Stream invertebrates and water temperature:

Evaluating thermal tolerances in the Cape Floristic Region (South Africa) – implications of climate change

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Abstract

Temperature is an important environmental variable for aquatic invertebrates as it affects their development, reproduction and survival. Temperature also affects the abundance and distribution of individuals in a stream or river. Stream temperatures are affected by human impacts which include not only impacts such as pollution, abstraction of water and the removal of riparian vegetation, but also the affects of climate change. In the Western Cape Province, South Africa, it is predicted that air temperatures will increase and winter rainfall will decrease. This in turn will affect water availability and water temperatures in streams and rivers. Thermally sensitive species are threatened by increases in water temperature, but currently very little information exists on the thermal tolerances of aquatic invertebrates in South Africa. In order to rectify this problem baseline data on thermal tolerances of aquatic species needs to be collected, together with stream temperature and associated invertebrate community data. An attempt was made to collect some of these much needed data in the Western Cape Province. Two methods, namely Critical Thermal Maxima (CTM) and LT_{50} experiments, were used to determine the thermal tolerances for a range of aquatic insect nymph species from the southwest fynbos bioregion and the south coast fynbos bioregion. The results from the experiments provided information on the relative thermal sensitivities of the species. Overall *Aphanicerca capensis* (form C and undescribed form), Notonemouridae ranked as the most thermally sensitive for both experiments. The *A. capensis* species complex (and possible other notonemourid stoneflies) may potentially be used as an indicator of changing stream temperatures in the Western Cape Province. To rapidly determine thermal sensitivities the CTM experiments are recommended rather than the more time consuming LT_{50} experiments as the relative thermal tolerance for the species tested ranked the same for both experiments. It is suggested that LT_{50} experiments of longer duration be investigated in order to compare the experiments to naturally-occurring thermal stress. Stream temperature and community composition data were collected from Window Gorge Stream, on Table Mountain, to provide baseline data for future monitoring and understanding of potential changes in thermal profiles.

Temperature loggers were placed at six sites along the stream. Stream temperatures were fairly low but the stream ran dry during the late summer months (February through to April/May). From the community composition data collected the highest diversity was found during the winter months, as expected. The community composition did change down the length of the stream, with the species composition found near the source being quite different to that of the species composition found lower down the mountain. Temperature and associated oxygen saturation were two important variables related to the community composition down the length of the stream. It is important that experimental data be combined with field data, enabling field sampling to focus on the collection of information on the abundance of the thermally sensitive species (e.g. *A. capensis* species complex). Recorded stream temperatures also provide reference conditions for the species tested in the laboratory. Climate change is likely to have an affect not only on stream temperatures but also on water availability, which will both influence stream communities and ecosystems and it is important to understand what these potential effects might be.

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Chapter 1 – Introduction

The lives of aquatic organisms are intimately linked to many abiotic factors including pH, temperature, conductivity and dissolved oxygen (Power *et al.* 1988, Dallas and Day 2004). Since aquatic species occupy a range of habitats, tolerance levels to these variables are expected to vary widely.

The importance of temperature

One of the most important physical variables affecting aquatic organisms is temperature. In streams water temperature varies with altitude, latitude and season, as well as longitudinally and even daily (Vannote and Sweeney 1980, Ward 1985, Dallas and Day 2004). Water temperature is also affected by hydrology, climate and the structure of the catchment area as well as by groundwater, nature of the substrate and riparian vegetation (Vannote and Sweeney 1980, Ward 1985, Beschta 1997, Naiman & Décamps 1997, Poole and Berman 2001, Dallas and Day 2004, Rivers-Moore *et al.* 2004, Caissie 2006, Dallas 2008). Aquatic organisms are adapted to particular temperature regimes of the stream in which they live and typically have a limited ability to acclimate to rapidly changing temperatures outside the range experienced within their habitat (Vannote and Sweeney 1980).

In fact, many aquatic organisms have a narrow range of temperatures which are optimal for growth, reproduction and general fitness (Brittain 1983, Sweeney and Vannote 1986, Quinn *et al.* 1994, Cox and Rutherford 2000). For instance, migration patterns and insect emergence of many species are influenced by seasonally changing temperatures (Nebeker and Lemke 1968, Brittain 1983, Dallas and Day 2004, Dallas 2008). Temperature is thus an important variable driving the ecology and life-history patterns of species, and ultimately affects the structure and function of these communities (Vannote and Sweeney 1980, Sweeney and Vannote 1986, Perry *et al.* 1987, Brittain and Campbell 1991, Quinn *et al.* 1994, Hawkins *et al.* 1997, Dallas and Day 2004).

Any unexpected and in particular, rapid changes in water temperature could potentially be lethal to these organisms. Unnatural changes in water temperature often result from anthropogenic causes such as thermal pollution, stream regulation and/or changes in riparian vegetation cover. Ultimately the predicted changes in global climate will also result in elevated air, and subsequently stream temperatures (Nebeker and Lemke 1968, Quinn *et al.* 1994, Dallas 2008). Increases in water temperature result in a decrease in the amount of dissolved oxygen and may also increase the toxicity of certain chemicals, both producing a source of physiological stress for aquatic organisms (Dallas and Day 2004).

Increased temperature could have further diverse and complicated effects on aquatic organisms (Nebeker and Lemke 1968). The growth potential of aquatic larvae may be reduced at high temperatures because their metabolic costs may be higher than their assimilation rate leading to insufficient energy and reduced growth (Heiman and Knight 1975, Vannote and Sweeney 1980). By increasing water temperature Vannote and Sweeney (1980) found that adult aquatic insects emerged earlier and at a smaller size. If adult size is reduced, this would in turn affect egg production, recruitment and thus the competitive ability of that taxon (Vannote and Sweeney 1980). Brittain (1983) found that temperature influenced the growth and development of stoneflies with approximately 90% of the variation in growth rates being explained by differences in temperature. Breeding and habitat requirements of species also influence their temperature tolerance (Ernst *et al.* 1984).

Temperature tolerance

Morphological, behavioural, ecological, physiological and biochemical adaptations allow aquatic organisms to survive certain temperatures (Lencioni 2004). An increase in temperature exceeding the tolerances of certain species may reduce species diversity (Hawkins *et al.* 1997, Cox and Rutherford 2000). There are various examples of insect temperature tolerance and the effect of increasing temperatures. Cox and Rutherford (2000) found several species of Plecoptera and Ephemeroptera to be sensitive to high temperatures as these species were not really found in unshaded streams. Hassal *et* al. (2007) studied the effect of temperature on odonate biology: in the egg stage diapause and hatching were affected; in the larval stage feeding rates, development time and larval ecdysis were affected; and in the adult stage flight periods and colour change were affected. Brittain and Campbell (1991) found a significant relationship between water temperature and the length of egg incubation and hatching success for *Coloburiscoides* (Ephemeroptera; Coloburiscidae), at temperatures between 10ºC and 25ºC. The number of degree-days necessary for egg development decreased with increasing temperature (Brittain and Campbell 1991).

Differences in temperature tolerance amongst taxa, and regional and seasonal temperature differences, should be considered when establishing guidelines for management of water temperature in streams and rivers (Dallas and Day 2004). Many biomonitoring techniques such as the SASS (South African Scoring System, version 5, Dickens and Graham 2002) commonly use macroinvertebrates as indicators of water quality. It would be useful for water resource managers to, in addition, also have an indication of the temperature tolerances of key species, which could then potentially be used as indicators of the stream's "thermal health". Unfortunately this concept is more complicated than it first appears, as the thermal tolerances of the most common aquatic invertebrate taxa in South African streams and rivers are unknown. There is, not only an urgent need to document lotic thermal regimes in South Africa, but also an urgency to study thermal responses of aquatic macroinvertebrates found in these South African lotic systems. This study provides some of the first data on thermal tolerances of selected aquatic invertebrates in South Africa.

There are two main methods used to determine the upper temperature tolerance of insects (Addo-Bediako *et al.* 2000) and can be applied to aquatic insect nymphs. The static lethal effect experiments involve either keeping the temperature constant and varying the duration of exposure or keeping the duration constant and varying the temperature. The dynamic method (critical thermal maxima experiments) involves increasing the temperature at a constant rate until an end-point is reached (often the loss of locomotory function).

The vulnerability of the stream fauna in Cape Floristic Region

The Cape Floristic Region (CFR) has been included as one of the top 25 global biodiversity hotspots (Myers *et al.* 2000). The CFR comprises an area of 87 892 km^2 and is located at the south-western tip of South Africa between 31 and 34.30ºS where it experiences a Mediterranean-type climate, characterized typically by cool, wet winters and warm, dry summers. The CFR has exceptionally high levels of plant endemism (70% of the region's 9000 species are endemic) but is not only important from the botanical perspective, as it also hosts a unique and highly endemic fauna (Stuckenberg 1962, Picker and Samways 1996, Giliomee 2003, Cowling *et al* 2003, Wishart *et al.* 2003). The CFR has long been recognized as a global priority for conservation action because of the high concentration of endemic taxa (Cowling *et al.* 2003).

One of the few parts of the CFR which has been profiled for invertebrates is the Cape Peninsula, where this study was conducted. The very high level of invertebrate endemism (at least 111 species) has an over-representation of Gondwanan fauna. Gondwanan fauna are relictual fauna from ancient lineages that currently inhabit palaeogenic habitats such as upper-reach forest streams, forests and caves (Picker and Samways 1996). Many of these taxa within Africa are concentrated in the CFR, which has provided refugia for these stenothermal fauna. The Mediterranean-type climate of the CFR may change due to climate change and the predicted increase in temperature and decrease in rainfall, especially in winter months (Midgley *et al.* 2005, De Wit and Stankiewicz 2006). This would have the effect of elevating water temperatures both directly and indirectly. The Gondwanan and other sensitive aquatic animals, that are restricted to the cold waters found in the headwaters of streams, may therefore be threatened by increasing temperatures in the streams and rivers in the CFR through climate change. These species are hypothesized to have a limited capacity to respond to elevated stream temperatures, and in addition already occupy the coldest part of streams viz. headwaters and upper-reach zones.

Objectives and aims

In light of the above-mentioned predictions for increasing temperatures for the CFR, this study aimed to evaluate temperature tolerances of several aquatic invertebrate species of both Gondwanan and non-Gondwanan origin from this region. The main objectives of the study were:

- To determine thermal tolerances of aquatic insect species from two different bioregions in the CFR using lethal (LT_{50}) and critical thermal maxima (CTM) experiments

- To investigate the hypothesis that Gondwanan fauna (restricted to cooler temperatures) are more sensitive to thermal stress than non-Gondwanan fauna (may be eurythermic – adapted to wide range of temperatures)
- To establish the relationship between invertebrate community composition and water temperature along a longitudinal and temporal gradient for a Cape Peninsula mountain stream.

Chapter 2 uses LT_{50} experiments to measure the relative thermal tolerance of aquatic insect nymphs across a range of taxonomic groups (Plecoptera, Ephemeroptera and Trichoptera), from CFR stream communities. It also compares the thermal tolerance of Plecoptera (Notonemouridae) from two different bioregions in the Western Cape Province of South Africa, viz. Table Mountain (southwest fynbos bioregion) and Tsitsikamma (south coast fynbos bioregion) (Rutherford *et al.* 2006).

Chapter 3 uses CTM experiments to measure the thermal tolerance of aquatic insect nymphs from a range of taxonomic groups (Plecoptera, Ephemeroptera and Trichoptera) selected to allow a comparison of CTM values among Gondwanan and non-Gondwanan species to provide further information on the relative thermal tolerances of these dominant CFR stream taxa. The chapter concludes with a comparison of results obtained from LT_{50} and CTM experiments.

Chapter 4 tracks the aquatic invertebrate community of a Table Mountain stream (Window Gorge Stream) over a number of seasons and along the reach of the river, thus providing two gradients along which to examine community responses. The community responses to increasing temperature are discussed in the light of implications of future climate change, and its impact on the CFR stream fauna. This is the first study to describe the aquatic community of a Table Mountain stream, and its responses to natural fluctuations in stream temperature.

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Chapter 2: Identifying thermally sensitive species from upper reach mountain streams in the Western Cape Province using LT_{50} trials

All organisms have an optimal temperature range where survivorship, growth, metabolism and fecundity are maximized. Temperature thus usually limits the distribution and abundance of species as many have narrow temperature ranges. Global warming, together with other anthropogenic activities such as over-abstraction of water from streams is likely to increase stream temperatures, and thus impact stream fauna. Baseline information on thermal tolerances of aquatic invertebrate species in South Africa is, however, limited. To provide insight into these thermal tolerances, LT_{50} experiments were conducted on some aquatic insect nymph species (*Aphanicerca capensis* (form C and an undescribed form), Notonemouridae; *Castanophlebia* sp., Leptophlebiidae; *Lestagella penicillata,* Teloganodidae; and *Athripsodes* sp., Leptoceridae). These were obtained from three Western Cape Province streams, namely Window Gorge and Skeleton Gorge streams on Table Mountain, and the Salt River in the Tsitsikamma area. Probit analysis and Trimmed Spearman-Karber analysis were used to calculate the LT_{50} values of the four species. The 96h-LT_{50} values ranged from 23.4°C for *A. capensis* (form C) (Skeleton Gorge), 25.0°C for *A. capensis* (undescribed form) (Salt River), 26.5°C for *Castanophlebia* sp. (Window Gorge), 29.5°C for *L. penicillata (*Window Gorge*)* and 29.9°C for *Athripsodes* sp. (Window Gorge). The survival of each species decreased with time exposure to a constant temperature. *A. capensis* (form C) was the most sensitive species in terms of upper thermal tolerances, and would be expected to respond to thermal gradients along streams and be restricted to upper reaches. These results provide some of the first data on upper thermal tolerances of aquatic insects in South Africa. Future studies should include a broader range of species as well as experiments of longer duration.

Keywords: temperature tolerance, LT_{50} experiments, aquatic insect nymphs, climate change

2.1 Introduction

Temperature is one of the most important abiotic factors influencing the structure and function of aquatic assemblages, affecting growth, metabolism, fecundity and survivorship (Brittain 1983, Sweeney and Vannote 1986, Quinn *et al.* 1994, Cox and Rutherford 2000a). For all species there is an optimum temperature for normal functioning of these parameters (Vannote and Sweeney 1980, McKie *et al.* 2004). Temperatures outside this optimum may result in changes in community composition, decreases in abundance and an increase in the probability of local extinctions (McKie *et al.* 2004). Temperatures during warmer months may limit the distribution and abundance of some stream invertebrates that prefer cooler temperatures (Quinn *et al.* 1994), especially if these temperatures are likely to increase dramatically due to anthropogenic activities.

Various human activities may influence the temperature in streams and rivers. These include the discharge of heated effluents (thermal pollution), land-use changes, abstraction of water, agricultural irrigation return-flows, flow modification, inter-basin water transfers, removal of riparian vegetation and ultimately the over-arching influence of potential global warming (Nebeker and Lemke 1968, Quinn *et al.* 1994, Dallas 2008). An increase in air temperatures is predicted for the Western Cape Province of South Africa where coastal areas are predicted to experience an increase of approximately 1.5°C by 2050 (Midgley *et al.* 2005). As stream temperatures are therefore likely to increase in tandem, it is important to understand what effect such increases may have on aquatic organisms, and whether they would be beneficial or harmful (Nebeker and Lemke 1968). For example, an increase in stream temperature may cause some thermally sensitive species to be eliminated, resulting in changes in community composition (Quinn *et al.* 1994, Cox and Rutherford 2000a).

The effect of increasing stream temperature on aquatic organisms

Temperature affects the distribution of stream animals in various ways. It can structure the distribution of aquatic invertebrates down the length of a river (Ernst *et al.* 1984, Quinn *et al.* 1994). The distribution of invertebrates in headwaters may be further affected if riparian vegetation is removed, allowing for an increase in stream temperature (Quinn *et al.* 1994). An increase in water temperature may then limit the occurrence of certain species, namely those adapted to cool water, and reduce species diversity (Hawkins *et al.* 1997, Cox and Rutherford 2000a). Species adapted to warmer temperatures on the other hand may be able to expand their distribution with an increase in temperature. Insects have changed their distribution in the past in response to an increase in temperature. An example of this is the increase in diversity and intensity of herbivory during the late Paleocence-early Eocene global warming shown in fossil leaves found in southwestern Wyoming (Wilf and Labandeira 1999).

Nebeker and Lemke (1968) found that adult emergence in the laboratory occurred earlier in several species of aquatic insects, when temperatures were increased but below the lethal limit. They predicted that an increase in stream temperature during winter would cause the stream organisms to develop faster, emerge earlier and die from the cold winter air temperatures, which may eliminate species (Nebeker and Lemke 1968). An increase in temperature also affects the size and fecundity of emerging adult insects, which impacts on reproduction and survival (Sweeney and Vannote 1986, Brittain and Campbell 1991, Quinn *et al.* 1994, Dallas and Day 2004).

Certain species are more sensitive to temperature changes than others (Quinn *et al.* 1994). For example Gondwanan species, which are usually restricted to cool upper reaches of mountain streams (Picker and Samways 1996, Day 2005), may be more sensitive to increases in temperature than non-Gondwanan fauna which could be eurythermic (adapted to a wide range of temperatures). The Gondwanan fauna would thus be predicted to have more conservative thermal tolerances, given that past climates were cooler, and that they seem to have been conservative in their thermal adaptation (Day 2005). For example Ernst *et al.* (1984) found that cold-adapted stoneflies were more susceptible to high temperatures than eurythermic stoneflies. On the contrary, McKie *et al.* (2004) compared the thermal tolerance of two Gondwanan chironomids species found in tropical and temperate areas and noted that they had broad temperature responses. Even though the species were the same and Gondwanan in origin, the individuals from the tropical area were more tolerant than those found in the temperate area (McKie *et al.* 2004).

LT_{50} experiments

There are a number of ways the effects of temperature change on aquatic organisms can be assessed. These include experiments on lethal limits (minimum and maximum), sublethal effects and behaviour avoidance preferences (Dallas and Day 2004, Dallas 2008). Lethal effect experiments are acute (short-term) experiments that involve exposure to a range of temperatures in order to measure the LT_{50} value (the temperature at which 50% of the test individuals die). Sublethal effects involve identifying the effects on growth, reproduction, survival, egg hatchability, etc., while behaviour avoidance preferences are used to determine the temperatures preferred or avoided by organisms.

The standard 96 hour LC_{50} (median lethal concentration – concentration at which half the test population dies) test for measuring toxicity of substances was developed by Hickey and Vickers (1992). Quinn *et al.* (1994) applied this method to determine and compare upper thermal tolerances (viz. $_{96}LT_{50}$) which provided information on the relative temperature tolerances of species. A stonefly and two mayflies were the most sensitive in their study of twelve New Zealand freshwater taxa (Quinn *et al.* 1994).

The effects of temperature on survivorship may thus be assessed using LT_{50} experiments run for a specific time period, typically 96 hours. Quinn *et al.* (1994) found that the LT_{50} experiments can be used to measure the thermal tolerance of a wide range of aquatic invertebrates (stoneflies, mayflies, beetle larvae, molluscs and crustaceans). These LT_{50} experiments can be conducted using inexpensive equipment, are easy to carry out and provide a standardised and repeatable methodology.

This study aimed to 1) investigate the use of LT_{50} experiments to measure the thermal tolerance of aquatic insect nymphs from a range of taxonomic groups (Plecoptera, Ephemeroptera and Trichoptera) 2) provide information on the tolerances of these common taxa found in streams in the CFR and 3) compare the thermal tolerance of Plecoptera nymphs from two different bioregions (Rutherford *et al.* 2006) in the Western Cape Province of South Africa, viz. Table Mountain (southwest fynbos bioregion) and Tsitsikamma (south coast fynbos bioregion) to test for any local adaptation of the same group.

2.2 Methods

Study sites

The streams selected for the purposes of this study were all located in the Cape Floristic Region (CFR) of South Africa. Nymphs were collected from Window Gorge (33°59’09”S, 18°26’01”E) and Skeleton Gorge (33°59’09”S, 18°25’45”E) streams, within Kirstenbosch National Botanical Garden (KNBG), located on the eastern slopes of Table Mountain, between April 2008 and July 2008. Both these streams originate on top of Table Mountain and flow through the Table Mountain National Park and KNBG and are typical CFR mountain streams in pristine condition. The streams flow over Table Mountain Sandstone at the top sites and over granite lower down. The main land use around the streams is the botanical gardens and hiking trails.

The Salt River (33°56’02”S, 23°29’29”E) is located in the Tsitsikamma area near Plettenberg Bay, about 600km east of Cape Town and flows through Kurland Polo Estate where it is in relatively good condition except for some alien riparian vegetation. The stream originates in the foothills of the Outeniqua mountains. Stonefly nymphs were collected from the Salt River in May 2009 and subsequently transported back to the Department of Zoology at the University of Cape Town where experiments were conducted after two days stabilisation.

Collection of nymphs

Cobbles were randomly selected from riffles and pools in each stream, placed in buckets and brushed gently to remove nymphs (care being taken not to harm the organisms). The nymphs were transported in stream water back to the laboratory, where the nymphs of different species were sorted while still alive. An additional twenty litres of stream water were collected and taken back to the laboratory for use in the experiments.

Individuals of the same species were placed in separate plastic containers. The three species that were most numerous in Window Gorge Stream were selected, namely *Castanophlebia* sp., Leptophlebiidae; *Lestagella penicillata,* Teloganodidae; and *Athripsodes* sp., Leptoceridae. Stonefly nymphs were collected from Skeleton Gorge Stream (*Aphanicerca capensis* (form C – Stevens 2009), Notonemouridae) and the Salt River (*A. capensis* (undescribed form), Notonemouridae). *A. capensis* has recently been shown to comprise a species complex (Stevens 2009). All the species tested were Gondwanan except for the caddisfly, *Athripsodes* sp. (See Appendix 1 for species description.)

Early to middle instar nymphs were used, as developmental stage is known to influences thermal tolerance (Bowler and Terblanche 2008). Containers with different species were placed in constant temperature rooms which had been set at the approximate stream temperature at the time of collection, with a 12 hour light/dark cycle. Individual containers were aerated using aquarium tubing and air-stones. Prior to experimentation, the nymphs were kept under the above-mentioned conditions for a minimum of 24 hours to ensure sufficient acclimation to the control temperature. Earlier trials in this study had shown that the nymphs could survive for several weeks without food, so the nymphs were not fed during acclimation or the four-day experiments.

LT_{50} experiments

Ten individuals of each species were placed in 500ml plastic tubs filled with stream water, with three or four replicates, depending on the species, giving a total of 30-40 individuals per species. Starting at the acclimation temperature, the temperature of the constant temperature room was gradually increased by 2-4ºC/hour until the appropriate target temperature was reached. By doing this, thermal shock and acclimation were avoided (Quinn *et al.* 1994).

The temperature of the room remained fairly constant, while the water temperature varied. The higher the air temperature the greater the difference was between the air and water temperatures. The water temperature in each tub varied due to water level and air flow. The different temperatures were chosen such as to attain a range of mortalities from 0 to 100%. A control temperature (the acclimation temperature) was included to test potential mortality factors such as influences from tubs, air, water or a lack of food.

The water temperature in each tub was measured with a mercury thermometer every day and averaged for all the tubs for each particular species (Table 2.1). Tubs were checked for survival every 24 hours for four days (96 hours) subsequent to the target temperature being reached. Nymphs that displayed no movement were either gently prodded or squirted with water from a pipette to check for survival. Dead individuals were removed and preserved in ethanol and their identities confirmed.

Table 2.1. Average water temperatures used to determine thermal tolerances of aquatic insect nymph species.

Constant Temperature	*Aphanicerca capensis* (form C) **(°C)**	*Aphanicerca capensis* (undescribed form) **(°C)**	*Castanophlebia* sp. **(°C)**	*Lestagella penicillata* **(°C)**	*Athripsodes* sp. **(°C)**
Control	13	14	14	14	14
1	17	19	25	25	25
2	24	23	27	27	27
3	25	25	29	29	29
4	28	29	32	32	32
5	-	-	-	33	33

Water temperature data

Water temperature data were collected from Window Gorge Stream using HOBO® Pendant temperature data loggers (accuracy 0.47°C at 25°C). These temperature data were used as a reference for nearby Skeleton Gorge Stream (approximately 300-600m from Window Gorge Stream) to compare to the Salt River temperatures for the stonefly comparison. The temperatures from Window Gorge Stream were from October 2008 to August 2009 from site 3 (chapter 4) as this was one of two sites that had data up until August. The water temperatures from the Salt River were from January 2008 to December 2008 (excluding September). The temperatures were thus each recorded for nearly a whole year at each location. The average, maximum and minimum temperatures were calculated. The average temperature for the months that the nymphs were collected in was calculated to ascertain the natural conditions the nymphs were likely to experience. The majority of the nymphs from Skeleton Gorge and Window Gorge were collected between May and June 2008, and therefore temperature logger data from May and June 2009 were used as a reference. Similarly for the Salt River the average May temperature in 2008 was used as a reference for the stonefly nymphs collected in May 2009.

Statistical analysis

The LT_{50} values and 95% confidence intervals were calculated for each time period (every 24 hours) by probit analysis (Finney 1971 cited in Quinn *et al.* 1994) in StatsDirect. Probit analysis is used for fitting probit sigmoid dose-response curves and for calculating confidence intervals for dose-response quantiles such as LT_{50}. Mortality was plotted against the range of water temperatures which usually formed a sigmoid curve. From this curve the temperature at which half of the test individuals died was calculated. Trimmed Spearman-Karber analysis was used to estimate the LT_{50} values in cases where the data did not fit the probit model (Hamilton *et al.* 1977).

2.3 Results

Thermal tolerances

Control mortality was zero for all species, except for *A. capensis* (form C) where 2.5% mortality occurred. No antagonistic behaviour was detected within tubs for any of the species. The controls therefore confirmed that the operational system employed was not a factor in death. Any deaths can thus be attributed to temperature change.

The LT_{50} values calculated for the four species tested (Table 2.2) showed that all four species differed in their upper thermal tolerances. The 96h LT_{50} values ranged from 23.4°C to 29.9°C (Table 2.2). *A. capensis* (form C) showed the lowest LT_{50} value of 23.4°C, followed by *A. capensis* (undescribed form) with an LT_{50} value of 25.0°C and *Castanophlebia* sp. with 26.5°C, while *L. penicillata* and *Athripsodes* sp. had similar LT_{50} values (29.5°C and 29.9°C respectively).

