HOST ASSOCIATIONS AND POLLUTION RESPONSES OF CILIATE EPIBIONTS OF MACROINVERTEBRATES IN THE SIX RIVERS IN THE WESTERN CAPE PROVINCE OF SOUTH AFRICA

By

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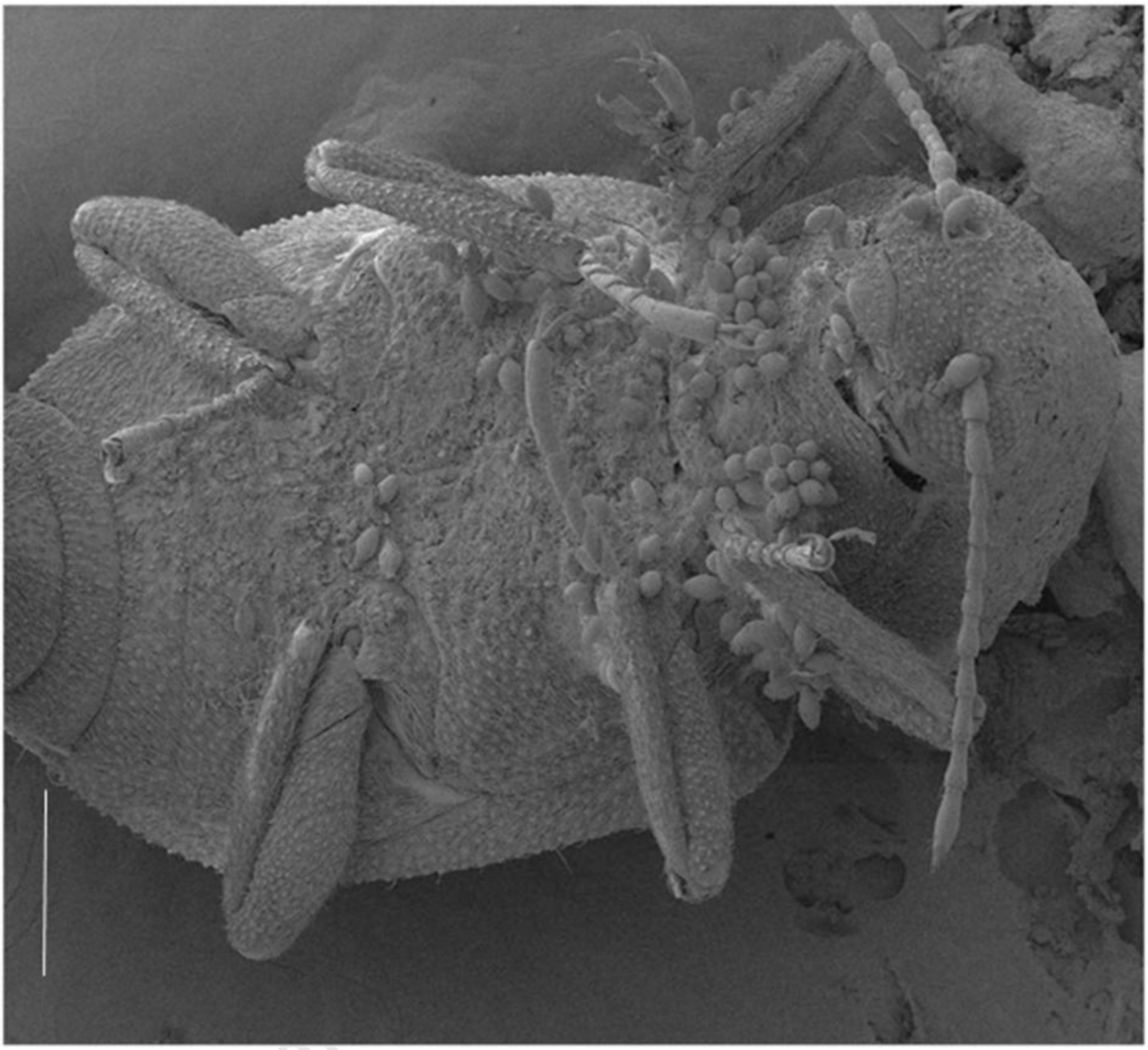

Short-stalked operculariad ciliate epibionts attached to the ventral thorax and legs of an adult elmid beetle (scale bar, 500µm).

PLAGIARISM DECLARATION

I know the meaning of plagiarism and declare that all the work in the document is my own, except that which has been properly acknowledged, including the phylogenetic trees (Chapter 3) which were constructed with the assistance of supervisor Dr. F. Roets.

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ABSTRACT

This study is the first documentation of ciliate epibiont-host associations in the Western Cape Province of South Africa. Benthic macroinvertebrates were sampled from six rivers in the Western Cape Province (Wolwekloof, Wit, Rooielskloof, Molenaars, Elandspad and Eerste) to determine epibiotic prevalence and to explore relationships between prevalence and host abundance. Ciliate morphology and 18S rDNA and ITS region sequence data were used to identify the different taxa present, and attachment preferences of epibiont colonies for host body part were determined. The response of ciliate epibionts to water pollution along a pollution gradient in the Eerste River was examined to assess their potential as bioindicators of water pollution.

