 *Cheumatopsyche afra* densities (side panel) and head-capsule size frequency by sampling period for each year. The number of head capsules used to create each frequency distribution represented for each year. The presence of pupae is noted for each month they were encountered.

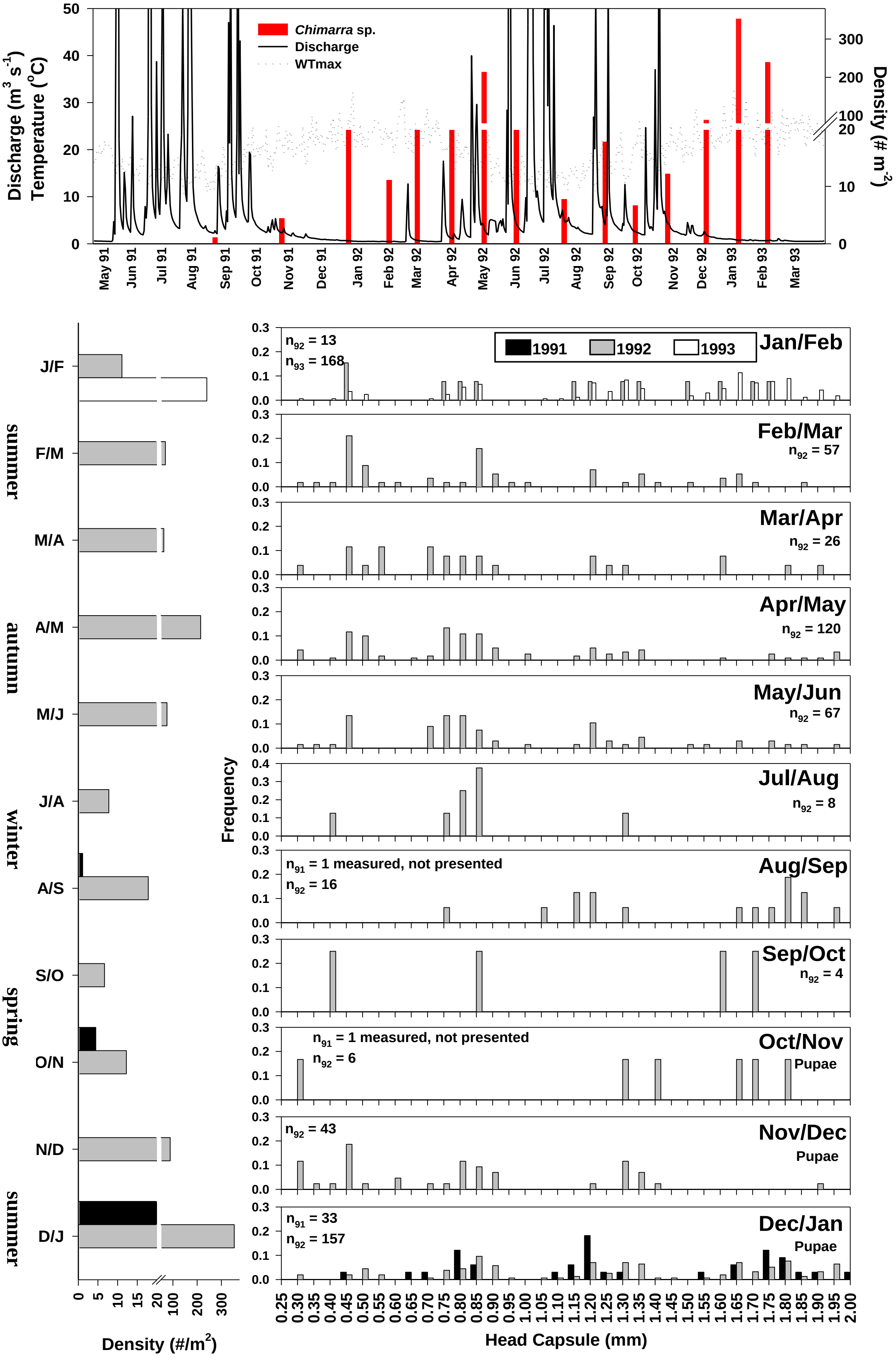


Figure 7.4 *Chimarra* spp. densities (side panel) and head-capsule size frequency by sampling period for each year. The number of head capsules used to create each frequency distribution represented for each year. The presence of pupae is noted for each month they were encountered.

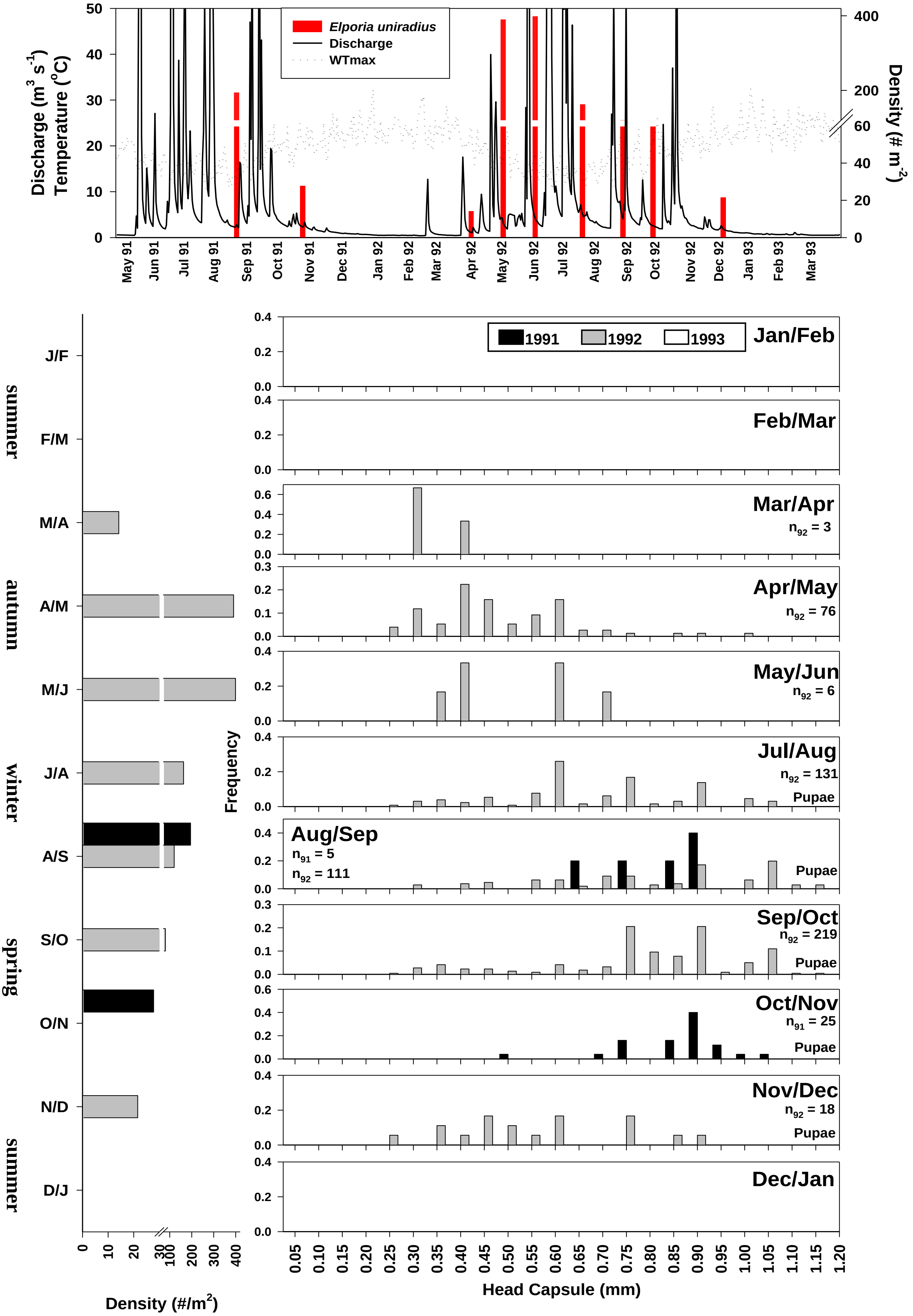


Figure 7.5 *Elporia uniradius* densities (side panel and top, red bars) and head-capsule size frequency by sampling period for each year. The number of head capsules used to create each frequency distribution is indicated for each month / year. The presence of pupae is noted for each month they were encountered.

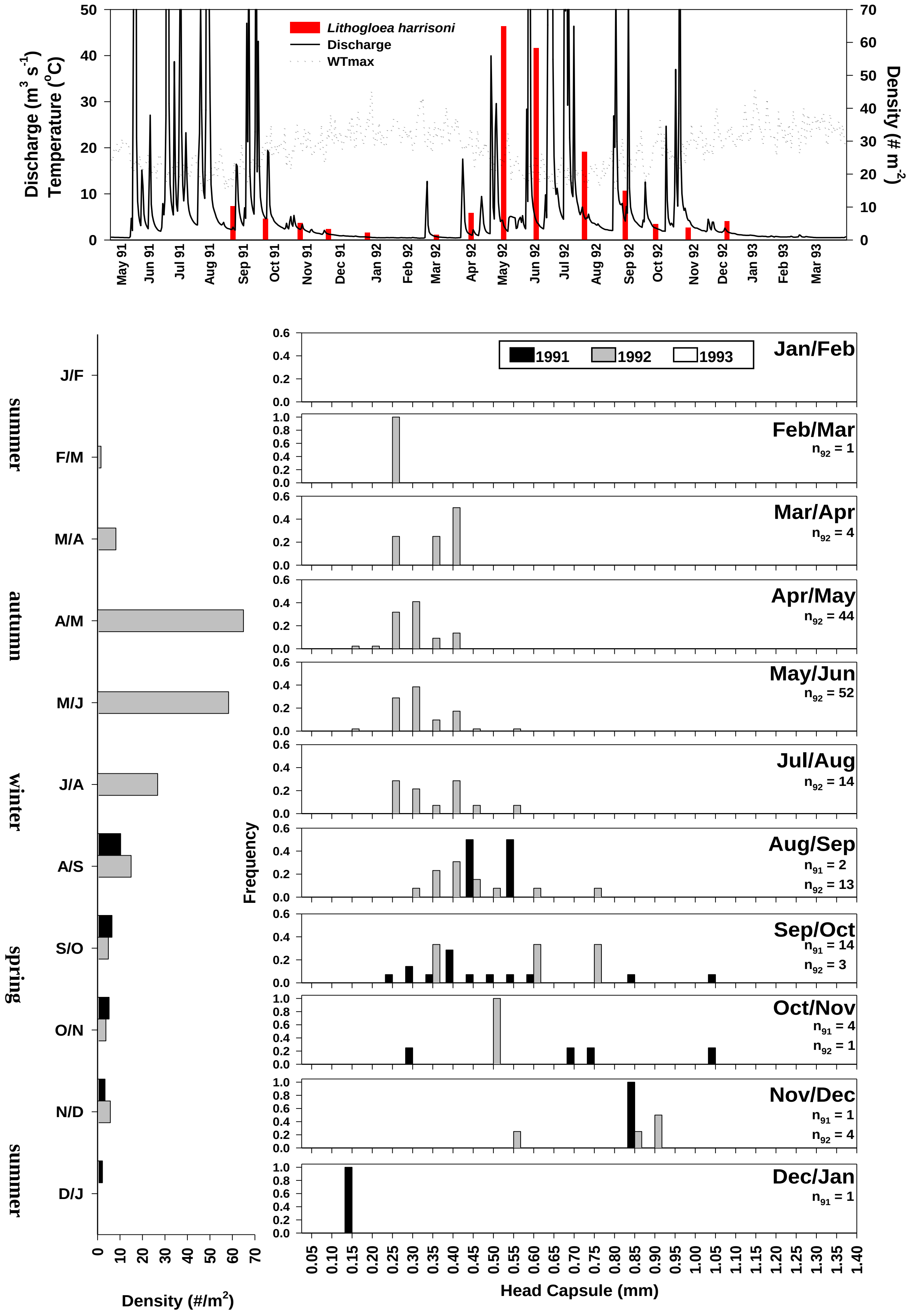


Figure 7.6 *Lithogloea harrisoni* densities (side panel and top, red bars) and head-capsule size frequency by sampling period for each year. The number of head capsules used to create each frequency distribution is indicated for each month / year.

flood just prior to the May / June 1992 sample (Figure 7.5). Continued recruitment, albeit at low levels, appeared to extend over the full period of this species' presence in the river. It is unclear as to whether this represents delayed hatching or facultative bivoltinism.

A similar pattern, of recruitment in autumn and early winter, April/ May and May/June, was observed for the ephemeropteran species *Lithogloea harrisoni* (Ephemeroptera: Telagonodidae), with once again minimal change in density following floods. Recruitment started in low densities at the height of summer, but in earnest in March /April, and mature nymphs were still present in late November / December in both years of the study (Figure 7.6). This nearly year-round presence, but with numbers tailing off at the height of summer, suggests somewhat less thermal inhibition of this species than in the case of *Elporia* spp. *Lithogloea harrisoni* densities were also not substantially altered even after the 1:20-year flood in early June, as with *Elporia* spp. Ongoing recruitment of new instars appeared to take place until July, with little overall change in the population size structure between April/May and July/August, except for the slight widening of the spread of the size frequency data, indicating increased representation of maturing nymphs (Figure 7.6). The data show a marked acceleration in development rates after early August coinciding with increasing temperature. This, combined with rapidly declining densities, suggests emergence of adults from August / September through to December, after which no large individuals were collected.

A similar life history, in terms of seasonality, was shown by *Lestagella penicillata* (Ephemeroptera: Telagonodidae) which was present nearly year-round (Figure 7.7). *Lestagella*, like *Lithogloea* matured and emerged in early to mid-spring, with no animals collected during late November / December in either year sampled. Although densities were highest in mid-summer, this was restricted to small individuals, with larger specimens absent over the summer period. Recruitment was discrete, over a period of a month or two, followed by continuous and synchronised growth by a single *L. penicillata* cohort through most of autumn and winter. More variable growth rates of mid-sized and mature instars coincided with the warming of temperatures from early September.