The upper thermal tolerances found for the species fall within the range of tolerances reported elsewhere in the world for Plecoptera, Ephemeroptera and Trichoptera (Table 2.2). There is, however, considerable variation between species and across families within an order. The majority of the stonefly species are more tolerant than both forms of *A. capensis*. For the family Leptophlebiidae there were four different species tested for their thermal tolerance and each species differed. *Castanophlebia* sp. from this study was the most tolerant of the four species.

Table 2.2. A comparison between the upper thermal tolerances found in this study (in bold) and studies elsewhere in the world.

Order	Family	Species	LT_{50} (°C)	Location	Reference
Plecoptera	**Notonemouridae**	***Aphanicerca capensis*** **(form C)**	**23.4**	**South Africa**	**This study**
	Notonemouridae	***Aphanicerca capensis*** **(undescribed form)**	**25**	**South Africa**	**This study**
	Perlidae	*Acroneuria californica*	29	North America	Heiman & Knight 1975
	Perlidae	*Acroneuria lycoria*	30	North America	Nebeker & Lemke 1968
	Perlidae	*Paragnetina media*	30.5	North America	Nebeker & Lemke 1968
	Capniidae	*Allocapnia granulate*	23	North America	Nebeker & Lemke 1968
	Perlodidae	*Isogenus fontalis*	22.5	North America	Nebeker & Lemke 1968
	Pteronarcyidae	*Pteronarcys dorsata*	29.5	North America	Nebeker & Lemke 1968
	Taeniopterygidae	*Taeniopteryx maura*	21	North America	Nebeker & Lemke 1968
	Gripopterygidae	*Zelandobius furcillatus*	26.5	New Zealand	Quinn *et al.* 1994
Ephemeroptera	**Teloganodidae**	***Lestagella penicillata***	**29.5**	**South Africa**	**This study**
	Leptophlebiidae	***Castanophlebia*** **sp.**	**26.5**	**South Africa**	**This study**
	Leptophlebiidae	*Deleatidium* sp.	22.6	New Zealand	Quinn *et al.* 1994
	Leptophlebiidae	*Sephlebia denata*	23.6	New Zealand	Quinn *et al.* 1994
	Leptophlebiidae	*Deleatidium autumnale*	24.2	New Zealand	Cox & Rutherford 2000a
	Ephemerellidae	*Ephemerella subvaria*	22.9	North America	Nebeker & Lemke 1968
	Heptageniidae	*Stenonema tripunctatum*	25.5	North America	Nebeker & Lemke 1968
Trichoptera	**Leptoceridae**	***Athripsodes*** **sp.**	**29.9**	**South Africa**	**This study**
	Brachycentridae	*Brachycentrus americanus*	29	North America	Nebeker & Lemke 1968
	Hydropsychidae	*Aoteapsyche colonica*	25.9	New Zealand	Quinn *et al.* 1994
	Conoesucidae	*Pycnocentrodea aureola*	32.4	New Zealand	Quinn *et al.* 1994
	Conoesucidae	*Pycnocentria eveta*	25	New Zealand	Quinn *et al.* 1994

The LT_{50} values decreased with exposure time for each of the species (Fig. 2.1) indicating that the longer a species is exposed to higher temperatures, the lower the temperature will need to be for 50% of the individuals to die.

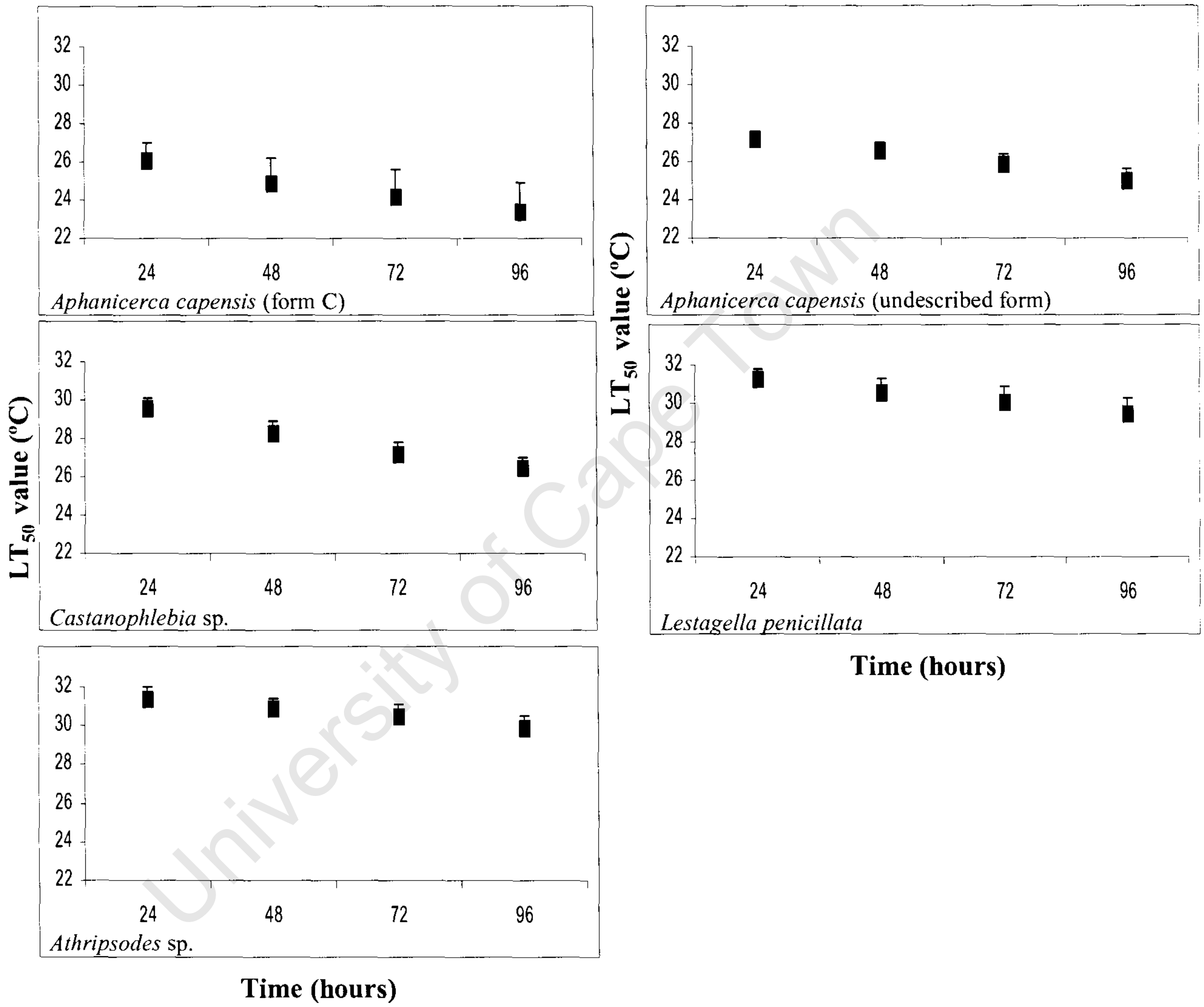

Figure 2.1. The LT_{50} values (°C) and standard deviation for the aquatic insect nymph species after each 24 hours of the 96 hour experiments.

Overall, the mean percentage survival of each species decreased with increasing temperature (Fig. 2.2). As the temperature increased, the individuals became more sensitive to temperature.

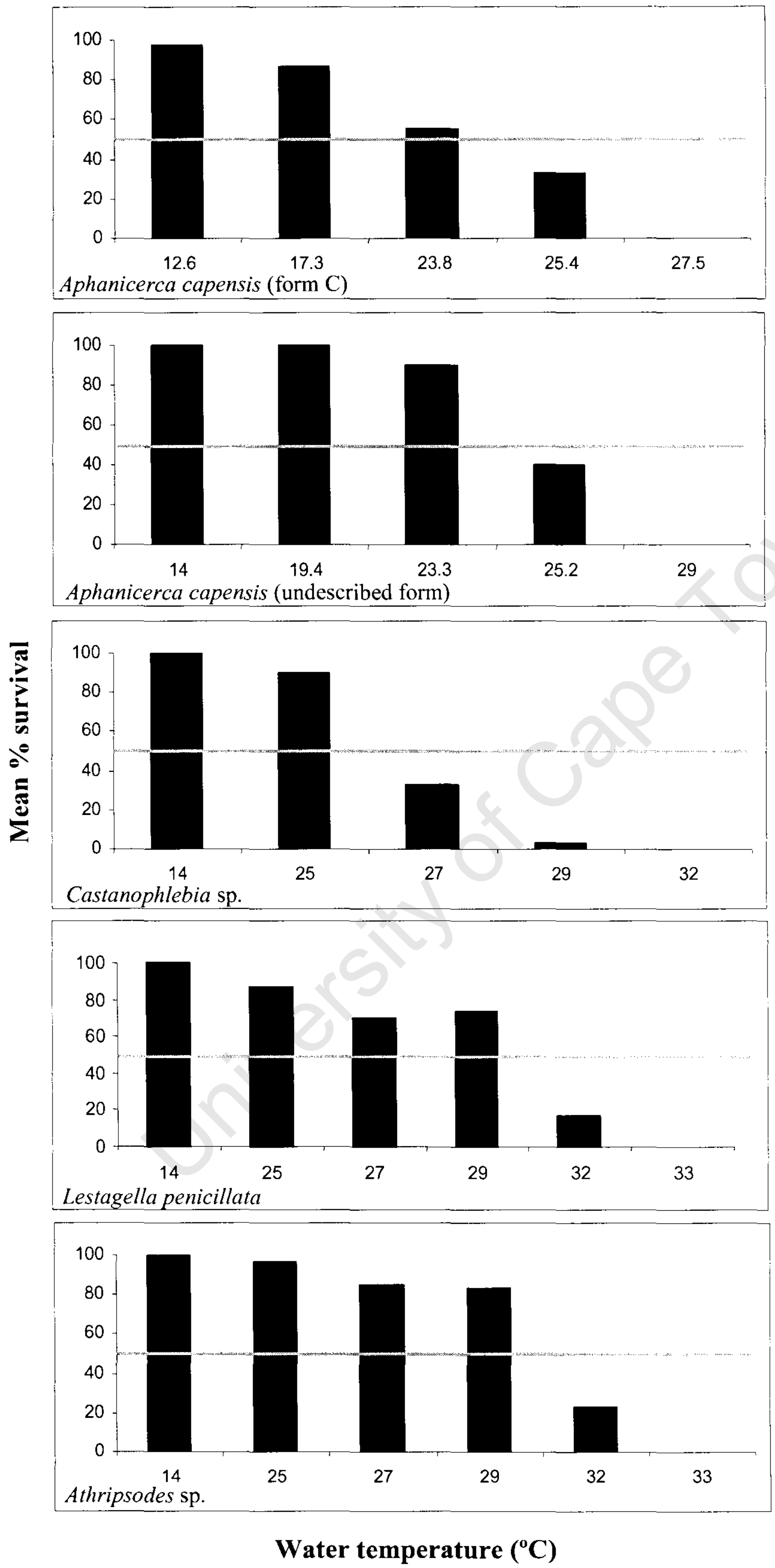

Figure 2.2. Mean percentage survival for the aquatic insect nymph species at different temperatures after 96 hours of the LT_{50} experiments. Grey line indicates the 50% survival.

Table Mountain vs. Tsitsikamma

The LT_{50} value of 23.4°C for *A. capensis* (form C) showed that they were more sensitive than *A. capensis* (undescribed form) with an LT_{50} value of 25°C. The temperature logger readings for Window Gorge Stream were used as a reference for very nearby Skeleton Gorge Stream (separated by approximately 300-600m). The temperature readings for Window Gorge Stream and the Salt River were similar. The average stream temperature for Window Gorge was 14.6°C and was 15.2°C for the Salt River. The maximum and minimum for Window Gorge Stream were 22.7°C and 7.7°C, while for the Salt River it was 23.0°C and 8.0°C respectively.

The average stream temperature around the time of collection of the nymphs was 13.4°C (May) and 11.6°C (June) for Window Gorge Stream, while the average May temperature for the Salt River was 14.1°C. These stream temperatures are somewhat below the thermal tolerances of the species tested.

2.4 Discussion

Thermal tolerances

A range of upper temperature limits was found for the species tested in these experiments (Table 2.2, Fig. 2.1). *Athripsodes* sp. (Trichoptera) appeared to be the most tolerant, showing the highest upper thermal tolerance of 29.9°C. The notonemourid stonefly *A. capensis* (form C) was most sensitive to an increase in temperature, with an upper temperature tolerance of 23.4°C and may therefore be the most restricted in its habitat requirements. *A. capensis* species complex is restricted to the cool upper parts of mountain streams.

Different families and species also have different thermal tolerances, as shown by the results of the LT_{50} experiments in this study, and those obtained by Quinn *et al.* (1994) (Table 2.2). Capniidae (*Allocapnia granulate*) which is in the same infraorder (Nemouromorpha) as Notonemouridae (Zwick 2000) also have a low temperature tolerance (LT_{50} value = 23°C) (Nebeker and Lemke 1968). Some of the other stoneflies tested elsewhere have higher temperature tolerances than the ones found for both forms of *A. capensis* (Table 2.2). This could partly be explained by voltinism – the number of generations per year for a particular species. Some stoneflies are univoltine (one generation per year), while others are bi- or trivoltine (two to three generations per year) (Fochetti and Tierno de Figueroa 2008). Multivoltine species would have to endure summer as well as winter temperatures. *A. capensis* species complex is univoltine and develops during the cold, wet winter months (Picker pers. comm.) and thus is likely to have a low tolerance of high temperatures.

As temperatures were increased during experiments, individuals became more sensitive to temperature (Fig. 2.2). The greater the rise in water temperature above the LT_{50} values, the greater the chance of the individuals dying. Increasing stream temperatures as a result of climate change, the removal of riparian vegetation or water abstraction, etc. could potentially impact on the development of nymphs and emergence patterns. For example, sublethal elevated temperatures are known to result in more rapid development and earlier emergence, placing adults at risk of being killed by the unexpected cold winter air temperatures in temperate regions (Nebeker and Lemke 1968).

Seasonal and environmental conditions should also be considered when interpreting the results of thermal sensitivity experiments (Nebeker and Lemke 1968). The nymphs used in this study were present nearly all year round with different instars occurring at different times of year except during the summer months when many of the nymphs were not found. It is thus important to use the same larval stage in all experiments, unless testing for tolerance of different stages. In a pilot study observations indicated that early instars of *A. capensis* (form C) may be more tolerant of higher temperatures than the later instars, as mature nymphs (with black wing pads) died rapidly with even the slightest increase in temperature.

Table Mountain vs. Tsitsikamma notonemourid stoneflies

Stoneflies were found to be the taxon most sensitive to increasing temperature. This was observed for both *A. capensis* (form C) and *A. capensis* (undescribed form). The LT_{50} values were similar for both, with the Salt River population being slightly less sensitive than the Table Mountain population of *A. capensis* (form C).

In Skeleton Gorge and Salt River the two *A. capensis* forms are restricted to upper reaches of the mountain streams. The average water temperatures were similar for

Window Gorge Stream and the Salt River. Window Gorge falls within the southwest bioregion which experiences winter rainfall, while the Salt River is in the south coast bioregion where there is rainfall all year round (Rutherford *et al.* 2006).

Different forms of *A. capensis* have been noted by Stevens and Picker (2003), with most of these considered to be separate biological species, or diverging populations of *A. capensis* and therefore the difference may lie in genetic aspects. *A. capensis* found on Table Mountain was described as form C by Stevens 2009. Stevens did not collect samples from the Salt River and therefore *A. capensis* found there may be another undescribed form. The COI gene was not efficient in separating the forms but morphology and mate choice were used to determine the different forms (Stevens 2009). The two forms could potentially be separate biological species and thus this and the geographic variation can explain the difference in their temperature tolerances. It has been suggested by Sweeney *et al.* (1992) that genetic variation may be a solution to environmental changes, viz. adaptive responses to climate change.

Thermal tolerance information

The results of this study provided information on upper temperature tolerances of some common aquatic invertebrates found in streams on Table Mountain and the Salt River. The species/families most sensitive to higher temperatures could be used as indicators of thermal pollution. For the species tested in this study, notonemourid stoneflies would be an appropriate taxon as they appear to be thermally conservative. Through continuous monitoring of them it may be found that over the years, as temperatures are expected to increase, their abundance and distributions may change.

Natural exposure period is likely to be far longer than the relatively acute (96h) time period used in these experiments. The results indicated that mortality increases with time at a set temperature (above the optimal), suggesting that the species tested might be far more sensitive to thermal stress than the acute trials indicated. Since thermal pollution is more likely to be subtle, even small increases in water temperature may have profound effects on survival. This should be evaluated in far longer tolerance (LT_{50}) trials and in the assessment of sublethal effects.

The equipment used in these experiments provides quite inexpensive, simple methodology for testing thermal tolerances of a variety of aquatic invertebrates. For longer term experiments food may have to be provided as not to add stress to the organisms.

Application of thermal tolerance information

Streams fluctuate in temperature diurnally and therefore it may be helpful if further studies were to take this into account. Cox and Rutherford (2000b) found a significant difference between the LT_{50} values estimated from experiments using constant and diurnally fluctuating temperatures, concluding that the LT_{50} value should be applied to a "temperature midway between the daily mean and daily maximum temperature".

From temperature logger data in Window Gorge Stream the average temperature was 14.6°C and the highest approximate water temperature was 22.7°C. The temperature midway between these values is 18.7°C. This temperature is lower than the LT_{50} value for *A. capensis* (form C) (23.4°C) which means that the stream temperatures are adequate for *A. capensis* (form C). The maximum temperature is near the LT_{50} value so there could be periods that the stoneflies cannot survive, but this is during the summer months when most stoneflies nymphs have emerged. From community composition data collected from August 2008 and May 2009 (Chapter 4), far fewer *A. capensis* (form C) nymphs were collected in the summer months (October to April) than during the winter months (May and August). The average winter temperature and maximum temperature are much lower than the overall average and maximum temperatures (12.1°C and 17.4°C respectively).

The predicted increase of approximately 1.5°C through climate change for the Western Cape Province (Midgley *et al.* 2005), would give a projected annual average stream temperature of 16.1°C for Window Gorge in summer. This is still somewhat lower than the LT_{50} value for *A. capensis* (form C). Given the various sublethal effects of thermal stress, and the probable lower LT_{50} value that would be obtained over a longer exposure period, *A. capensis* (form C) (and possibly other notonemourid stoneflies) could still potentially be at risk of thermal pollution from climate change.

Thermal tolerance information can be used in constructing management plans for streams and rivers with regards to water temperature. The removal of riparian vegetation, water abstraction and climate change could all increase water temperature which could affect important temperature-sensitive aquatic invertebrates. This is especially true if they act synergistically. Thermal tolerance data for sensitive aquatic invertebrates would therefore be useful in understanding which species are important to monitor and how they would be affected by thermal pollution. Management plans should thus include water temperature and aquatic invertebrate monitoring.

In future studies more species from the same orders and families should be tested to see if thermal tolerance is species specific or whether it can be generalized at family/order level. Additionally of particular interest, in the Western Cape, would be a comparison between thermal tolerances of non-Gondwanan and Gondwanan relict species to identify whether Gondwanan fauna are more sensitive to increases in temperature.

Conclusions

A. capensis (form C) was found to be the most thermally sensitive species and *Athripsodes* sp. the most tolerant in the LT_{50} experiments. *A. capensis* (form C) is only found in the cool parts of upper mountain streams and may therefore be restricted in its habitat requirements. The LT_{50} value for *A. capensis* (form C) was similar but lower than that for *A. capensis* (undefined form). The two forms could potentially be two different species and together with the geographic variation could explain the difference in the LT_{50} values. Thermally sensitive species (e.g. *A. capensis* (form C) in this study) could potentially be used as indicators of increases in stream temperature. Monitoring of stream temperatures and thermally sensitive species is required to form a baseline against which the changes resulting from global warming can be compared.

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Chapter 3: Identifying thermally sensitive species from upper reach mountain streams in the Western Cape Province using Critical Thermal Maxima (CTM) trials

Temperature is an important physical variable influencing all aquatic organisms. Some aquatic insects such as the relictual Gondwanan fauna are restricted to cool streams occurring mostly in high-lying, upper mountain or torrent sections of some Southern Hemisphere rivers. As a result of their habitat specificity, these species may be more sensitive to abrupt, unexpected or rapid increases in water temperature than other species occupying rivers that are subject to greater temperature fluctuations. Critical Thermal Maxima (CTM) experiments investigate the sensitivity of organisms to increasing temperatures using behavioural endpoints. The CTM value is the mean endpoint at which an organism loses the ability to retain locomotory function. No data exist for CTM values of aquatic insect nymphs in the Southern Hemisphere. In this study, six species recognised as Gondwanan/relictual and two non-Gondwanan species were tested using CTM experiments. The recommended rate of temperature increase (0.34°C/minute) was used. Two stonefly species (*Aphanicerca capensis* (form C and undescribed form) and *Aphanicercopsis tabularis,* Notonemouridae) were the most sensitive species, and *Barbarochthon brunneum*, Barbarochthonidae was the most tolerant with a mean CTM value of 37.12°C. The Gondwanan mayflies (*Castanophlebia* sp., Leptophlebiidae and *Lestagella penicillata*, Teloganodidae) were more thermally sensitive than the non-Gondwanan mayfly (*Acanthiops* sp., Baetidae) tested. Each species displayed different behavioural responses with increasing temperatures. The Gondwanan caddisfly, *B. brunneum* was, however, more tolerant than the non-Gondwanan caddisfly, *Athripsodes* sp., Leptoceridae. The hypothesis that Gondwanan species are more sensitive than non-Gondwanan species therefore may not hold true. These CTM experiments provided the first data from the Southern Hemisphere on the sensitivity of aquatic insect species to constant increases in temperature.

Key words: temperature tolerance, critical thermal maxima, aquatic insect nymphs, climate change, Gondwanan insects

3.1 Introduction

Migration, reproduction, emergence and growth of most aquatic organisms are influenced by temperature (Brittain 1983, Sweeney and Vannote 1986, Quinn *et al.* 1994, Cox and Rutherford 2000, Dallas and Day 2004). Anthropogenic influences such as global warming have the potential to upset this equilibrium, potentially bringing about disastrous consequences for those organisms, in particular, with narrow temperature tolerances.

Within optimal temperature ranges, which are usually related to geographic distribution, an organism's growth rate will increase as temperatures increase (Perry *et al.* 1987). Any unnatural increase in temperature, i.e. falling outside the optimum species tolerance range, may limit the distribution of some species to certain areas and will likely reduce species diversity in areas where temperature regimes shifts (Hawkins *et al.* 1997, Cox and Rutherford 2000). Some organisms have become adapted to survive temperature conditions outside their optimal range, which they accomplish by employing morphological, behavioural, ecological, physiological and biochemical strategies (Lencioni 2004). However, this phenotypic plasticity is likely to be expressed in varying degrees in different species.

Gondwanan fauna

The Gondwanan fauna are a relictual fauna which represent survivors of ancient lineages and are restricted to the upper reaches of streams and rivers where temperature conditions have remained stable for millions of years (Stuckenberg 1962). By definition, the southern Gondwanan taxa are currently restricted to the southern parts of a few southern continents, such as New Zealand, South America, Madagascar and South Africa. For some of these species, their current distribution could be argued to be relictual (as opposed to the strict interpretation of the Gondwanan taxa whose distribution is believed to be caused by events associated with continental drift). Streams in the Cape Fold Mountains of the Western Cape of South Africa contain several of these Gondwanan taxa, which also occur in the Eastern Highlands of South Africa (Stuckenberg 1962). These aquatic 'palaeorelicts' are adapted to cool, acid to neutral waters with low conductivities and occur mostly in the high-lying, upper mountain streams or torrent sections of rivers (Day 2005). There is a large number of Gondwanan species found in South Africa and Day (2005) studied the distribution of these palaeorelictual invertebrates which exhibits a "strong westerly bias". Some Gondwanan insect families that are important in freshwater systems include Leptophlebiidae, Teloganodidae, Notonemouridae, Barbarochthonidae, Hydrosalpingidae, Petrothrincidae, Corydalidae and Synlestidae.

These ancient groups are of Gondwanan or Pangaean origin and have been isolated for between 65 and 150 million years (Day 2005). Their low genetic variability may limit these organisms' ability to respond to relatively rapid environmental fluctuations (Wishart *et al.* 2003). This in turn has wide-ranging, serious implications for almost any change in environmental conditions such as changes in temperature relating to climate change. A decrease in species richness, as found by Durance and Ormerod (2007), with an increase in stream temperature, has particularly important implications for the cooler-water species (e.g. Gondwanan fauna) which are limited to higher altitude streams or streams running through forests.

South Africa lay approximately in the centre of Gondwanaland where a far more temperate climate existed than today (Day 2005). The climate of South Africa has been slowly warming since the break-up of Gondwanaland (Stuckenberg 1962, Day 2005). The Gondwanan fauna appear not to have changed their thermal tolerances, judging by their inability to invade warmer water systems in South Africa. These species may therefore be conservative in terms of their thermal tolerance and therefore may be more sensitive to increases in temperature than non-Gondwanan species. In Australia, however, Gondwanan chironomid species are found in both temperate and tropical areas and the species found in the tropical area are more tolerant of increases in temperature than the same species in the temperate area (McKie *et al.* 2004).

Critical Thermal Maxima

If an organism does not undergo diapause it needs to remain active even under extreme conditions in order to survive. Therefore to understand how environmental fluctuations will affect the dynamics of a population the limits to activity need to be determined (Terblanche *et al.* 2007). In the case of stream organisms, the ability to remain attached to the substrate is important to their survival. Critical Thermal

Maxima (CTM) experiments have been described as a good method for determining the thermal requirements of different species (Lutterschmidt and Hutchison 1997).