Peritrichian ciliates colonised adult Elmidae, Hydraenidae, and Corixidae, and larval Dryopidae, Ptylodactylidae, and Notonemouridae. Suctorian ciliates were found mainly on adult Hydraenidae. Morphological and molecular studies identified three different taxa of peritrichs (within the *Opercularia* clade). Epibiotic prevalence of peritrichs showed variation among river sites. There was a strong positive relationship between epibiotic prevalence and abundance of elmid beetle host in Molenaars, Elandspad and Eerste rivers; and between epibiotic prevalence and abundance of hydraenid beetle hosts in Molenaars River. Peritrichs were only present on larger nymphs of Notonemouridae, and absent on the earlier instars. Peritrichs were almost always attached to the ventral aspect of all beetle hosts examined. Infestation density of ciliates was higher on the ventral side of the thorax of elmid beetles (10.56 colonies per unit area of host body part) in comparison to the ventral aspects of legs (5.68), abdomen (0.00) and head (0.00) suggesting that these peritrichs not only prefer the less mobile body parts but also body parts that offer shelter and protection from predation by higher organisms. On hydraenid beetles, infestation density was highest on the head (8.89 colonies per unit area of host body part) and zero on the thorax, legs and abdomen, suggesting that availability of food (nutrients) may influence colonisation site preferences.

There were differences in prevalence and abundance of peritrichs and suctorians at the sites above, and below the Kleinplaas dam along the Eerste River. The dam is a source of water pollution for downstream sites, with conductivity and turbidity increasing in sites below the dam, and oxygen concentration decreasing in sites below the dam. Peritrich infestation prevalence and zooid abundance decreased at sites below the dam, while infestation prevalence and zooid abundance of suctorian ciliates increased below the dam. The peritrich species thus appear to be pollution-sensitive, while the suctorian species appear to tolerate water pollution, as they had higher abundances below the pollution source. Macroinvertebrate diversity was lower at sites below the dam, with the disappearance of pollution-sensitive species (e.g., Notonemouridae, Leptophlebidae, Blepharoceridae). Also, SASS5 and ASPT (average score per taxon) values were significantly higher above the dam than below the dam indicating a change in the health status of pollution-impacted sites below the dam.

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CHAPTER 1. OVERVIEW

There exists in nature a widespread biological phenomenon in which two or more different biological species coexist together for long periods. This phemonenon is known as Symbiosis. It was first defined by Anton de Bary as “the living together of different species” (Douglas 1994). Though it has been argued that symbiosis must confer mutual benefit to the interactive species, Douglas (1994) maintained that symbionts must not necessarily benefit from the association, but rather that there should be an acquisition of a new metabolic property by at least one of the partners. Taking the symbiosis between *Festuca ovina* and their mycorrhizal fungi (Koucheki & Read 1976) as an example, the fungi obtain carbon requirements from the plant. In return, the fungi facilitate the plant’s mineral nutrient uptake (especially phosphorus) from the soil. This is of great benefit to the plant, particularly in nutrient-poor soils. Interestingly, when there were enough nutrients, performance of both mycorrhizal plants and non-mycorrhizal plants was similar, implying that plants do not always benefit from this association. Mycorrhizal fungi were observed to retard the growth of young seedlings by diverting the photosynthetic carbon needed by the plant seedlings for their own use, thus causing the affected seedlings to become stunted. It was then deduced that environmental conditions (concentration of phosphorus in the soil) and age of plant (seedlings or mature plants) determined whether a plant benefits from its association with the mycorrhizal fungi. In the light of this understanding, Douglas (1994) argued that benefit is not an inherent feature of symbiosis, but is determined by the interaction of the association with the environment. As mentioned earlier, symbiosis entails the acquisition of a new metabolic property by at least one of the interactive species. This is illustrated in the association between herbivorous mammals and their gut microorganisms where the cellulose degrading capability of the latter is gained by the former (Stevens & Hume 1998). Similarly, nitrogen-fixation is acquired by many leguminous plants by virtue of their association with the bacteria e.g., *Rhizobia, Frankia* (Cocking 2003). Also, essential amino acids are gained by aphids from their symbiotic associations with the bacteria *Buchnera* (Douglas 1998).

Different types of symbioses exist in nature and comprise all associations between and among living organisms. They include:

- mutualism in which the interactive species both benefit from the association as seen in symbiosis between the territorial clownfish and sea anemone. The clownfish lives among the stinging tentacles of the anemone and protects it from predators e.g., butterfly fishes. The anemone, in turn, protects the clownfish from predation by wrasses and other fishes (Davenport & Norris 1958, Rosenberg & Cruz 1988).
- commensalism which is described as an association in which one organism benefits while the other is neither harmed nor benefiting. It could be phoretic where one organism is transporting the other as seen between the millipede *Polyxenus lagurus* and birds (Tajovsky *et al*. 2001); inquinilistic where one organism houses the other as illustrated in the relationship between the gall aphid *Tamalia* and its host plant *Arctostaphylos* (Miller & Crespi 2003); or metabiotic where the symbiont uses substances produced by, or lives in a favourable environment created by its partner (metabiont) before death. This association is seen in symbiosis between pathogenic organisms (bacteria and fungi) and raw fruits and fruit products (Richards & Beuchat 2005).
- parasitism where the symbiont benefits from the association to the detriment of the host. Endoparasites live within their host's body, e.g., gastrointestinal worms (Bilal *et al*. 2009) while ectoparasites live on their host's body surface, e.g., ticks and fleas (Akucewich *et al*. 2002).
- amensalism, which is the competitive interaction where an organism (the amensal) completely inhibits the activities of its partner, as shown by the interaction between Nazca boobies and blue-footed boobies during nesting season, in the Galápagos islands, Southern America. Non-breeding adult Nazca boobies disrupt the nesting activities of the blue-footed boobies, preventing nestlings from fledging. Consequently, the latter is forced to nest in the unfavourable conditions of hinterlands away from the preferred cliff edges to avoid the attacks by Nazca boobies (Townsend *et al*. 2002).

On the whole, symbioses could be obligate or facultative; and can either occur within the body of a host (endosymbiosis) or on the body of the host (ectosymbiosis) depending on the interactive organisms.