Three genera of plecopterans were identified from the Molenaars River, *Aphanicerca*, *Desmonemoura* and *Aphanicercella*, the last of these present at very low densities and thus excluded from this analysis. The young nymphs of the different genera, without adult genitalia, are impossible to distinguish (Mr Duncan Stevens, plecopteran taxonomist, University of Cape Town, pers. comm.). However, two species of *Aphanicerca* (*A. capensis* and *A. bicornis*) and one of *Desmonemoura* (*D. pulchellum*) are thought to occur in the Molenaars River, based on adult collections.

Aphanicerca spp. and *D. pulchellum* showed year-round presence (Figures 7.8 and 7.9) peak densities in spring and early summer. Late winter / spring emergence overlapped with recruitment of new instars. New instars of *Aphanicerca* spp. showed fairly rapid development during spring and early summer, but a mid- to late-summer growth hiatus, from January through to May, indicated by little change in size distribution. No head capsule measurements were available for the two genera for December, but density data for the Plecoptera overall (inserted in Figure 7.8 and 7.9, left panel) indicated that the spring peak extended to this time. Density declined quite substantially over the remainder of summer, but then remained steady over the winter months. Unfortunately, June data were also missing, but the increases in head-capsule widths between April / May and July / August indicates rapid growth during winter. A substantial drop in density in August / September, after the main floods, appeared to be the consequence of emergence, with most of the remaining population comprising very large specimens, along with a few new instars (Figure 7.8).

D. pulchellum showed a longer period of overlap between generations, with mature instars continuing beyond September, to December. Recruitment of young was concentrated over generally the same period as for *Aphanicerca* spp. but continued through to November (December data missing). Data for the mid and late summer months were very sparse, making interpretation of monthly growth difficult. Figure 7.9 shows what appears to be a loss of bigger mid-instars over the summer, along with the appearance of smaller animals, which are assumed to have relocated from other habitats rather than be newly hatched individuals, since they were larger than the first instar recruits from September / October.

7.3.3 Temperature and flow resistant life cycles

Euthralus elegans (Ephemeroptera: Leptophlebiidae) and *Demoreptus capensis* were both present year-round (Figures 7.10 and 7.11). Recruitment in *E. elegans* occurred over a single month in mid-summer. Growth rates were variable however, with a wide spread of size classes extending over the full year's development period. Growth rate was continuous in summer, but slowed substantially in winter.

D. capensis appeared to have two recruitment episodes, a rather extended one from August to November and a shorter one in February / March. The larger size classes in February and March represent mature and emerging nymphs, which indicate bivoltinism in this species.

Both species showed accelerated growth and development of mature instars in spring. *E. elegans* emerged synchronously and were represented by small instars in the following month, whilst *D. capensis* had overlapping generations in late spring, new recruits coinciding with final instar nymphs.

A different life history altogether, although one also capitalising on resistance characteristics of the organism is that of *Agapetus agilis* (Trichoptera: Glossosomatidae). Although sample sizes for head-capsule measurements were at times small, the size frequency distribution, combined with density peaks in both mid-summer and early winter (Figure 7.12) indicate bivoltinism, with recruitment at the start of winter (early June 1992, immediately after the 1:20-year flood), and a second, spring recruitment in late October / November. Densities were greatest in October through January, reflecting the simultaneous presence of the spring generation and the maturing winter generation. Winter development from new instars to pupation took six to seven months, whilst the dwindling numbers and the presence of pupae in March through May suggest that the spring generation has a less synchronised, faster development trajectory, of some five to six months.

Another species for which life-history patterns may be obscured by the fact that sampling was restricted to riffles is *Athripsodes bergensis* (Trichoptera: Leptoceridae). As with *Agapetus agilis*, recruitment occurred twice, in winter (May / June) and in spring (November / December) (Figure 7.13). After initial recruitment at the start of winter, however, few animals were collected from riffle samples until spring, when mid-size instars re-appeared and completed their development. The summer population was much larger, densities peaking in December / January when new recruits coincided with large numbers of the mature winter generation. Despite rapid growth of the summer cohort, and attainment of maximum size by April / May, no pupae were recorded from riffles in late summer or autumn.

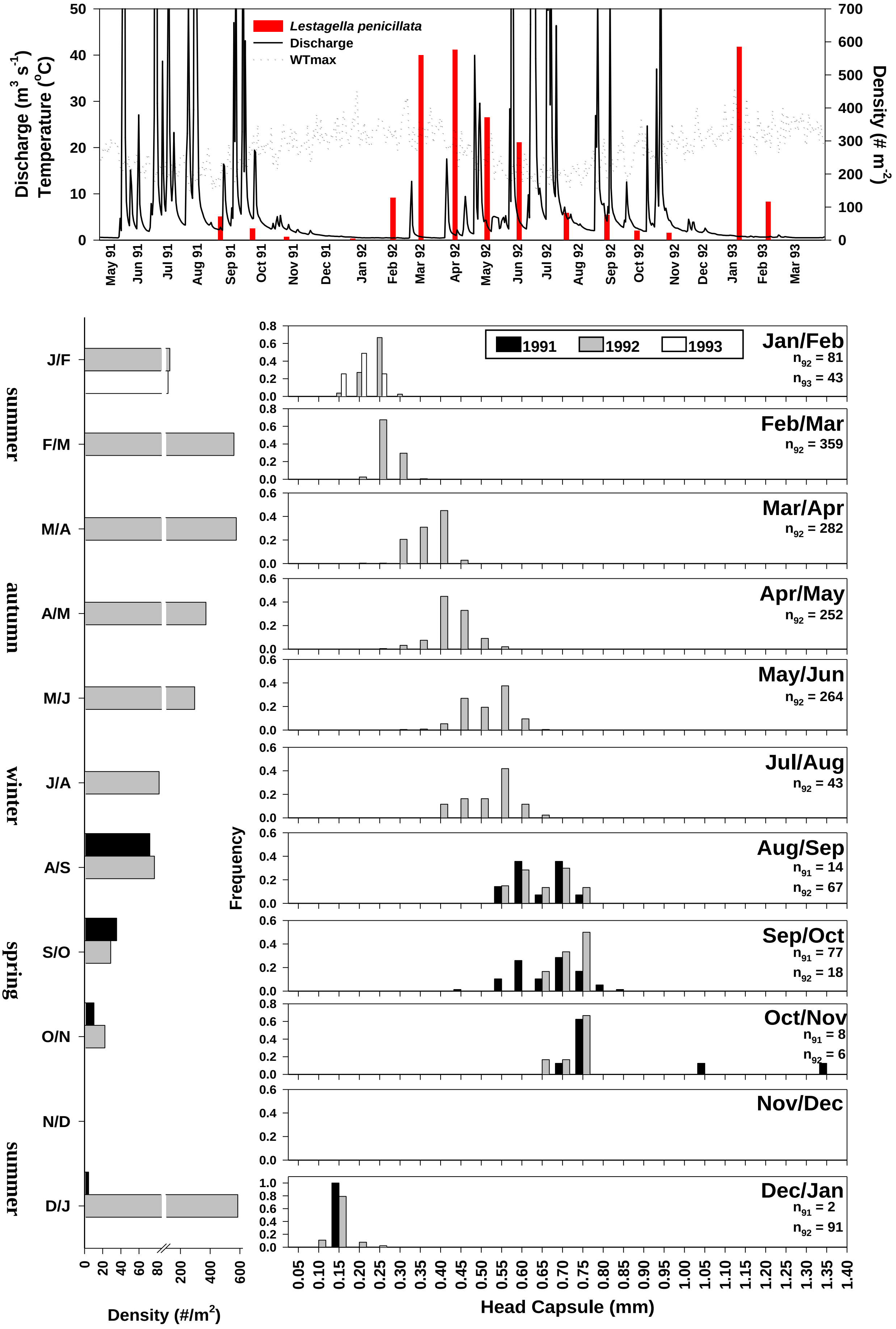


Figure 7.7 *Lestagella penicillata* densities (side panel and top, red bars) and head-capsule size frequency by sampling period for each year. The number of head capsules used to create each frequency distribution is indicated for each sample.

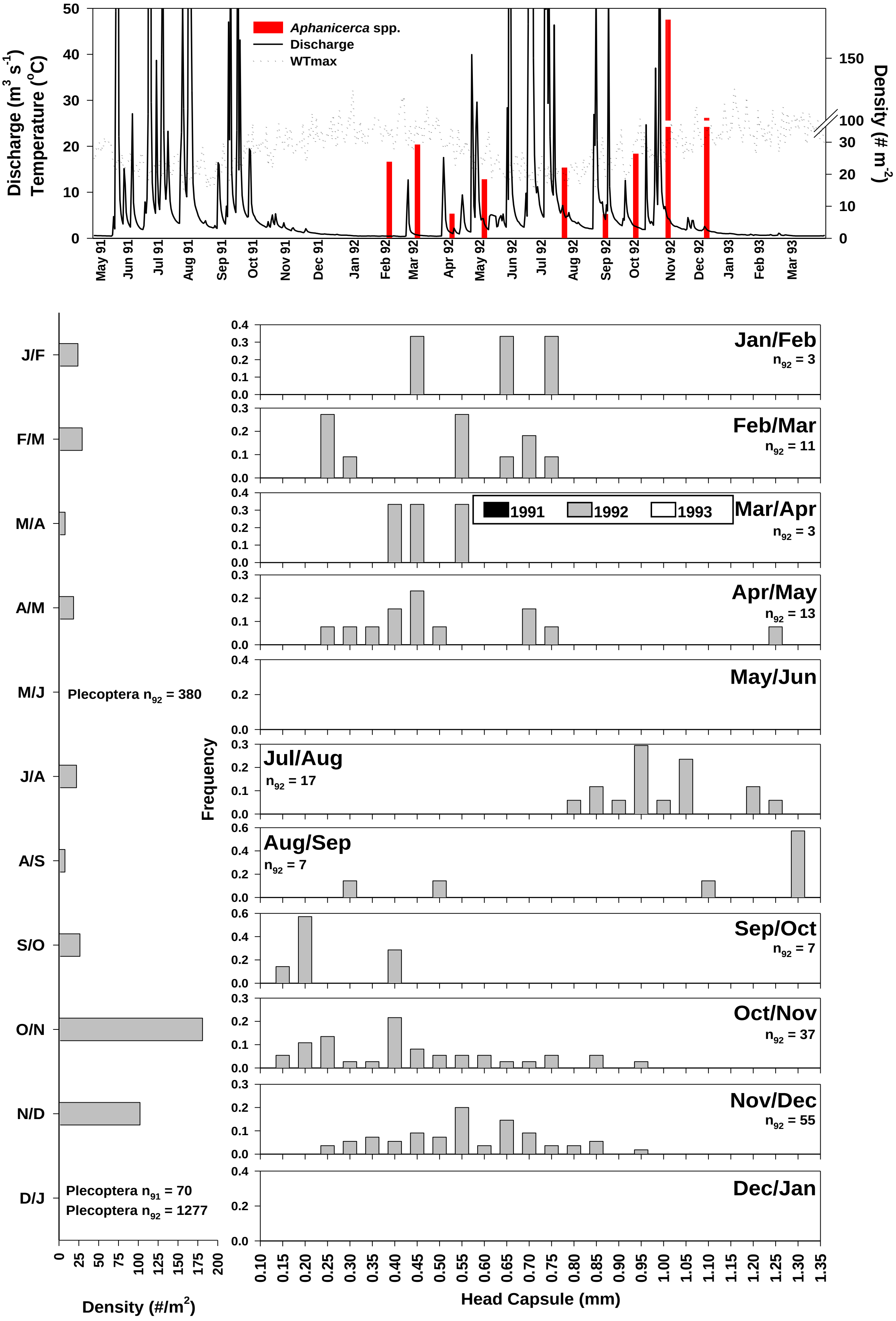


Figure 7.8 *Aphanicerca* spp. densities (side panel and top, red bars) and head-capsule size frequency by sampling period for each year. The number of head capsules used to create each frequency distribution is indicated for each month / year. No head capsules were measured for 1991 and 1993 samples or for the Dec/Jan sampling period.

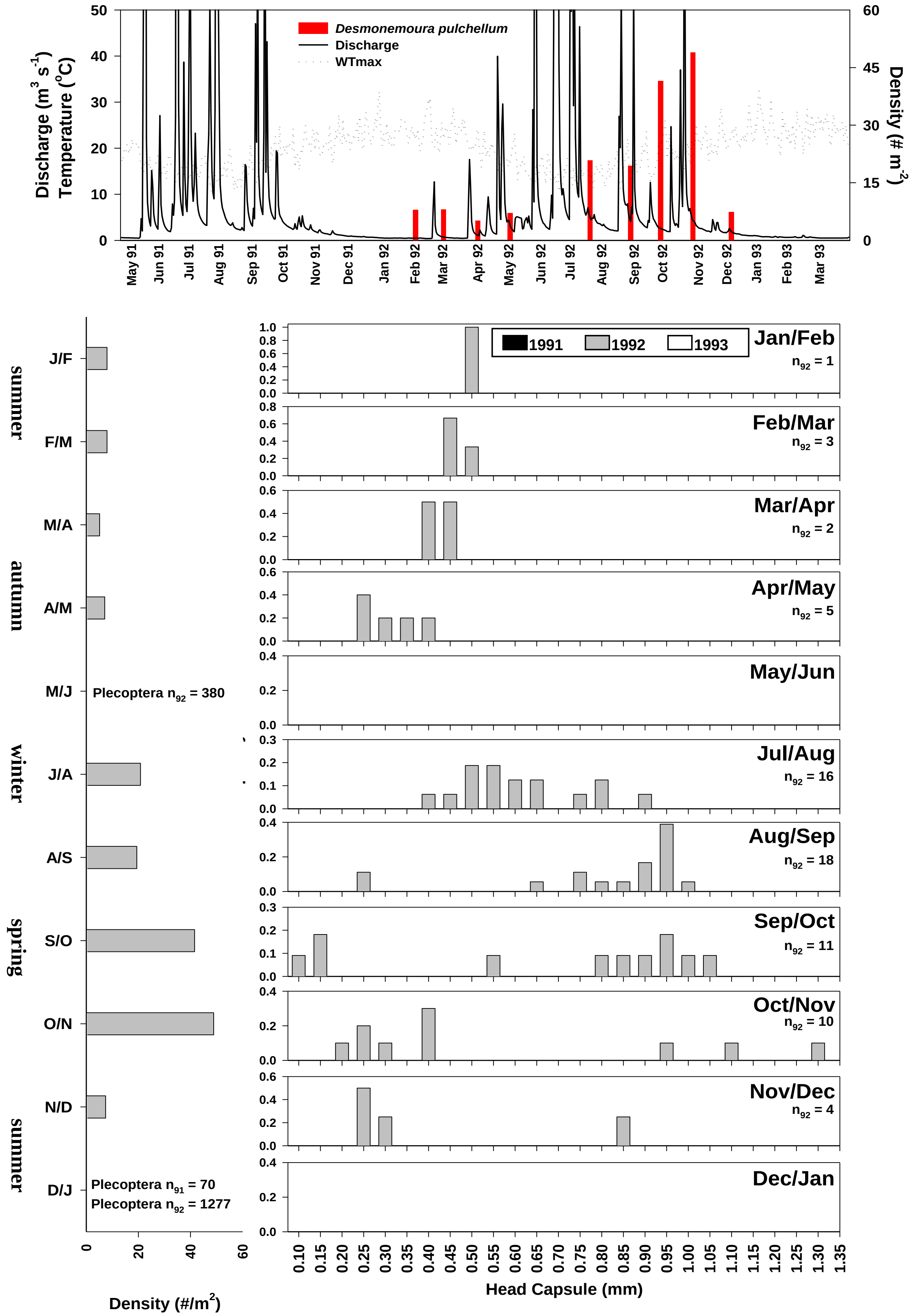


Figure 7.9 ***Desmonemoura pulchellum*** **densities (side panel and top, red bars) and head-capsule size frequency by sampling period for each year. The number of head capsules used to create each frequency distribution is indicated for each month / year. No head capsules were measured for 1991 and 1993 samples or for the Dec/Jan sampling period.**