CTM experiments require the temperature to be increased at constant rate from an acclimation temperature, until an end point is reached where the organisms lose their locomotory functions which will ultimately lead to its death (Ernst *et al.* 1984). The CTM endpoint is sublethal and may include a loss of equilibrium and the start of muscle spasms (Beitinger *et al.* 2000). The CTM of a species is thus the arithmetic mean of the end point temperatures recorded for that specific species (Ernst *et al.* 1984).

The CTM method shows some advantages over LT_{50} experiments and other lethal methods, in that organisms mostly survive once they are returned to pre-test temperatures. This means these experiments can be conducted on endangered species. The rate of temperature change is, however, very important. The rate of increase needs to be fast enough to avoid acclimation, but slow enough to ensure that the core temperature of the test animal approximates the ambient temperature (Ernst *et al.* 1984). A rate of 0.3°C per minute was recommended for fish (Becker and Genoway 1979, Beitinger *et al.* 2000) while Ernst *et al.* (1984) used a rate of 0.34°C per minute for Plecoptera nymphs.

Determining the endpoint is quite difficult, as for most species the point of locomotory disorganization has not been defined (Ernst *et al.* 1984). It is likely that different taxa have different responses to increases in temperature and will therefore have different endpoints (Becker and Genoway 1979). Even though it may be difficult to determine the endpoint, animals do display a number of responses to thermal stress and these responses are usually the same for all individuals of a species (Ernst *et al.* 1984). As an example, Ernst *et al.* (1984) noted that when stonefly nymphs lost their grasp of a meshwork, they could not reattach. They used this as an indication of the endpoint as it might be indicating loss of muscular coordination. This behaviour may be used to avoid rising temperatures, as in streams it would allow nymphs to detach and drift downstream where temperatures might be cooler (Ernst *et al.* 1984).

Several studies in the Northern Hemisphere have documented CTMs for aquatic invertebrates. Ernst *et al.* (1984) recorded mean CTM values of 31.5°C to 36.3°C for Plecoptera nymphs in Delaware County, Oklahoma and Moulton *et al.* (1993) recorded mean CTM values of 31.4°C to 38.5°C for Trichoptera nymphs from Missouri and Texas. No studies have yet been done on Southern Hemisphere taxa using traditional CTM methods. This study therefore aims to 1) use CTM experiments to measure the thermal tolerance of aquatic insect nymphs from a range of taxonomic groups (Plecoptera, Ephemeroptera and Trichoptera) occurring in mountain streams of the Cape Floristic Region (CFR); 2) contrast CTM values for Gondwanan and non-Gondwanan species to provide further information on the thermal tolerances of these common taxa found in CFR streams; and 3) compare the results obtained from the LT_{50} experiments and CTM experiments on the same taxa.

3.2 Methods

Study sites

The streams selected for this study were located in the CFR of South Africa. Aquatic insect nymphs were collected from Window Gorge (33°59'09"S, 18°26'01"E) and Skeleton Gorge (33°59'09"S, 18°25'45"E) streams, within Kirstenbosch National Botanical Garden (KNBG) between April 2008 and July 2008. Both these streams originate on top of Table Mountain and flow through the Table Mountain National Park and KNBG and are typical CFR mountain streams in pristine condition.

Additional nymphs were collected from the Salt and Keur Rivers in May 2009, representing a second broad locality in the CFR of the Western Cape Province. These nymphs were compared to those tested from Table Mountain. The Salt River (33°56'02"S, 23°29'29"E) is located in the Tsitsikamma area near Plettenberg Bay about 600km east of Cape Town and flows through Kurland Polo Estate where it is in relatively good condition except for some alien riparian vegetation. The Salt River originates in the foothills of the Outeniqua mountains. The Keur River (33°54'25"S, 22°25'04"E) is north-west of George, about 450km east of Cape Town, and also originates in the Outeniqua Mountains. The site sampled along the Keur River is in a fairly pristine condition near the old Montague Pass road. The nymphs were transported directly back to the laboratory where they were kept at 16°C prior to testing.

Sampling

The collection of nymphs from the stream and the sorting of them in the laboratory were conducted in the same way as in chapter 2. Oxygen saturation was measured using the YSI 550A dissolved oxygen meter (accuracy ± 2%) at the time the nymphs were collected. Different species were collected from the four streams/rivers (Table 3.1). The three species that were most numerous in number from Window Gorge Stream were selected namely *Castanophlebia* sp., Leptophlebiidae; *Lestagella penicillata*, Teloganodidae; and *Athripsodes* sp. Leptoceridae. Stonefly nymphs were collected from Skeleton Gorge and Window Gorge Stream, (*Aphanicerca capensis* (form C – Stevens 2009), Notonemouridae and *Aphanicercopsis tabularis*, Notonemouridae) and the Salt River (*A. capensis* (undescribed form), Notonemouridae). Other nymphs were collected from the Salt River (*Acanthiops* sp., Baetidae) and from the Keur River (*Barbarochthon brunneum*, Barbarochthonidae). (See appendix 1 for species description.)

Early to middle instars were used in experiments in order to keep the age of the nymphs relatively constant, additionally, these instars were the most numerous at the time of sampling. Containers holding each of the different species were placed into a constant temperature room, set at a temperature near stream temperature (16 °C) and with a 12 hour light/dark cycle. Each individual container was aerated using aquarium tubing and air-stones. The nymphs were kept under the above-mentioned conditions for a minimum of 24 hours to ensure acclimation to the control temperature. Earlier trials, in this study, had shown that the nymphs could survive for several weeks without feeding, so they were not fed during acclimation or the experiments.

Table 3.1. Aquatic insect nymph species collected from Western Cape streams for Critical Thermal Maxima experiments.

Order	**Family**	**Species**	**Gondwanan (from Day 2005)**	**Stream/River**
Plecoptera	Notonemouridae	*Aphanicerca capensis* (undescribed form)	Gondwanan	Salt River
		Aphanicerca capensis (form C)	Gondwanan	Window & Skeleton Gorge
		Aphanicercopsis tabularis	Gondwanan	Window & Skeleton Gorge
Ephemeroptera	Leptophlebiidae	*Castanophlebia* sp.	Gondwanan	Window Gorge
	Teloganodidae	*Lestagella penicillata*	Gondwanan	Window Gorge
	Baetidae	*Acanthiops* sp.	Non-Gondwanan	Salt River
Trichoptera	Barbarochthonidae	*Barbarochthon brunneum*	Gondwanan	Keur River
	Leptoceridae	*Athripsodes* sp.	Non-Gondwanan	Window Gorge

CTM experiments

A Julabo MC Heating immersion circulator was used for the CTM experiments. This heating immersion circulator was attached to one side of a water bath to heat and circulate the water, ensuring a constant temperature was maintained throughout the water bath. Increases in temperature were set at the suggested rate of 0.34°C/minute (Ernst *et al.* 1984). The water bath was kept together with the organisms in the same constant temperature room set at 16°C. During the experiment nymphs were transferred to four 500ml plastic sampling containers (in the water) that had walls of

1mm mesh to allow water to flow freely through the container. The mesh also allowed the nymphs to cling to sides of the container and move around. The floor of the container was sanded so that the nymphs could attach to the floor too.

Each experiment involved the testing of ten individuals at a time (two to three nymphs per container). All experiments were repeated three times (thirty individuals in total) for each species. Terblanche *et al.* (2007) noted that the organisms should be held at constant temperature before heating to allow for the body temperature to approximate the ambient temperature. The control water temperature in the water bath itself was heated from 17°C to 17.34°C over a 30 minute period. During trials, the behaviour of each individual was noted every minute (for example 'sitting' (stationary), walking and swimming). The oxygen saturation was measured with the YSI 550A oxygen meter (accuracy ± 2%) for the duration of the control and the experiment and recordings taken every ten minutes.

Both the start temperature and the rate of increase were kept constant (Terblanche *et al.* 2007, Chown *et al.* 2009). Individuals were removed when the behavioural end points were observed. For the stoneflies and mayflies the end point was when the individual was floating and immobile for five seconds, and for caddisflies the end point was when their heads were tucked into their cases for more than five to ten seconds when squirted with water. The water temperature at which the end point was observed was recorded as the critical temperature. The organisms were then removed and placed in stream water at 16°C and allowed to recover. Data used in the analyses were taken only from organisms that survived the experiment.

The temperatures recorded as the critical temperature for each individual had to be adjusted to account for variation within the containers. There was a time lag between the temperature given by the Julabo MC Heating immersion circulator instrument and the temperature recorded in the containers (measured with Hobo TidbiT® v2 loggers). The rate of increase in temperature (0.34°C/minute) was constant between the instrument and the loggers ($r^2 = 0.9994$). The equation determined from the different temperature readings from the heating immersion circulator and loggers was: $y = 0.9212x + 0.9358$. This equation was used to adjust the recorded end point temperatures for each individual.

Statistical analysis

The mean critical thermal maximum as well as the standard error was calculated for each of the species to indicate the uncertainty around the estimate of the mean measurement. Thirty individuals were used for each species to ensure sufficient replication. One-way Analysis of Variance (ANOVA) in STATISTICA 8 (2009) was used to compare end point temperatures for the eight aquatic insect nymph species as the data were normally distributed. A post-hoc Tukey HSD test was used to determine any significant differences between the CTM's of the various species. A non-parametric Kendall Tau Correlation (STATISTICA 8, 2009) was used to test whether the same species tested in both the LT_{50} and CTM experiments ranked the same in terms of their thermal sensitivity. It was difficult to tell the end point of *Athripsodes* sp. for the CTM experiments and thus the Kendall Tau Correlation was done with and without *Athripsodes* sp. The Kendall Tau Correlation gives a result between 0 and 1, where 0 means the species are not ranked the same while 1 means the species are ranked exactly the same.

3.3 Results

Oxygen saturation

The oxygen saturation in the water bath remained high for the duration of the experiments of approximately ninety minutes (Fig. 3.1). It increased over time due to the aeration associated with the water pumping action of the immersion heater, and plateaued at about 30°C, after which the oxygen saturation began to decrease (Fig. 3.2). The experiments typically ended before 40°C was reached, and the oxygen saturation was still around 100%.

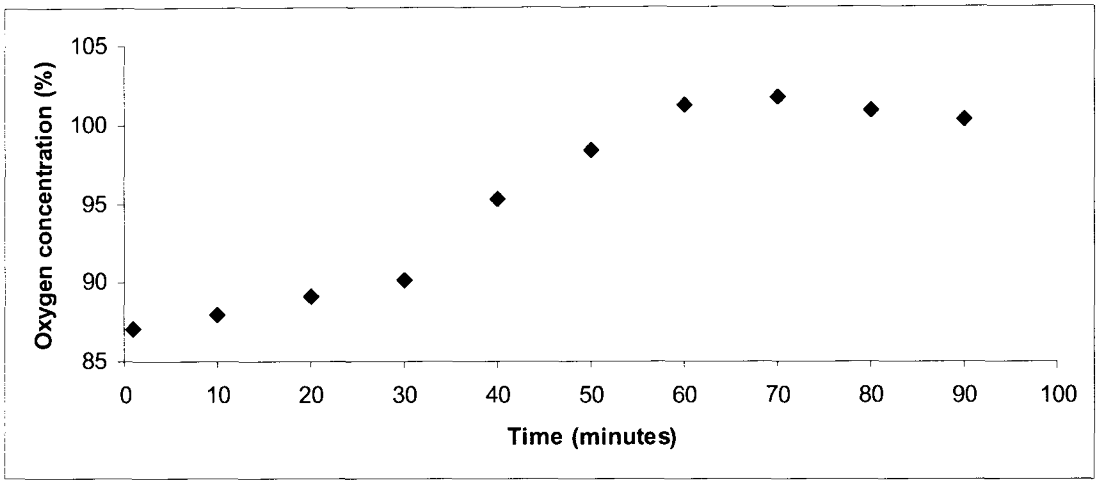

Figure 3.1. Oxygen saturation for the duration of the CTM experiments.

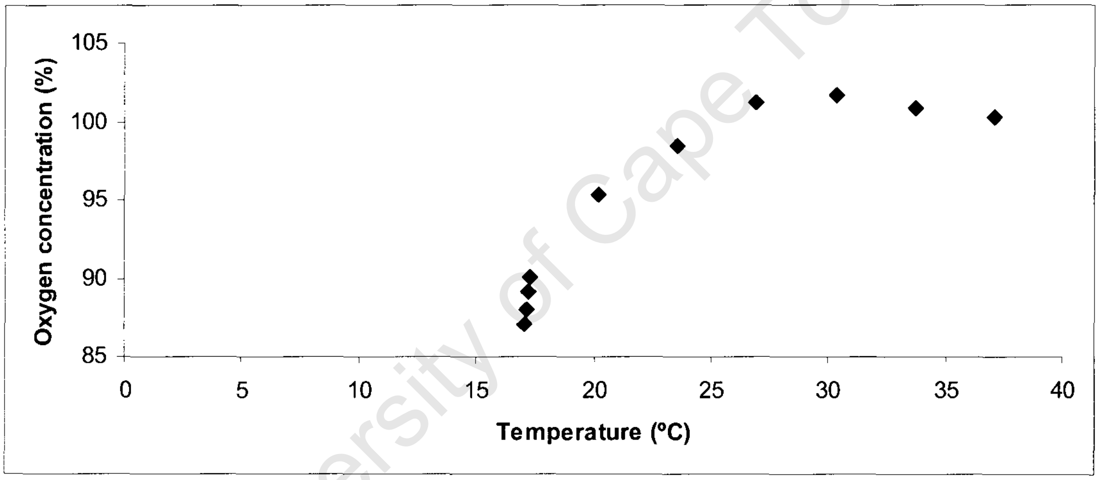

Figure 3.2. The relationship between oxygen saturation and temperature during the course of CTM experiments.

Behavioural response

The different aquatic insect nymph species exhibited different behavioural responses to increases in water temperature (Table 3.2). The stonefly and mayfly nymphs displayed similar end points to increases in temperature, namely immobile (floating) and unable to reattach to the mesh. The caddisfly nymphs tucked their heads into their cases.

Table 3.2. The different behavioural responses noted for the eight aquatic insect nymph species

Species	**General behaviour**	**End point behaviour**
Aphanicercopsis tabularis	Sitting, walking, swimming	On back, floating
Aphanicerca capensis (form C)	Sitting, walking, swimming	On back, floating
Aphanicerca capensis (undescribed form)	Sitting, walking, swimming	On back, floating
Athripsodes sp.	Sitting, walking	Head tucked into case
Castanophlebia sp.	Sitting, walking, walking backwards, swimming	Floating
Lestagella penicillata	Sitting, walking, swimming	On back, floating
Acanthiops sp.	Sitting, walking, swimming	Floating
Barbarochthon brunneum	Sitting, walking, clinging to mesh, walking up silk thread	Head tucked into case, climbed out of case

The three stonefly species (*A. Capensis* (form C), *A. tabularis* and *A. capensis* (undescribed form)) reacted in a similar way (Fig. 3.3). The stonefly nymphs became less active (less walking and swimming) as the temperature increased. Near the critical temperature the nymphs all started floating around and going onto their backs. The critical temperature was determined as the temperature when the nymphs were not moving and floating for five seconds or more, at which point they were removed and placed in stream water at 16°C to recover.

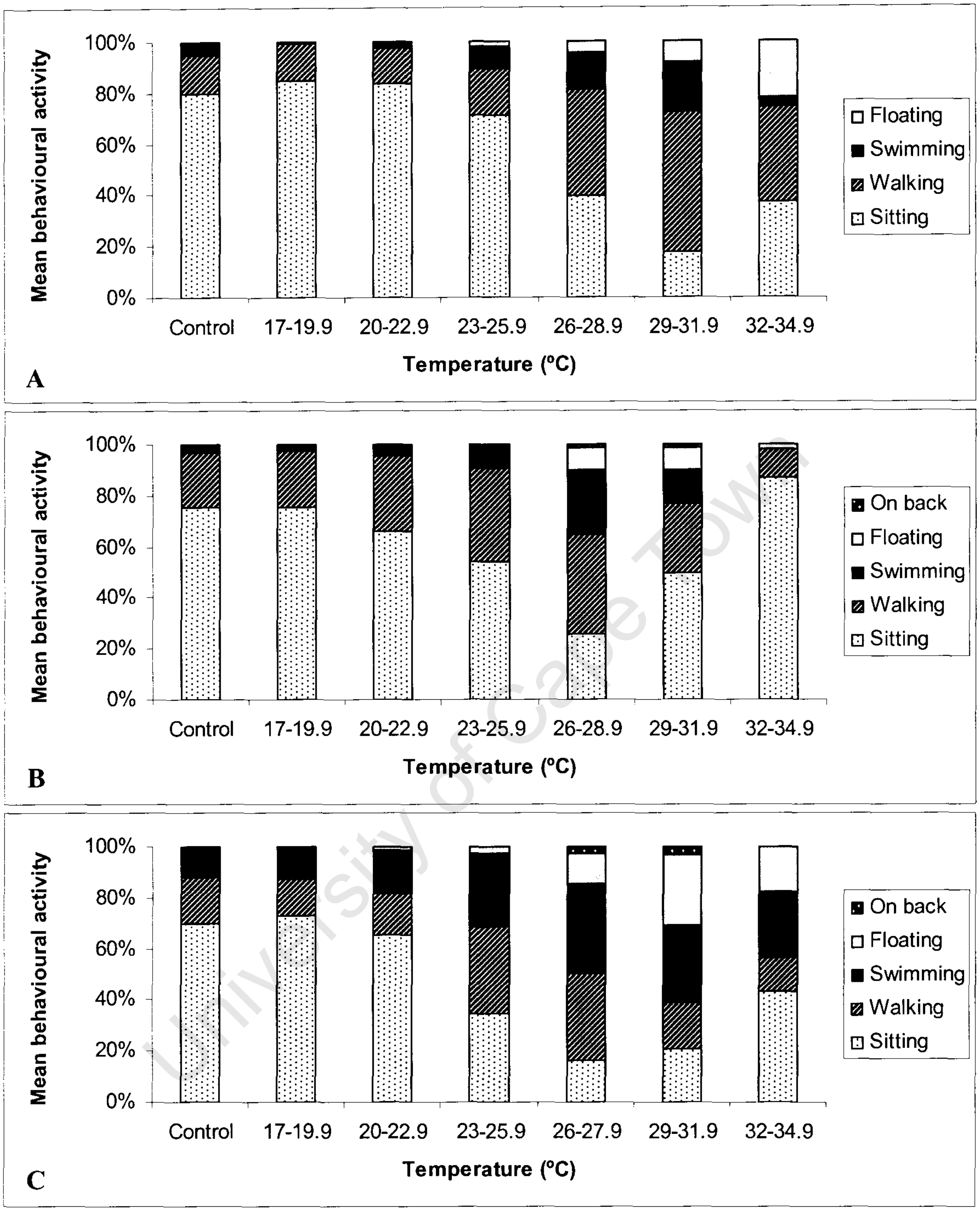

Figure 3.3. The mean percentage of each behavioural activity for 30 individuals of Plecoptera as the temperature increased during the CTM experiments A*)* *Aphanicerca capensis* (undescribed form), B) *Aphanicerca capensis* (form C) and C) *Aphanicercopsis tabularis*

The mayflies showed a similar behavioural pattern (Fig. 3.4). The organisms were fairly inactive with 80-90% being stationary at all experimental temperatures. *Acanthiops* sp. did, however, become slightly more active as the temperature increased. There was an increase in 'floating' near the critical temperature for *Castanophlebia* sp. and *L. penicillata* (floating was also noted for *Acanthiops* sp.). The critical temperature was determined as the temperature when the organisms were not moving while floating for five seconds or more, at which point they were removed and allowed to recover.

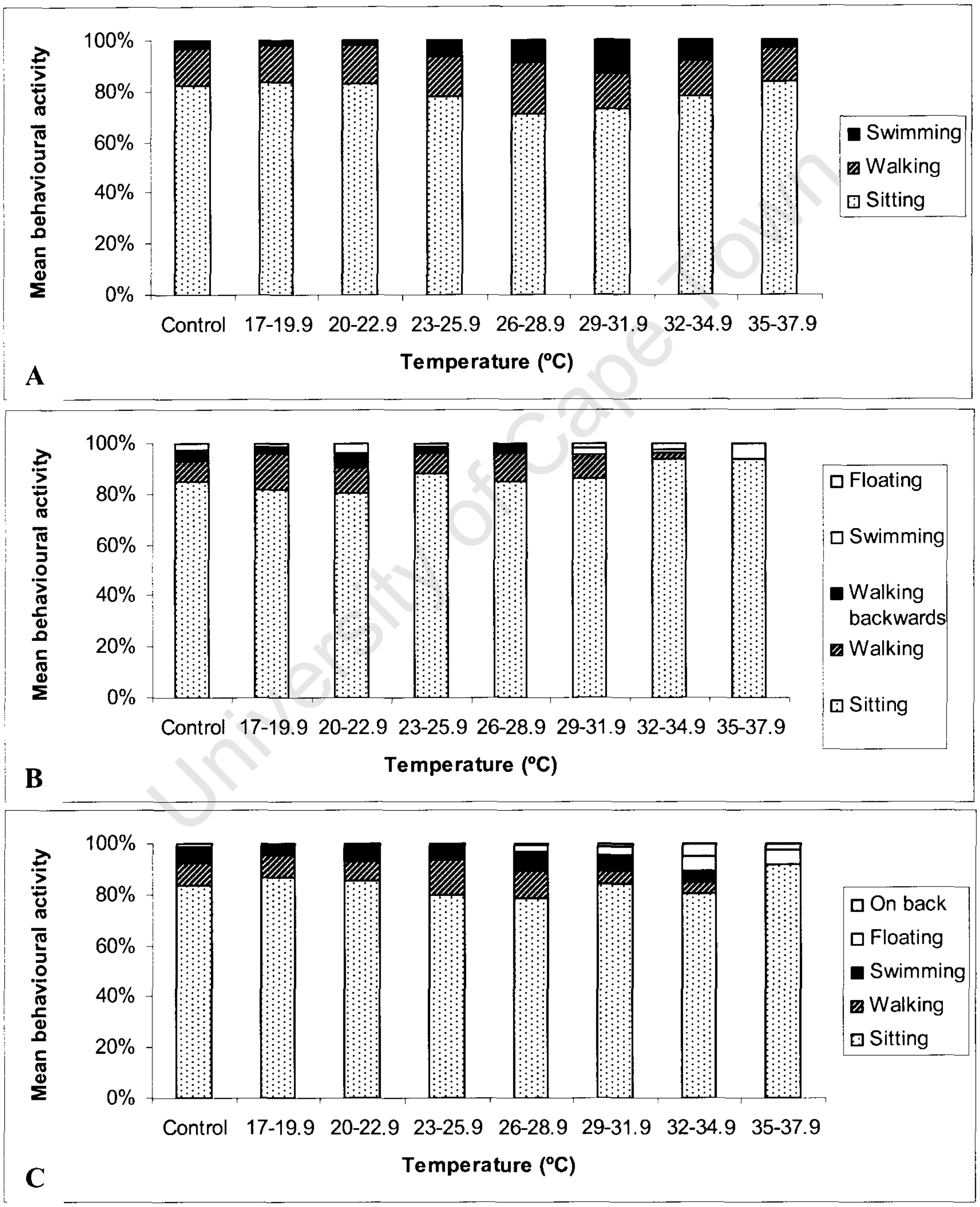

Figure 3.4. The mean percentage of each behavioural activity for 30 individuals of Ephemeroptera as the temperature increased during the CTM experiments A) *Acanthiops* sp., B) *Castanophlebia* sp. and C) *Lestagella penicillata*.

The caddisfly species compared to one another differed slightly in their response to increasing temperature (Fig. 3.5). Both *B. brunneum* and *Athripsodes* sp. became less active (less walking) as the temperature increased and tucked their heads in more. Some nymphs of *B. brunneum* climbed out of their cases near the critical temperature. The caddisflies were removed when they did not come out of their cases for five to ten seconds, when squirted with water. This was determined as the critical temperature and then the individuals were allowed to recover.

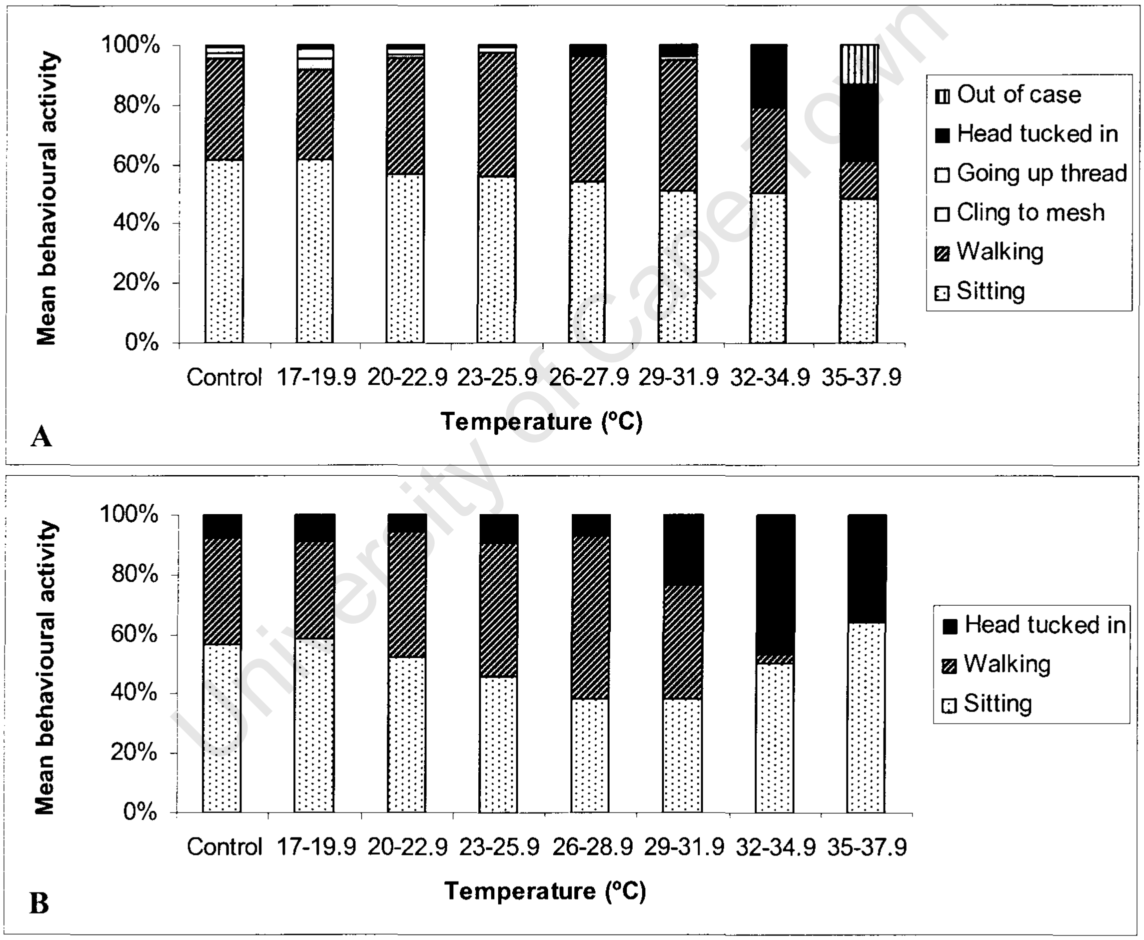

Figure 3.5. The mean percentage of each behavioural activity for 30 individuals of Trichoptera as the temperature increased during the CTM experiments A) *Barbarochthon brunneum* and B) *Athripsodes* sp.