1.1 EPIBIOSIS

A particular form of ecto-commensalism occurs in aquatic systems and is called Epibiosis. Epibioisis is a symbiotic association between organisms (epibionts) that settle and live on hosts (basibionts) (Wahl 1989, Wahl & Mark 1999). It is a widespread phenomenon in both marine and freshwater systems (Green 1974, Wahl 1989, Fernández-Leborans & Tato-Porto 2000a,2000b). The terms “epibiont” and “basibiont” describe the ecological roles of the two interactive organisms (Wahl 1989). Epibionts are organisms that, during the sessile phase of their life cycle, attach to and live on the body surface of another living organism. The sessile phase is a period of epibiont growth and reproduction following active dispersion from an old substrate or habitat (Green 1974). Meanwhile, basibionts (substrate organisms) are organisms that host the epibiont (Wahl 1989, Threlkeld *et al*. 1993).

A similar association, epiphytism, occurs in the terrestrial ecosystem between plants and epiphytes (Barkman 1958). Epiphytes are non-parasitic plants which grow mainly on trees which serve as substrates for support and for epiphytic growth and development (Barkman 1958, Zotz & Heitz 2001). Epiphytic communities are important components of rain forests contributing to species richness and diversity (Ter Steege & Cornelissen 1989), hence, they are used in the biomonitoring of forest air as well as the assessment of forest health (McCune 1993). In temperate climates, epiphytes include lichens, mosses, liverworts and algae (Zotz 2005) while ferns, cacti, orchids and bromeliads (e.g. Spanish moss) are typically associated with tropical climates (Johansson 1974, Zotz & Heitz 2001). Also, microepibionts, e.g., fungi, have been found to colonise terrestrial plants (Dickinson & Preece 1976, Legault *et al.* 1989).

Aquatic epibionts consist of two major groups namely the epiphytes (plants, algae) which attach to aquatic plants, and the epizoans (animals) (Harder 2008). Epizoans include:

- ciliophoran protozoans: suctorians, chonotrichids, apostomatids, heterotrichs and peritirich (Fernández-Leborans & Tato-Porto 2000a, 2000b)
- bacteria (Holland & Hergenrader 1981, Sar & Rosenberg 1987, Poltz *et al*. 1998)
- rotifers (Green 1974)
- algae (Barea-Arco *et al*. 2001)
- diatoms (Gaiser & Bachmann 1993)
- hydrozoans (Williams & McDermott 2004)
- entoprocts, cirripeds and polychaetes (Fernández-Leborans 2006, Cupul-Magaña *et al.* 2011), all of which attach to marine and freshwater plants and animals.

Epibiosis has long been regarded as a commensal relationship between two or more organisms but Nenninger (1948) and Lust (1950) (cited in Cook *et al.* 1998) suggested that the relationship could either be facultative in which epibionts are unspecialised and colonise both living and non-living substrates, or completely obligate and highly specific where epibionts and basibionts have undergone morphological and behavioural changes required for the association. In the case of the latter, there would be some degree of coevolution between the two species (Al-Dhaheri & Willey 1996). The term "Epibiosis" also spans a wide range of symbiotic relationships from parasitism to mutualism (Barea-Arco *et al*. 2001). For example, Green (1974) reported a decrease in egg production by *Daphnia magna* colonised by the rotifer *Branchionus rubens*. In this system, the epibionts compete with *D. magna* for food and the high densities of infestation also hindered the feeding mechanism of *D. magna*. Consequently, the increase in energy expenditure due to epibiont load, in addition to depletion of food resources by the epibiont, translated to decreased egg production in *D. magna*. In the light of the above, the association can be regarded as being a parasitic symbiosis. On the other hand, in large numbers and in their motile stage, epibionts have been reported to be important food sources for their hosts, in return for the support and shelter provided by the latter (Green 1974, Threlkeld & Willey 1993, Al-Dhaheri & Willey 1996, Holland & Hergenrader 1981, and Barea-Arco *et al*. 2001). This is also illustrated in the nutritional symbiosis between shrimps in hydrothermal vents and their epibiotic bacteria, in which bacteria serve as sources of nitrogen and carbon for the shrimps (Polz *et al*. 1998).

The movements of the shrimps in the vents, in turn, create a nutrient-rich environment for the epibiotic bacteria.

1.2 FACTORS THAT INFLUENCE EPIBIONT ABUNDANCE AND PREVALENCE IN AQUATIC SYSTEMS

Various factors influence the prevalence of epibionts in any given system. Epibiosis has been shown to be positively correlated with host abundance (Chiavelli *et al*. 1993). As an example, Xu (1992) observed maximum prevalence of epibionts when zooplankton hosts were highly abundant. Reduction in host zooplanktons due to predation by fish or invertebrates can lead to a reduction in epibiont abundance (Willey *et al*. 1990, Chiavelli *et al.* 1993). Increase in host population without a resultant increase in the epibiont population also affects epibiont abundance and prevalence (Henebry & Ridgeway 1979). On the other hand, high epibiont burdens can alter the seasonal occurrence of host population. Weissman *et al*. (1993) reported that high epibiont burden contributed to the seasonal decline in *Acartia hudsonica* populations in Stony Brook Harbour, Long Island Sound, New York.

Interspecific competition among epibionts for attachment space and predation by carnivorous epibionts (e.g., suctorian epibionts) can also influence epibiont abundance when these force weak or slow colonisers out of preferred substrates (Threlked *et al*. 1993, Willey & Threlkeld 1993, Threlkeld & Willey 1993). This is especially obvious among epibionts that share the same ecological niche, for example, Utz and Coats (2005) observed that *Epistylis* occurred in high abundances on *Acartia tonsa* in Chesapeake Bay at specific times of the year but was never present at these times when high abundances of *Zoothamnium* were recorded. They suggested that this pattern of occurrence could be as a result of competition for space between *Epistylis* and *Zoothamnium*. Unlike *Epistylis*, *Zoothamnium* is a contractile, colonial peritrich. As a result, it uses more space than *Epistylis* and the contraction of its colony disturbs the microenvironment, making it a better competitor than *Epistylis*. In Finland, Ebert *et al.* (2001) reported an interspecific competition among rock pool epibionts *Vorticella octava*, *Epistylis helenae* and *Colacium vesiculosum*. *E. helenae* and *C. vesiculosum* do not compete strongly with each other because they are spatially separated; rather, both epibionts compete with *V. octava* for space. *V. octava* has a contractile stalk unlike *E. helenae* and *C. vesiculosum*, hence, its contraction takes up space and interferes with the non-contractile epibionts to its detriment. This could explain the low prevalence values recorded for *V. octava* in low conductivity rock pools in the Tvaerminne Archipelago, Finland.