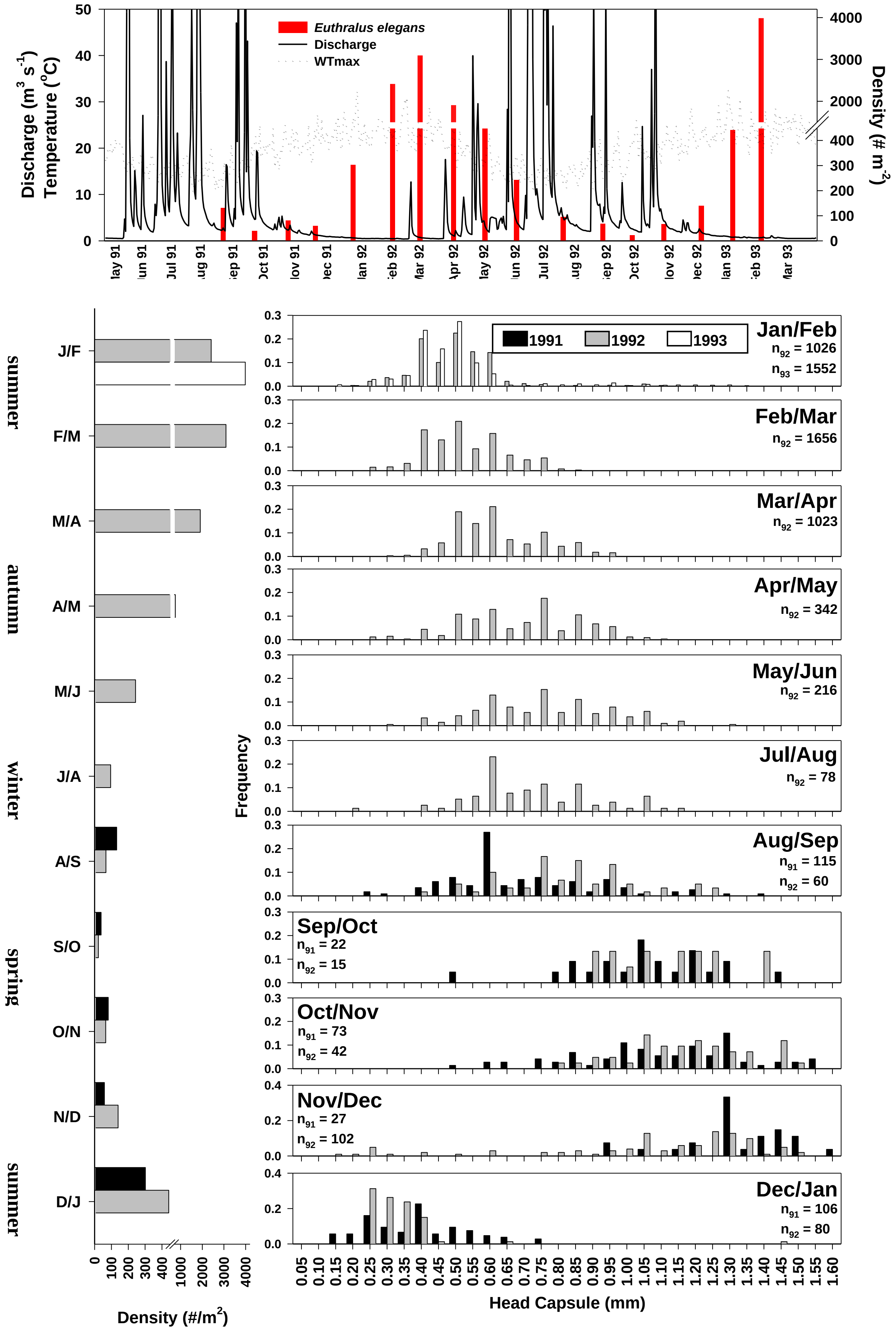


Figure 7.10 *Euthralus elegans* densities (side panel and top, red bars) and head-capsule size frequency by sampling period for each year. The number of head capsules used to create each frequency distribution is indicated for each month / year.

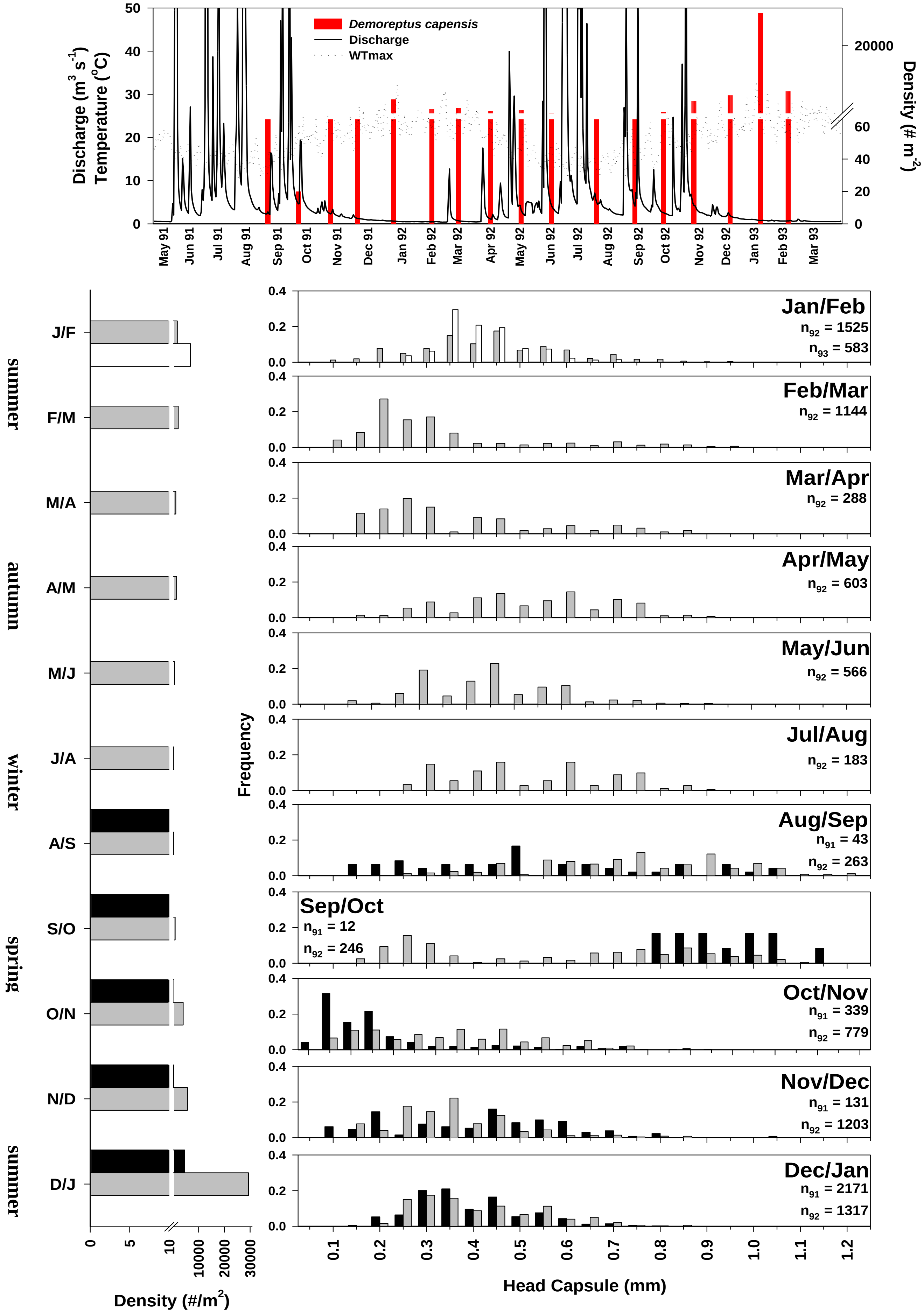


Figure 7.11 *Demoreptus capensis* densities (side panel and top, red bars) and head-capsule size frequency by sampling period for each year. The number of head capsules used to create each frequency distribution is indicated for each month / year.

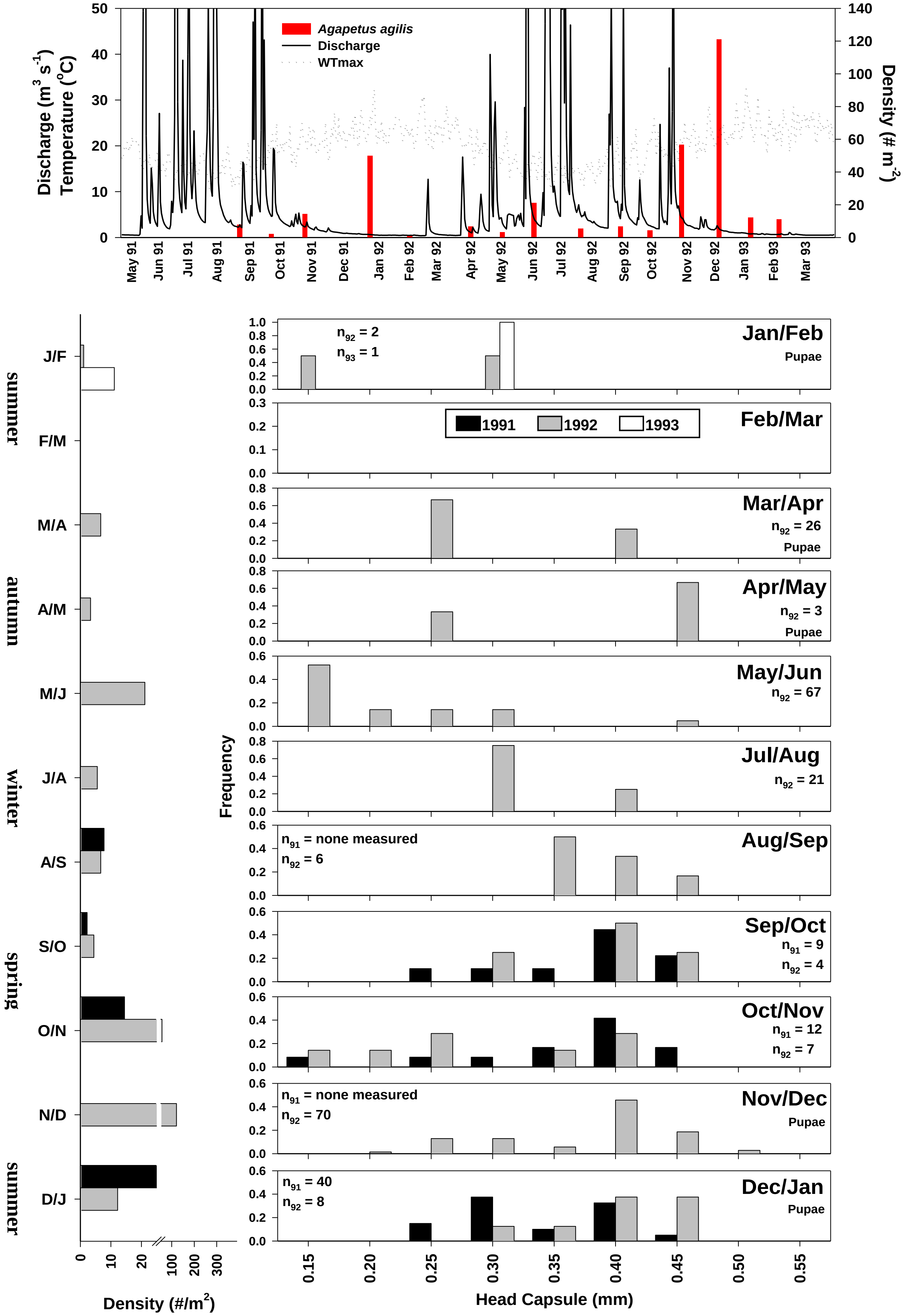


Figure 7.12 *Agapetus agilis* densities (side panel) and head-capsule size frequency by sampling period for each year. The number of head capsules used to create each frequency distribution represented for each year. The presence of pupae is noted for each month they were encountered.