Comparing end points

The eight aquatic insect nymph species tested showed differences in their mean CTM end points (Table 3.3). The three Gondwanan stonefly species appeared to be the most sensitive, with *A. tabularis* being most sensitive (the greatest change in behaviour over the shortest temperature range) with a mean end point of 28.8°C. *B. brunnuem* was the least sensitive species with a mean end point of 35.1°C.

Acanthiops sp. (non-Gondwanan) had a higher end point compared to the two Gondwanan mayflies (*Castanophlebia* sp. and *L. penicillata*). The opposite was true for the caddisflies as *Athripsodes* sp. (non-Gondwanan) had a lower end point than *B. brunneum* (Gondwanan).

Table 3.3. A comparison of the mean end points for the eight organisms (in ascending order) (N=30).

Family	**Species**	**Mean end point (°C)**	**Standard error**
Notonemouridae	*Aphanicercopsis tabularis*	28.8	0.34
Notonemouridae	*Aphanicerca capensis* (form C)	29.8	0.35
Notonemouridae	*Aphanicerca capensis* (undescribed form)	31.3	0.27
Leptoceridae	*Athripsodes* sp.	32.0	0.28
Leptophlebiidae	*Castanophlebia* sp.	32.1	0.29
Teloganodidae	*Lestagella penicillata*	32.9	0.31
Baetidae	*Acanthiops* sp.	34.1	0.21
Barbarochthonidae	*Barbarochthon brunneum*	35.1	0.23

There was a significant difference between the eight species tested (F = 62.65, d.f. = 232, $p < 0.01$). A post-hoc Tukey HSD test showed the significant differences, in terms of their CTM end points, between the eight species (Table 3.4). The CTMs of *A. capensis* (form C) and *A. tabularis* were not significantly different from each other, but significantly different from that of all the other species, including *A. capensis* (undescribed form). The CTM of the two Gondwanan mayflies, *L. penicillata* and *Castanophlebia* sp. were not significantly different. While the CTM of *Castnophlebia* sp. was significantly different from that of the non-Gondwanan mayfly *Acanthiops* sp., the CTM of *L. penicillata* was not. The CTM of *B. brunneum* was significantly different from that of all the other species, except *Acanthiops* sp.

Table 3.4. Tukey HSD post-hoc test to show significant differences in terms of the CTM end points for the aquatic insect nymph species. (Bold – significantly different, not bold – not significantly different)

Species	*A. capensis* (undescribed form)	*Acanthiops*	*B. brunneum*	*Athripsodes*	*Castanophlebia*	*L. penicillata*	*A. capensis* (form C)	*A. tabularis*
A. capensis (undescribed form)		**0.00003**	**0.00003**	0.47945	0.25767	**0.00048**	**0.00109**	**0.00003**
Acanthiops	**0.00003**		0.14751	**0.00003**	**0.00004**	0.02483	**0.00003**	**0.00003**
B. brunneum	**0.00003**	0.14751		**0.00003**	**0.00003**	**0.00003**	**0.00003**	**0.00003**
Athripsodes	0.47945	**0.00003**	**0.00003**		0.99996	0.29692	**0.00003**	**0.00003**
Castanophlebia	0.25767	**0.00004**	**0.00003**	0.99996		0.53002	**0.00003**	**0.00003**
L. penicillata	**0.00048**	0.02483	**0.00003**	0.29692	0.53002		**0.00003**	**0.00003**
A. capensis (form C)	**0.00109**	**0.00003**	**0.00003**	**0.00003**	**0.00003**	**0.00003**		0.172237
A. tabularis	**0.00003**	**0.00003**	**0.00003**	**0.00003**	**0.00003**	**0.00003**	0.17224	

CTM experiments vs. LT_{50} experiments

The CTM values were higher than the LT_{50} values recorded for the same species tested (Table 3.5). The species' sensitivity ranked the same except for *Athripsodes* sp. whose CTM value was lower than the two mayflies but was the most tolerant according to the LT_{50} experiments. This is confirmed by the Kendall Tau Correlation results. When the results from all the species were compared for the two experiments, the Kendall Tau Correlation was 0.6, but when *Athripsodes* sp. was removed the Kendall Tau Correlation was 1. The species, except *Athripsodes* sp., have the same thermal tolerance according to both experiments. Overall *A. capensis* (form C) was the most sensitive species in both sets of experiments.

Table 3.5. A comparison between the LT_{50} values from the LT_{50} experiments and the CTM values from the CTM experiments for the same species

Species	**LT_{50} value (°C)**	**CTM value (°C)**
Aphanicerca capensis (form C)	23.4	29.75
Aphanicerca capensis (undescribed form)	25.0	31.29
Castanophlebia sp.	26.5	32.18
Lestagella penicillata	29.5	32.91
Athripsodes sp.	29.9	32.04

3.4 Discussion

Thermal sensitivity of Gondwanan vs. non-Gondwanan taxa

All three stonefly species tested (*A. capensis* (form C), *A. tabularis* and *A. capensis* (undescribed form)) were representatives of the Gondwanan family Notonemouridae, and were the most sensitive of all the taxa tested to thermal stress. This sensitivity correlates with the distribution of these species as they are restricted to upper reaches of sandstone mountain streams in South Africa (Stevens & Picker 2003). They currently occupy the coldest and most thermally buffered portions of the streams in which they occur, and thus cannot retreat to cooler refugia in response to elevated stream temperatures that might arise from anthropogenic activities. Such species might thus be considered especially vulnerable to the threat of elevated water temperatures resulting from climate change. Unfortunately, no non-Gondwanan stoneflies occupy the same habitat as Notonemouridae, making contrasts from distantly related members of the order not possible. A useful group with which to compare thermal tolerances would be *Neoperla* (Perlidae), which occur in warmer, more sluggish rivers in certain parts of South Africa.

The Gondwanan mayflies (*Castanophlebia* sp. and *L. penicillata*) were more thermally sensitive than the non-Gondwanan mayfly (*Acanthiops* sp.) (Table 3.3). The *Acanthiops* sp. nymphs were, however, from the Salt River and not Skeleton Gorge Stream, where the Gondwanan mayflies were from. The Gondwanan caddisfly (*B. brunneum*) nymphs from the Keur River were less sensitive to increased temperatures than the non-Gondwanan *Athripsodes* sp. from Window Gorge Stream. The behavioural criteria for the CTM endpoint were not as clear as those of other taxa, due to the complicating factor of the caddisfly case, which prevented them from releasing and floating (as in stoneflies and mayflies). This may have complicated the accurate determination of the endpoint.

Factors affecting CTM values

Oxygen concentration

The effect of an increase in water temperature on the behaviour of aquatic insect nymphs is not well documented (Sweeney *et al.* 1992), even though there is a close relationship between the oxygen holding capacity of water and its temperature. Oxygen concentration is an important factor for the survival of the organisms which inhabit well-oxygenated streams/rivers. Oxygen saturation measured in the streams when test organisms were collected was high for both streams. The average oxygen saturation in the Salt River was 104%, and 81% for Window Gorge Stream.

As oxygen concentration decreases with an increase in temperature, gaseous exchange will potentially be affected and might require behavioural adaptations (e.g. active ventilation) to deal with this change. Oxygen concentration may become a problem in CTM experiments, as test temperatures increase. Under low oxygen conditions caddisflies undulate their abdomens, stoneflies do "push ups" and some mayflies beat their abdominal gills (Sweeney *et al.* 1992). However, none of the end point behaviours were characteristic of active ventilatory movements, and one of the three groups tested (notonemourid stoneflies) does not have any external gills, obviating a possibility of active ventilation under conditions of oxygen stress. Finally, during the CTM experiments oxygen levels remained high for the duration of the experiment. The behavioural responses noted were thus due to increases in temperature, rather than decreases in oxygen concentration.

Rate of increase and start temperature

Start temperature and rate of increase are known to affect the outcome of CTM experiments (Terblanche *et al.* 2007, Chown *et al.* 2009). The start temperature and rate of increase were kept constant in the CTM experiments in this study, for each species tested. The start temperature was 17.34°C (average stream temperature) and the rate of increase was 0.34°C per minute for all the experiments.

The effect of the rate of increase in temperature has not been widely explored (Terblanche *et al.* 2007), especially for aquatic species. Terblanche *et al.* (2007) conducted CTM experiments on adult *Glossina pallidipes* (tsetse fly) and found that a higher start temperature and a faster rate of increase resulted in higher CTM values. They concluded that the duration of exposure affected the CTM value, as longer duration exposures resulted in a decrease in the CTM value (Terblanche *et al.* 2007). Chown *et al.* (2009) also found that an increase in the rate resulted in higher CTM values for both *Drosophila melanogaster* (fruit fly) and *Linepithema humile* (Argentine ant). It is therefore important to consider both the start temperature and rate of increase when using CTM experiments. Future studies should identify what affect varying the start temperature and rate of increase could have on CTM results for aquatic insect nymphs.

Influence of age of test organism and intraspecific variation on CTM values

Bowler and Terblanche (2008) noted that life stage (typically instar) influenced temperature tolerance and in particular, that the upper temperature limits decreased with age. When comparing thermal tolerances of organisms it is therefore important to standardise for age. An attempt was made to standardise age in the CTM experiments, with early to middle instars being used in the experiments – however in the absence of specific information about the life history of each tested species, the best approximation is to use similar-sized individuals for each species tested. Further work should be conducted to track ontogenetic changes in thermal tolerance.

Local thermal adaptation

A. capensis (form C) from Window Gorge Stream on Table Mountain had a lower CTM than *A. capensis* (undescribed form) from the Salt River in the Tsitsikamma area. As mentioned in chapter 2 there is no genetic difference, according to the COI gene, between the different forms of *A. capensis* but there are fairly large morphological differences between them. The different forms may potentially be different species (Stevens and Picker 2003). It can be expected that there would be some geographic variation in the temperature tolerance within a species due to genetic differences and phenotypic plasticity.

Comparison with other studies

The results from CTM experiments reflect the upper range of sublethal temperatures that can be tolerated under acute exposure conditions, and therefore may be considered relevant from an ecological perspective (Terblanche *et al.* 2007). However, it is apparent that acute exposure may be the exception, and that natural behavioural avoidance may appear at different temperatures to those obtained under laboratory conditions.

Few studies exist for CTMs of aquatic species and some results were found from two studies on insect nymphs in North America. For Plecoptera, Ernst *et al.* (1984) in Delaware County, Oklahoma found CTM values of 33.2°C and 33.8°C for Perlodidae, 33.4°C and 36.3°C for Perlidae, and 31.5°C for Nemouridae. The higher CTM value occurred when the acclimation temperature was higher (Ernst *et al.* 1984). In this study the CTM values obtained for Plecoptera were slightly lower (28.80-31.29ºC). Two quite different CTM values were obtained for the two Trichoptera species in this study (32.04 and 35.13ºC), but these were within the range reported by Moulton *et al.* (1993) (between 33.6 and 36.5ºC for Hydropsychidae and Philopotamidae) for North American Trichoptera in Texas and Missouri. The CTM value for a particular species is therefore dependent on the specific species and geographic variation.

CTM experiments vs. LT_{50} experiments

LT_{50} experiments determine the temperature at which 50% of the test organisms are predicted to die. Chapter two of this thesis presented the results of LT_{50} experiments conducted on a subset of the same species used in the CTM experiments. The CTM values recorded in this study were higher than the LT_{50} values (Table 3.5), for the same species, suggesting that the test animals could withstand higher temperatures for a short period of time. For the species tested in both experiments, their tolerance levels ranked the same, except for *Athripsodes* sp. *Athripsodes* sp. ranked as the most tolerant in the LT_{50} experiments but less tolerant in the CTM experiments. This could be due to the difficulty in identifying the end point for caddisflies in their cases in the CTM experiments. *Athripsodes* sp. was thus excluded from the Kendall Tau Correlation, which resulted in the species thermal tolerance ranked exactly the same for both experiments. *A capensis* (form C) and *A. capensis* (undescribed form) were the most sensitive in both sets of experiments. However, it is important to treat the results from the two methods separately as it had been noted that static experiments (LT_{50}) and dynamic (CTM) experiments are likely to be assessing different genetic bases (Rako *et al.* 2007).

It is apparent that there are advantages and disadvantages to methods (Table 3.6). CTM experiments were simpler and faster to do than the LT_{50} experiments. The CTM experiments required fewer individuals and most of the individuals survive the experiments. CTM experiments can thus be used to test the thermal tolerance of endangered species. The equipment used for the LT_{50} experiments was fairly cheap (plastic tubs, tubing and air stones), while for the CTM experiments the water bath and heating immersion circulator were fairly expensive items in comparison.

Table 3.6. A comparison between the CTM and LT_{50} experiments

CTM experiments	**LT_{50} experiments**
Simpler and faster (water bath and about one and half days work)	Longer (four days at one temperature and repeated for different temperatures)
Less individuals (about thirty)	More individuals (about thirty individuals per temperature – about 150 total)
More survive (most of the individuals survive the experiment – can be used for endangered species)	Less survive (most of the individuals die in the experiments)
More expensive (the water bath and heating immersion circulator were expensive) Constant temperature room at the university	Cheaper (the plastic tubs, tubing and air stones were fairly cheap) Constant temperature rooms at the university

Of the two methods, CTM experiments rank species sensitivity, and might be a more sensitive indicator. However, in terms of a reflection of what happens *in situ*, the experiments are compromised by the unnatural, acute exposure period. LT_{50} experiments also rank species sensitivity, but are more likely to give improved predictability for natural temperature changes, including that which is anthropogenic in origin. Neither matches the types of naturally-occurring thermal stress, which is typically of longer duration and less severe. The best approach might be to run LT_{50} experiments of far longer duration, and to use match rankings of thermal sensitivity as obtained by both methods. The limitations of both methods are a consequence of their design for rapid testing.

Conclusions

The three stonefly species tested [*A. tabularis, A. capensis* (form C) and *A. capensis* (undefined form)] were the most sensitive in the CTM experiments. The Gondwanan mayflies (*Castanophlebia* and *L. penicillata*) were more sensitive than the non-Gondwanan mayfly (*Acanthiops* sp.). The opposite was, however, found for the caddisflies as it was difficult to identify the endpoint for cased caddisflies. The results obtained from the LT_{50} and CTM experiments were compared and found that the thermal sensitivity of the species tested ranked the same expect for the caddisfly. The CTM experiments have advantages over the LT_{50} experiments as they are quicker and use fewer animals which survive the experiments. The CTM experiments, however, do not reflect what happens *in situ*.

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Chapter 4 – Baseline stream temperatures and community composition: implications of climate change for Window Gorge Stream, Western Cape of South Africa

Southern Africa largely falls within an unstable regime with regards to predicted climate change. The Western Cape Province of South Africa is predicted to experience an increase in air temperature and a decrease in winter rainfall which may result in a decrease in water availability and an increase in water temperature in streams/rivers. This will in turn have implications for the aquatic organisms, especially those that are thermally sensitive. The imminent effects of climate change increase the need to establish baseline monitoring, collect experimental data and quantify relationships between community composition and environmental variables. Window Gorge Stream, originating on Table Mountain in the Western Cape Province, was selected to establish baseline information on stream temperature and community composition along altitudinal (six sites) and seasonal gradients. Overall the average water temperature was low (12°C to 18°C), increasing along the length of the stream, except for when sections of the stream ran dry from between February and May 2009. It is important to maintain riparian vegetation and manage abstraction properly along the stream to keep water temperatures low. Site 4 in the mountain stream zone had the highest species richness and diversity, where a greater attachment area, more nutrients and high oxygen levels are available for the aquatic organisms. The highest diversity was found during the cooler months. The community composition was best explained by a combination of water quality variables including pH, oxygen saturation and water temperature. The community composition data coupled with the water temperature data provide an important reference for future monitoring of changes, e.g. changes in abundance of thermally sensitive species, related to climate change.

Key words: climate change, community analysis, water temperature, future monitoring

4.1 Introduction

Climate change

Current consensus is that the anthropogenic emission of carbon dioxide and other greenhouse gases are the ultimate cause of climate change and that the effects on fauna and flora will be quite significant (Hogg and Williams 1996, de Wit and Stankiewicz 2006, Menéndez 2007, Hassall *et* al. 2007). Major anthropogenic contributions to increased greenhouse gases include combustion of fossil fuels and biomass, burning of forests, and human and animal waste production (Watson *et al.* 1992).

The average global surface temperature has increased by about 0.6°C over the last century and is predicted to continue to rise during the next century by up to 5.8°C in 2100 (Menéndez 2007). This has profound implications for Southern Africa which largely falls within an unstable regime with regards to predicted climate change. For the region it is predicted that by 2050 rainfall could decrease by 10%, leading to serious droughts in certain parts of the country (de Wit and Stankiewicz 2006).

Projections for the Western Cape Province of South Africa include increased summer rainfall from January onwards, especially inland and towards the east, with delayed winter rainfall, especially towards the southwest (Midgley *et al.* 2005). By 2050, the temperature in the Western Cape Province is predicted to rise by approximately 1.5°C at the coast, and 2-3°C inland (Midgley *et al.* 2005). There is always, however, some uncertainty in the models. The predicted increase in air temperature and decrease in rainfall may consequently result in less water being available to streams and rivers as well as an increase in water temperature.

Freshwater ecosystems and temperature

Freshwater ecosystems are important to the earth physically, chemically and biologically and provide varied goods and services to the human population (Allan and Flecker 1993). When the ecosystem cannot function properly and the basic ecosystem processes are disrupted (e.g. nutrient cycling, primary and secondary production and decomposition) these goods and services cannot be provided. The nature and rates of these processes and the condition of ecosystems are impacted by water temperature (Meyer *et al.* 1999, Morrill *et al.* 2005) and are thus potentially threatened by predicted future climate change.

The effects of increased water temperature through climate change could be positive and/or negative (Meyer *et al.* 1999). Elevated water temperatures may lead to a decrease in the volume of cooler water in upper mountain streams which may impact on the entire stream system (Durance and Ormerod 2007). The distribution for cooler-water species is likely to shrink, while the distribution for warmer-water species (typically restricted to lower reaches) is likely to expand (Allan and Flecker 1993, Meyer *et al.* 1999, Day 2005, Durance and Ormerod 2007, Haidekker and Hering 2008). The vulnerability of species occupying upper reach mountain streams to climate change has already been observed to result in local extinctions (Parmesan 2006). It is thus likely that species restricted to upper reach mountain streams in the Western Cape Province, such as Gondwanan and relictual species, will suffer range restrictions or even extinction (Day 2005).

Freshwater communities and temperature

Temperature plays an important role in shaping the community composition of aquatic invertebrates (Vannote and Sweeney 1980, Perry *et al.* 1987, Hawkins *et al.* 1997, Dallas 2008, Haidekker and Hering 2008). Durance and Ormerod (2007) found a change in the composition as well as a decrease in macroinvertebrate abundances when temperature increased. Haidekker and Hering (2008) found that the differences in the longitudinal distribution of aquatic invertebrates from nineteen sampled streams were partly explained by water temperature (e.g. most of the Plecoptera were restricted to parts of the stream with the lowest temperature).

While a range of parameters such as drought and flood influence the life history of aquatic invertebrates, temperature tends to be the most important factor (Brittain 1983, Sweeney & Vannote 1986, Quinn *et al.* 1994, Dallas and Day 2004). Climate change, with a change in precipitation and an increase in air temperature, is likely to bring about considerable changes in the distribution, behaviour and life history of aquatic invertebrates (Hogg and Williams 1996, Menéndez 2007, Hassall *et al.* 2007, Barber-James *et al.* 2008). Responses are likely to be rapid, given the short generation times and high reproductive rates of insects (Menéndez 2007). For these reasons aquatic insects are suitable indicators for evaluating the biological impact of climate change (Burgmer *et al.* 2007, Menéndez 2007).

Importance of monitoring

The imminent effects of climate change increase the urgency to establish baseline monitoring, collect experimental data and quantify relationships between species richness and environmental variables (Sweeney *et al.* 1992, Allan and Flecker 1993, Meyer *et al.* 1999, Fleishman and Murphy 2000). Only then can the thresholds for ecological change be determined. Burgmer *et al.* (2007) have predicted that the effects of global warming are likely to be species-specific. They found no direct relationship between diversity and temperature, but noted significant changes in species composition of benthic macroinvertebrates with changes in temperature.

In Southern Africa, the SASS rapid bioassessment method (South African Scoring System, version 5, Dickens and Graham 2002) is uses macroinvertebrates to indicate water quality and requires a specific sampling protocol. It is based on the identification of invertebrates to family level in the field, and thus requires little prior knowledge (Davies and Day 1998). SASS is based on the principle that some invertebrate taxa are more sensitive than others to pollutants. Each taxon of invertebrates has been allocated a score from 1 to 15 depending on their sensitivity to pollution, with 15 being the most sensitive. The sampling protocol used for SASS can be applied to collect and identify macroinvertebrates in the field and may be able to be used in the future to rapidly assess the community composition down the length of the stream in relation to thermal pollution.

This study aimed to 1) collect baseline stream temperature and community composition data for Window Gorge Stream in the Western Cape Province of South Africa 2) compare community composition along a stream temperature gradient 3) compare SASS scores taken in the field to those obtained using more detailed species identification done in the laboratory; and 4) use patterns of communities response to water temperature to make predictions about the impact of global climate change on stream communities.

4.2 Methods

Study site

The stream selected for the purposes of this study was located on Table Mountain (Cape Peninsula) in the Cape Floristic Region (CFR) of South Africa. Aquatic invertebrates were collected from Window Gorge Stream, within the Table Mountain National Park (TMNP) and Kirstenbosch National Botanical Garden (KNBG) between August 2008 and May 2009. This stream originates on top of Table Mountain and, flows through the TMNP and KNBG and is a typical CFR mountain stream in good condition. The area along the upper sites (except site 1) is partially shaded by Southern Afrotemperate Forest (Mucina and Geldenhuys 2006), with little or no alien vegetation. The lower sites are impacted to a small degree by alien vegetation, the abstraction of water and fertilizer from KNBG.

Invertebrate sampling

Six sites were selected down the length of Window Gorge Stream from near the source to the start of the 'upper foothill' zone (Fig. 4.1, Table 4.1). There was a large altitudinal difference between site 1 and the other sites (Fig. 4.2). The approximate horizontal distance between each site was determined using Google Earth (Fig. 4.2).

Aquatic invertebrates were collected at each of the sites using a net (30cmx30cm) with a 0.5mm mesh. The samples were collected by vigorously kicking rocks upstream of the net allowing the organisms to drift into the net. The kicks were conducted for three minutes at a time and three replicates were collected at each of the sites. Each of the samples was drained and transferred to a 500ml sampling jar with 96% alcohol. Water quality was measured using a Crison pH 25 pH meter (accuracy ≤ 0.01pH), YSI EC300 conductivity meter (accuracy ± 0.002mS/cm), YSI 550A dissolved oxygen meter (accuracy ± 2%) and a mercury thermometer.

Invertebrate samples were collected every two months from August 2008 to April 2009, and in May 2009. As Window Gorge Stream was dry for part of summer, no samples were collected at site 6 in February, and at most of the sites in April (except for one sample at sites 3 and 4, taken from small pools). The samples were identified at least to morphospecies, but in many cases further identification was possible, sometimes to species level (Appendix 2). The number of individuals for each species was recorded.

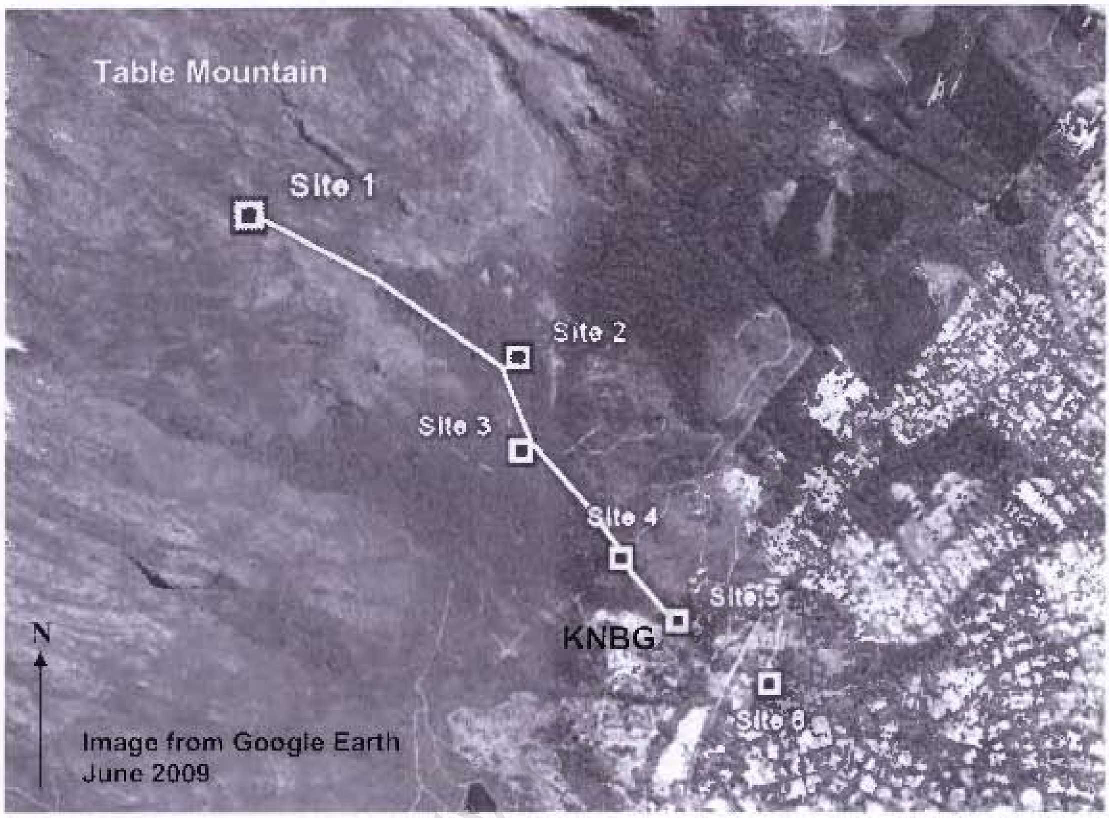

Figure 4.1. The location of each of the sites sampled along Window Gorge Stream in relation to Table Mountain and Kirstenbosch National Botanical Gardens (KNBG). Path of stream indicated by white line.