Abiotic conditions, such as conductivity, salinity, turbidity, temperature, pH, dissolved oxygen concentration and nutrient levels of carbon and nitrogen, in the aquatic systems can either favour or hinder the prevalence of epibionts. For example, water clarity, by affecting light penetration, determines the prevalence of phototropic epibionts. In Oneida Lake, New York, Chiavelli *et al*. (1993) reported that prevalence levels for *Synedra* were highest during periods of high water clarity. However, the prevalence was observed to be limited by high temperatures because *Synedra* appeared in spring and disappeared in early summer although its preferred daphnid host was still present. Also, epibiotic abundance was not affected by nutrient concentrations in the lake. A similar finding was reported in North American lakes where the high *Synedra* prevalence and densities recorded in spring were attributed not only to cold water temperatures, but also nutrient availability and temperature-related reduction in the host's moulting frequency (Gaiser & Bachman 1993). Though López *et al.* (1998) reported a negative correlation of *Epistylis* prevalence with dissolved oxygen, no relationship was found between water temperature and prevalence in the Tulé Reservoir, Western Venezuela. High salinity levels constitute environmental stress for freshwater organisms, resulting in population decline. For example, in the solar saltern of Sfax (Tunisia), abundances of aquatic communities, including ciliates, decreased with increasing salinity (Elloumi *et al.* 2006). Similar findings have been reported in a solar saltern in Spain (Pedrós-Alio *et al*. 2000) and in the Antarctic saline lakes (Periss & Laybourn-Parry 1997). In the Merbok mangrove of north-western Malaysia, prevalence of zoothamnid ciliates on mysids was found to increase as salinity decreased (Hanamura *et al.* 2010). In the rock pools of the Tvaerminne Archipelago (Baltic Sea), epibiont richness was found to correlate with water conductivity. Epibiont species recorded in pools with lower water conductivity were more than was found in pools with higher water conductivity (Ebert *et al*. 2001). However, in southern Spain, Barea-Arco *et al*. (2001) found no relationship between abiotic conditions and the seasonal prevalence of *Korshikoviella gracilipes* in the Río Seco Lake.

Abiotic conditions can also determine the geographic distribution of epibionts, such that epibionts that survive under a broad range of conditions will have a wider distribution than their restricted counterparts (Utz & Coats 2005), e.g., *Zoothamnium* have been found in broad ranges of temperatures and salinities. In 1964, zoothamnid ciliates colonised copepods at salinities between 0.9‰ to 14.22‰ and a temperature range from 2.34°C to 14.10°C in the Chesapeake Bay (Herman & Mihursky 1964). Four decades later, these ciliates were still found to survive in a broad range of salinities (3 – 22psu) and temperatures (4°C – 26°C) in the same aquatic system (Utz & Coats 2005). Similarly, zoothamnid ciliates colonising shrimps in an Indian estuary was found to survive in salinities ranging from 0 – 34psu (Jayasree *et al.* 2001). In rock pools surrounding the Baltic Sea, the epibiont *Vorticella octava* was found to colonise daphnid hosts under a wide conductivity range (0.043 – 12.4 $mS.cm^{-1}$) unlike *Colacium vesiculosum* and *Epistylis helenae* which were only able to colonise in pools within a limited conductivity range (<3 $mS.cm^{-1}$) (Ebert *et al.* 2001).

1.3 FACTORS THAT INFLUENCE HOST SPECIFICITY AND ATTACHMENT SITE PREFERENCE OF EPIBIONTS IN AQUATIC SYSTEMS

Epibionts have been reported to exhibit host specificity (Herman & Mihursky 1964, Henbry & Ridgeway 1979, Willey & Threlkeld 1993, Gilbert & Schröder 2003) as well as attachment site (body part) preference on their substrate organisms (Willey & Threlkeld 1993, Cook *et al*. 1998, Mayén-Estrada & Aladro-Lubel 2001). Consequently, this specificity favours their prevalence in any given system. This is seen in the case of *Colacium calvum* which has been reported to consistently prefer the anal region of cladoceran hosts where it feeds off the nutrient-rich host excreta (Chiavelli *et al*. 1993). In the case of peritrichs, though they have been observed to be evenly distributed all over the zooplankton, certain crustacean body parts such as setae, antennal segments, scales etc. were found more favourable than others because they were able to either provide shelter from predation or trap bacteria and debris (Henebry & Ridgeway 1979, Mayén-Estrada & Aladro-Lubel 2001). The suctorian epibiont *Tokophrya cyclopum* has consistently been found on the anterior aspect of the head and the first antennae of cyclopoid copepods because at these positions, it has a higher probability of coming into contact with potential prey (Henebry & Ridgeway 1979).