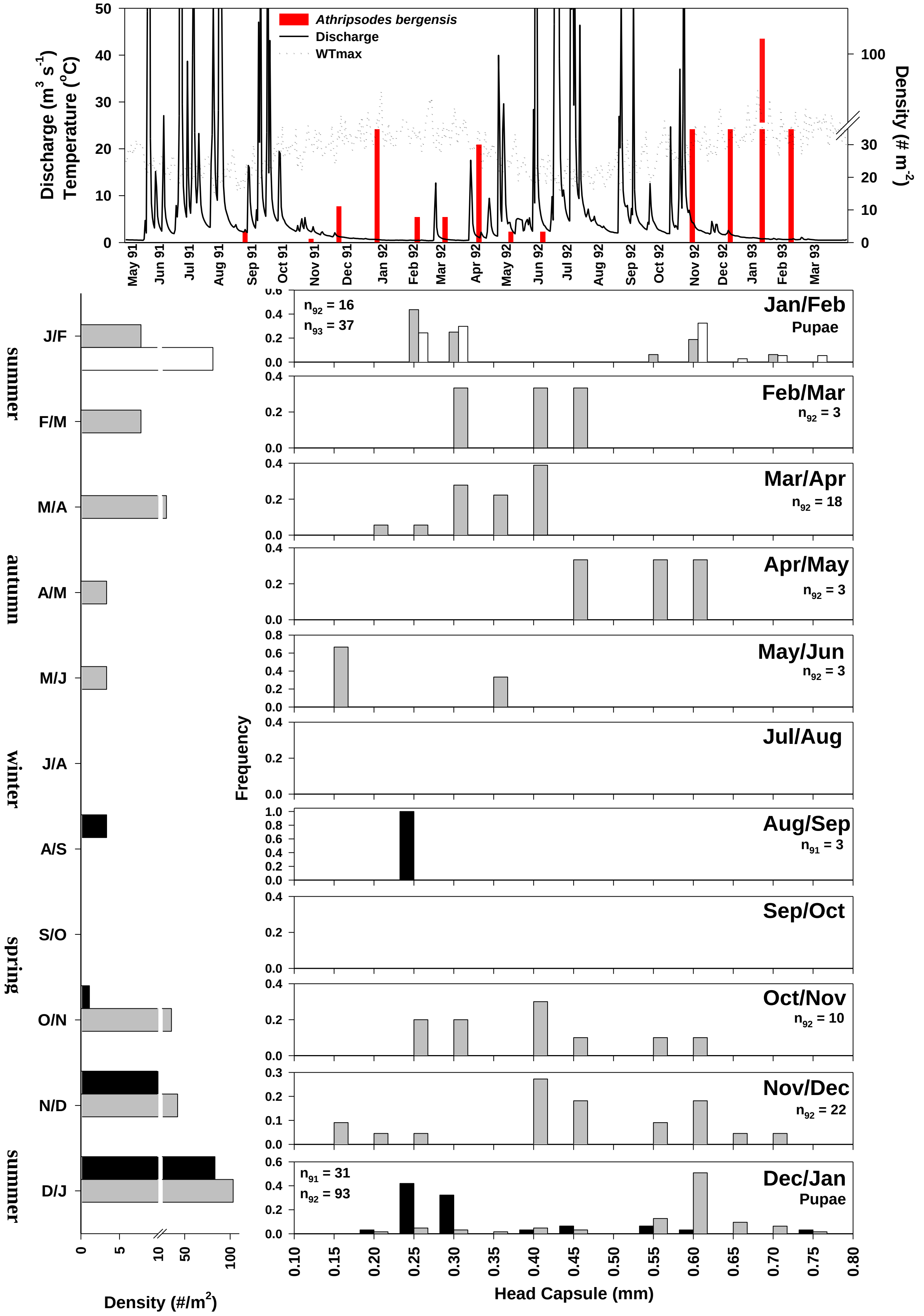


Figure 7.13 *Athripsodes bergensis* densities (side panel) and head-capsule size frequency by sampling period for each year. The number of head capsules used to create each frequency distribution represented for each year. The presence of pupae is noted for each month they were encountered.

7.3.4 High resilience, unsynchronised life histories

Small instars of *Baetis harrisoni* and four other unnamed *Baetis* species were impossible to differentiate, which, coupled with the absence of synchrony in larval development, made interpretation of the size frequency data impossible. Because of this, data for the three species were combined, and the size frequency distribution for the genus presented in Figure 7.14. This is problematic from the perspective of defining life history, but can nevertheless provide some insight into seasonal patterns of recruitment within the group. Densities were highest in the spring and summer months, dominated by recruitment of new instars each month from late September through to late November. After a summer lull, recruitment increased from March, continuing through autumn and most of winter (July / August). Although summer recruitment of young was a small proportion of the total population, this nevertheless represents large numbers of new instars, given overall densities recorded then. The largest mature instars were present in spring. Maximum size declined over the summer, although the continuous recruitment and constant densities are considered to be indicative of emergence throughout summer.

Of the Simuliidae, *Simulium* (species complex Metomphalus) spp. were by far the dominant group in the Molenaars River. Differentiation of species level beyond the species complex is difficult, and impossible for smaller instars. Recruitment peaked during the winter and spring months (Figure 7.15), with multi-cohort development. As with *Baetis* spp., the high overall densities in summer and the multiple cohorts still indicate a large recruitment despite new instars being a relatively small proportion of the population. An obvious characteristic of the life-history was the dominance of young instars in the winter months. Given the continuous recruitment of new instars, and continuous presence of pupae over the year, it is difficult to separate out the issue of voltinism from asynchronous development. However, an examination of cohort development in the August to December 1991 period, when densities were low compared with the following year, suggests a four-month larval development period in spring / summer, with multiple generations per annum.

Similarly, the Chironomidae demonstrated very high recruitment on a nearly year-round basis, thus maintaining high densities despite the effects of floods. All four species examined showed overlapping cohorts and development rates indicative of multivoltinism. *Cricotopus* spp. (Figure 7.16) had peak recruitment in October / November, with rapid growth of the population over a one- to two-month period. All size classes were present over the summer months, with the data suggesting at least four generations between October / November and April / May, and a decline in recruitment over the autumn period given the reduced densities and skew in size distributions, indicating a single cohort at the latter date. However new recruitment over June (size frequency data missing) is indicated by the dominance of mid-instar individuals in the following month. The other numerically dominant species, *Notocladius capicola* (Figure 7.17), was represented by overlapping cohorts in nearly all months, indicated by the multi-modal size distribution and full range of size classes present. Densities of this species never declined below 1000 ind. m^{-2}, but peaked in September through November. The low representation of the smallest instars is somewhat puzzling, since this size class is well represented in the other species, suggesting that sampling mesh-size is not the reason for this. It may, however, represent either recruitment in other biotopes such as runs, or the movement of this species to the bed surfaces at a larger instar size than with other species.

Thienemaniella spp. and *Synorthocladius* spp. (Figures 7.18 and 7.19) were both less abundant in the Molenaars River than the two afore-mentioned genera. Like *Cricotopus* spp. the data for *Thienemaniella* spp. suggested four generations between October / November and April / May, along with recruitment

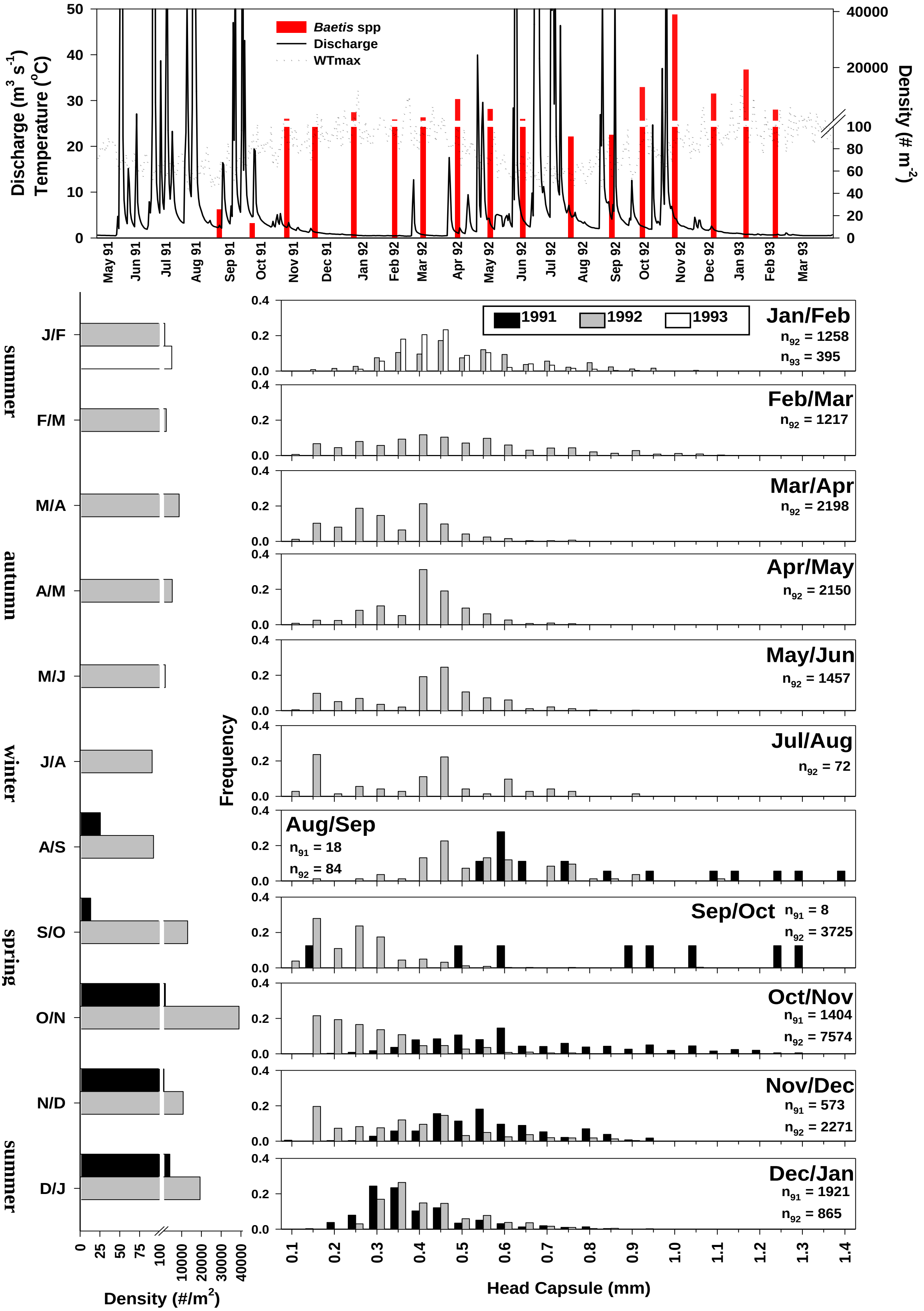


Figure 7.14 *Baetis* spp. densities (side panel and top, red bars) and head-capsule size frequency by sampling period for each year. The number of head capsules used to create each frequency distribution is indicated for each month / year.

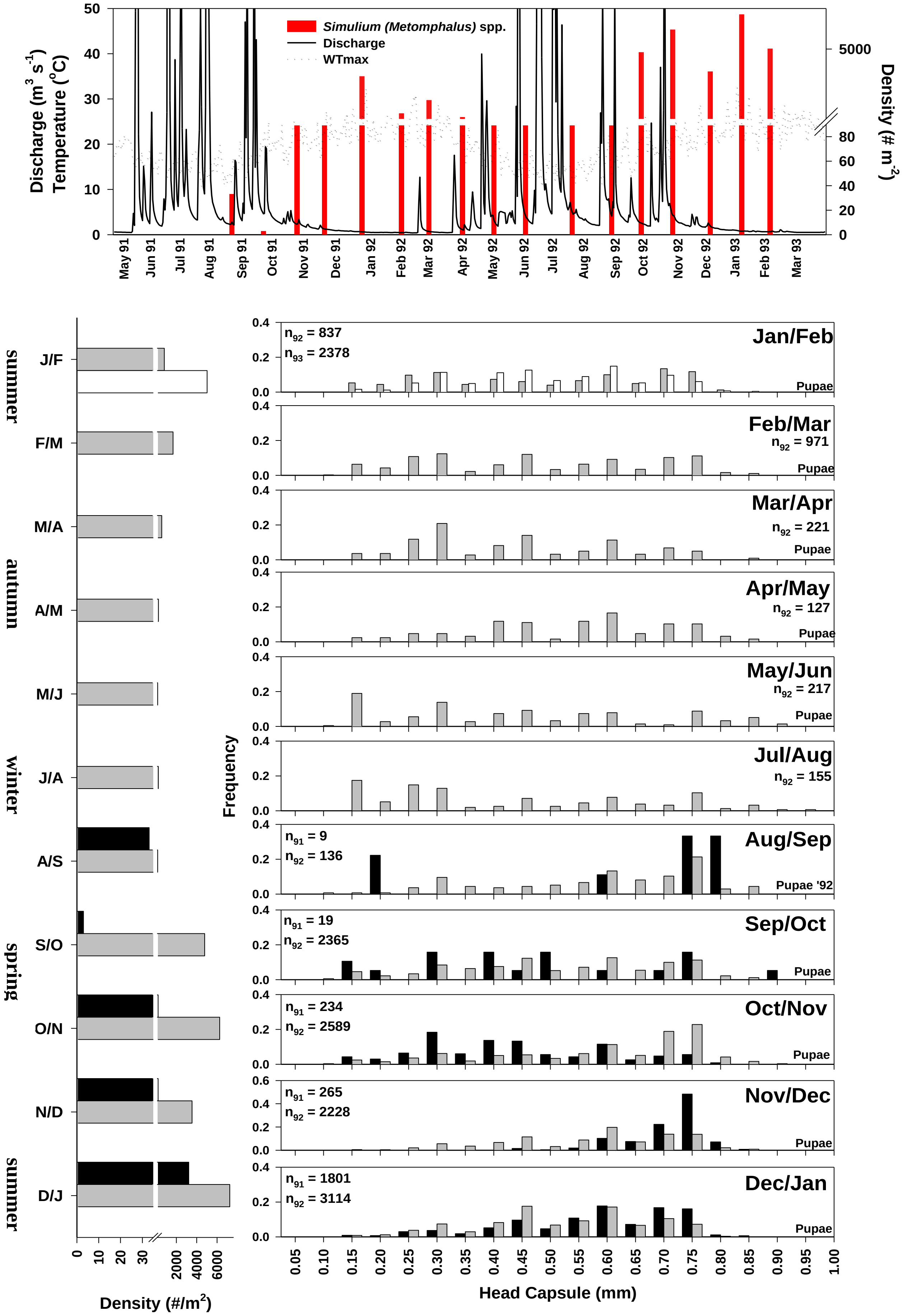


Figure 7.15 ***Simulium (Metomphalus)*** **spp. densities (side panel and top, red bars) and head-capsule size frequency by sampling period for each year. The number of head capsules used to create each frequency distribution is indicated for each month / year. The presence of pupae is listed for each month they were recorded in the samples.**