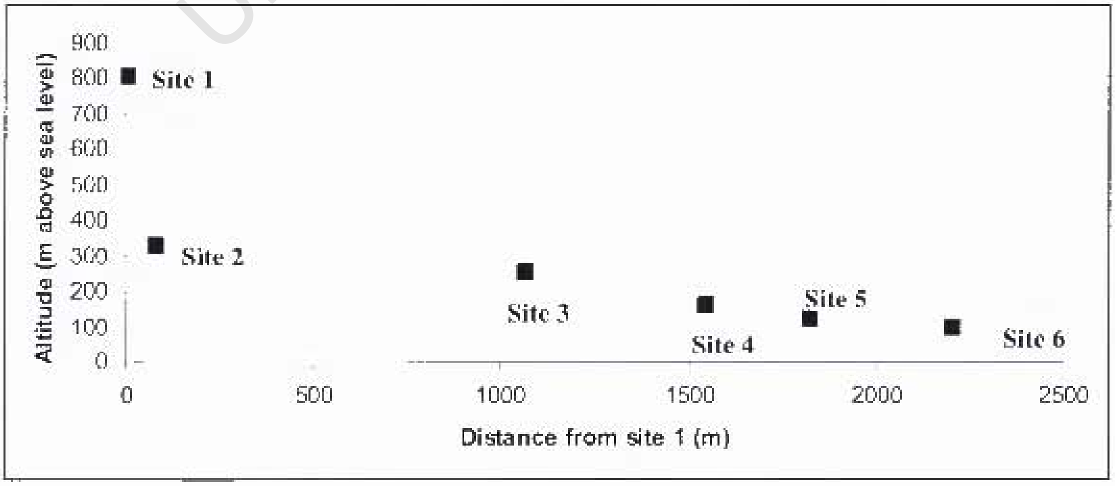

Figure 4.2. The longitudinal profile for Window Gorge Stream where the markers represent the six sites sampled down the length of Window Gorge Stream.

Table 4.1 GPS coordinates and details of each of the six sites sampled down the length of Window Gorge Stream.

Site	Coordinates	Details
1	33° 58' 31.01" S 18° 25' 14.38" E	Near the source of the stream
2	33° 58' 41.30" S 18° 25' 41.45"E	'Mountain torrent' zone as this part of the streams consists of large boulders and a steep slope
3	33° 58' 50.66" S 18° 25' 41.74" E	'Mountain stream' zone as these parts of the stream were narrow and consist mostly of medium sized boulders and cobbles
4	33° 59' 2.18" S 18° 25' 54.62" E	'Mountain stream' zone as these parts of the stream were narrow and consist mostly of medium sized boulders and cobbles
5	33° 59' 9.02" S 18° 26' 2.08" E	'Mountain stream' zone as these parts of the stream were narrow and consist mostly of medium sized boulders and cobbles
6	33° 59' 16.08" S 18° 26' 14.42" E	'Upper foothill' zone where the stream flattens out a bit (below Kirstenbosch National Botanical Gardens)

Temperature recordings

Two HOBO® Pendant temperature data loggers (accuracy 0.47°C at 25°C) were placed at each site, one in a shaded area and one in an open area of the stream in October 2008. The loggers were secured in a metal tube via screw braces, to protect them from mechanical damage, and were set to record temperature every hour. Metal cabling attached the metal housing to a large, secure rock via a bolt screwed into a hole drilled into the rock. All the loggers were downloaded using the HOBO® Waterproof Shuttle in February and May 2009. In August only one logger at site 2 (sun) and site 3 (shade) were able to be downloaded as the other loggers were buried under rocks in fast flowing water and were not able to be retrieved to be downloaded.

SASS (South African Scoring System)

The SASS protocol was used in February (Sites 1 and 3) and May 2009 (Sites 1, 3 and 5) to identify the invertebrates to family level in the field. Only the 'stone-in-current' habitat was sampled, as this was the only habitat present at the sites. Kick sampling was done for three minutes as for previous samples taken back to the laboratory. The sample was examined in the field for five minutes to identify as many individuals as possible to family level and estimate the numbers of individuals. The same sample was taken back to the laboratory for more accurate species identification and quantification. This was done for five samples at three of the sites in February and May 2009.

Statistical analysis

Temperature and water quality data

The temperature data downloaded from the temperature loggers were used to determine the maximum, minimum and average temperature for each day. The overall average for the eight months for each logger was then calculated. Temperatures recorded when the stream was dry had to be removed from the dataset. Based on logger data, this was fairly difficult to determine, and was different for each logger at each site. Therefore in cases where the logger recorded an abrupt and steep rise in temperature, all subsequent temperatures were removed from the dataset, as these were likely air temperatures. Logger data was again included when day temperatures were again low.

For the temperature loggers downloaded at sites 2 (sun) and 3 (shade) some of the winter temperatures were included. Thus temperature data from October 2008 through to early August 2009 were analysed in more depth. It constituted nearly a year's worth of data and thus an overall mean and standard deviation were calculated. The mean minimum, mean maximum, average diel range and maximum diel range were also calculated for the two sites. An average temperature for the months of spring (October), summer (November- March), autumn (April and May) and winter (June-August) were estimated.

Nearby air temperature data were provided by KNBG and were compared to the water temperatures from the loggers using the Product Moment Correlation (STATISTICA 8) to determine a relationship between air and water temperature.

A t-test (STATISTICA 8) was used to test the difference between the temperatures in the sun and the shade at each site using the average daily temperatures as the data were normal and had equal variance. ANOVA (STATISTICA 8) was used to test the difference between the daily average temperature at each site and the post hoc Tukey test was used to identify which sites were significantly different from each other. Kruskal-Wallis (STATISTICA 8) and the post hoc test (multiple comparisons) were used to test the difference in the water quality data between the sites along Window Gorge Stream.

Community composition data

The highest number of species and the Margalef's index (*d*) (Primer6, Clarke and Warwick 1994) were used to describe species richness. The Margalef's index is a measure of the number of species present for a given number of individuals. The Shannon-Wiener ($\log_e$) diversity index (Primer6, Clarke and Warwick 1994) was used to describe species diversity down the length of the stream as well as across the months. The Shannon-Wiener diversity index incorporates both species richness and equitability (or evenness).

It is important to include all species present at each site for diversity measures as the omission of some of the species will affect the result of the analysis (Clarke and Warwick 1994). However, in the production of ordinations, rare species need to be excluded as their occurrence at a site may just be due to chance (Clarke and Warwick 1994). For the multivariate analyses used those species whose numbers comprised less than 1% of the total number of individuals were excluded. Thus 16 species out of a total of 67 were used in the multivariate analyses.

An MDS plot and cluster analysis was constructed using Primer6 (Clarke and Warwick 1994). An MDS plot with all the replicates for each site for each month gave a stress level of 0.24 which means that the points are close to being arbitrarily placed and should be treated with a great deal of scepticism (Clarke and Warwick 1994). The replicates were thus pooled which gave a stress level of 0.17 which is more useful. Data were 4throot transformed, but since analyses did not differ from untransformed data, the latter was retained for all subsequent analyses.

The ANOSIM function in Primer6 was used to show if there were significant differences between sites. The SIMPER function in Primer6 was used to determine which taxa were primarily responsible for the groupings in the MDS plot and cluster analysis. The three species that contributed the most to the observed similarity between the groups were noted.

The BIO-ENV procedure in Primer6 was used to compare the ordination of the community data to environmental variables (water quality). The water quality data included average water temperature, pH, conductivity (mS/cm) and oxygen saturation (%). The water quality data were normalized and compared to ordinations done using the 16 most common species. All sites and months (except April) were compared to the water quality data to identify which water quality variables best explain the community data. Environmental variables were superimposed on points of the MDS plot using circles of varying size to match water quality variables.

4.3 Results

Stream temperatures

The air temperatures measured at KNBG were compared to the water temperatures measured at site 5 (situated within KNBG) with sun logger (5.1) and shade (logger 5.2). This site was the closest to that where air temperatures were measured. There was a significant correlation between air temperature and water temperatures taken from both shaded and sunny sites for minimum and maximum temperatures (Table 4.2) – as air temperature increased so did the water temperature but did not increase at a 1:1 relationship with air temperature. The change in stream temperature therefore reflected the change in air temperature, but was at a reduced amplitude (Fig. 4.3). It appears that water temperatures are buffered at night compared to air temperatures (Fig. 4.3A), with the reverse being true for maximum temperatures (Fig. 4.3B).

Table 4.2. Product Moment Correlations between air and water temperature for site 5 along Window Gorge Stream

Temperature logger	Min/Max	N	Correlation	p-value
5.1 (sun)	Min	195	0.78	$p < 0.05$
5.1 (sun)	Max	195	0.66	$p < 0.05$
5.2 (shade)	Min	176	0.79	$p < 0.05$
5.2 (shade)	Max	176	0.67	$p < 0.05$

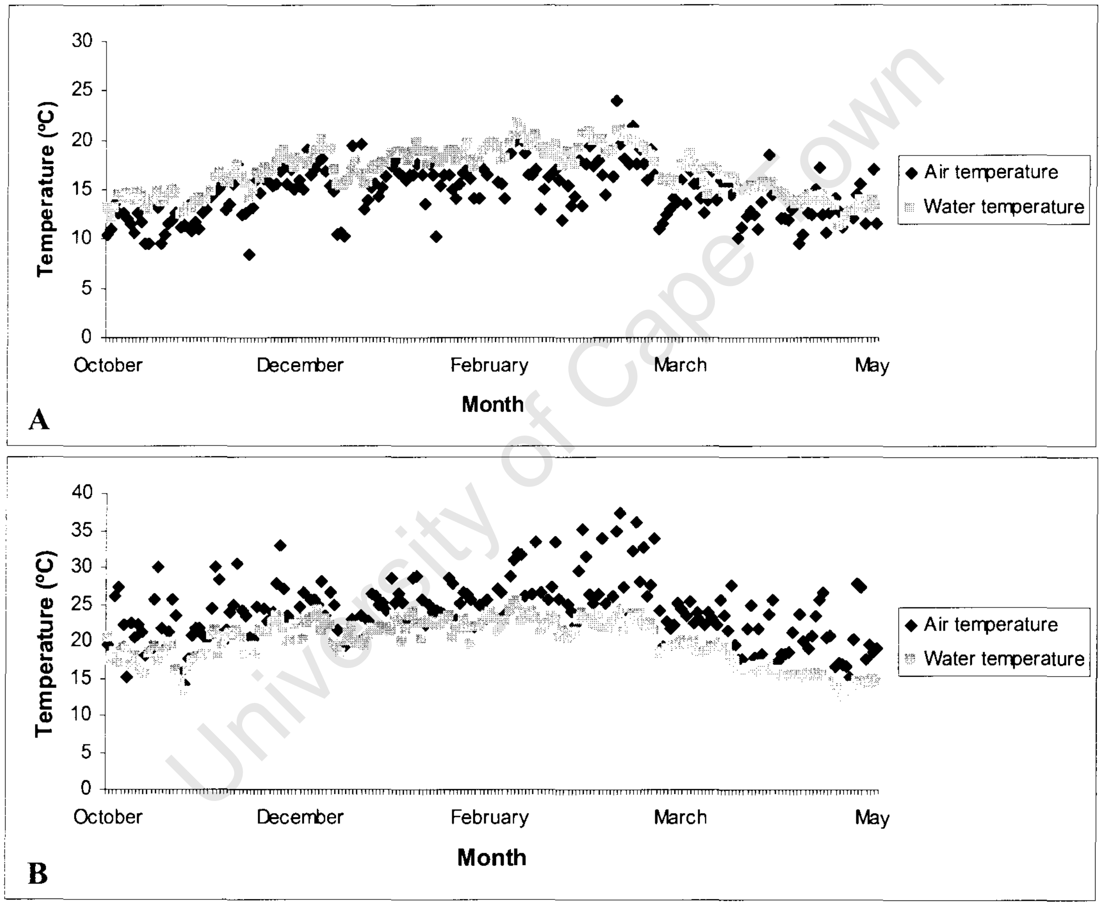

Figure 4.3. The relationship between the A) minimum and B) maximum air and water temperatures at site 5 in the sun for the months October 2008 to May 2009.

The average water temperature increased down the length of the stream from October 2008 to May 2009 (Fig. 4.4). The difference in water temperature between the sites was significant (F = 349, d.f. = 1947 $p<0.01$). There were significant differences in water temperature between all of the sites except sites 5 and 6 ($p = 0.70$).

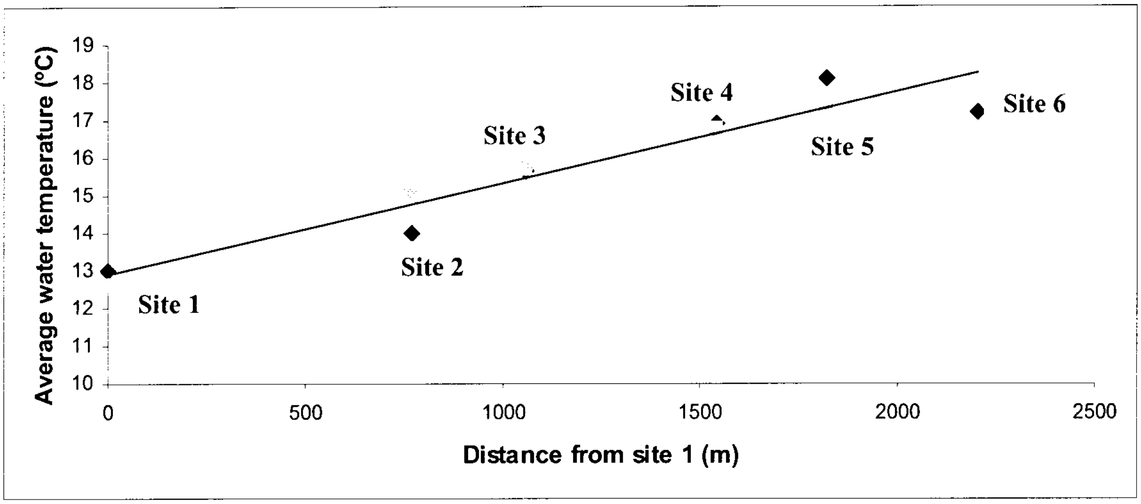

Figure 4.4. The average water temperature from twelve temperature loggers at the six sites along Window Gorge Stream (black diamond: sun, grey square: shade).

When comparing the average water temperatures found in the sun and in the shade at each site (Fig 4.4), a significant difference was found at sites 1, 2, 5 and 6 (Table 4.3). The difference, however, between these water temperatures was expectedly very small – the maximum difference was found at site 2 and was only 0.98°C. From the sites that were significantly different, the loggers in the sun at sites 1 and 5 were warmer than the ones in the shade, but the loggers in the shade at sites 2 and 6 were warmer than the ones in the sun. It is, therefore, difficult to make a conclusion regarding a difference in average water temperature between the loggers placed in the sun and the shade at each site.

Further water temperature analyses were done for logger 2.1 (sun) and 3.2 (shade) (Table 4.4). Logger 2.1 experienced lower temperatures on average and for each season than logger 3.2, consistent with its position higher up the reach of the stream, but the average and maximum diel range was higher for logger 2.1.

Table 4.3. The difference between the average water temperatures in the sun and the shade at each site along Window Gorge Stream from October 2008 to May 2009 (bold – significant difference).

Sites	**Mean (sun)**	**N (sun)**	**Mean (shade)**	**N (shade)**	**t-value**	**df**	**p-value**
1.1 (sun) vs. 1.2 (shade)	13.0	166	12.6	215	2.33	379	**0.0204**
2.1 (sun) vs. 2.2 (shade)	14.0	75	15.0	151	-4.05	224	**0.0001**
3.1 (sun) vs. 3.2 (shade)	15.7	109	15.7	215	-0.08	322	0.9333
4.1 (sun) vs. 4.2 (shade)	16.9	205	16.8	215	0.77	418	0.4417
5.1 (sun) vs. 5.2 (shade)	18.1	202	17.3	57	2.07	257	**0.0394**
6.1 (sun) vs. 6.2 (shade)	17.2	132	18.0	211	-3.16	341	**0.0017**

Table 4.4. Analysis of temperature data from sites 2 and 3 along Window Gorge Stream from October 2008 to August 2009 (Spring: October; summer: November-March; autumn: April-May; and winter: June-August).

Water temperature analysis	**Logger 2.1 (ºC) (site 2, sun)**	**Logger 3.2 (ºC) (site 3, shade)**
Overall mean	12.1	14.6
Overall standard deviation	2.08	2.31
Mean minimum	11.4	14.2
Mean maximum	12.9	15.1
Average diel range	1.4	0.8
Maximum diel range	9.5	5.5
Average spring temperature	13.1	13.8
Average summer temperature	13.8	16.1
Average autumn temperature	13.4	14.7
Average winter temperature	10.5	11.5

Water quality

There was no significant difference between the sites for conductivity [H = 3.53 (df = 5, N=23), p = 0.6,], nor for oxygen saturation [H = 1.77 (df = 5, N=23), p=0.9]. There was, however, a significant difference between sites for pH [H = 18.12 (df = 5, N=23), p = 0.003). This significant difference was only between sites 1 and 6 with the mean pH for site 1 being 4.03 and for site 6 being 5.78. The average oxygen saturation values were fairly high for the months October, December and May (between 88% and 93%), but were low for February (52.5%) when the stream flow was slower as there was less water in the stream. There was no water at site 6 in February and no flowing water except for some small pools at sites 3 and 4 in April. (See appendix 3 for average water quality variables.)

Comparison of field-based and laboratory-based identifications

Of the species identified in the laboratory for five samples, on average 77.5% of the families were identified in the field using the SASS guide. The number of individuals per taxon estimated in the field was slightly under- or over-estimated, on average 13% of the time. The families not noted in the field included those with low abundance in the samples (e.g. one or two individuals). The only family that was not noted with more than a few individuals present was Chironomidae. This only occurred for two samples out of the five samples taken and could be because the chironomids are fairly small in size.

Community composition

The highest number of species and the Margalef's index showed the differences in species richness at each of the sites (Table 4.5). Site 4 had the highest species richness (*d* = 2.22), while site 3 had the lowest species richness (*d* = 1.78). The number of species recorded at sites 1 to 4 was similar (between 18 and 19 different species), while sites 5 and 6 had a lower number of species (15 species).

Table 4.5. The highest number of species and Margalef's index as a measure of species richness recorded at each site along Window Gorge Stream from August 2008 to May 2009.

Site	Number of species (S)	Margalef's index (*d*)
1	19	2.17
2	18	1.93
3	18	1.78
4	19	2.22
5	15	1.86
6	15	2.19

The Shannon diversity index showed differences between the six sites for the months sampled from August 2008 to May 2009 (Fig. 4.5). Site 1, near the source of the stream, had the lowest measure of diversity, while sites 4-6 had the highest values. The diversity increased slightly from site 1 to 4 and then leveled out after site 4.

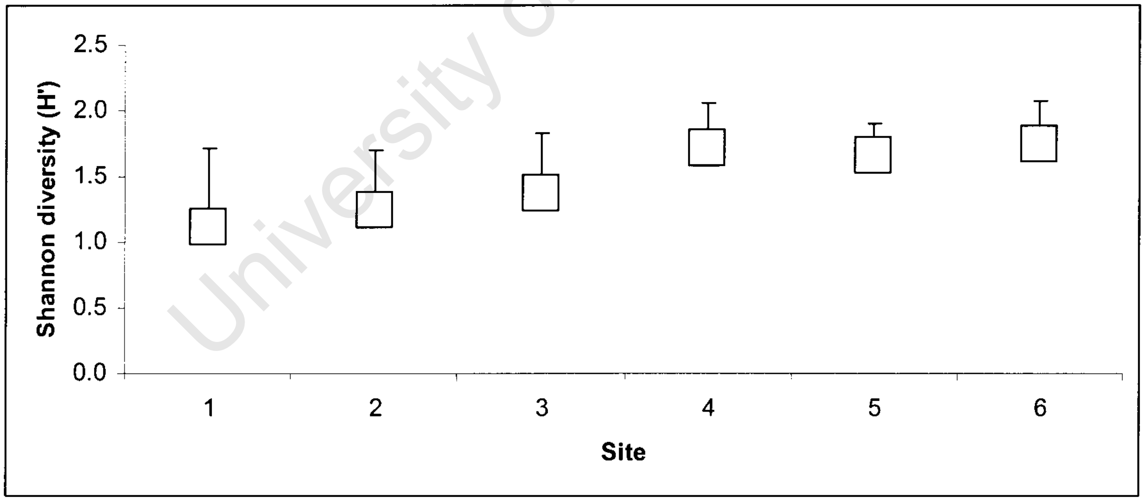

Figure 4.5. Average Shannon diversity (H') and standard deviation for each site along Window Gorge stream sampled every two months from August 2008 through to May 2009. (Site 1 is near the source, site 6 the mountain foothill site).

Since there were already differences in species diversity among sites, the seasonal variation for each site had to be calculated separately (Fig. 4.6). Species diversity was highest in the months August, October and May for the six sites. During December the diversity was very low at the top three sites, but higher at sites 4, 5 and 6. In

February and April the species diversity was lower at all sites compared to the other months. Samples in February and April were less as the stream was dry in parts during these months of sampling.

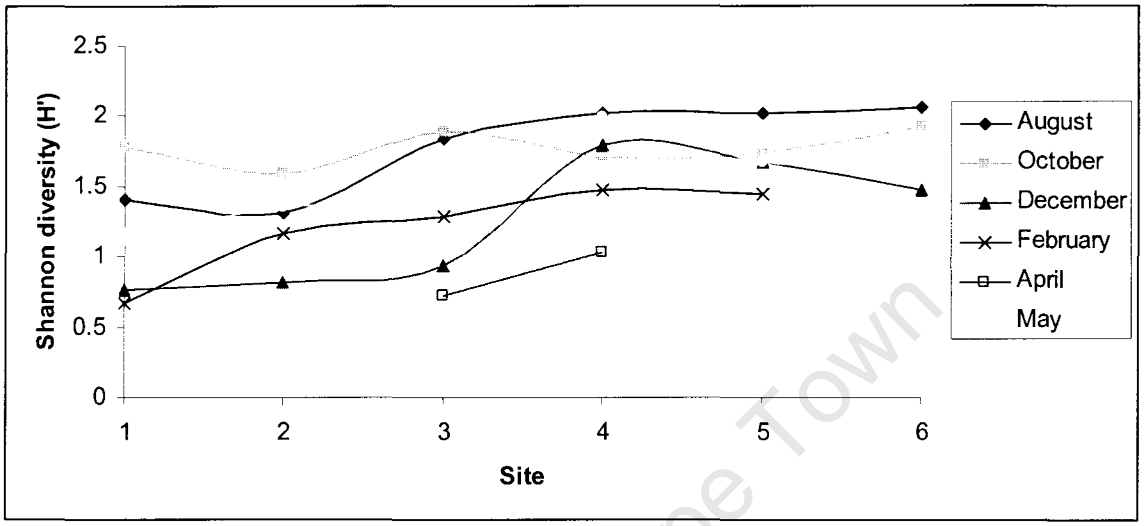

Figure 4.6. Average Shannon diversity (H') for each site for each month from August 2008 through to May 2009 sampled along Window Gorge Stream. (Site 1 is near the source, site 6 the mountain foothill site).

The MDS plot and cluster analysis show that site 1 had a species composition very different to the rest of the sites (Figs. 4.7, 4.8), while sites 4, 5 and some of 6 formed a discrete grouping. Sites 2 and 3 did not separate clearly from one another based on species composition, and appeared to represent transitional communities between sites 1 and 4.