Also, some ciliate epibionts were found to be confined to particular sites due to the feeding habits and behavioural patterns, for example, grooming and burrowing, of their substrate organisms (Green 1974, Clamp 1987). Fernández-Leborans *et al*. (1997) attributed the low numbers of epibionts found on the portunid crab to the latter's burying behaviour. Nagasawa (1988) found that *Zoothamnium* preferred *Centropages abdominalis* over *Acartia clausi* because the former can ascend to a higher position in the water column, and therefore was more phototropic than the latter. In addition, some sites are constantly being avoided to prevent mechanical damage, interference with feeding current and general disturbance associated with the substrate's movement (Green 1974). On the other hand, some ciliates have been found to have undergone minor morphological changes such as production of shorter stalks (Laird 1959, Gilbert & Schröder 2003), reduction or flattening of lorica as well as secretion of robust stalk (Cook *et al*. 1998) to adapt to life on less-preferred attachment sites.

1.4 EPIBIOSIS: BENEFITS AND COSTS

As mentioned earlier, the specific association between the epibiont and its host can vary. Often, the association cannot be regarded as entirely favourable or unfavourable for any of the participants. For an epibiont, life on a motile habitat has certain advantages. Not only does the basibiont provide an attachment surface required for epibiont growth and reproduction, its growth can ensure the availability of expanding surfaces for colonisation (Wahl 1989). Also, the epibiotic habitat serves as shelter, protecting epibionts from predation by zooplankton (Al-Dhaheri & Willey 1996). In addition, phototrophic epibionts have increased access to light (Green 1974) and dissolved nutrients (Threlkeld *et al*. 1993) when attached to positively phototactic basibionts. Heterotrophic epibionts benefit by the constant renewal of nutrient availability zones either through the swimming movement of their substrate hosts (Kankaala & Eloranta 1987, Polz *et al*. 1998), from the feeding currents of filter feeding hosts (Threlkeld *et al*. 1993), from host's excreta (Willey & Threlkeld 1993) or from dissolved organic substances deposited on the basibiont's exoskeleton (Holland &

Hergenrader 1981). Finally, transport of epibionts by their motile basibionts to new sites with improved nutrient status facilitates dispersal and gene-flow among epibiont populations (Wahl 1989). However, epibionts can be faced with the unstable nature of their living substrates, which are subject to collapse and dispersion due to moulting and death or predation of the host (Holland & Hergenrader 1981, Threlkeld & Willey 1993, Threlkeld *et al*. 1993, Poltz *et al*. 1998). During basibiont migration, epibionts may be transported to unfavourable environments, resulting in physiological stress for the epibiotic population (Wahl 1989).

There are various advantages to the host organism in this relationship. In addition to utilising epibionts as a food resource by some basibionts, basibionts also benefit from the interaction through the ingestion of specific nutrients, e.g., vitamins, carbon and nitrogenous compounds excreted by epibionts (Holland & Hergenrader 1981, Poltz *et al*. 1998). Also, bacterial epibionts prevent accumulation of food debris on calanoid copepods through degradation of these organic materials as seen in Nebraska lakes (Holland & Hergenrader 1981). Epibiotic cover has also been reported to confer protection to the substrate organism in various ways. In periods of low tides, water-retaining marine epibionts may reduce dessication rate of inter-tidal organisms as in the case of the eelgrass and its epiphytic community (Penhale & Smith 1977). Toxic metabolites secreted by marine epibionts and surface properties (e.g., spines) of certain epibionts provide defence for the substrate organism (Christophersen 1985, Dyrynda 1986). The hydrophobic, drag-reducing bacteria on the skin of the fast-swimming fishes (spanish mackerel*,* great barracuda*,* and gilt-head) have been reported to have surface-modifying properties, thus, making the fish more streamlined, and increasing their swimming efficiency (Sar & Rosenberg 1987).

Although certain epibionts can be viewed as having a positive effect on their hosts, the majority of studies report negative effects of epibionts on individual survivorship and population dynamics of their substrate organisms. Large populations of epibionts constitute impediments to host movement, and increases in weight reduces buoyancy of zooplankton, affecting swimming efficiency and making it more difficult for zooplankton to maintain their position in the water column (Herman & Mihursky 1964, Wahl 1989, Allen *et al*. 1993, Weissman *et al*. 1993, Barea-Arco *et al*. 2001). Also, the increase in size and visual contrast enhances the visibility of substrate organisms to visually-oriented fish and decreases their evasive ability against filter-feeding fish (Willey, *et al.* 1990). In addition, epibionts can compete with their substrate organisms for food (Green 1974, Wahl 1989). For example, in the polyhumic Mekkojärvi Lake, Southern Finland, *Vorticella* was reported to compete with its daphnid host for food (Kankaala & Eloranta 1987). Moreover, competition for food may increase the death rate of hosts especially in cases of heavy epibiotic infestation, in which the feeding rates of basibionts would have to increase in order to offset the infestation-related increase in energy expenditure (Allen *et al*. 1993). Furthermore, high epibiotic burden reduces the reproductive rate of zooplankton hosts because the energy reserved for reproduction is used to offset the increased energy demand for locomotion (Green 1974, Threlkeld & Willey 1993). This is illustrated by Stirnadel and Ebert (1997) who found a reduction in the clutch size of heavily infested *Daphnia magna* in eutrophic ponds in southern England.

Attachment sites of ciliates can also serve as sites of bacterial, fungal, as well as protozoan colonisation and multiplication, resulting in secondary infection (Esch *et al*. 1976). Nutrients in the fluid which leaks out of lesions at these sites are thought to be utilised by the invading colony resulting in increased death rate of host organisms, as illustrated in Escambia Bay, Florida. In the afore-mentioned system, secondary bacterial infection of, and loss of fluid from, lesions on *Acartia tonsa* exoskeleton at attachment sites of *Epistylis* stalks increased the copepod's death rate (Turner *et al.* 1979). In shrimp aquacultures, *Zoothamnium*, *Epistylis* and *Vorticella* have been associated with pathological conditions of shrimps (surface and gill fouling) (Brock & Leamaster 1992, Turnbull *et al.* 1994, Ambipillai *et al*. 2003). Similarly, in Lake Erie, Ohio, fishes (smallmouth bass, rock bass and freshwater-drum) were found to be infested by *Epistylis niagarae* (Crites 1977). Penetration of the stalks through the epidermis into the hypodentine of fish scales resulted in: scale erosion and ulcers at the sites of *Epistylis* attachment, secondary infection by the protozoa *Trichodina* and *Ichthyophthirius*, damage to fish, and in severe cases, death. Also, in Tennessee, channel catfish mortality has been attributed to the erosion of epithelial tissues and underlying bone structure caused by *Epistylis* (Wayne & Warner 1975).