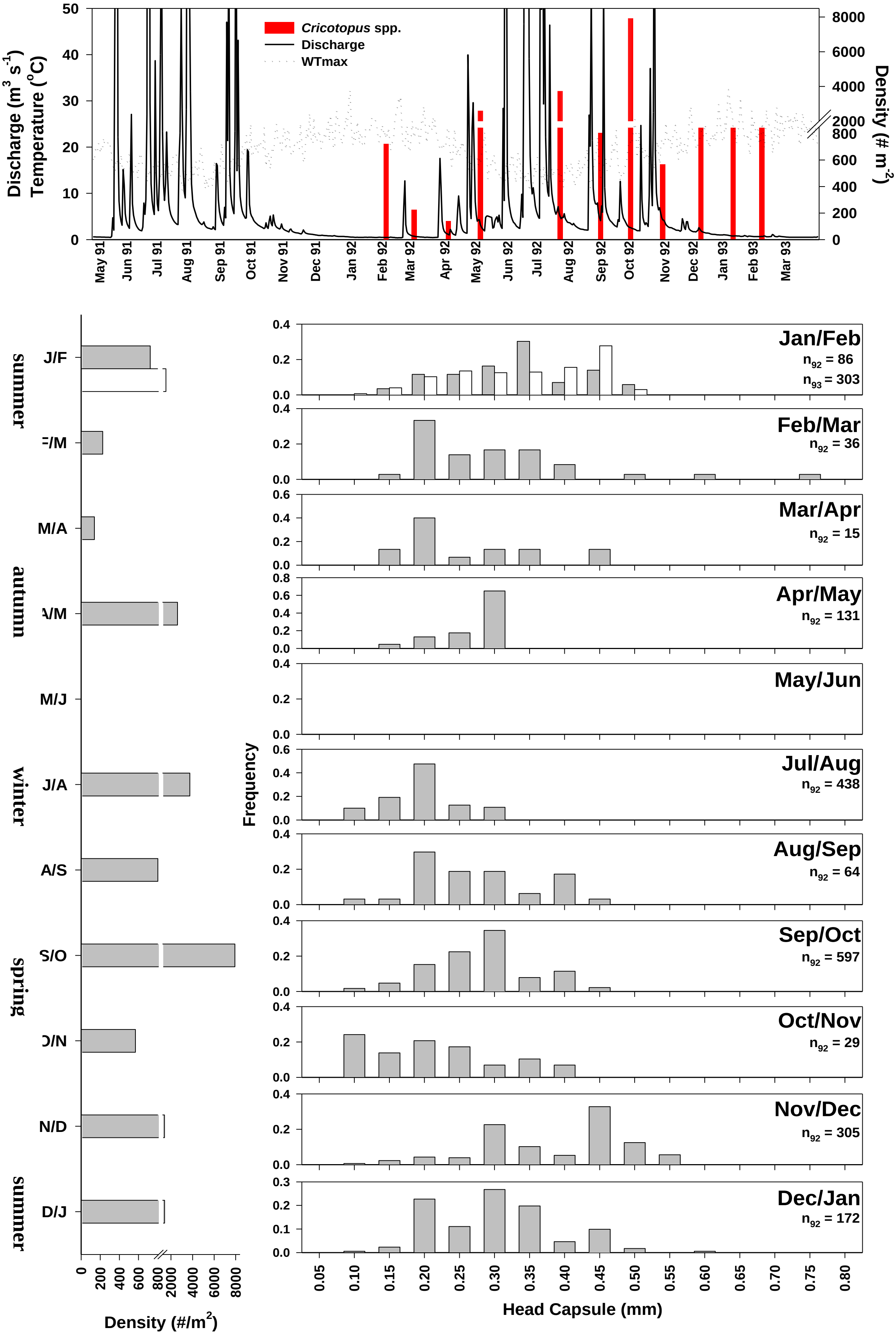
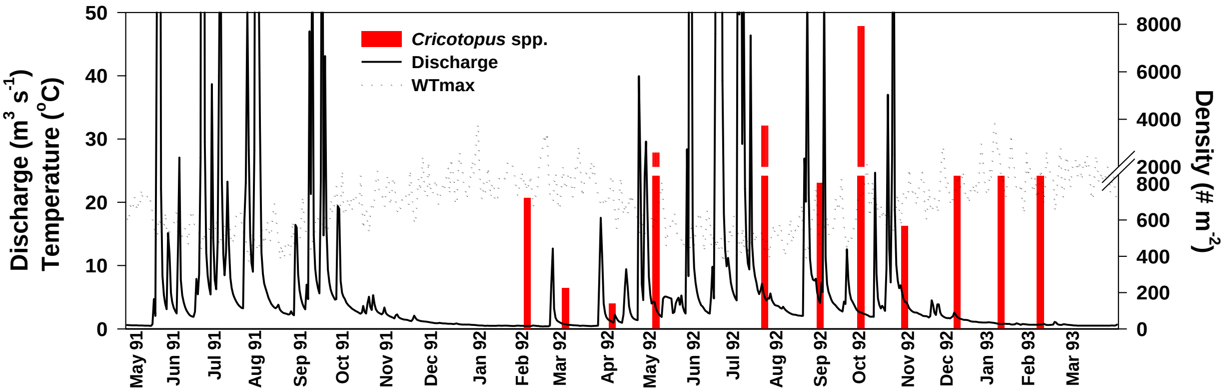


Figure 7.16 ***Cricotopus*** **spp. (Chironomidae: Orthocladinae) densities (side panel) and head-capsule size frequency by sampling period for each year. The number of head capsules used to create each frequency distribution is indicated for each month / year.**



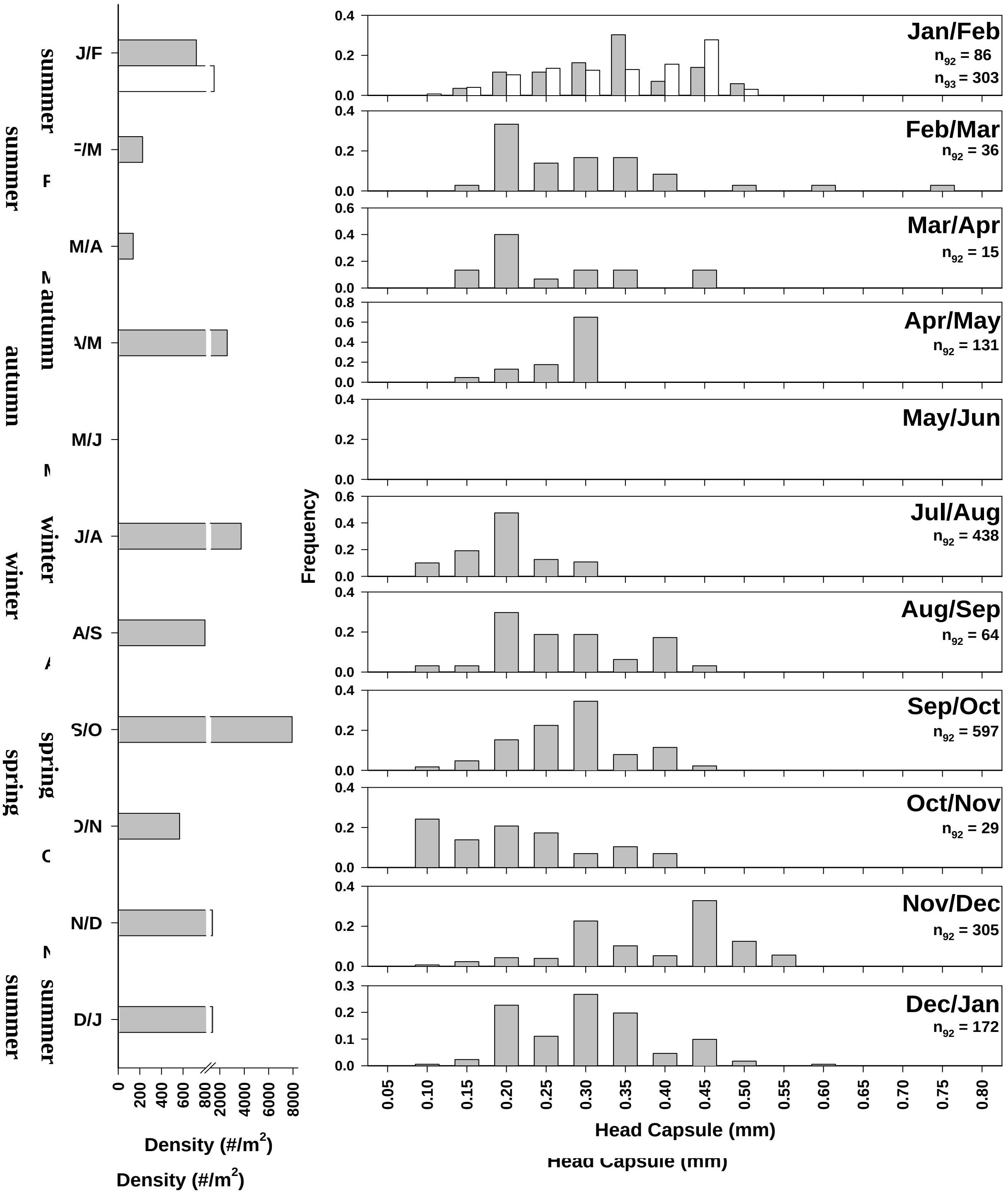


Figure 7.17 *Notocladius capicola* (Chironomidae: Orthocladinae densities (side panel) and head-capsule size frequency by sampling period for each year. The number of head capsules used to create each frequency distribution is indicated for each month / year.

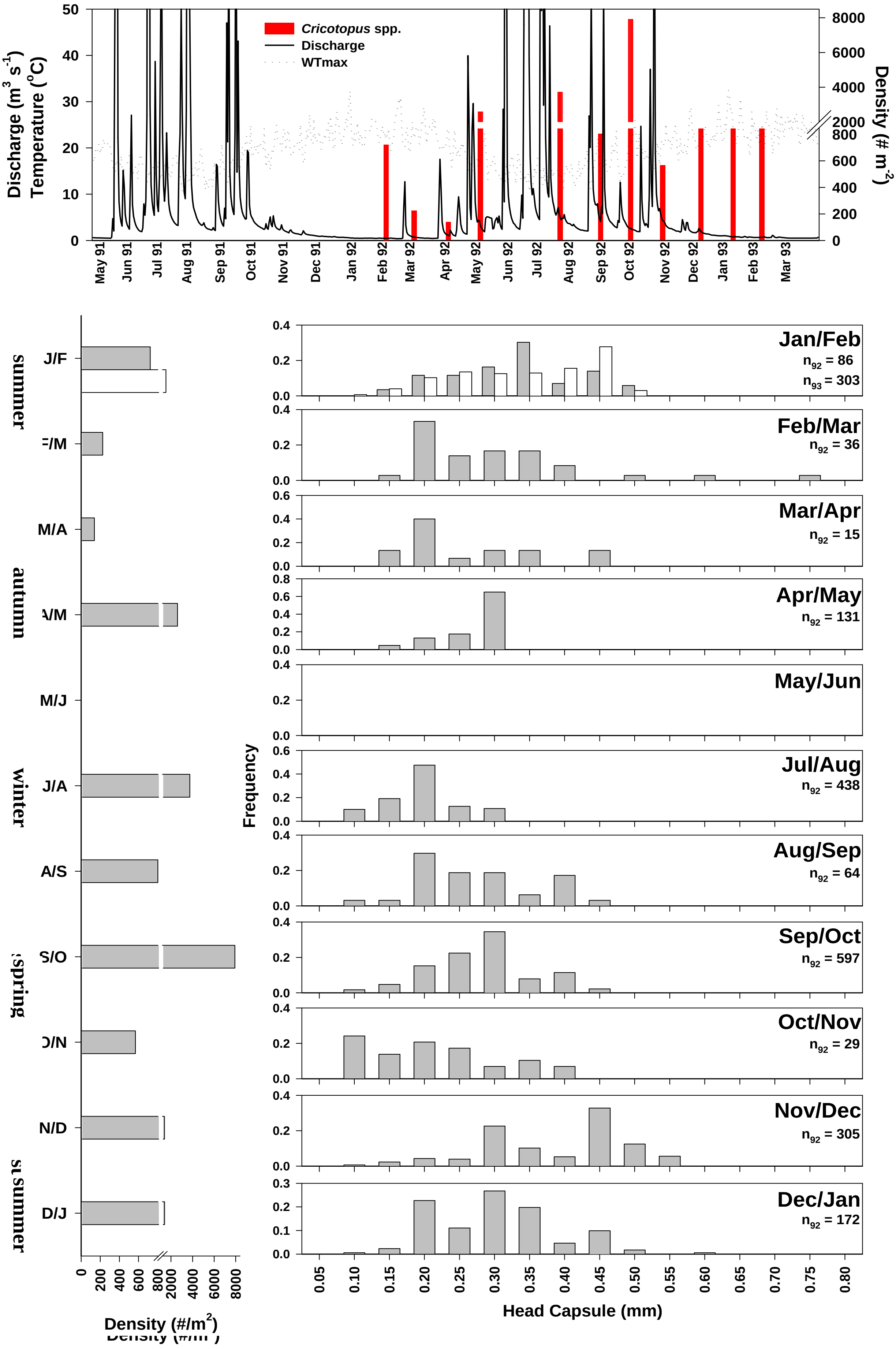
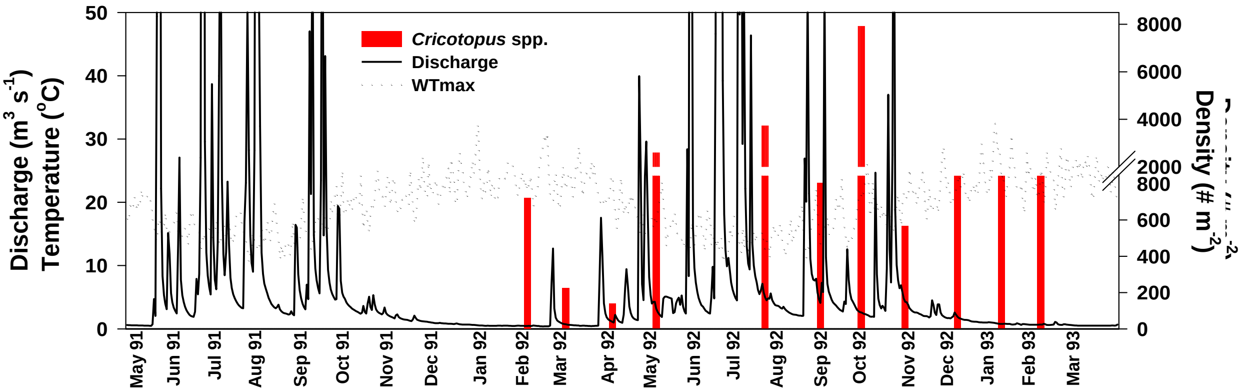


Figure 7.18 *Thienemaniella* spp. (Chironomidae: Orthocladinae) densities (side panel) and head-capsule size frequency by sampling period for each year. The number of head capsules used to create each frequency distribution is indicated for each month / year.