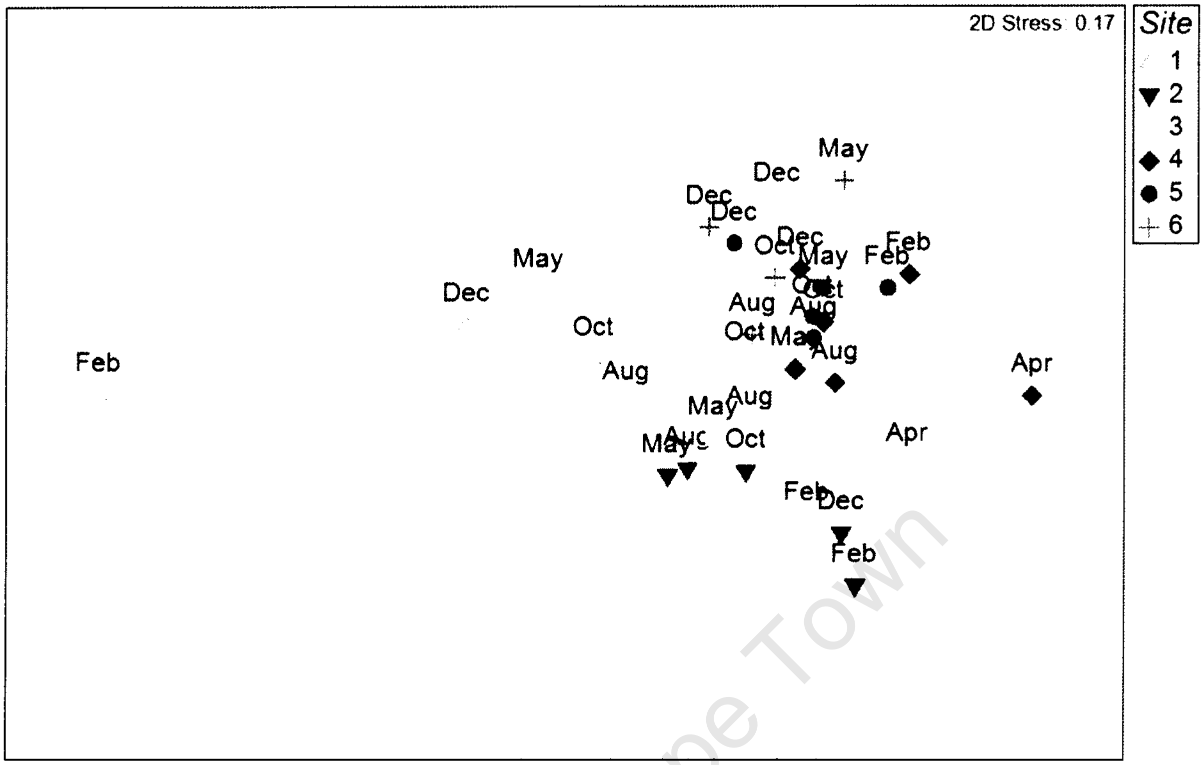

Figure 4.7. 2-dimensional MDS plot of the abundances of the 16 most common species for the sites down the length of Window Gorge Stream sampled every two months from August 2008 to May 2009.

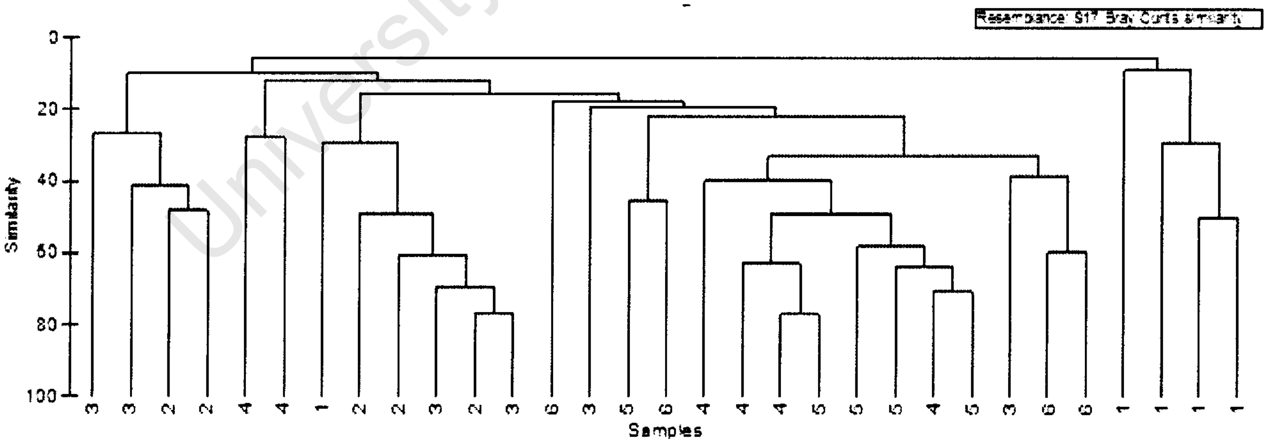

Figure 4.8. Cluster analysis of the abundances of the 16 most common species for the sites down the length of Window Gorge Stream sampled every two months from August 2008 to May 2009.

Site 1 was very different from the other sites (average dissimilarity between site 1 and other sites was 94.24%) as there were large numbers of *Nebrioporus* sp., Dytiscidae in December, February and May (up to 20 000 individuals in the February samples). The dissimilarity between sites 2 and 3 was only 69.27% and these two sites had the highest numbers of amphipods (*Paramelita nigroculus*). Sites 4 and 5 were very similar in species composition, reinforced 1) by the ANOSIM result indicating that sites 4 and 5 did not have significantly different species composition ($p > 0.05$) and 2) by having the lowest dissimilarity value of 58.23% in the SIMPER analysis. The two most important species linking these sites are *Lestagella penicillata* (Teloganodidae), and *Simulium (*subg: *Nevermannia)* sp. (Simuliidae). Sites 5 and 6 also had a low dissimilarity value of 67.95% and shared the species *L. penicillata* and *Acanthiops* sp.

Community structure of the sites differed seasonally and showed different degrees of inter-seasonal species turnover (Fig. 4.7). The community of site 1 in February was quite different than the other monthly communities for that site; sites 4 and 5 had uniform community composition for most of the year, while sites 2, 3 and 6 all had variable community composition across seasons.

When the patterns seen in the community clustering (Fig. 4.8) were related to the water quality data, the best fit was a combination of pH and oxygen saturation ($r = 0.524$, $n = 23$, $p < 0.05$). The second best fit was the combination of water temperature, pH and oxygen saturation ($r = 0.478$, $n = 23$, $p < 0.05$). Therefore these three variables can best describe the changes in community composition 1) down the length of the stream and 2) across seasons.

Oxygen saturation, pH and water temperature were associated with the biotic clusters for sites and months (except April) graphically (Fig. 4.9). At most sites oxygen seemed to be fairly uniformly high with unusually low levels at only two sites, during the month of February. The pH and water temperature both increased along the altitudinal gradient (Fig. 4.9). There was no definite seasonal pattern of pH variation at each of the sites. The lowest water temperatures were recorded at site 1 during the months of October and May.

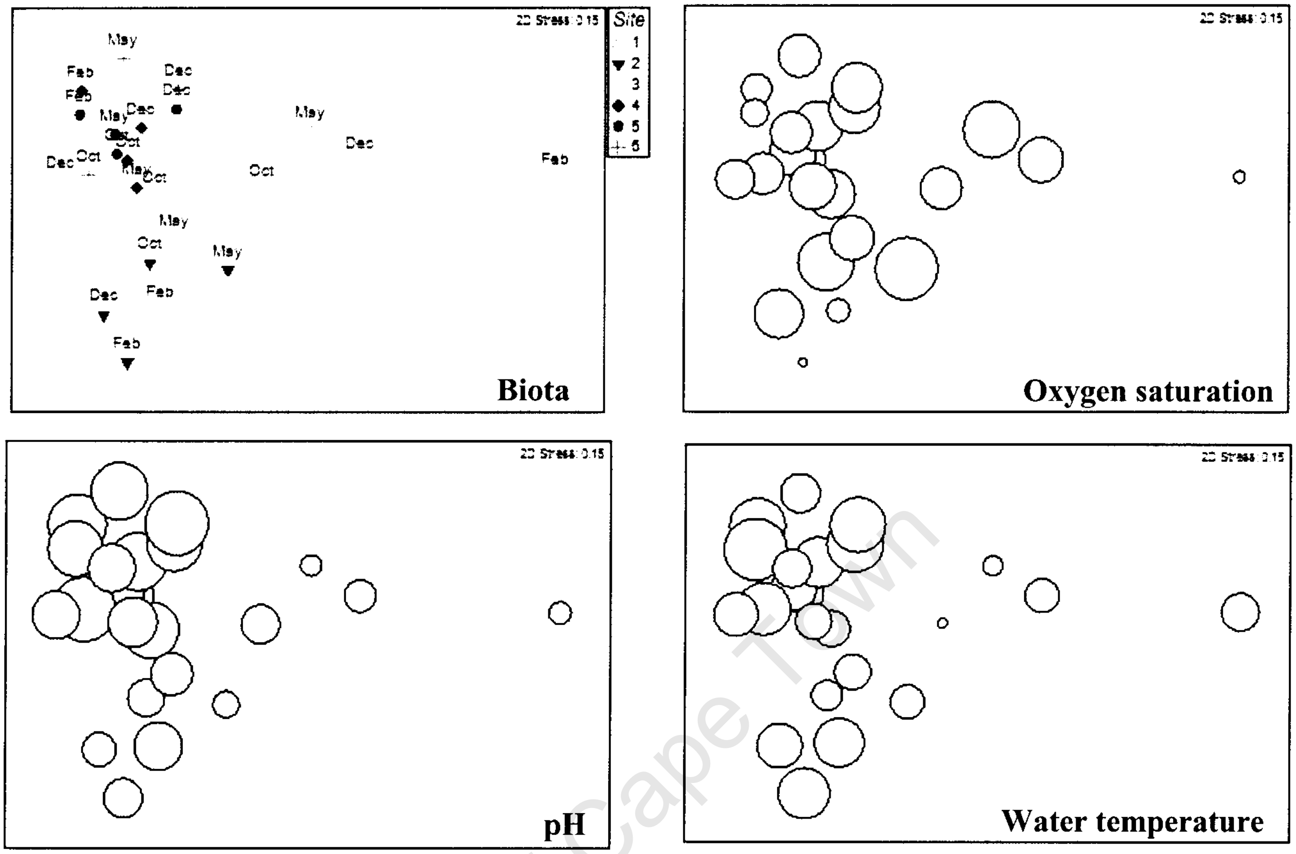

Figure 4.9. 2-dimensional MDS plot for the sites down the length of Window Gorge Stream sampled over an eight month period, with superimposed circles of increasing size representing increasing oxygen saturation, pH and water temperature.

4.4 Discussion

Stream temperatures

Stream temperature is of prime importance for water quality and the 'health' of the stream ecosystem (Meyer *et al.* 1999, Morrill *et al.* 2005). Overall the average stream temperatures were found to be fairly low at each of the six sites along Window Gorge Stream (between 12.6 and 18.1°C) for the months of October through to May when there was water in the stream. Temperatures for developing nymphs were fairly uniform and low. Winter stream temperatures were collected for two loggers and the average was 10.5°C at site 2 (sun) and 11.5°C at site 3 (shade).

Although the overall average temperature at site 2 was lower than at site 3, while the diel range was higher at site 2. The site 2 logger was in an unshaded part of the river, and thus would be expected to have a higher diel range. The average temperature for site 2 does not cover all months, as a large portion of the summer data had to be removed as the logger was out of the water most of the time when the stream began to dry out.

Overall, the average stream temperatures along the length of the stream increased from site 1 to site 6 for the period from August 2008 to May 2009. There are many factors that influence stream temperature – land-use, riparian vegetation, the temperature of incoming water, abstraction and potentially climate change (Nebeker and Lemke 1968, Quinn *et al.* 1994, Morrill *et al.* 2005, Dallas 2008). The land-use around Window Gorge Stream is very low and consistent down the length of the sampled section of the stream, and includes hiking paths and KNBG. Site 6 is, however, different as it is situated below KNBG, within the residential suburb of Bishopscourt and is no longer protected and is therefore impacted upon by outside pollution from litter and run-off.

With regards to riparian vegetation, parts of the stream are covered by Southern Afrotemperate Forest (Mucina and Geldenhuys 2006) while others are not. However, the canopy is fairly uniform, and gaps are small. There were no large differences in the average stream temperature between the shade and unshaded areas at a site, which is not unexpected, given the small horizontal distance between these contrasts at any site. The maximum difference was found at site 2 and was only 0.98°C. For sites 1 and 5 the loggers in the sun were significantly warmer than the ones in the shade, but for sites 2 and 6 the loggers in the shade were significantly warmer than the ones in the sun. The removal of riparian vegetation is known to increase stream temperatures (Beschta 1997, Naiman and Décamps 1997, Poole and Berman 2001, Dallas and Day 2004), pointing to the importance of maintaining naturally occurring forest along Window Gorge Stream. The only break in the canopy, at site 2 for example, is currently through natural tree death. However, other streams in the Cape Floristic region may suffer loss of canopy cover through fire or active deforestation.

The temperature of source water also influences stream temperature. Window Gorge stream is fed by groundwater from the Table Mountain Group Aquifer, which is cool. This probably maintains the constant low temperatures recorded for this stream. Poole and Berman (2001) noted that the baseline temperature of streams fed by groundwater approximated the groundwater temperature. However, with reduced water flow in the summer months, warming can occur, and might be a factor in unshaded parts of the stream.

Abstraction of water is an important factor influencing water temperature for most of South Africa's rivers and streams. KNBG uses some water from streams flowing through their garden for irrigation purposes. The linear relationship between abstraction volumes and increases in water temperature (Poole and Berman 2001) suggests that the temperatures in Window Gorge stream may already be affected by this abstraction. It is therefore important that this abstraction is managed properly, and includes monitoring that enables an assessment of this potential impact.

Climate change will potentially influence all stream temperatures as air temperatures rise. For the majority of the 43 streams in 13 countries compared by Morrill *et al.* (2005) the stream temperature increased about 0.6-0.8°C for every 1°C increase in air temperature. The predicted increase in air temperature of approximately 1.5°C for the coastal parts of the Western Cape Province by 2050 (Midgley *et al.* 2005) is likely lead to an increase in water temperature for the streams in the area. However, streams that are largely fed by groundwater as opposed to precipitation are likely to have a dampened temperature response.

Community changes along altitudinal and seasonal gradients

The species richness was highest at site 4 and lowest at site 3. Diversity was greatest in the 'mountain stream' part of the river (site 4) and lowest at the 'source' (site 1) and 'mountain torrent' (site 2) zones. Site 1 is located on the plateau of Table Mountain, near the source of the stream and is therefore fairly isolated. While it is covered by low shrubs, it does not benefit from the allochthonous leaf input of other sites which are covered by forest comprising large (10m high) trees. Site 2 is situated in the 'mountain torrent' zone with large boulders and steep gradient, thus few organisms can attach and survive here. Sites 3, 4 and 5 are in the 'mountain stream' zone where the flow of water is somewhat slower than in the 'torrent' zone. This area offers greater attachment area, more nutrients in the form of allochthonously-derived leaves, and high oxygen levels. The species diversity was more or less the same at sites 4, 5 and 6. Site 6 is in the upper foothill zone which has similar conditions to the mountain stream zone, but is prone to warming in summer when flows are reduced considerably, and the stream runs in the rithron for most of its length, only appearing occasionally in the form of rock pools.

The highest species diversity was found in months August, October and May while the lowest was found in April. In April most of the stream was dry, with the river flowing in the rithron during parts of February, March and April, with only two sites being sampled in April. Window Gorge Stream receives winter rainfall, with life cycles being initiated after the first autumn rains (Picker pers. comm). King (1983) found abundance levels to be lower in winter and peak in spring in the Eerste River also in the Western Cape of South Africa. Similarly therefore diversity would be expected to peak in spring. This was confirmed with the highest diversity levels recorded for all sites in August, October but high diversity levels were also found in May (samples were not collected during June or July). High levels of diversity are therefore also found in the winter months in Window Gorge Stream.

In addition to sites 4, 5 and 6 having the highest diversity scores, they also clustered in terms of species composition. In particular, the communities of sites 4 and 5 were very similar (Fig. 4.7). Sites 2 and 3 were also very similar to one another in species composition, and appear to represent a transitional community between that present at site 1, and sites 4 and 5. There was also a difference in the community structure at the sites over the different months where the summer communities (December and February) were different from the autumn and winter communities. Temperature and/ or rainfall differences may explain this.

The community structure was best explained by a combination of pH and oxygen saturation, but the combination of water temperature, pH and oxygen saturation also correlated significantly with the clustering patterns of the sites by season. Even though a correlation is found between community structure and the water quality variables it is difficult to make a conclusion as both may be affected by other unknown co-varying variables. Since oxygen levels were fairly high at all times, slight variations appear to be biologically important, as they influence community structure. Water temperature increased with decreasing altitude, and pH may be influenced by the release of humic compounds from decaying leaves. The latter is probably most important at sites 4-6, where the effect of allochthonous organic input is expected to be greatest. Water temperature also affects dissolved oxygen as the concentration of dissolved oxygen decreases with an increase in temperature and therefore temperature may be one of the most important variables for aquatic organisms.

Future monitoring and climate change

The community composition data coupled with the water temperature data provide an important reference for future monitoring of possible changes to the ecology of Window Gorge stream related to climate change. The temperature tolerance information and the identification of thermally sensitive species (Chapters 2 and 3) provide a useful reference for future monitoring. Thermally sensitive species can be used as indicator species (e.g. *Aphanicerca capensis* species complex), where changes in abundance and distribution could be used to track impacts of climate change. *A. capensis* was found at all six sites and therefore all the sites are vulnerable to climate change impacts.

Thermal sensitivity experiments should be expanded to include other potentially sensitive indicator species. However, the *A. capensis* species complex has representatives in most of the Western Cape Province streams (Stevens 2009), and might provide a very useful species in the detection of thermal pollution, or thermally altered stream profiles.

For rapid onsite monitoring of stream fauna, the SASS protocol has been shown to be a useful bioassessment tool for streams. The comparison of field-based to laboratory-based identifications of the same sample showed that on average 77.5% of the individuals present were identified in the field, and the number of individuals was comparable, which is fairly acceptable. For a quick assessment of the macroinvertebrate community composition at a site the SASS protocol can therefore be applied and the individuals identified and counted in the field. *A. capensis* species complex would possibly be confused with other genera of Notonemouridae, so further thermal sensitivity tests would have to done on these before the family itself could be used as an indicator of a thermally sensitive species. Measurement of water temperatures would need to be carried out in combination with SASS to evaluate changes in the presence and abundance of thermally sensitive taxa.

In 2009 large sections of Window Gorge Stream ran dry from February until May. This could be due to the very hot and extended summer experienced in Cape Town at the start of 2009, where late summer (April) air temperatures reached 35°C. During the February sampling period was not all sites were able to be sampled, as site 6, was completely dry at this time of year. In April only sites 3 and site 4 retained some water in the form of small pools. While this may be an abnormal flow pattern (in April 2008 there was water in the stream), more detailed records of flow rates need to be added to the baseline data measurements for this stream. This will enable the effects of climate change on stream flow and its influence on the biota, to be evaluated more accurately.

Climate change is predicted to cause longer and drier summers for the Western Cape (Midgley *et al.* 2005), which suggest that the pattern observed in 2009 of the stream running dry for an extended period of time will most likely become the norm, not only for Window Gorge stream, but possibly for other streams in the Western Cape Province. Longer periods of drying out will have consequences for the ecosystem as a whole as the conditions change. The organisms living in these streams may not be able to survive the dry conditions, with semivoltine species being impacted most directly as they take a number of years to develop. The dry conditions may have a far more dramatic impact than gradually rising water temperature. The two are, however, related and slower flow rates preceding drying out will exert their own effects on developmental rates, life history parameters and community structure. The full suite of interacting physical and chemical water parameters that are altered by reduced rainfall and rising temperatures need to be considered in their impact on the biota.

Conclusions

This is the first study to describe the aquatic community of a Table Mountain stream, and together with the recorded stream temperatures provides an important baseline for future monitoring of possible changes related to climate change. The community composition differed between the sites and sites 2 and 3 appeared to represent a transitional community between site 1, and sites 4 and 5. The community composition also differed between the summer and winter months sampled. Overall stream temperatures were fairly low but did increase from site 1 to site 6. The combination of autocorrelated variables temperature and oxygen best explained community structure down the length of the stream. It is important that abstraction from this stream be managed properly because together with dry, hot, long summers it may result in very little to no water in the stream and predicted climate change can potentially worsen this.

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Chapter 5 – Summary and conclusions

Water temperature influences survivorship, growth, metabolism, fecundity, abundance and the distribution of aquatic organisms (Brittain 1983, Sweeney and Vannote 1986, Quinn *et al.* 1994, Cox and Rutherford 2000a). Stream temperatures are affected by human impacts such as pollution, water abstraction and removal of riparian vegetation). Climate change, through increased greenhouse gas emissions, will also affect stream temperatures (Hogg and Williams 1996, de Wit and Stankiewicz 2006, Menéndez 2007, Hassall *et al.* 2007). An increase in temperature and a change in precipitation patterns, including more extreme flooding and droughts, have been predicted worldwide (Milly *et al.* 2005, Alcamo *et al.* 2007). In the Western Cape Province, in particular, temperatures are predicted to increase, and the winter rainfall decrease (Midgley *et al.* 2005), factors conducive to warming of river and stream temperatures.

Thermally sensitive species are threatened by increases in water temperature (Quinn *et al.* 1994, McKie *et al.* 2004), but currently very little information exists on the thermal tolerances of aquatic invertebrate species in South Africa. Variation in thermal tolerance is to be expected, with Gondwanan and other relictual species likely to be limited in their thermal tolerance. The collection of baseline data on the thermal tolerances of a range of aquatic species as well as the recording of stream temperatures and associated invertebrate communities is fundamental to establishing a baseline for South Africa against which to measure future change of environmental parameters and biotic responses.

This project attempted to produce some baseline data for streams in the Western Cape Province. The main objectives of the study were:

- To determine thermal tolerances of a range of aquatic insect species from two different bioregions in the CFR using lethal (LT_{50}) and critical thermal maxima (CTM) experiments

- To investigate the hypothesis that Gondwanan fauna (currently restricted to cooler temperatures) are more sensitive to thermal stress than non-Gondwanan fauna (may be eurythermic – adapted to wide range of temperatures)
- To establish the relationship between invertebrate community composition and water temperature along longitudinal and temporal gradients in a Cape Peninsula mountain stream.

Temperature tolerance of stream fauna

Two methods were used to determine the temperature tolerance of a number of aquatic insect nymph species from three families, namely Plecoptera, Ephemeroptera and Trichoptera. These methods were the static lethal temperature (LT_{50}) experiments and the dynamic Critical Thermal Maxima (CTM) experiments.

In addition to comparing thermal tolerances of different species within two nearby stream systems, thermal tolerances of the same taxon (the stonefly *Aphanicerca*, family Notonemouridae) were compared in two populations, each drawn from different bioregions. The streams from the southwest fynbos bioregion were Window Gorge and Skeleton Gorge streams on Table Mountain, and the south coast fynbos bioregion streams sampled were the Keur River near George and the Salt River near Plettenberg Bay (Rutherford *et al.* 2006).

From the *A. capensis* species complex (Stevens 2009), form C (Table Mountain) and undescribed form (Salt River) ranked as the most thermally sensitive species for both types of experiments. For *A. capensis* (form C) the LT_{50} and CTM values were 23.4°C and 29.75°C respectively, while for *A. capensis* (undescribed form), the values were 25°C and 31.29°C respectively. This Gondwanan family, Notonemouridae, is restricted to the cool, upper parts of mountain streams, and thus exists at the limits of its thermal range. It has not managed to invade any other thermal habitats indicating that temperature is probably the limiting factor governing their distribution but this may not be the sole factor. *A. capensis* (and likely other notonemourid stoneflies) can thus be considered to be thermally conservative in terms of their current habitat usage and may potentially be used as an early indicator of changing stream temperatures in the Western Cape Province.

Of the two types of experiments used the CTM experiments were the easiest to conduct and have many advantages as fewer individuals are needed and most individuals survive the experiment. For the species tested in both experiments, their tolerance levels ranked the same, except for *Athripsodes* sp. but this could be due to the difficulty in identifying the end point for caddisflies in their cases in the CTM experiments. Either of the two experiments can be used to determine the relative thermal sensitivity of species, but attention needs to be given to those species' whose end points are difficult to determine using the CTM experiments.

The results of the CTM experiments indicate the thermal sensitivity of species but use unnaturally acute exposures to temperatures far greater than would ever likely occur in nature. The results from LT_{50} experiments can be compared better to the natural conditions than the CTM experiment results (Cox and Rutherford 2000b), however, both fail to address the influence of slightly elevated temperatures on life history parameters other than mortality. Neither approximates the natural exposure conditions of lengthy exposure at slightly elevated temperatures. Consequently, the main conclusions from such tests relate only to the relative sensitivity of various species. In order to compare the experiments to naturally-occurring thermal stress, which is typically of longer duration and less severe, it is recommended that LT_{50} experiments of longer duration and sublethal effects be investigated.

Relative thermal sensitivities of Gondwanan vs. non-Gondwanan fauna

From the CTM experiments it was found that the Gondwanan mayflies (*Lestagella penicillata* and *Castanophlebia* sp.) were more sensitive than the non-Gondwanan mayfly (*Acanthiops* sp.). The CTM value for *Castanophlebia* sp. was 32.18ºC, for *L. penicillata* it was 32.91ºC and for *Acanthiops* sp. it was 34.14ºC. The CTM value for the Gondwanan caddisfly (*Barbarochthon brunneum*) was 35.13ºC which was, therefore, more tolerant than the non-Gondwanan caddisfly (*Athripsodes* sp.) with a CTM of 32.04ºC. This suggests that thermal tolerances may be related both to habitat adaptations and the evolutionary history of the taxon.

Within a single stream different species showed varying thermal tolerance, suggesting that genetics and evolutionary history also play a role in this trait. One would expect that species would demonstrate some local thermal adaptability, and although very few studies have addressed this, McKie *et al.* (2004) identified Gondwanan chironomid species in both temperate and tropical areas and found the species in the tropical area were more tolerant than the same species in the temperate area. Two forms from the *A. capensis* species complex (Stevens 2009) were tested for their thermal tolerance, namely form C from the western and an undescribed form from the southern Cape. These two forms differed in their thermal tolerances with the undescribed form of *A. capensis* being slightly less sensitive than *A. capensis* (form C). This further confirms local thermal adaptability and the southern Cape population could tolerate slightly higher temperatures. Unfortunately it was not possible to obtain adequate numbers of individuals to enable a contrast of Gondwanan and non-Gondwanan species from the same stream/river.

Stream temperatures and community composition

Continuous monitoring of stream temperatures and associated invertebrate communities is another crucial process in the establishment of baseline data against which changes resulting from global warming can be compared. Temperature loggers installed at six sites in Window Gorge Stream in October 2008 showed that water temperatures were fairly low (averaging between 12 and 18ºC) at all the sites when there was water in the stream. For most of the summer months (particularly from February through to April/May) the stream was dry and flowed slowly in the rithron, as a result of high summer temperatures and relatively little rainfall.