Interestingly, the negative effects of epibiosis on basibionts seem to be more evident in the absence of food for the basibiont. Xu and Burns (1991) found no major negative effect on host fitness when there is enough food to meet the increased energy demand generated by epibionts. But in the absence of food, they observed a shorter survival period for, and increased death rate of, *Epistylis*-infested copepodites, in comparison to uninfested ones.

1.5 PROTOZOAN EPIBIOSIS AS AN INDICATOR OF WATER POLLUTION

Ciliated protozoa are commonly found attached to freshwater plants and animals as well as non-living substrates. In addition to being a major component of the aquatic food chain and an important ecosystem study tool for understanding zooplankton population dynamics and community structure, ciliates have been used as bioindicators of water quality (Burbanck and Spoon 1967, Bick 1972, Small 1973, Cairns 1974, 1978, Sládecek 1981). Though doubts have been raised regarding their feasibility in use as bioindicators (Hynes 1960), the unicellular nature of protozoa makes them highly responsive to small environmental changes. Additionally, they have a fast rate of reproduction, are widespread, and can be maintained with relative ease in culture. They are therefore well-suited as indicator taxa for pollution assessment and environmental studies (Cairns 1974). Laird (1959) suggested that the occurrence and abundance of certain species of protozoa on mosquito larvae could be related to pollution of lakes in Singapore. Also, Antipa (1977) showed that commensal ciliates attached to molluscan hosts may serve as very sensitive indicators of organic pollution, thus facilitating early detection of changes in water quality. He reported that, unlike in the control/unpolluted stations, the epibiont *Heterocinetopsis* disappeared completely from its host, the mussel *Anodonta grandis* in the polluted stations of the Salt Fork (Vermillion River), Illinois. Furthermore, a survey of the Ashmore Lake in Illinois showed that prevalence level of ciliates on copepods and cladocerans was higher than levels recorded on these hosts in other aquatic systems in the USA. Hence, this occurrence was thought to be as a result of the eutrophic condition of the Ashmore Lake (Henebry & Ridgeway 1979).

Protozoan colonisation of artificial substrates has also been used to assess water quality in aquatic systems (Henebry & Cairns 1980, Plafkin *et al*. 1980). For example, in Northern

Ireland, Xu and Wood (2011) studied protozoan communities colonising an artificial substrate, polyurethane foam, in order to assess water quality in Kinnego Bay. The low species richness recorded in polluted stations, when compared with unpolluted stations, indicate that the various protozoan species in the bay are strongly affected by pollution. Both species richness and diversity were found to be higher in unpolluted stations. In addition, certain protozoan species (*Cryptomonas erosa*, *Opercularia coarctata*, *Vorticella microstoma* and *Spirostomum ambiguum*) were recognised as good indicators of organic pollution (Bick 1972, Sládecek 1981, Villegas & Giner 1973).

Water quality in South African rivers has been negatively impacted by anthropogenic activities, posing serious dangers to human health and the natural environment. A report by the Council for Scientific and Industrial Research (CSIR) (2010) on water quality in South Africa noted that five of the six key global threats to freshwater biodiversity (Dudgeon *et al*. 2006) are applicable to South African eco-regions. One of these was water pollution. In addition, assessment of ecosystems showed that the aquatic ecosystems are more negatively impacted than terrestrial ecosystems, with fresh water systems categorised as moderately to highly eutrophic as result of the excessive nutrient enrichment (CSIR 2010). In South Africa, water pollution occurs as a result of intensive agriculture and human habitation. Aquatic systems (rivers, dams, wetlands and reservoirs) receive contaminated runoffs from different sources of pollution which include (CSIR 2010):

- high concentrations of fluoride and nitrate in soils and groundwater
- runoff from irrigation containing high levels of dissolved salts and agro-chemicals (fertilizers and pesticides)
- contaminated runoff from human settlements containing high concentrations of metal ions, nutrients, salts and Endocrine Disrupting Compounds (EDCs) as well as pathogenic organisms
- domestic and industrial effluent (treated and untreated)
- saline effluents from tanneries
- highly acidic seepage from mines and coal-powered industrial plants

Also, silt and clay sediments from easily erodible rocks and soils accumulate in water storage facilities, e.g., dams. Consequently, there is a steady deterioration of water quality in freshwater and reservoir systems, particularly in areas with naturally saline soils and groundwater (CSIR 2010).

The excessive input of nutrients, mainly nitrogen and phosphorus forms, results in eutrophication, or simply put, nutrient enrichment of water systems. Changes in phytoplankton species composition, due to eutrophication, lead to the development of algal blooms of toxic species, e.g., cyanobacteria (blue-green algae). In eutrophic reservoirs in South Africa, the cyanobacteria *Microcystis* and *Anabaena* have been identified as dominant phytoplankton genera (Van Ginkel 2004). These harmful blooms negatively impact the environment by (Fleming *et al*. 2002):

- depletion of oxygen through bloom respiration and degradation
- production of harmful compounds (cyanotoxins, allergens, odour-causing compounds)
- interference with food web dynamics by the displacement of normal phytoplankton species which decreases ecosystem stability
- their increased absorption of sunlight which reduces light penetration to submerged aquatic plants.