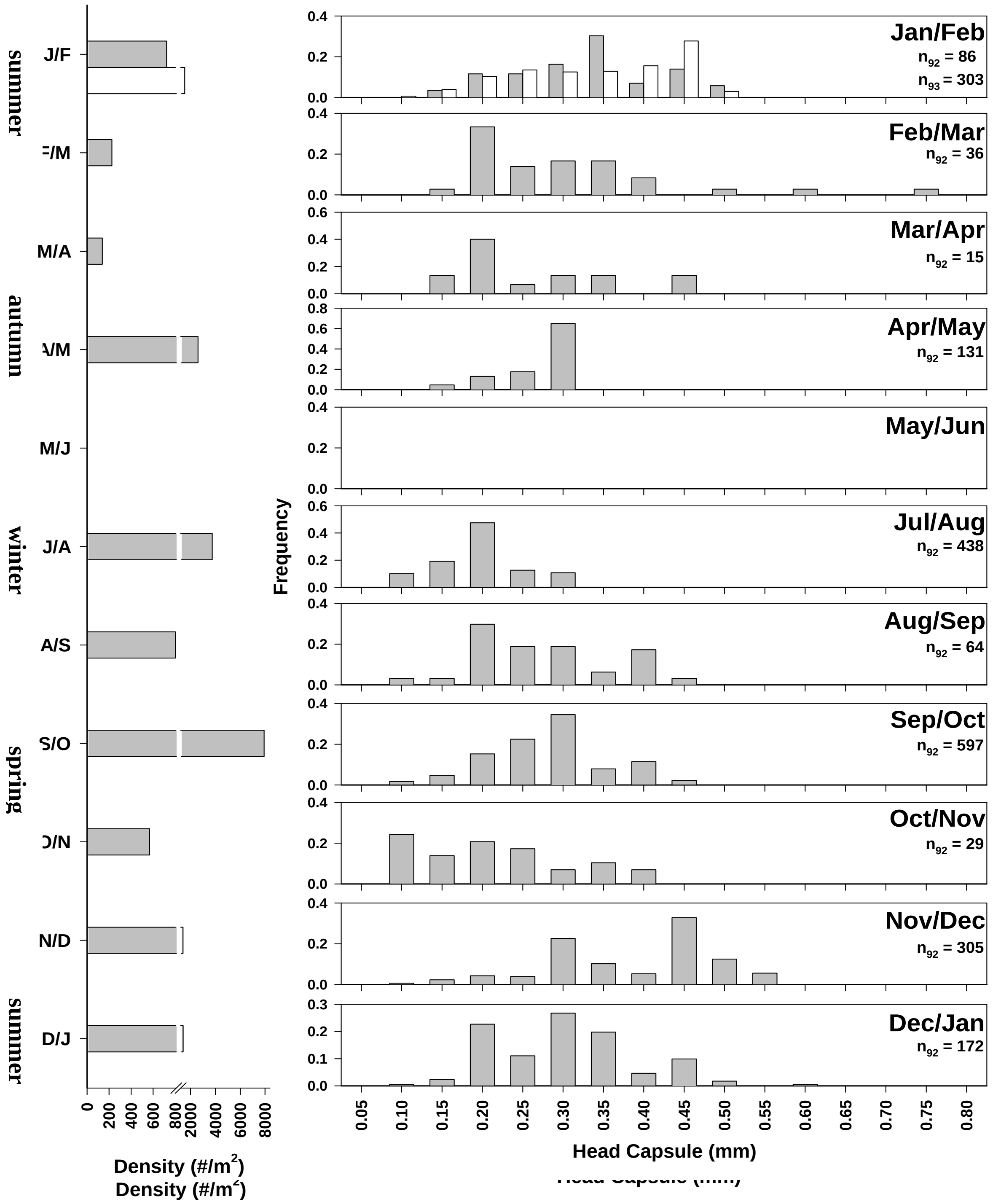


Figure 7.19 *Synorthocladius* spp. (Chironomidae: Orthocladinae) densities (side panel) and head-capsule size frequency by sampling period for each year. The number of head capsules used to create each frequency distribution is indicated for each month / year.

during the winter months, albeit at low densities. *Synorthocladius* spp. was the only chironomid whose life-history data suggested no winter recruitment of new instars, with recruitment being delayed until August (Figure 7.19).

7.4 DISCUSSION

Life-history attributes provide a framework for the ability of aquatic insects to persist in the face of disturbance, and are the trade-offs of the dual imperatives to maximise reproductive output and yet avoid mortality. The timing of life-history events, such as age at reproduction, should therefore match the timing of mortality-inducing processes, of which floods that disturb bed sediments are a major component (Lytle & Poff 2004). Whilst floods that cause near-complete population losses and that occur predictably on an annual basis must certainly rank as an overriding force driving the evolution of insects with life cycles of a year or two, floods with less severe impacts, per event or over a flood season, will act as one of the risks of mortality, along with predation, resource limitation, competition or adverse physico-chemistry. All these may have a seasonal dimension, as in the Molenaars River.

In a discussion of major life-history patterns, Giller & Malmqvist (1998) refer to three life-history types first described by Hynes (1970), namely slow seasonal, fast seasonal[6] and non seasonal. Slow seasonal life cycles show distinct and synchronised change in size distributions over time, from egg-hatching to adult emergence, with completion of life cycles over the course of approximately one year cued to seasonal phenomena (Merrit & Cummins 1996). Fast seasonal life histories involve rapid growth cued to some seasonal shift, after a long egg or larval diapause that is associated with adverse instream conditions. Non-seasonal life histories are present when individuals of many different life stages coexist over an annual cycle, which may be a consequence of life spans that exceed a year, or taxa that have multiple overlapping generations.

Four life history types were present in the Molenaars River, that bore some correlation in their timing with attributes of the disturbance and seasonal temperature regime. These are discussed under the headings fast summer seasonal development, a fast winter development, non-seasonal, asynchronised development. The slow seasonal life cycle of Hynes (1970) was not expected in the Molenaars River.

7.4.1 Summer life histories with restricted exposure to winter floods

An unexpected result of this study was the small number of taxa that were restricted to the summer period, or had life-history patterns where developmental stages were clearly timed so as to avoid the flood period altogether, which was unexpected because avoidance of a seasonal flood period could be considered to be of primary importance for survival. Two leptophlebiid species, *Aprionyx* spp. and *Adenophlebia peringueyella*, did fit this life-history pattern, and were substantially more abundant during summer than other seasons. In the only other life-history work on mayflies in the Western Cape, King (1981) showed summer-restricted nymphal growth in these species. The current study suggests that nymphs from both species shift habitat from riffles to slackwaters at some development threshold, possibly as an adaptation to optimise growth, as slackwaters are highest in deposits of fine organic matter, the principal food for these species (Schael & King 2005). Barnard (1932) gives the adult flight period for *Adenophlebia peringueyella* as June to October, winter / spring, whilst that for *A. peterseni* was given as spring through autumn, October to April, and others in the latter genus as September to June.

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[6] The present study separated fast seasonal life cycles into two – fast winter and fast summer cycles.

The flood-response study (Chapter 4) showed that floods reduced densities of both *Aprionyx* and *Adenophlebia* to near zero, in the immediate post-flood period. This apparent population loss, however, was more likely because of the fact that most of the population had already emerged in autumn. It is speculated that this might be an adaptation in the species to avoid the winter period, a phenomenon also recorded by Lytle (2002) showed that 86 % of the population of a case-building caddisfly emerged prior to the long-term mean date of the first monsoon flood in Arizonan desert streams. What was not evident in flood-response study was the smaller portion of the autumn population that remains in the river over winter, more markedly in the case of *Aprionyx*, emerging after winter. This phenomenon of cohort splitting by differential growth rates is fairly common in aquatic invertebrates (e.g. Brittain 1982; Bunn 1988; Schultheis *et al*. 2002; Lieske & Zwick 2008), and is often a response to temperature or food resource differences within the stream, resource limitation or intra-specific competition. Cohort splitting maximises overall fitness, since development time and adult size is correlated in most species with increased reproductive output, but comes at the risk of mortality with exposure to predation or adverse conditions (Lytle 2002, 2008). In years when few floods occur, or where patchy disturbance effects might be low, extending the growth period would thus have clear advantages over emergence at a small size for that portion of the population that has had poor summer / autumn growth rates, for example as a result of resource limitation.

Scott *et al*. (1988) collected *Cheumatopsyche afra* (Trichoptera: Hydropsychidae) imagos from August to December and also in March and concluded that the species may be bivoltine or have an extended emergence period. In the Molenaars River *C. afra* was present year-round, with asynchronous larval development, but probably two generations: a large summer population was present in overlapping cohorts over some seven months, each taking some four month to complete larval development. Late summer recruits formed a second generation, and developed through six to eight months during winter, to pupate and emerge in spring. Although pupae were found continuously from October / November through to March / April, pupal abundances were highest in November/December (the winter generation) and again in January / February (the peak of the summer generation). Pupal cases containing dead specimens often remain present after the peak of emergence, and may obscure separate periods of pupation (Rutherford & Mackay 1986). Bivoltinism is reported for other members of the genus *Cheumatopsyche* (e.g. Smock 1988; Sanchez & Hendricks 1997). Rutherford & Mackay (1986) calculated the development period from egg to adult to be 50 days (1000 degree-days) for species of *Hydropsyche* in a temperate mid-order river, and concurred with other studies that proposed that bi- and tri-voltinism should be expected in summer-warm rivers. Other studies of total development time in various species of Hydropsychidae range from 50 d, 1100 degree-days to 127 d 2540 degree-days (Jackson & Sweeney 1995).

The bivoltine life-history pattern of this species may arise from a combination of a “seasonal time constraint” *sensu* Rowe & Ludwig (1991) in maximising summer development, and a winter development strategy that bets against the possibility of mortality from winter floods. Larvae are tolerant of fairly high velocities, but not resistant to rock tumbling, whilst the extended pupal stage, generally between two and three weeks in the Trichoptera (Rutherford & Mackay 1986; Jackson & Sweeney 1995; Lytle 2002), represents a particularly vulnerable period in the life-cycle. In years with early or intense flooding, summer development may be considered to be adaptively advantageous. Summertime is not without its own constraints, however. The highly specific habitat requirements of these animals for sites at which they can construct collecting nets increases the likelihood that competition for space (e.g. Malas & Wallace 1977; Plague 1999; Funakoshi 2005) will affect development success. For example, Georgian & Thorp

(1992) showed up to 50 % differences in hydropsychid prey capture in optimal vs. suboptimal microhabitats. The continuous or serial hatching of eggs in the Molenaars River and asynchronous larval development may be one adaptation to reduce intra-specific competition, and the fact that temperatures are warm and food abundant may allow for the rapid development that allows multiple cohorts to complete their development between November and April.

Despite increased mortality from floods, the life-history data indicated up to four cohorts that develop through winter. It is not completely clear from the data whether the winter population is the offspring of a spring / summer-emerging generation, or a split cohort of larvae that did not complete their development in summer, or both. At least some of the summer generation grew increasingly slowly with declining temperatures but reached adulthood in mid-winter, as indicated by the collection of pupae in early August. This is evidence for cohort splitting. However, at least two winter cohorts suggest that they represent the progeny of the summer generation developing throughout winter and pupating in October / November. Split-cohort development is reported for some Hydropsychidae in both bivoltine and trivoltine species (e.g. Rutherford & Mackay 1986; Winterbourn & Harding 1993), in response to a range of factors including temperature, food quality and photoperiod.

Philopotamids occupy more sheltered areas of the stream bed and build finer nets with smaller openings than do hydropsychids, which have flimsy, coarser nets. This is ascribed to resource partitioning as a result of competition (Malas & Wallace 1977) since net mesh size and particle capture are closely correlated with velocity (Georgian & Thorp 1992) but an alternative explanation is provided by Miller (1984) relating velocity preferences and net characteristics to the dual requirements to avoid displacement from stone surfaces and ensure protection from benthic predators. There are no data on any species interactions in the Molenaars River to allow for evaluation of either possibilities, but either way, *Chimarra* spp. (Philopotamidae) and *Cheumatopsyche afra* appear to be both spatially separated and different in the size classes of particulate matter (including small animals) that they collect. The life-history data presented for *Chimarra* spp. is less clear than that for *C. afra*, particularly given that pupae were collected only between November and January, which would suggest that this species is univoltine. However, this is somewhat at odds with the apparent peaks in recruitment in November / December and February / March, coupled with rapid development of young over the summer. These are more suggestive of bivoltinism, possibly with split cohort development.

The four species assigned to a summer-development life-history in fact show a range of strategies, focused on a large and dominant, fast-developing summer generation, but with either a second smaller generation or a portion of the initial generation continuing its development through winter, despite the increased threat of mortality from floods.

7.4.2 Winter-resistant and / or summer intolerant life histories

Unlike the previous species which were susceptible to flood disturbance, a suite of species in the Molenaars River displayed moderate to very high levels of resistance to floods, and were characterised by having life histories where larval development occurred predominantly during the very period that should theoretically be avoided, that of frequent bed-moving floods. The blephariceerid species *Elporia uniradius*, best exemplified the short seasonal winter development life history, with a larval stage of only some three months, followed by pupation, a development period similar to that reported for blephariceerid species in the European Alps, of seven to twenty weeks (Frutiger & Buergisser 2002). In that study, larval development rates were independent of temperature, although the species themselves are cold stenotherms with larval distribution restricted to a temperature range of 0-16 °C. In contrast, despite a general description of *Elporia* spp. as being restricted to cool and highly oxygenated habitats (Wishart & Hughes 2001), it would appear that it nevertheless has a higher tolerance for warmer temperatures than the European species – average temperatures in Western Cape foothill rivers remain well above zero, and generally above 10 °C. Only summer temperatures in the Molenaars River, in excess of 20 °C, appeared to exceed the tolerance of these animals.