The same temperature loggers will be used to continue to monitor stream temperature in the future to identify any changes due to human impact or climate change. South African National Parks Board (SANParks) will take over the monitoring of the stream, and use it a representative stream for the many that flow off Table Mountain. Since there is interest in tapping the Table Mountain aquifer, this extends the importance of establishing a baseline stream for future monitoring. Flow readings should be incorporated in future monitoring of the stream.

Community composition data was collected from August 2008 to May 2009 at the six sites along Window Gorge Stream. The highest number of species was found at sites 1-4, while sites 5 and 6 had less species. The Margelef's index indicated that site 4 had the highest species richness. The Shannon diversity index indicated that sites 4-6 had the highest species diversity, while site 1 had the lowest. Further more species diversity was higher in the months of May, August and October, while lower species diversity was found in February and April.

Temperature was one of three measured variables that explained community structure across all six sites. The combination of temperature and oxygen best explained community structure down the length of the stream, however, these two variables are probably auto-correlated as temperature controls the concentration of dissolved oxygen.

The species used in the thermal tolerance experiments from Skeleton Gorge and Window Gorge Streams can be compared to the community composition data collected, to identify where and when they occurred in the stream. *Aphanicercopsis tabularis* was the most sensitive species in the CTM experiments and was found only during August, October and May never at site 6. *A. capensis* the second most sensitive species in the CTM experiments was found at all sites and for most of the months. *Castanophlebia* sp. and *Lestagella penicillata* displayed intermediate thermal sensitivities. *Castanophlebia* sp. was found only at sites 3-6 and mostly in the cooler months, August, October and May. *L. penicillata* was found during all the months, but never at site 1. *Athripsodes* sp., the most tolerant in the LT_{50} experiments, was found at all sites and in all months.

The South Africa Scoring System (SASS) protocol (version 5, Dickens and Graham 2002) was applied to some of the samples in the field. The results from comparing in-field identifications to those identified in the laboratory indicate that SASS can be used as a tool to estimate community responses that might be related to thermal stress. SASS gave a fairly good approximation of the community composition. The field identifications identified 77.5% of the taxa and the estimated numbers of individuals was under- or over-estimated 13% of the time. Identifications to family level in SASS may appropriate as thermal stress may be specific for a family, but this still needs to be tested by widening the range of species used in the thermal tolerance tests.

Combining experimental and field data on thermal responses

Both the experimental and field data are important in establishing baseline information for monitoring trends in the face of climate change. The experiments identify which species are most thermally sensitive. Field sampling can then focus on the collection of information on the abundance of the most thermally sensitive species (viz. *A. capensis* species complex), which could thus be used as indicators of future thermal changes in the stream.

Stream temperatures taken during the developmental period of nymphs should be used as a reference for the temperature at which control experiments are run in the laboratory. For Window Gorge Stream the average stream temperatures in May and June were 13.4°C and 11.6°C respectively. *A. capensis* (undescribed form) from the Salt River was collected in May and the average stream temperature was 14.1°C. These stream temperatures are somewhat below the thermal tolerances of the species tested. For example the LT_{50} value for *A. capensis* (form C), the most sensitive species, was 23.4°C.

It is possible that some species may be forced to endure elevated temperatures in nature for brief periods, which approximate LT_{50} values for that species. In the case of Window Gorge Stream, this would include days that the water temperature is above the specified temperature or when the stream is dry, both of which are unsuitable. From the temperature logger data collected from Window Gorge the number of days above the LT_{50} value for *A. capensis* (23.4°C) was averaged for the six loggers down the length of the stream in the sun. The average number of days above 23.4°C was 46 days (between October 2008 and May 2009). However, for the majority of this time the stream was flowing very slowly or in the rithron. However, life cycles are typically timed to coincide with the maximum stream volumes, viz. winter, avoiding periods of slower flow rates. This would buffer the stream fauna from elevated temperatures, which are likely to have their greatest impact in summer.

Climate change

It has been predicted that temperatures will increase and winter rainfall will decrease in the Western Cape Province (Midgley *et al.* 2005). The summer of 2008/2009 in

Cape Town was exceptionally warm and long, leaving most of Window Gorge Stream dry from February until the first rains in May. Nymphs first made their appearance at different times in 2008 and 2009. They were present in April in 2008, but in 2009 were only present towards the end of May and beginning of June. This would influence development time for univoltine species, which would impact on their emergence time.

In cases of late rainfall triggering late hatching, life cycles could continue into that part of the year (early summer) when drying out or warming could negatively impact on development. Both factors could result from climate change. However, it should be noted that many of the mountain streams in the Western Cape Province are temporary, yet share the same univoltine taxa as perennial streams. More importantly semivoltine species, which take a few years to develop, may be eliminated when the stream runs dry. With climate change these dry conditions are likely to become the norm meaning that the organisms in the stream may not be able to survive the unpredictable rainfall and dry conditions. Univoltine species, normally found in predictable environments, may be replaced by multivoltine species which characterise unpredictable environments. Monitoring communities which include both uni- and multivoltine species (as is the case for Window Gorge) would reveal such possible impacts. The interaction and auto-correlation between water oxygen levels, temperature and flow rate require that all of these variables be considered when evaluating the impact of climate change.

Conclusions

- *A. capensis* was the most thermally sensitive species compared to the other species tested in the LT_{50} experiments, while *A. tabularis* was the most sensitive in the CTM experiments.
- *A. capensis* species complex members can potentially be used as indicators of changes in stream temperature.
- The CTM experiments were easier to conduct and ranked the thermal sensitivities of the species tested. The LT_{50} experiments can be used to further examine the responses of the more thermally sensitive species.

- The study has established baseline stream temperature recordings and associated community data against which changes resulting from global warming can be compared.
- The combination of autocorrelated variables temperature and oxygen best explained community structure down the length of the stream.
- The combination of the predicted decrease in rainfall and increase in temperature due to climate change will have an effect on physical and chemical water parameters and consequently impact on stream biota.

Future research

As an outcome of this project the temperature loggers will provide continuous thermal data, and ongoing community composition monitoring will take place along Window Gorge Stream by SANParks. This monitoring may potentially be expanded to include other streams on Table Mountain. The community composition monitoring can be more focussed to include indicator species sensitive to thermal stress instead of monitoring the entire community. Monitoring the entire community would, however, provide a better understanding of ecological responses to climate change. It is also important to monitor the flow rate of the stream as this could potentially show when the stream starts drying out and to identify what effect abstraction and the change in climate has on the stream.

Thermal tolerance experiments in the future should include more species to identify more thermally sensitive species. LT_{50} experiments of longer duration as well as sublethal effects need to be examined. A more detailed comparison of the thermal tolerances of Gondwanan and non-Gondwanan species should be undertaken, using a wider suite of species. For the CTM experiments different rates of temperature increase and different acclimation temperatures can be included to identify what effects this might have on the CTM value for a specific species.

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Appendix 1 – Species used in thermal tolerance experiments

Gondwanan

Plecoptera (stoneflies):

Plecoptera are one of the most important and dominant insect components of stream fauna (Wishart *et al.* 2003). The nymphs typically require cool, aerated waters, thus their distribution is limited to fast-flowing reaches of high altitude streams (Wishart *et al.* 2003). Stoneflies may be important for detecting changes in lotic temperature regimes as they form an important part of the stream food web and have been found to be good indicators of water quality (Ernst *et al.* 1984). Water temperature has been shown to influence growth in stoneflies. The temperature threshold for growth in stoneflies is generally low (Brittain 1983).

- **Notonemouridae**

The family Notonemouridae is endemic to the Southern Hemisphere. They are typically Gondwanan and have with relatives of the same family on the other southern continents (Stevens & Picker 2003). The nymphs are relatively small and don't possess gills (Stevens & Picker 2003).

- *Aphanicerca capensis* species complex (Tillyard 1931)

In *Aphanicerca* nymphs, the hairs on the inner margin of the antennae are longer than the other antennal hairs (Fig. 2). *A. capensis* (Common Cape stonefly) has recently been shown to comprise of a species complex (Stevens 2009). Members of this complex are the most common southern African notonemourids. The *A. capensis* complex occurs across the Western Cape of South Africa from the Cederberg in the north, through Table Mountain and the mountains of the south-western parts of the Western Cape to the Tsitsikamma Mountains in the east. Nymphs of this species are relatively large (9-12.8mm). The form of *A. capensis* found in Window Gorge stream is form C (Fig. 1). The abdomen appears smooth with some fine setae on the posterior margins of segments (Stevens & Picker 2003).

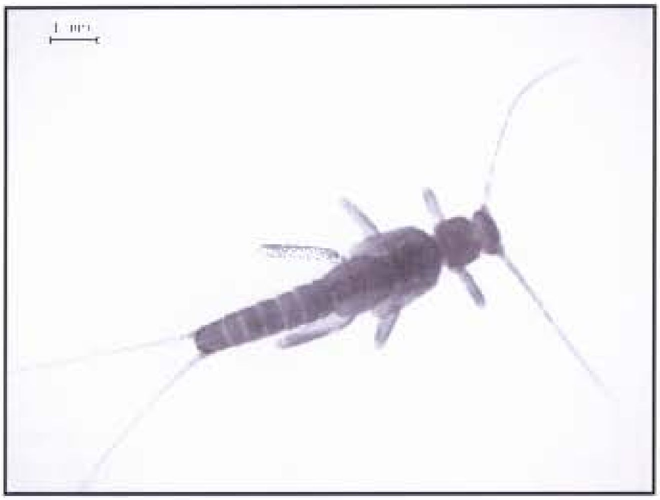

Figure 1. ***Aphanicerca capensis*** **(form C)**

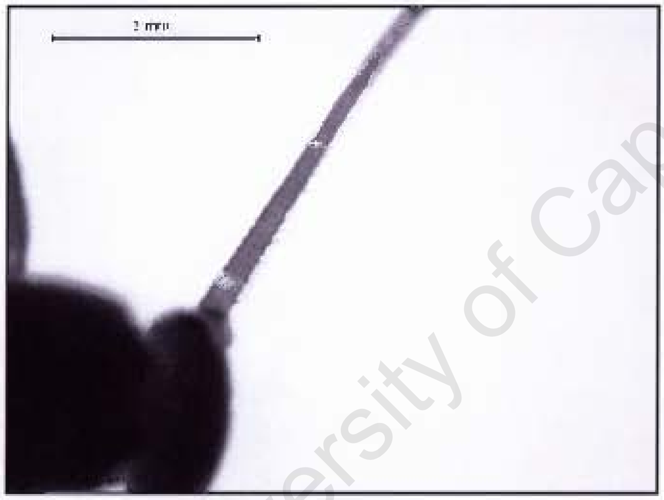

Figure 2. ***Aphanicerca capensis*** **(form C) – hairs on inner margin of antennae**

- ***Aphanicercopsis tabularis*** (Barnard 1934)

The nymphs of this species are small to medium in size (5.6-6.8mm) (Fig. 3). Short, stout bristles are found on the posterior margin of the abdominal segments (Stevens & Picker 2003). *A. tabularis* is endemic to the Cape Peninsula in the Western Cape of South Africa (Stevens & Picker 2003).

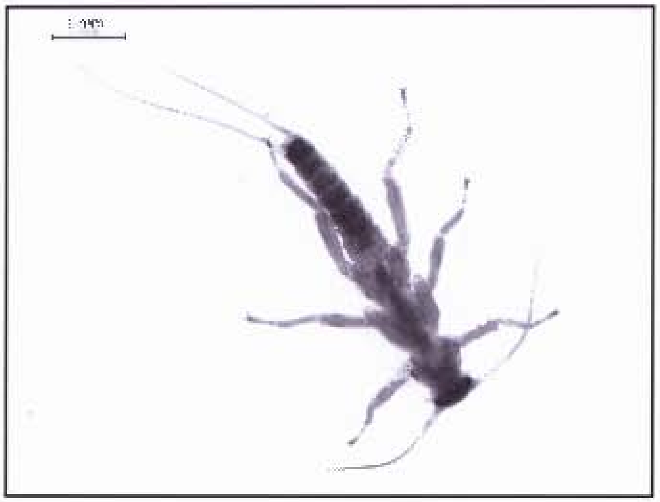

Figure 3. ***Aphanicercopsis tabularis***

Ephemeroptera (mayflies):

Changes in the climate in the past may have changed the availability of habitats and thus the pattern of mayflies (Barber-James *et al.* 2008). The only remnants of Gondwanan, cold adapted, taxa may be some of the endemic genera of the Leptophlebiidae, in the southern and south-western tip of Africa (Barber-James *et al.* 2008). Many studies have been carried out lately on the effects of climate change on mayflies. Climate change does have an effect on the behaviour and ecology of some mayflies, including early emergence (Barber-James *et al.* 2008).

- **Leptophlebiidae**

The family Leptophlebiidae (or 'prong gills') has a worldwide distribution and in the Afrotropics there are 16 genera (Barber-James & Lugo-Ortiz 2003). The different nymph species in this family are easily distinguished by their gills. Leptophlebid nymphs generally occur in flowing water, and are found on rocks, gravel, woody debris or roots (Barber-James & Lugo-Ortiz 2003). Leptophlebiidae nymphs are variable in their feeding habits as there are scrapers, collector-gatherers and a few are shredders.

- ***Castanophlebia*** (Barnard 1932)

There are only two known species, *C. albicauda* and *C. calida*, which are endemic to southern Africa and are common in the fast-flowing waters of mountain streams throughout South Africa and Lesotho (Barber-James & Lugo-Ortiz 2003). Mature nymphs are relatively large (11mm and cerci up to 18mm). A distinguishing characteristic of the nymphs is their long, slender sword-shaped gills (Fig. 4).

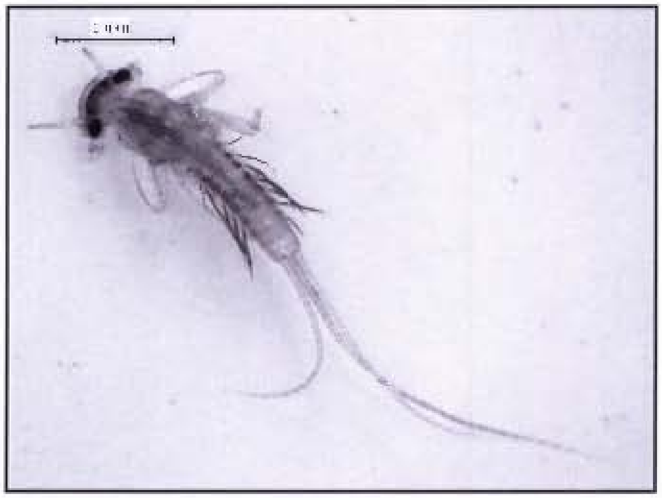

Figure 4. *Castanophlebia* sp.

- **Teloganodidae**

This family is Gondwanan in origin. Teloganodid nymphs generally have lateral, simple filamentous gills present on abdominal segment 1 and have a squat, almost dorsoventrally-flattened body (Barber-James & Lugo-Ortiz 2003). The nymphs are found in clean, fast-flowing mountain streams.

- *Lestagella penicillata* (Barnard 1940)

There is only one species of *Lestagella* (*L. penicillata*) known from the Western Cape of South Africa (Barber-James & Lugo-Ortiz 2003). A distinguishing feature of the nymphs is long setae on the anterior part of the head (Fig. 5). Mature nymphs are relatively small (8mm and cerci 0.4mm). Nymphs are found on the underside of stones in fast-flowing streams.

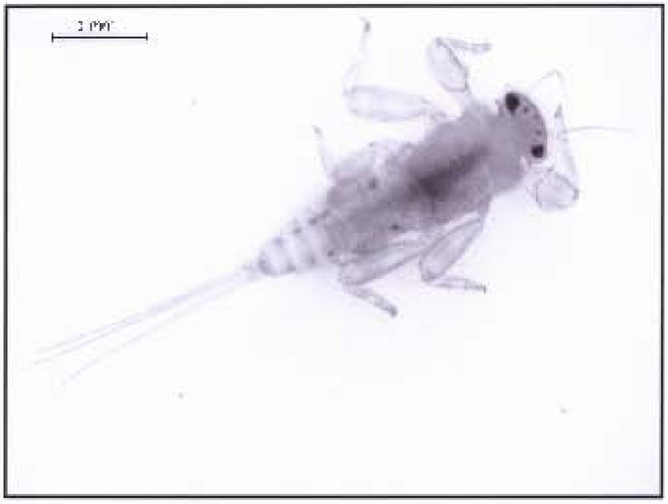

Figure 5. ***Lestagella penicillata***

Trichoptera (caddiflies):

Caddisflies are good indicators of the condition of aquatic ecosystems (de Moor and Ivanov 2008). There are three endemic families restricted to the south-western region of South Africa and are considered relicts from temperate Gondwana, including Barbarochthonidae (de Moor and Ivanov 2008).

- **Barbarochthonidae**

Barbarochthon is the sole southern African genus in the family Barbarochthonidae (de Moor & Scott 2003).

- ***Barbarochthon brunneum*** (Barnard 1934)

The nymphs of *B. brunneum* make their portable, tubular tusk-shaped cases out of dark-brown silk embedded with minute sand grains (de Moor & Scott 2003) (Fig. 6).

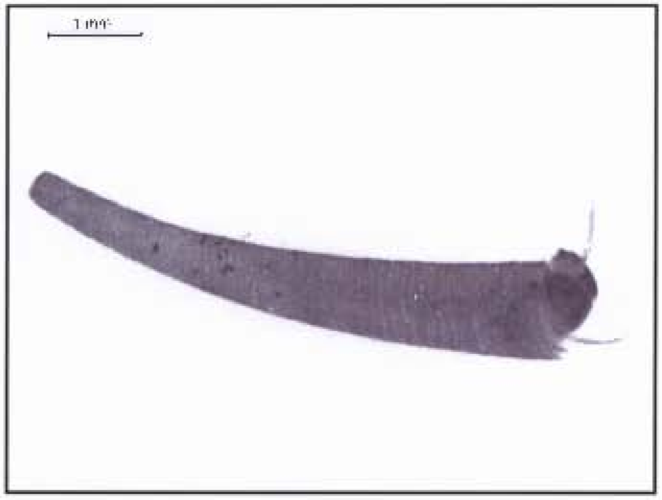

Figure 6. *Barbarochthon brunneum*

Non-Gondwanan

Ephemeroptera (mayflies):

- **Baetidae**

The family Baetidae is also known as small minnow mayflies. They form a diverse and important component of the Afrotropical freshwater biota with 18 out 40 genera occurring in South Africa (Barber-James & Lugo-Ortiz 2003). Baetid nymphs usually have seven simple gills including gill 1 (Barber-James & Lugo-Ortiz 2003). They are generally found in flowing waters, but some genera are found in still waters. Most of the baetid nymphs are collector-gathers of detritus but some genera are carnivorous or filterers.

***Acanthiops* sp.**

The genus *Acanthiops* is endemic to the Afrotropics, where 13 species are widely distributed (Barber-James & Lugo-Ortiz 2003). There are three known species in South Africa. The medial caudal filament is half to two thirds the length of the lateral cerci (Barber-James & Lugo-Ortiz 2003) (Fig. 7). The nymphs of *Acanthiops* sp. are usually found under small- to medium-sized stones in fast- and moderate-flowing streams at middle to high elevations.

Figure 7. ***Acanthiops* sp.**

Trichoptera (caddisflies):

- **Leptoceridae**

Leptoceridae is the dominant Trichopteran family with 12 genera known from the southern African region (de Moor & Scott 2003). The antennae of leptocerids are relatively long and their cases come in a diversity of shapes constructed of almost every possible type of plant or mineral (de Moor & Scott 2003).

***Athripsodes* sp.** (Billberg 1820)

The dorsal plates on the mid section of the thorax do not completely cover the mesonotum (de Moor & Scott 2003). The case is made from sand grains (Fig. 8).

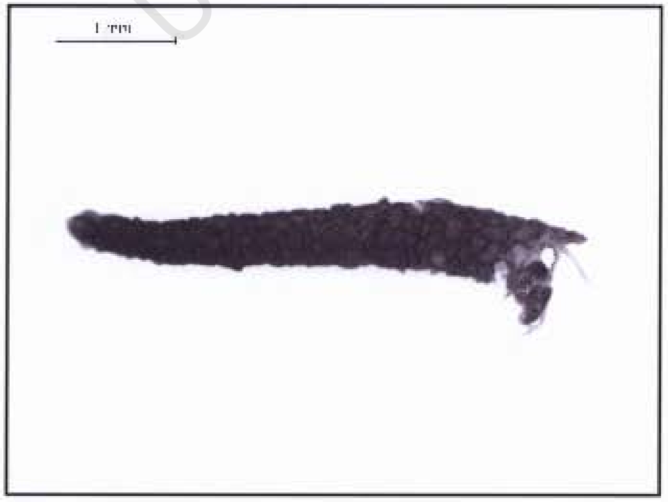

Figure 8. ***Athripsodes* sp.**

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Appendix 2 – Community composition

Appendix 2. The total number of individuals for each species collected from three replicates per site from Window Gorge Stream from the source to below Kirstenbosch National Botanical Gardens between August 2008 and May 2009.

Order	Family	Species	Site 1 Aug	Site 2 Aug	Site 3 Aug	Site 4 Aug	Site 5 Aug	Site 6 Aug
Odonata	Synlestidae	*Chlorolestes* sp.	4	0	0	0	0	0
Odonata	Aeshnidae	*Aeshna* sp.	0	0	0	1	0	0
Odonata	Libellulidae	*Orthetrum* sp.	0	0	0	0	0	0
Ephemeroptera	Teloganodidae	*Lestagella penicillata*	0	1	66	346	238	18
Ephemeroptera	Teloganodidae	*Lithoglea harrisoni*	0	0	0	0	0	1
Ephemeroptera	Leptophlebidae	*Castanophlebia* sp.	0	0	0	131	52	35
Ephemeroptera	Baetidae	*Acanthiops* sp.	0	0	0	0	0	25
Plecoptera	Notonemouridae	*Aphanicerca capensis*	133	386	178	26	11	101
Plecoptera	Notonemouridae	*Aphanicercopsis tabularis*	99	225	339	83	48	0
Plecoptera	Notonemouridae	*Aphanicercella clavata*	0	0	0	0	0	0
Trichoptera	Pisuliidae	*Dyschimus* sp.	5	12	0	0	0	0
Trichoptera	Leptoceridae	*Athripsodes* sp.	13	13	73	265	84	86
Trichoptera	Barbarochthonidae	*Barbarochthon brunneum*	6	0	0	0	0	0
Trichoptera	Philopotamidae	*Chimarra* sp.	0	0	0	0	0	0
Hemiptera	Notonectidae	*Notonecta lactitans*	0	0	0	0	0	0
Hemiptera	Gerridae	*Limnogonus* sp.	0	0	0	0	0	0
Hemiptera	Veliidae	Species 1	0	0	0	0	0	0
Hemiptera	Veliidae	*Xiphoveloidea major*	0	0	0	0	0	0
Hemiptera	Saldidae	*Capitonisalda ripa*	0	0	0	0	0	0
Diptera	Blephariceridae	*Elporia* sp.	0	0	7	3	2	0
Diptera	Athericidae		0	0	3	32	31	71
Diptera	Ceratopogonidae	*Atrichopogon* sp. 1	0	0	0	0	0	0
Diptera	Ceratopogonidae	*Atrichopogon* sp. 2	0	0	0	0	0	0
Diptera	Psychodidae	*Pericoma* sp.	0	0	0	0	0	0
Diptera	Dixidae	*Dixa* sp.	0	0	0	0	0	0
Diptera	Empididae	Species 1	0	0	0	0	0	0
Diptera	Empididae	Species 2	0	2	0	0	0	0
Diptera	Empididae	Species 3	0	0	0	0	0	0
Diptera	Empididae	Species 4	0	0	0	0	0	0
Diptera	Tabanidae	Species 1	0	0	0	0	0	0
Diptera	Tabanidae	Species 2	0	0	0	1	0	0
Diptera	Tabanidae	Species 3	0	0	0	0	0	0
Diptera	Tipulidae	*Rhabdiomastix* sp.	2	0	0	0	0	0
Diptera	Tipulidae	*Limonia capicola*	0	0	0	0	0	0
Diptera	Tipulidae	*Tipula* sp. 1	0	0	0	0	0	0
Diptera	Tipulidae	*Tipula* sp. 2	0	0	0	0	0	0
Diptera	Simuliidae	*Simulium (Nevermannia)* sp.	694	9	7	34	70	125
Diptera	Chironomidae	Species 1	0	2	3	0	0	40
Diptera	Chironomidae	Species 2	7	3	1	10	86	300
Diptera	Chironomidae	Species 3	0	0	0	0	0	0
Diptera	Chironomidae	Species 4	23	3	2	0	0	0
Diptera	Chironomidae	Species 5	0	0	0	14	1	0
Diptera	Chironomidae	Species 6	0	0	0	0	0	0
Coleoptera	Helodidae (larvae)	Species 1	0	49	85	251	25	72
Coleoptera	Helodidae (larvae)	Species 2	0	1	14	294	168	16
Coleoptera	Gyrinidae (adults)	*Orectogrus* sp.	0	0	0	0	0	0
Coleoptera	Dryopidae (adults)	*Strina* sp.	1	0	1	0	0	0
Coleoptera	Dytiscidae (adults)	*Nebrioporus* sp.	37	0	0	0	0	0
Coleoptera	Dytiscidae (adults)	*Hydropeplus* sp.	2	0	2	0	0	0
Coleoptera	Hydrophilidae (larvae)	*Amphiops* sp.	0	1	1	1	0	0
Coleoptera	Hydrophilidae (larvae)	*Helochares* sp.	0	0	0	0	0	0
Coleoptera	Ptilodactylidae (larvae)		0	0	0	2	0	0
Coleoptera	Hydraenidae (adults)	*Prosthetops* sp.	3	0	0	0	0	0
Coleoptera	Hydraenidae (adults)	*Hydraena monikae*	0	1	0	0	0	0
Coleoptera	Elmidae (adults)	*Leielmis georyssoides*	12	1	0	4	0	0
Coleoptera	Elmidae (adults)	*Peloriolus* sp.	0	0	0	0	0	0
Coleoptera	Elmidae (adults)	*Elpidelmis* sp.	3	0	0	0	0	0
Coleoptera	Curculionidae (larvae)	*Neohydronomus*	0	0	0	0	0	1
Amphipoda	Paramelitidae	*Paramelita nigroculus*	0	13	79	0	0	0
Amphipoda	Paramelitidae	*Paramelita capensis*	12	1	3	0	0	0
Isopoda	Amphisopodidae	*Mesamphisopus* sp.	0	0	0	1	0	0
Acarina	Hydrodromidae	Species 1	0	0	0	0	0	0
Acarina		Water mite sp. 1	0	0	0	0	0	1
Acarina		Water mite sp. 2	0	0	0	0	0	0
Acarina		Water mite sp. 3	0	0	0	0	0	0
Oligochaeta			20	0	6	3	0	0
Planaria (Polycladida)			12	4	0	0	0	0