Additionally, affected waters lose aesthetic and recreational values due to foul odour, bad taste and discolouration. The presence of toxic compounds makes the purification of eutrophic water systems problematic and expensive because conventional water treatment processes cannot remove cyanobacterial biotoxins (CSIR 2010). Ultimately, the highly unfavourable environment leads to mortality of fish and other aquatic animals either as a result of direct poisoning or indirectly from diseases and environmental stress (Paerl 1988, Carmicheal 1994, CSIR 2010). Likewise, human populations and terrestrial animals are faced with the risk of toxicity and medical disorders caused by pathogens and noxious substances in eutrophic water systems.

Traditionally, water monitoring techniques have included the physical assessment and the chemical analysis of water variables, as well as the use of macroinvertebrates (insects, gastropods, annelids, crustaceans, and molluscs) as bioindicators. Biological indicators have been widely used, not only to detect contamination, but also to evaluate the health status of water systems, especially rivers (Burbanck & Spoon 1967, Dickens and Graham 2002). Formerly, occurrence of “fish kills” or the absence of fish or other aquatic animals in water systems indicated water pollution. But, the response of aquatic animals to pollution was found to be complex, and as a result, was not easily observed. This is because their internal regulatory systems allow adaptation to a broad range of environmental conditions, making them weak bioindicators of water quality (Hynes 1960). Conversely, benthic macroinvertebrates have been found to be better bioindicators because of their limited mobility, rapid life cycle, ease of identification and visibility. The use of macroinvertebrates is based on their varying tolerance levels to pollution such that highly sensitive organisms e.g. stoneflies are completely absent from polluted waters. They have been used in various river bioassessment tools such as River Invertebrate Prediction and Classification System (RIVPACS), Biological Monitoring Working Party (BMWP) method, Rapid Bioassessment Protocols and in the South African Scoring System (SASS) (Dickens & Graham 2002).

In 1972, Chutter developed the Biotic Index for river health assessment. But, it was not widely used because it was labour-intensive (Dickens & Graham 2002). This led to the development of SASS which is both fast and economical (Chutter 1994, 1998). Over the years, SASS has become the standard technique in bioassessment of river health and other impact assessments (Dickens & Graham 1998). Because macroinvertebrate taxa have varying pollution-tolerance levels, SASS assigns tolerance scores to each taxon to generate the total SASS score and the average score per taxon (ASPT). The afore-mentioned scores, in addition to the number of taxa recorded, are the commonly used SASS bioindices. However, field trials have shown ASPT to be the most consistent among the three indices (Dickens & Graham 2002).

On the whole, these methods require a long-term and substantial change in the environmental parameters (Antipa 1977) such that the early environmental changes may not be detected. The intimate contact which protozoa maintain with their immediate environment due to their unicellular nature makes them more susceptible to subtle eco-environmental stress which may not be picked up by any of the afore-mentioned methods (Burbanck & Spoon 1967, Cairns 1974, Antipa 1977).

Although protozoan epibiosis has been recorded in South African water systems, little is known about the ecology and use of protozoans as bioindicators of water quality. Though there was no information on the health status of the Westdene dam, Johannesburg, Viljoen and Van As (1983) identified 15 species of ciliate protozoa, representing nine genera (*Carchesium*, *Vorticella*, *Apiosoma*, *Epistylis*, *Opercularia*, *Scyphidia*, *Cothurnia* and *Vaginicola*) in their survey. These were attached to both biotic (fish, snail, dragon fly larvae, water bug, crab, algae) and non-biotic substrates (plastic petri-dishes and glass slides). Interestingly, five sessile ciliates found on fish were not only morphologically distinct, but host-specific as well.

There is apparently no published work on the occurrence of ciliate protozoa in rivers in the Western Cape Province, including their use as bioindicators of water pollution. This study is therefore the first examination of ciliate diversity and host associations in the region. The aims of the study were:

a) To investigate the prevalence of epibiosis of ciliate protozoa on benthic macroinvertebrates in six rivers in the winter rainfall fynbos biome of the Western Cape Province
b) To identify the genera involved using light and scanning electron microscopy and DNA sequencing
c) To determine the response of ciliate protozoa to water pollution along a pollution gradient in a single river (the Eerste) in the Province, and to thus evaluate their role as potential bioindicator taxa for freshwater pollution.

Chapter 2 gives the site information on the six rivers in the Western Cape Province of South Africa sampled and a general description of materials and methods used in the study.

Chapter 3 uses morphological and molecular markers to identify ciliate epibiont taxa found on aquatic hosts in the Western Cape rivers sampled for the study.

Chapter 4 determines the epibiotic prevalence of the ciliate epibionts of aquatic hosts in the six Western Cape rivers and reports on the patterns of prevalence identified with respect to seasonality, host preference and host body part preference of ciliate epibionts.

Chapter 5 evaluates the response of ciliates to environmental changes as reflected in changes in the physico-chemical water characteristics. Their responses are discussed in the light of their possible roles in water bio-monitoring. This study provides the first record of ciliate-host association in Western Cape rivers and their responses to changes in the aquatic environment.

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CHAPTER 2. GENERAL MATERIALS AND METHODS

2.1 FYNBOS BIOME

The study was carried out in the Cape Floristic Region (CFR) of the Western Cape Province, South Africa. The CFR, also referred to as the Fynbos Biome, is a global biodiversity hotspot (Cowling *et al.* 2003). It is a fire-driven biome which occurs largely in the winter-rainfall areas of South Africa (Rebelo *et al.* 2006). Geological substrates in the fynbos biome include: sandstone, quartzite, granite, shales and limestone sediments eroded from quartzite-sandstone Table Mountain. This unique soil structure and type have also been linked to the biodiversity and diverse vegetation types seen in the fynbos biome (Rebelo *et al.* 2006).