Recruitment extended over most of an eight-month period during which this species was encountered in the river, presumably as a result of extended hatching of eggs. This may be a resilience adaptation in the event of population losses from severe floods, although the density data suggest that this species is highly resistant to hydraulic stress. On the other hand, some Blephariceridae are algal grazers (Frutiger & Buergisser 2002; Harrison *et al.* 2003), with growth rates that have been shown to be strongly related to periphyton availability (Frutiger & Buergisser 2002). Because of their tolerance of extreme hydraulic conditions, they are considered to remain relatively unaffected by competition with other invertebrate groups or by predation (Frutiger & Buergisser 2002). Indeed in the Molenaars River, the spatial distribution of *Elporia* spp. densities was positively correlated with velocity, and these organisms are generally found with a clumped distribution on large stable boulders exposed to the strongest of sheer stresses (Wishart & Hughes 2001). Whilst these habitats might provide for low predation levels and inter-specific competition, synchronised development of a large population in these preferred habitats may reduce food availability (*sensu* Lieske & Zwick (2008) for Plecoptera). Continuous recruitment of young larvae may thus prevent resource limitation. Furthermore, late winter hatching in the Molenaars River could expose *E. uniradius* to improved food resources associated with increased periphyton abundance at this time, as shown in Chapter 6. Alternatively, the squeezing in of a second generation from September, taking advantage of more abundant food resources, albeit at the risk of mortality through increased temperatures, might equally explain the observed patterns. The limitations of this study make it impossible to differentiate between these alternate explanations, or even to test the validity of either, and both are mere speculation. Extended egg-hatching was also a feature of the species described by Frutiger & Buergisser (2002), but these authors concluded their data did not support the suggestion of bivoltinism made by earlier researchers, since egg hatching ended at the start of peak pupation. In the Molenaars River, young individuals were present long after pupae were first observed in the river, which would be consistent with the recruitment of a second generation, or facultative bivoltinism in this species.

The life-history patterns of *Lithogloea harrisoni* and *Lestagella penicillata*, both mayflies of the family Telagonodidae, are interesting to compare, since both are regarded as inhabitants of swift mountain streams (Barber-James & Lugo-Ortiz 2003), and both had low densities over the peak of summer. *Lithogloea* was present in order-of-magnitude lower densities than *Lestagella* and may be slightly more restricted in distribution to the upper foothill and mountain stream reaches, whereas *Lestagella* extends further downstream to lower-foothill gravel reaches (pers. obs.).

Like *Elporia uniradius, Lithogloea harrisoni* also showed a winter recruitment and development life history, with the highest densities of this organism coinciding with the period immediately after the onset of bed-moving floods. Indeed, the first appearance of *Lithogloea* in the Molenaars River in 1992 was following a small fresh, in early March, with numbers growing by the following month, after a second fresh, but increasing exponentially in early May 1992 following larger floods. In contrast, in the 2003 flood study, there were no autumn freshes at all prior to the sampling programme. June sampling in that study component followed single, minor rainfall events which resulted in short pulses of increased flow above low base flow levels but not exceeding 4 $m^3 s^{-1}$, and the invertebrate samples contained extremely low densities of *Lithogloea*. These only increased to appreciable densities following small floods of 6 and 10 $m^3 s^{-1}$, in July and August 2003, some two to three calendar months later than in 1992. Although the two studies are not comparable because of different sampling techniques, these differences do offer the speculation that *Lithogloea* recruitment may occur in response to, and in proportion to, the magnitude of flow pulses, which perhaps bring hyporheic water, and newly hatched instars, to the surficial sediments.

The flood study (Chapter 4) found both continued recruitment of new instars of *Lithogloea* during winter combined with increased representation of larger size classes, the latter indicating high levels of resistance to floods. The life-history data presented here agree with this interpretation, although the rather low representation of mature instars in the population in May / June and July / August suggests that continuous recruitment of young instars might obscure possibly more substantial population losses as a result of the 1992 floods. Here the compensation provided by asynchronous recruitment of young would amount to an effective resilience strategy in the event of larger floods which might incur population losses, allowing immediate recolonisation from hyporheic refugia (Huryn & Wallace 2000).

Barnard (1932) described mature *Lithogloea harrisoni* nymphs as being present from September through March, based on field observations in mountain stream reaches. My data suggest that initiation of the nymphal life stage may be cued to the onset of higher flows in the river, and it would be expected therefore that emergence patterns would vary in accordance with initial recruitment. The fact that month-on-month densities declined by greater percentages from May / June to July / August 1992 than from April / May to May / June, the latter being after the 1:20-year flood, suggests that emergence of mature nymphs may have commenced in August 1992, with very low densities remaining by late October. In both 1991 and 1992, no mature instars remained in the river from late December onward. This difference from that offered in Barnard (1932) is probably due to higher temperatures in the Molenaars River foothill zone than those in the mountain stream reaches, which should result in earlier development and emergence of nymphs (Brittain 1982). In any event, the species may be regarded as being univoltine, with winter development, and a life span, which falls just short of an annual cycle.

Lestagella penicillata had both mid-summertime and a more temporally restricted recruitment of young instars than did *Lithogloea*. This extended over a period of about two months, from middle to late summer (January to March), with rapid autumn growth, in contrast to the massive autumn recruitment and very slow initial growth of *Lithogloea*. Development of nymphs was the most synchronised out of all the Molenaars River species, with one discrete cohort developing progressively through winter, more fitting of the "slow seasonal" life cycle of Hynes (1970) than a fast seasonal one. However, population losses were high during the period of nymphal growth: this species has been shown to have only a moderate overall resistance to floods (Chapter 4) and low resistance in stream patches where floods overturn stones, but it does find refugium under unmoved stones. Only a sufficiently large initial population combined with patchy flood disturbance allowed sufficient numbers to survive through to spring to emerge and produce the next generation. This study was not long enough to allow population size to be monitored over years when floods fail, but it is suggested that substantial inter-annual variation in population size might be the result of variability in the specific flood patterns of any year.

Mature instars of both species did not persist beyond early December. King (1981, 1982) included both these species in her "winter group" of mayflies, occurring predominantly from March / April to December. The data for *Lithogloea harrisoni* in the present study agree with this, but *Lestagella* in the Molenaars

River has a definitely longer growth period as a result of earlier recruitment.

The plecopteran species in the Molenaars river also fitted the "slow seasonal" life-history pattern, with approximately annual life cycles. Recruitment and early growth occurred in spring. However, this was followed by retardation of growth in summer, along with a decline in densities that suggests mortality. Alternatively, many species of Plecoptera have evolved nymphal diapause to escape the effects of elevated summer temperatures (Giller & Malmqvist 1998). The reductions in plecopteran density in mid and late summer in the Molenaars River, along with the lower representation of mid-instars in the population, may therefore represent the retreat of these species into temperature refugia, rather than mortality, since densities increased again as the temperature declined in autumn and winter, without additional recruitment of young instars.

High growth rates and the sustained densities throughout winter add to the evidence already provided in Chapter 4 of the high resistance to floods of this group. Winter development ends with the emergence of *Aphanicerca* spp. between late July and late August, but later emergence occurs in *Desmonemoura pulchellum*, around October / November. Adult *Aphanicerca bicornis* have been recorded from April to August in the Western Cape. *A. capensis* adults have been recorded from May to November. Adult *Desmonemoura pulchellum* have been collected from the Molenaars River in October but more often in November (unpublished PhD data, Mr Duncan Stevens, University of Cape Town). These observations are consistent with the life histories presented in this study.

7.4.3 Temperature and flow resistant life cycles

A third suite of species was characterised also by moderate to high levels of flood resistance along with apparent tolerance of summer lowflows. A slow seasonal, univoltine life-history pattern was exemplified by the mayfly *Euthralus elegans*, characterised by a distinct change in size distributions over time and long (annual) larval development periods. Fast seasonal, bivoltine life histories with both summer and winter generations were characteristic of the mayfly *Demoreptus capensis* and the cased caddisflies *Agapetus agilis* and *Athripsodes bergensis*.

King (1982) included *Euthralus elegans* in her "winter group" of mayflies (April to December growth period), but the current data suggest a broader temporal window during which the nymphs of this species are present, with earlier recruitment of young instars in the Molenaars River than in the study river of King (1982). Densities over winter were reduced, but not nearly as dramatically as the other leptophlebiid species. This, combined with the fact that mid-sized instars rather than new recruits coincided with the flood period, indicates moderate resistance to floods in these species. This is unlike in the case of *Lithogloea harrisoni*, where a combination of resistance to floods is augmented by a resilience strategy of continuous recruitment of new instars over winter – a "belts and braces" approach to ensuring survival.

The resistance to floods indicated for *E. elegans* by these life-history data appears to be greater than that suggested by the flood study (Chapter 4). That study sampled invertebrates associated with individual stones on the uppermost bed layer, whilst the 1991-1993 data removed all bed material to the underlying gravel. The obvious implication is that many taxa are afforded much greater refugia during floods in deeper hyporheic interstitial spaces, even when bed particles are moved, than may be indicated by sampling of the upper stone surfaces. Most hyporheic fauna reported in the literature are smaller taxa, rather than the rather fragile leptophlebiids, but these data point to the interesting and under-researched topic of refugium use in South African rivers.

Of all the mayflies in the Western Cape rivers, *D. capensis* is probably the most resistant to increased shear stress associated with elevated flows, with a preference for fast flowing areas such as the upper surfaces of large boulders (database of King & Schael 2001). Its simple, small gills and high oxygen requirements (Dallas & Janssens 1999) are testimony to the trade-off associated with allocating resources to resisting hydraulic forces rather than overcoming adverse conditions in slow-flowing habitats. The strong perennial flow in the Molenaars River probably ensures sufficient high quality habitat through summer to support this species.

Size distribution changes over summer suggest that *D. capensis* recruits from August or September emerge as adults by January or February, with a second generation developing over the winter. The low frequency of mature instars in summer might at first suggest otherwise, but consideration of two factors adds weight to this conclusion: overall densities were very high in December 1991 and 1992, and even a low proportion of mature instars would represent a sizeable number of individuals. These densities also declined by some 60% by the following month, whilst population growth continued. The Molenaars River is stocked with trout, and their feeding preferences for baetid mayflies (McPeek & Peckarsky 1998) are likely to be the cause of both massive predation mortality and reduced size on emergence (McPeek & Peckarsky 1998).

The winter generation of *D capensis* includes a fairly wide size-distribution, suggesting serial hatching of cohorts in autumn, despite a peak in recruitment in February / March. Adult emergence is indicated between August and October, and overlaps with recruitment of the summer generation, indicated in this study by a clearly bimodal distribution in the size frequency data. A notable observation in the Berg River flood study of 2004 (Chapter 4) was of such a bimodal distribution in the early August sampling, immediately post-floods, suggesting that recruitment may be earlier in that river or in drier years.

The glossosomatid caddisfly *Agapetus agilis* constructs larval and pupal cases with heavy sand grain ballasts, an investment in morphological resistance to hydraulic forces and in predator defence. This allows it, like *Demoreptus capensis*, to occupy the more exposed surfaces of larger boulders. Here, faster flows in summer increase algal productivity, its main food source. Similarly, the survival of this species is high on unmoved stones during floods (Chapter 4), and these boulder habitats thus minimise the risk of flood-induced mortality in winter. No other life-history information exists for *A. agilis*. Whilst many authors have shown other species of this genus to be univoltine (e.g. Machant & Hehir 2001; Becker 2005), Alvarez & Pardo (2005) demonstrated trivoltinism in *Agapetus quadratus* in a seasonal, spring-fed Mediterranean stream, with winter, spring and summer generations. The size-frequency data in this study suggest bivoltinism in *A. agilis.* This species is as small as the smaller of the chironomids, a feature which enables rapid development (Giller & Malmqvist 1998). Also, the high levels of resistance to winter floods and to summer stress or competition already suggested would be consistent with the alternating of a winter and a summer generation.

The life history of *Athripsodes bergensis* is difficult to piece together without greater sample sizes and wider sampling, beyond simply riffle areas. A puzzling feature of these data was the absence of pupae from riffles in June, when pupation of the summer generation would have been expected. In the autumn baseline study of June 2003 (Chapter 6), this species was more abundant in slower-flowing runs than riffles. It is speculated therefore that mature instars pupating at the start of winter might move to sheltered stream margins, since these are less prone to flood disturbance.

7.4.4 High resilience, unsynchronised life histories

The three groups that demonstrated resilience in the face of floods, viz. *Baetis* spp., *Simulium* spp. and the Orthocladinae as a whole, showed both non-significant decreases in density combined with population structure changes that showed strong recruitment of new instars after bed-moving floods in the Berg River in 2004 (Chapter 4). This finding is consistent with the life-history data presented here, in which winter recruitment was such a marked feature of five of the six taxa in this category, with the exception of the chironomid *Synorthocladius* spp.

Rapid development rates and multivoltinism is well demonstrated in studies of simuliid (e.g. Smock 1988; Huryn & Wallace 2000), chironomid (e.g. Huryn 1990) and baetid species (e.g. Brittain 1982; Robinson *et al.* 1992) life histories.

All the five species comprising the genus *Baetis* in the Molenaars River were present year-round, and showed similar changes in density from month to month. It is unfortunate that separation of this genus into its component species was not possible, as competition for resources is highly likely given the very high densities, and a more detailed analysis of life histories could shed light on this. Unlike *Demoreptus capensis*, whose microhabitat preferences separate it from other baetids, little information exists on the difference in habitat requirements of *Baetis* spp. in the Molenaars River. The relatively high number of species (five), from just riffle samples, would make this a productive avenue for research. Two aspects from this life-history study that bear consideration are the recruitment of new instars all through winter, and the huge recruitment spike in spring. These provide, respectively, a ready source of individuals to offset population losses during winter floods and a very large population that would increase its chances of survival through predator saturation in summer (*sensu* Brittain 1990; Huryn & Wallace 2000).

Densities of *Simulium* (Metomphalus) spp. in riffles in the Molenaars River were very high values in summer, but were seldom lower than 500 indiv. m^{-2} at any other time. Although all life stages, including pupae, were present year-round, small instars dominated during winter, presumably recruited from the hyporheos to offset flood-induced mortality of conspecifics and of other species. Higher velocities associated with winter base flows also suit this family: similar species have been shown to occur in velocities in excess of 0.9 m s^{-1} (Rivers-Moore *et al.* 2007), as a result of better food capture rates.