Order	**Family**	**Species**	**Site 1 Oct**	**Site 2 Oct**	**Site 3 Oct**	**Site 4 Oct**	**Site 5 Oct**	**Site 6 Oct**
Odonata	Synlestidae	*Chlorolestes* sp.	7	0	0	0	0	0
Odonata	Aeshnidae	*Aeshna* sp.	0	0	0	0	0	0
Odonata	Libellulidae	*Orthetrum* sp.	0	0	0	0	0	0
Ephemeroptera	Teloganodidae	*Lestagella penicillata*	0	1	92	723	577	47
Ephemeroptera	Teloganodidae	*Lithoglea harrisoni*	0	0	0	1	0	0
Ephemeroptera	Leptophlebidae	*Castanophlebia* sp.	0	0	0	69	93	14
Ephemeroptera	Baetidae	*Acanthiops* sp.	0	0	0	0	0	228
Plecoptera	Notonemouridae	*Aphanicerca capensis*	80	400	106	12	6	6
Plecoptera	Notonemouridae	*Aphanicercopsis tabularis*	0	20	0	0	0	0
Plecoptera	Notonemouridae	*Aphanicercella clavata*	0	0	0	0	0	0
Trichoptera	Pisuliidae	*Dyschimus* sp.	13	10	4	0	1	0
Trichoptera	Leptoceridae	*Athripsodes* sp.	12	61	72	83	90	116
Trichoptera	Barbarochthonidae	*Barbarochthon brunneum*	1	0	0	0	0	0
Trichoptera	Philopotamidae	*Chimarra* sp.	0	0	0	1	0	0
Hemiptera	Notonectidae	*Notonecta lactitans*	0	0	0	0	0	0
Hemiptera	Gerridae	*Limnogonus* sp.	0	0	0	0	0	0
Hemiptera	Veliidae	Species 1	0	0	0	0	0	0
Hemiptera	Veliidae	*Xiphoveloidea major*	0	0	0	0	0	0
Hemiptera	Saldidae	*Capitonisalda ripa*	0	0	0	0	1	0
Diptera	Blephariceridae	*Elporia* sp.	0	15	53	8	0	0
Diptera	Athericidae		0	0	2	9	40	34
Diptera	Ceratopogonidae	*Atrichopogon* sp. 1	0	0	0	0	0	0
Diptera	Ceratopogonidae	*Atrichopogon* sp. 2	0	0	0	0	0	0
Diptera	Psychodidae	*Pericoma* sp.	0	0	0	0	0	1
Diptera	Dixidae	*Dixa* sp.	0	0	0	0	0	0
Diptera	Empididae	Species 1	0	0	0	0	0	0
Diptera	Empididae	Species 2	0	0	0	0	0	0
Diptera	Empididae	Species 3	0	0	0	0	0	1
Diptera	Empididae	Species 4	0	0	1	0	0	0
Diptera	Tabanidae	Species 1	0	0	0	0	0	0
Diptera	Tabanidae	Species 2	0	0	0	0	0	0
Diptera	Tabanidae	Species 3	0	0	0	0	0	0
Diptera	Tipulidae	*Rhabdiomastix* sp.	2	0	0	0	0	0
Diptera	Tipulidae	*Limonia capicola*	0	0	0	0	0	0
Diptera	Tipulidae	*Tipula* sp. 1	1	0	0	0	0	0
Diptera	Tipulidae	*Tipula* sp. 2	0	0	0	0	0	0
Diptera	Simuliidae	*Simulium (Nevermannia)* sp.	6	16	149	48	170	67
Diptera	Chironomidae	Species 1	19	4	8	43	10	246
Diptera	Chironomidae	Species 2	1	9	25	141	212	439
Diptera	Chironomidae	Species 3	8	2	0	0	0	12
Diptera	Chironomidae	Species 4	195	16	0	71	3	0
Diptera	Chironomidae	Species 5	0	0	0	12	7	0
Diptera	Chironomidae	Species 6	0	0	0	0	0	0
Coleoptera	Helodidae (larvae)	Species 1	0	34	3	254	104	122
Coleoptera	Helodidae (larvae)	Species 2	0	32	11	162	220	60
Coleoptera	Gyrinidae (adults)	*Orectogrus* sp.	0	0	0	0	0	0
Coleoptera	Dryopidae (adults)	*Strina* sp.	0	3	1	1	0	0
Coleoptera	Dytiscidae (adults)	*Nebrioporus* sp.	251	0	1	0	0	0
Coleoptera	Dytiscidae (adults)	*Hydropeplus* sp.	46	0	0	0	0	0
Coleoptera	Hydrophilidae (larvae)	*Amphiops* sp.	0	0	0	0	0	1
Coleoptera	Hydrophilidae (larvae)	*Helochares* sp.	0	0	1	1	0	0
Coleoptera	Ptilodactylidae (larvae)		0	0	0	0	0	0
Coleoptera	Hydraenidae (adults)	*Prosthetops* sp.	59	0	0	0	0	0
Coleoptera	Hydraenidae (adults)	*Hydraena monikae*	0	1	0	0	0	0
Coleoptera	Elmidae (adults)	*Leielmis georyssoides*	33	4	1	0	0	0
Coleoptera	Elmidae (adults)	*Peloriolus* sp.	7	0	4	3	0	0
Coleoptera	Elmidae (adults)	*Elpidelmis* sp.	9	4	1	0	0	0
Coleoptera	Curculionidae (larvae)	*Neohydronomus*	0	0	0	0	0	0
Amphipoda	Paramelitidae	*Paramelita nigroculus*	0	294	47	0	1	0
Amphipoda	Paramelitidae	*Paramelita capensis*	1	2	2	0	0	0
Isopoda	Amphisopodidae	*Mesamphisopus* sp.	0	0	0	0	0	0
Acarina	Hydrodromidae	Species 1	0	0	0	1	0	0
Acarina		Water mite sp. 1	0	0	0	0	0	0
Acarina		Water mite sp. 2	0	1	0	0	0	0
Acarina		Water mite sp. 3	0	1	0	0	0	0
Oligochaeta			2	4	1	1	2	10
Planaria (Polycladida)			9	1	0	0	0	1

Order	**Family**	**Species**	**Site 1 Dec**	**Site 2 Dec**	**Site 3 Dec**	**Site 4 Dec**	**Site 5 Dec**	**Site 6 Dec**
Odonata	Synlestidae	*Chlorolestes* sp.	19	0	0	0	0	0
Odonata	Aeshnidae	*Aeshna* sp.	0	0	0	0	1	0
Odonata	Libellulidae	*Orthetrum* sp.	0	0	0	0	0	0
Ephemeroptera	Teloganodidae	*Lestagella penicillata*	0	0	52	173	12	5
Ephemeroptera	Teloganodidae	*Lithoglea harrisoni*	0	0	0	0	0	0
Ephemeroptera	Leptophlebidae	*Castanophlebia* sp.	0	0	0	0	0	4
Ephemeroptera	Baetidae	*Acanthiops* sp.	0	0	0	54	81	432
Plecoptera	Notonemouridae	*Aphanicerca capensis*	3	3	17	12	8	38
Plecoptera	Notonemouridae	*Aphanicercopsis tabularis*	0	0	0	0	0	0
Plecoptera	Notonemouridae	*Aphanicercella clavata*	0	0	0	0	0	0
Trichoptera	Pisuliidae	*Dyschimus* sp.	5	16	1	0	0	0
Trichoptera	Leptoceridae	*Athripsodes* sp.	7	123	35	1	3	1
Trichoptera	Barbarochthonidae	*Barbarochthon brunneum*	1	0	0	0	0	0
Trichoptera	Philopotamidae	*Chimarra* sp.	0	0	0	1	0	0
Hemiptera	Notonectidae	*Notonecta lactitans*	4	0	0	0	0	0
Hemiptera	Gerridae	*Limnogonus* sp.	0	0	0	0	0	0
Hemiptera	Veliidae	Species 1	1	0	0	0	0	0
Hemiptera	Veliidae	*Xiphoveloidea major*	0	0	0	0	1	0
Hemiptera	Saldidae	*Capitonisalda ripa*	0	0	0	0	0	1
Diptera	Blephariceridae	*Elporia* sp.	0	0	0	0	0	0
Diptera	Athericidae		0	2	0	4	2	86
Diptera	Ceratopogonidae	*Atrichopogon* sp. 1	0	0	4	0	0	0
Diptera	Ceratopogonidae	*Atrichopogon* sp. 2	0	0	0	0	0	0
Diptera	Psychodidae	*Pericoma* sp.	0	0	0	0	0	0
Diptera	Dixidae	*Dixa* sp.	0	0	0	1	2	8
Diptera	Empididae	Species 1	0	0	1	0	0	0
Diptera	Empididae	Species 2	0	0	0	0	0	0
Diptera	Empididae	Species 3	0	0	0	0	0	0
Diptera	Empididae	Species 4	0	0	0	0	0	0
Diptera	Tabanidae	Species 1	0	0	0	0	0	0
Diptera	Tabanidae	Species 2	0	0	0	0	0	0
Diptera	Tabanidae	Species 3	0	0	0	0	0	0
Diptera	Tipulidae	*Rhabdiomastix* sp.	0	0	0	0	0	0
Diptera	Tipulidae	*Limonia capicola*	0	0	0	0	0	1
Diptera	Tipulidae	*Tipula* sp. 1	0	0	0	0	0	0
Diptera	Tipulidae	*Tipula* sp. 2	0	0	0	0	0	0
Diptera	Simuliidae	*Simulium (Nevermannia)* sp.	1	3	18	20	88	77
Diptera	Chironomidae	Species 1	10	2	432	78	15	33
Diptera	Chironomidae	Species 2	2	0	0	0	0	0
Diptera	Chironomidae	Species 3	0	0	0	1	0	0
Diptera	Chironomidae	Species 4	145	11	0	55	28	48
Diptera	Chironomidae	Species 5	6	3	0	10	25	12
Diptera	Chironomidae	Species 6	0	0	0	1	0	1
Coleoptera	Helodidae (larvae)	Species 1	1	5	0	52	20	5
Coleoptera	Helodidae (larvae)	Species 2	0	0	0	22	1	0
Coleoptera	Gyrinidae (adults)	*Orectogrus* sp.	0	0	0	0	0	0
Coleoptera	Dryopidae (adults)	*Strina* sp.	1	3	0	0	0	0
Coleoptera	Dytiscidae (adults)	*Nebrioporus* sp.	1810	1	3	0	0	1
Coleoptera	Dytiscidae (adults)	*Hydropeplus* sp.	151	0	0	0	0	0
Coleoptera	Hydrophilidae (larvae)	*Amphiops* sp.	0	0	0	0	1	0
Coleoptera	Hydrophilidae (larvae)	*Helochares* sp.	0	0	0	0	0	1
Coleoptera	Ptilodactylidae (larvae)		0	1	0	0	1	0
Coleoptera	Hydraenidae (adults)	*Prosthetops* sp.	5	0	0	0	0	0
Coleoptera	Hydraenidae (adults)	*Hydraena monikae*	0	0	0	0	0	0
Coleoptera	Elmidae (adults)	*Leielmis georyssoides*	23	1	0	0	0	0
Coleoptera	Elmidae (adults)	*Peloriolus* sp.	4	3	0	0	0	0
Coleoptera	Elmidae (adults)	*Elpidelmis* sp.	1	0	0	0	0	0
Coleoptera	Curculionidae (larvae)	*Neohydronomus*	0	0	0	0	0	0
Amphipoda	Paramelitidae	*Paramelita nigroculus*	0	691	11	0	0	0
Amphipoda	Paramelitidae	*Paramelita capensis*	6	0	0	3	0	0
Isopoda	Amphisopodidae	*Mesamphisopus* sp.	2	0	0	0	0	0
Acarina	Hydrodromidae	Species 1	0	0	0	0	0	0
Acarina		Water mite sp. 1	0	0	0	0	0	0
Acarina		Water mite sp. 2	0	1	0	0	0	0
Acarina		Water mite sp. 3	0	0	0	0	0	0
Oligochaeta			0	1	0	0	0	14
Planaria (Polycladida)			1	6	1	0	0	1

Order	Family	Species	Site 1 Feb	Site 2 Feb	Site 3 Feb	Site 4 Feb	Site 5 Feb	Site 3 Apr	Site 4 Apr
Odonata	Synlestidae	*Chlorolestes* sp.	85	0	0	0	0	1	0
Odonata	Aeshnidae	*Aeshna* sp.	0	0	0	1	6	0	0
Odonata	Libellulidae	*Orthetrum* sp.	0	0	0	5	163	2	8
Ephemeroptera	Teloganodidae	*Lestagella penicillata*	0	0	0	64	291	0	1
Ephemeroptera	Teloganodidae	*Lithoglea harrisoni*	0	0	0	0	0	0	0
Ephemeroptera	Leptophlebidae	*Castanophlebia* sp.	0	0	0	0	0	0	0
Ephemeroptera	Baetidae	*Acanthiops* sp.	0	0	0	13	4	0	0
Plecoptera	Notonemouridae	*Aphanicerca capensis*	0	1	86	2	9	1	0
Plecoptera	Notonemouridae	*Aphanicercopsis tabularis*	0	0	0	0	0	0	0
Plecoptera	Notonemouridae	*Aphanicercella clavata*	0	0	0	0	0	0	0
Trichoptera	Pisuliidae	*Dyschimus* sp.	23	47	92	0	0	1	0
Trichoptera	Leptoceridae	*Athripsodes* sp.	21	677	366	0	1	251	1
Trichoptera	Barbarochthonidae	*Barbarochthon brunneum*	6	0	0	0	0	0	0
Trichoptera	Philopotamidae	*Chimarra* sp.	0	0	0	0	0	0	0
Hemiptera	Notonectidae	*Notonecta lactitans*	13	0	0	0	0	0	0
Hemiptera	Gerridae	*Limnogonus* sp.	0	0	0	0	1	0	0
Hemiptera	Veliidae	Species 1	0	0	0	0	0	0	0
Hemiptera	Veliidae	*Xiphoveloidea major*	0	0	0	1	0	0	0
Hemiptera	Saldidae	*Capitonisalda ripa*	0	0	0	0	0	0	0
Diptera	Blephariceridae	*Elporia* sp.	0	0	0	0	0	0	0
Diptera	Athericidae		0	4	5	34	46	2	6
Diptera	Ceratopogonidae	*Atrichopogon* sp. 1	0	0	1	0	0	0	0
Diptera	Ceratopogonidae	*Atrichopogon* sp. 2	0	0	0	0	0	0	4
Diptera	Psychodidae	*Pericoma* sp.	0	0	0	0	0	0	0
Diptera	Dixidae	*Dixa* sp.	0	0	0	0	0	0	0
Diptera	Empididae	Species 1	0	0	0	0	0	0	0
Diptera	Empididae	Species 2	0	0	0	0	0	0	0
Diptera	Empididae	Species 3	0	0	0	0	0	0	0
Diptera	Empididae	Species 4	0	0	0	0	0	0	0
Diptera	Tabanidae	Species 1	0	0	0	0	0	0	0
Diptera	Tabanidae	Species 2	0	0	0	0	0	0	0
Diptera	Tabanidae	Species 3	0	2	0	0	0	0	0
Diptera	Tipulidae	*Rhabdiomastix* sp.	0	0	0	0	0	0	0
Diptera	Tipulidae	*Limonia capicola*	0	0	0	0	0	0	0
Diptera	Tipulidae	*Tipula* sp. 1	0	0	0	0	0	0	0
Diptera	Tipulidae	*Tipula* sp. 2	0	0	3	0	0	0	0
Diptera	Simuliidae	*Simulium (Nevermannia)* sp.	0	0	0	9	2	0	0
Diptera	Chironomidae	Species 1	3	0	0	2	0	0	0
Diptera	Chironomidae	Species 2	0	0	0	0	0	0	0
Diptera	Chironomidae	Species 3	0	0	0	1	0	0	0
Diptera	Chironomidae	Species 4	2	30	61	8	15	6	4
Diptera	Chironomidae	Species 5	10	85	570	25	20	44	89
Diptera	Chironomidae	Species 6	0	0	0	1	0	0	0
Coleoptera	Helodidae (larvae)	Species 1	0	0	8	0	16	0	5
Coleoptera	Helodidae (larvae)	Species 2	0	0	0	2	2	0	1
Coleoptera	Gyrinidae (adults)	*Orectogrus* sp.	0	0	0	0	2	0	0
Coleoptera	Dryopidae (adults)	*Strina* sp.	2	119	3	0	0	0	0
Coleoptera	Dytiscidae (adults)	*Nebrioporus* sp.	20033	16	0	0	0	0	0
Coleoptera	Dytiscidae (adults)	*Hydropeplus* sp.	270	2	0	0	0	0	0
Coleoptera	Hydrophilidae (larvae)	*Amphiops* sp.	0	0	0	0	0	0	0
Coleoptera	Hydrophilidae (larvae)	*Helochares* sp.	0	0	0	0	0	0	0
Coleoptera	Ptilodactylidae (larvae)		0	0	0	0	0	0	0
Coleoptera	Hydraenidae (adults)	*Prosthetops* sp.	27	0	0	0	0	0	0
Coleoptera	Hydraenidae (adults)	*Hydraena monikae*	0	0	0	0	0	0	0
Coleoptera	Elmidae (adults)	*Leielmis georyssoides*	72	131	1	0	0	0	0
Coleoptera	Elmidae (adults)	*Peloriolus* sp.	1	3	0	2	1	0	0
Coleoptera	Elmidae (adults)	*Elpidelmis* sp.	1	0	0	0	0	0	0
Coleoptera	Curculionidae (larvae)	*Neohydronomus*	0	0	0	0	0	0	0
Amphipoda	Paramelitidae	*Paramelita nigroculus*	0	1795	330	0	0	4	0
Amphipoda	Paramelitidae	*Paramelita capensis*	2	2	1	1	0	1	0
Isopoda	Amphisopodidae	*Mesamphisopus* sp.	3	0	3	0	0	0	0
Acarina	Hydrodromidae	Species 1	0	0	0	0	0	0	0
Acarina		Water mite sp. 1	0	0	0	0	0	0	0
Acarina		Water mite sp. 2	0	0	0	0	0	0	0
Acarina		Water mite sp. 3	0	0	0	0	0	0	0
Oligochaeta			0	0	0	0	11	0	0
Planaria (Polycladida)			1	0	0	0	0	0	0

Order	Family	Species	Site 1 May	Site 2 May	Site 3 May	Site 4 May	Site 5 May	Site 6 May
Odonata	Synlestidae	*Chlorolestes* sp.	0	0	0	0	0	0
Odonata	Aeshnidae	*Aeshna* sp.	0	0	0	0	2	0
Odonata	Libellulidae	*Orthetrum* sp.	0	0	0	0	0	1
Ephemeroptera	Teloganodidae	*Lestagella penicillata*	0	0	45	282	277	10
Ephemeroptera	Teloganodidae	*Lithoglea harrisoni*	0	0	0	0	0	0
Ephemeroptera	Leptophlebidae	*Castanophlebia* sp.	0	0	2	110	45	4
Ephemeroptera	Baetidae	*Acanthiops* sp.	0	0	0	0	0	0
Plecoptera	Notonemouridae	*Aphanicerca capensis*	4	191	344	68	23	2
Plecoptera	Notonemouridae	*Aphanicercopsis tabularis*	24	141	348	112	0	0
Plecoptera	Notonemouridae	*Aphanicercella clavata*	0	0	3	15	2	4
Trichoptera	Pisuliidae	*Dyschimus* sp.	32	0	1	0	0	0
Trichoptera	Leptoceridae	*Athripsodes* sp.	0	11	46	141	27	3
Trichoptera	Barbarochthonidae	*Barbarochthon brunneum*	1	0	0	0	0	0
Trichoptera	Philopotamidae	*Chimarra* sp.	0	0	0	1	0	0
Hemiptera	Notonectidae	*Notonecta lactitans*	0	0	0	0	0	0
Hemiptera	Gerridae	*Limnogonus* sp.	0	0	0	0	0	0
Hemiptera	Veliidae	Species 1	0	0	0	0	0	0
Hemiptera	Veliidae	*Xiphoveloidea major*	0	0	0	0	0	0
Hemiptera	Saldidae	*Capitonisalda ripa*	0	0	0	0	0	0
Diptera	Blephariceridae	*Elporia* sp.	0	0	0	0	0	0
Diptera	Athericidae		1	1	6	40	35	13
Diptera	Ceratopogonidae	*Atrichopogon* sp. 1	0	0	0	0	0	1
Diptera	Ceratopogonidae	*Atrichopogon* sp. 2	0	0	0	0	0	1
Diptera	Psychodidae	*Pericoma* sp.	0	0	0	0	0	0
Diptera	Dixidae	*Dixa* sp.	0	0	0	3	0	0
Diptera	Empididae	Species 1	0	0	0	0	0	0
Diptera	Empididae	Species 2	0	0	0	0	0	0
Diptera	Empididae	Species 3	0	0	0	0	0	0
Diptera	Empididae	Species 4	0	0	0	0	0	0
Diptera	Tabanidae	Species 1	1	0	0	0	0	0
Diptera	Tabanidae	Species 2	0	0	0	0	0	0
Diptera	Tabanidae	Species 3	0	0	0	0	0	0
Diptera	Tipulidae	*Rhabdiomastix* sp.	0	0	0	0	0	0
Diptera	Tipulidae	*Limonia capicola*	0	0	0	0	0	0
Diptera	Tipulidae	*Tipula* sp. 1	0	0	0	0	0	0
Diptera	Tipulidae	*Tipula* sp. 2	0	0	0	0	0	0
Diptera	Simuliidae	*Simulium (Nevermannia)* sp.	505	0	0	14	25	11
Diptera	Chironomidae	Species 1	0	2	2	35	28	13
Diptera	Chironomidae	Species 2	65	2	20	29	50	84
Diptera	Chironomidae	Species 3	0	0	0	0	1	0
Diptera	Chironomidae	Species 4	0	2	0	1	1	1
Diptera	Chironomidae	Species 5	2	1	1	15	0	2
Diptera	Chironomidae	Species 6	0	0	0	1	0	1
Coleoptera	Helodidae (larvae)	Species 1	6	0	1	4	1	0
Coleoptera	Helodidae (larvae)	Species 2	7	5	16	15	13	1
Coleoptera	Gyrinidae (adults)	*Orectogrus* sp.	0	0	0	1	0	0
Coleoptera	Dryopidae (adults)	*Strina* sp.	10	0	0	0	0	0
Coleoptera	Dytiscidae (adults)	*Nebrioporus* sp.	860	14	11	5	3	4
Coleoptera	Dytiscidae (adults)	*Hydropeplus* sp.	1	2	0	0	0	0
Coleoptera	Hydrophilidae (larvae)	*Amphiops* sp.	0	0	0	0	0	0
Coleoptera	Hydrophilidae (larvae)	*Helochares* sp.	0	0	0	0	0	0
Coleoptera	Ptilodactylidae (larvae)		0	0	0	0	0	0
Coleoptera	Hydraenidae (adults)	*Prosthetops* sp.	0	0	0	0	0	0
Coleoptera	Hydraenidae (adults)	*Hydraena monikae*	0	0	0	0	0	0
Coleoptera	Elmidae (adults)	*Leielmis georyssoides*	29	6	0	2	0	0
Coleoptera	Elmidae (adults)	*Peloriolus* sp.	20	0	11	0	1	0
Coleoptera	Elmidae (adults)	*Elpidelmis* sp.	7	0	2	0	0	0
Coleoptera	Curculionidae (larvae)	*Neohydronomus*	0	0	0	0	0	0
Amphipoda	Paramelitidae	*Paramelita nigroculus*	0	3	16	3	0	0
Amphipoda	Paramelitidae	*Paramelita capensis*	4	0	0	3	0	0
Isopoda	Amphisopodidae	*Mesamphisopus* sp.	0	0	0	0	0	0
Acarina	Hydrodromidae	Species 1	0	0	0	0	0	0
Acarina		Water mite sp. 1	0	0	0	0	0	0
Acarina		Water mite sp. 2	0	0	0	0	0	0
Acarina		Water mite sp. 3	0	0	0	0	0	0
Oligochaeta			1	1	0	1	0	7
Planaria (Polycladida)			18	2	0	0	0	0

Appendix 3 – Water quality variables

Appendix 3. The water quality variables at each site along Window Gorge Stream measured between October 2008 and May 2009.

Month	Site	Oxygen saturation (%)	Conductivity (mS/cm)	pH
October	1	78.4	0.0468	4.4
	2	109.2	0.0526	4.3
	3	91.8	0.0701	5.4
	4	83.0	0.0703	5.7
	5	87.8	0.0733	5.9
	6	78.0	0.0764	6.0
December	1	84.6	0.0694	4.1
	2	92.3	0.076	4.2
	3	73.9	0.0856	4.9
	4	94.3	0.086	5.5
	5	96.8	0.0859	5.3
	6	94.0	0.0889	5.9
February	1	45.2	0.0706	3.8
	2	43.6	0.0971	4.4
	3	53.6	0.074	4.9
	4	61.8	0.0731	5.6
	5	58.2	0.0731	5.3
	6	No water		
April	1	No water		
	2	No water		
	3	39.9	100.2	5.13
	4	95.0	89.7	5.72
	5	No water		
	6	No water		
May	1	109.7	60.9	3.78
	2	124.0	59.4	3.94
	3	83.3	52.2	4.57
	4	87.3	52.6	4.98
	5	77.7	53.3	4.86
	6	79.2	55.9	5.46