These life-history and physical adaptation makes the simuliids better adapted to winter conditions than their hydropsychid competitors, and may be a response to competitive displacement and predation of this family by the Hydropsychidae where these groups co-occur, as discussed in Chapter 4. Winter mortality of hydropsychids is much greater than that of simuliid and probably the key factor ensuring their ongoing coexistence.

Asynchronous development and univoltinism in orthoclad Chironomidae appears to be a dominant life-history pattern (e.g. Lindegard & Mortensen 1988; Huryn 1990; Boothroyd 1999), and certainly all records of life cycles show four or more generations per annum in this subfamily (Armitage *et al.* 1995). Thermal regime and terminal size of the species was shown by Huryn (1990) to be the key factors in determining the number of generations per annum, whilst rapid growth was related by Boothroyd (1999) to warmer temperatures and high food quality. Because chironomids are capable of rapid development, the number of generations per year may be misrepresented where the time-step of life-history studies is too big to capture population changes adequately (Armitage *et al.* 1995), and this may pertain to the present study, where cohort development appears to shift quite dramatically during the spring and summer months, even where size frequency data are presented for one species alone, such as *Notocladius capicola*. This species is the smallest of the chironomids in the Molenaars River, with a maximum head-capsule width of 0.4 mm, and a length generally under 2 mm. Despite uncertainty about the number of generations in a year, the data show a combination of multiple cohorts and asynchronous development. These combined with the year-round presence of pupae in the Molenaars River do suggest that whilst the spring generation in all four groups is the largest, additional generations occur throughout the year.

The success of these three groups in terms of year-round population density and possibly also production in the Molenaars River may thus be attributed to a life-history "strategy" that combines small size and rapid turnover with asynchronous development and multivoltinism. This, along with high mobility in the case of *Simulium* spp. and *Baetis* spp. allow these species to persist in the face of disturbance, predation and possibly competition.

In conclusion, species traits and environmental conditions may be regarded as being connected through the suit of life-history attributes that represent a species' solution to the environmental problems of resource accumulation, avoidance of predation, reproduction and dispersal (Verberk *et al.* 2008). This chapter has examined life-history patterns in the Molenaars River as an example of the flood-prone, winter disturbance regime of Western Cape rivers. A range of life histories were found. Some, but few, were constrained to a summer development period. Most life-history patterns suggested that floods are but one of a suite of environmental variables that may influence life histories, and that their impacts may be overcome by other mechanisms than life histories which time development to avoid floods through terrestrial or egg stages. These mechanisms include high resistance through morphological adaptations, enabling a suite of species to exploit the resources available during winter, such as high algal growth; and resilience by the larval stage, probably through temporary refuge in the interstitial spaces that extend some depth in these coarse-bedded rivers, thus minimising mortality. Specific life-history attributes that confer on aquatic invertebrates a measure of resilience to floods include continuous recruitment of new instars from the hyporheos, as a constant source of replacement following population losses; and asynchronous development, which results in all size classes in a population being represented, thus offsetting size-related effects of floods. Many life histories showed cohort splitting and /or bi- or multi-voltinism, with winter generations as a hedged bet against the possibility that floods of sufficient magnitude and / or sufficient frequency will occur in that year. The benefits of such behaviour are evident in this being a dominant feature of invertebrate life histories in the Molenaars River.

All life histories are a trade-off between the adaptive value of one trait that solves one problem, compared with the value of other traits that an organism possesses as a solution to other challenges to its survival. This study presents some insight into traits that solve part of these challenges, specifically those associated with the effects of floods. Further investigation of species interactions, resource limitation, thermal tolerances and predation effects is required to complete the picture.

8 GENERAL DISCUSSION AND CONCLUSION

8.1 DISCUSSION OF THE THEMES COVERED IN THIS THESIS

This thesis study emanated from the proposition that disturbance asserts an overwhelming influence on invertebrate community characteristics and life history traits in Western Cape foothill rivers. In the perennial systems within this region, floods, more than droughts are the dominant form of disturbance, defined as discrete events that reduce populations of one or more species, through removal or mortality, and that alter resource availability and / or physical characteristics of invertebrate habitats.

The themes investigated included firstly the definition of what sort of flow constitutes a disturbance to invertebrate assemblages, and the most appropriate scales at which this should be measured. The second theme was the characterisation of the flood regime, i.e. the temporal dimensions of floods over the threshold considered to represent a disturbance, and particularly included quantifying the predictability of the flood regime. Thirdly, the effects of floods were examined in a broader context, one of temporal fluctuation in invertebrate assemblages related to environmental variables other than or in addition to floods. A focal point of this theme was the attempt to differentiate between the importance of floods per se versus the role of monthly, seasonal and inter-annual shifts in ambient conditions or resources on river invertebrates. Linked to this theme of temporal variability was the exploration of concepts of stability and persistence of invertebrate assemblages, specifically in relation to the predictability or otherwise of floods that constitute disturbances. Finally, species life-history attributes were examined in terms of the extent to which they might be shaped by attributes of the flood regime, but also in relation to other drivers shown to be equally important in determining community patterns in these rivers.

Recent studies of how the hydraulic forces at work during a flood affect biota show that both the distribution of shear forces over the stream bed and bed particle entrainment are patchy. In this study (Chapters 3 and 4) the spatial scale of invertebrate sampling before and after floods, and the hydraulic surveys for the calculation of shear forces and measurement of particle movement, was that of individual stones. This allowed the distinction to be made between stones that moved or did not, so that changes in invertebrate community or species density could be related to the possible refugium offered by stable stones. This approach also allowed invertebrate densities on a stone immediately after a flood to be related to the shear stress acting on that stone during the flood.

The field investigation of the impacts of individual floods on stream invertebrates in this thesis found a positive relationship between the magnitude of a disturbance event and the proportion of the bed disturbed. One of the intentions was to establish a relationship between flood magnitude or the percentage of stones that were moved and the degree of reduction in invertebrate population size immediately after the flood. This was not possible because not enough individual floods were sampled in a before-after sampling design to deduce such a relationship, in part because of field conditions. In the end, the consequences for invertebrates of very small floods and of a set of two substantially larger floods were quantified. Since the former were associated with variable responses, from increased densities in some species to mild, mostly non-significant decreases in others, this category flood was rejected as a threshold for disturbance. The size of the larger flood was then used as a disturbance threshold, since this size was associated with significant reductions in almost all species of invertebrates, with decline in total invertebrate density of some 60% immediately after the flood. Death (2008) argued that there is general agreement that the threshold for a flood being a disturbance is some percentage of bed movement. The threshold flood established in this flood study moved 43 % of the river stones. I consider this to be a conservative estimate, since the associated reductions in invertebrate densities were large. Floods somewhere between the very small and substantially larger ones recorded would probably be more appropriately defined as a *threshold* of disturbance, but these were not studied. Of note for other disturbance studies is that the threshold flood defined in this study was a flood that on average occurs seven to eight times per annum. A lower threshold, as per the argument made above, would be even smaller and represent a more common event in the flow regime.

The massive differences in responses of different species to floods both big and small makes the question of what constitutes a disturbance a more complex one. The very small floods in the Molenaars River did cause reductions in some species, whilst they appeared to have facilitated the distribution of others. This finding is important: if the definition of disturbance advanced by Townsend (1989) is accepted, as an event that "opens up space that can be colonised by individuals of the same or different species", then even the small floods in the Molenaars River should be called disturbances. Even the large floods did not reduce the densities of all species across the board, and the 1:20-year flood recorded during the longer-term study in the Molenaars River, which could be assumed to be associated with more than 43% bed movement, did not decimate invertebrate populations. The relative change in species densities that are the result of floods, of all sizes, as was shown in this study may be the crucial effect of floods, particularly where species compete for space or resources. This relative change might be as a result of one species' increase and another's decline or different degrees of population reduction, or indeed simply changes in the relative proportions of different life stages. These species-specific effects may thus be an important aspect of autecological studies and those examining interspecific interactions in streams.

Despite the agreement between studies that have linked reductions in invertebrates and algae to bed-moving floods, this study found that movement of the substratum is not a threshold for population losses to occur, since almost all taxa were reduced on unmoved as well as moved stones, presumably because of the shear forces associated with the peak discharge of the floods. This finding supports the conclusion of Bond & Downes (2000) that hydraulic forces acting on even fixed stones may play as great a role in disturbance as stone movement. Measuring the actual hydraulic forces acting during a flood, therefore, is an essential component of flood studies: the large size of the river bed elements in this study, much larger than in most other flood studies, means that on average stones will move only as a result of substantially greater flow forces than in rivers with smaller stones, and dislodgement of animals as a result of these strong forces may preceded bed movement. The hydraulic model distinguished between moved and unmoved stones of the previously unmarked control samples in the Berg River study fairly successfully, but the descriptions of the hydraulic forces during a flood appeared less so: the calculated hydraulic forces acting on the stones during a flood were not successfully linked to post-flood invertebrate densities. Actual measurement of shear stress during a flood is impossible, and most studies have extrapolated from shear stress measurements at low and moderate discharges to those at high discharge (e.g. Gjerløv *et al.* 2003; Townsend *et al.* 1997c; Lancaster & Hildrew 1993b). More refined techniques for modelling hydraulic forces acting at a point during high flows would be a fruitful avenue for research in flood studies.

Stable stones nevertheless provided a relative refugium for many taxa over the course of a flood, with lower population losses on these stones than on stones that moved. What was not anticipated was that there would be such a wide difference in the relative refugium afforded to different taxa, with unmoved stones offering no advantage to many species examined in the study. Also, less mobile taxa did not especially enjoy this relative refugium, contrary to what was hypothesised. Two such taxa, elmid beetles and chironomid larvae probably use finer-scale refugia, such as micro-crevices in the surface roughness of stones. In other species the recruitment of new instars immediately after a flood may also obscure any preference patterns, since new recruits might settle either randomly over the stream, or in hydraulically sheltered areas that develop as flood flows recede.

In relation to stream hydraulics more generally, it was noticed that slower-flowing areas of the stream at low flow were not necessarily protected from scour at elevated discharges. This implies that biotopes, which were shown in this study to have strong associations with invertebrate community differences, do not change consistently during a flood. Not all riffles become runs, for example. This particular spatial dimension of disturbance was not taken further in this study, because of its focus on identifying the response of invertebrates on stones that moved versus those that did not, but consideration of how well different patches are worked by floods, and what this implies for the characterisation of biotopes, is worthy of further investigation.

The characterisation of rivers in the Western Cape as "predictably unpredictable" by Davies *et al.* (1994) is borne out by the analysis of the flow regimes of the Molenaars and Berg Rivers (Chapter 5). Strong seasonality of flood disturbance is a feature of the regimes. Winter disturbance is unpredictable on a month to month basis. In contrast, the summer period is characterised by a high predictability of disturbance, because floods are almost non-existent. Three aspects of the examination of temporal change in invertebrates in the context of the flood regime that emerged from this study are particularly relevant for the applied field of environmental water requirements. The first is that the frequency of floods above the disturbance threshold was a far more important driver of community patterns than the degree to which a flood exceeds this threshold. Both periphyton and invertebrate densities were reduced by floods, but not in proportion to flood size. In contrast, total invertebrate densities and those of a number of individual species was strongly and linearly related to the frequency of floods. The implications for managing modified flow regimes in dammed rivers are substantial. Whilst a few, large releases per annum may meet requirements for channel geometry and sediment movement, they are unlikely to be associated with adequate disturbance signals to maintain natural population dynamics in regulated rivers with flow regimes similar to these study rivers. These tentative findings require more research, since they are based on a small sample of pre- and post-flood data.

The second noteworthy result of this study was the demonstration of very strong month-by-month changes in invertebrate assemblages. Individual months were strongly differentiated from those that preceded or followed them at all times of the year except summer, when monthly changes were less significant than seasonal ones. This indicates that the time scale of variation in invertebrate community pattern is that of a month, or maybe even less, which has implications for long-term studies. This intra-annual variability is seldom examined; even where monthly samples are collected, these are often grouped by season, which is a construct to aid in interpretation of pattern, but which may obscure relationships between invertebrate communities and physical drivers.

The third remarkable finding was the indication that temperature was at least as important as were floods in determining invertebrate patterns, both at the level of monthly changes in density and community structure, and in terms of life histories. Life histories of almost all species were timed to take advantage of the fast development rates that are possible between spring and autumn as a result of warmer temperatures. For some species, life-history patterns showed avoidance larval development during summer. For all but a few species, maximising development time meant betting against the chances of mortality during the flood season.

8.2 CONCLUSION

This thesis set out to test the notion that the disturbance regime exerts a dominant role in ecosystem functioning in the rocky foothill rivers of the Western Cape region of South Africa and on the evolution of life history attributes their invertebrates. One of the hypotheses formulated at the start of the thesis was that there would be a threshold above which flows act as disturbances, and that this threshold would be quite high, perhaps equating to an event occurring once or twice per year, since this threshold has been considered to be the bankful flood event in many international studies.

What was demonstrated was that species respond to their environment differently, and that there is no one threshold discharge, or shear stress, at which populations of different species are reduced in density. Even different life stages may respond differently to the same magnitude flood. Whilst this may appear rather obvious, the implications are profound. Floods of all magnitudes effect different degrees of population reduction and thus determine the proportions of species that comprise invertebrate assemblages. The regulation of population dynamics by flow has largely been ignored in studies aimed at the management of regulated rivers, where hydraulic modelling of habitat requirements has perhaps been emphasised to the exclusion of examining the direct role of discharge on stream fauna and stream algae. This thesis show that there is great potential for this research field to contribute to a better understanding of environmental flow requirements.

Moreover, quantifying the complex interactions of flow with other variable seems to be an important direction that should be pursued by future studies. This thesis has illustrated the interaction between flows, temperature and algal availability, which appears to play a large role in invertebrate population dynamics. The exploration of this interaction was tentative, because of the restricted nature of the temperature and algal (periphyton) data sets. However the work performed on top-down controls of invertebrates by fish predators indicates that algal biomass is probably the major food resource for invertebrates, even in these oligotrophic systems. The community aspect of stream algae is now a subject of extensive international focus, but in South Africa algal research has largely been restricted to the development of biotic indices. Similarly, further investigation of the ecological effects of temperature on both algal and invertebrate population and community dynamics is needed. Consideration of temperature effects must be an essential component of any flow management strategy. Long-term collection flow-linked temperature data is needed to support these needs.

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