The Fynbos biome has three broad vegetation types namely: strandveld, renosterveld and fynbos vegetation (Rebelo *et al.* 2006). The strandveld vegetation consists of dense shrublands dominated by sclerophyllous, broad-leaved shrubs (Moll *et al.* 1984). It is also known as beach vegetation because it is usually found close to the sea. It occurs in well-drained sandy soils with high concentrations of calcium and it is less fire-prone than the renosterveld and fynbos vegetations (Rebelo *et al.* 2006). Renosterveld, is an evergreen, fire-prone shrubland predominated by small-leaved asteraceous shrubs growing among grasses (Poaceae), and a high biomass and diversity of geophytes. It occurs predominantly on clay-rich soils which are more fertile than the soils of the other vegetation types, hence, croplands and farms are major features of renosterveld (Moll *et al.* 1984, Rebelo *et al.* 2006). The Cape fynbos is a fire-prone restioland dominated by fine-leaved shrubs (Restionaceae, Eriaceae and Proteaceae). It occurs on soils poor in nutrients: sandy soils, limestone, leached clay soils from shale and granite, gravelly soils from duricrust outcrops and alluvial sediments (Rebelo *et al*. 2006).

In the CFR, the freshwater systems consist mainly of headwater rivers (Galatowitsch & Richardson 2004). Headwaters are key components of river systems which support a diverse array of unique species (Meyer *et al.* 2007). In their upper reaches or mountain zones, rivers in the Western Cape Region are clear, acidic (pH 3.5 – 6), foamy and stained black or brown by certain dissolved organic carbon compounds (phenols, tannins, humic/fulvic acids and saponins) produced by the undisturbed fynbos vegetation (Noble & Hemens 1978, King *et al.* 1979, Midgley & Schafer 1992). Midgley and Schafer (1992) found dark stream colour to correlate with paleness of catchment soil colour. The nutrient-poor, bleached, sandy fynbos soil are low in sorption sites (bases and clay minerals), and thus, the organic compounds are not adsorbed out as water flows through the soil (Midgley & Schafer 1992). Also, upper reaches have low nutrient concentrations (oligotropic), low levels of total dissolved solids (TDS), are sediment-free and poorly buffered (King *et al.* 1979).

Additionally, in longer rivers, the five river zones are well-developed (Noble & Hemens 1978) and include: mountain source (high-altitude sponges) and cliff waterfall; mountain stream, foothill sandbed; low and midland stream and river; and estuary. But, river zonation depends on the topography (Noble & Hemens 1978); hence, short rivers have fewer zones than the long ones, e.g., the Rooiels exhibits two zones, descending from its mountain zone to an estuary zone (King *et al.* 1979).

Riparian ecosystems are highly susceptible to invasion by alien plants as a result of their dynamic hydrology, nutrient levels and ability to disperse propagules (Planty-Tabacchi *et al.* 1996). Additionally, interference with hydrology, e.g., altered river flow, as seen in dam construction, contribute to invasions by alien species (Tickner *et al.* 2001). Alien invasion rates have been found to be directly related with degree of degradation of natural riparian vegetation, e.g., by agricultural activities, human disturbances and land cover transformation (Le Maitre *et al.* 2000). Originally, the riparian vegetation of Western Cape rivers consists indigenous woody plants, e.g., Silky Bark (*Maytenus acuminata*), Wild Almond (*Brabejum stellatifolum*), Breede River Yellowwood (*Podocarpus elongatus*) etc. However, over the years, they have been replaced by invasive alien tree species, e.g., Black wattle (*Acacia spp.),* Albizia (*Paraserianthes lophanthas*), eucalypts (e.g., *Eucalyptus camaldulensis*) amongst others. Le Maitre *et al.* (2000) identified the Western Cape as the most heavily invaded region in South Africa with the catchment areas of the Breede and the Berg Rivers having the highest invasion rates. The *Acacia spp.* was introduced into South Africa from Australia and has spread widely through the Western Cape Province (Richardson *et al*. 1992) from upper catchments of Western Cape rivers (Le Maitre *et al.* 2000). Unfortunately, just like the indigenous riparian woody plants, its regeneration is triggered by fire (Pieterse & Boucher 1997). And, the seeds, which are produced regularly in large numbers, are dispersed rapidly downstream of the invaded river systems (Galatowitsch & Richardson 2004), hence, its wide geographical distribution along watercourses in the Western Cape. Following establishment, dense stands of *Acacia* serve as sediment traps, thus creating a favourable environment for stand expansion (Van der Heyden 1998) and altering ecosystem functioning as seen in reduced streamflow in alien-invaded systems (Scott *et al*. 1998).

In October 1996, the "Working for Water" programme was initiated by Department of Water Affairs and Forestry (DWAF). It was aimed at clearing of alien vegetation to increase water production, improve water quality, conserve biodiversity and facilitate the return of invaded areas to their pre-invasion status (DWAF 1997). This removal involves the felling, use of herbicides and prescribed burning of aliens, followed by regular checks to remove re-invaders and progagule remnants (Van Wilgen *et al.* 1998). Variations were recorded in the success rate of the clearing program. While some cleared riparian zones returned to their pre-invasion status without further management intervention, in other sites, indigenous plants failed to regenerate and there was further reinvasion of aliens (Galatowitsch & Richardson 2004). This was as a result of the slow regeneration rates of indigenous plants when compared with alien plants. Also, alien invasion is disturbance-triggered such that the unstable post-clearing conditions (open canopy sites and unstable soil conditions) favour the re-invasion of these plants rather than recovery of indigenous plants. Nevertheless, there are indigenous species that have been found to thrive in both open and closed canopy zones, e.g., the Wild Almond, water heath (*Erica caffra*) etc., indicating their adaptation to growth in unstable sites. On the whole, to increase the rate of regeneration, repeated follow-up clearing of aliens is needed; though reversal to pre-invasion status may be protracted in highly invaded riparian zones (Galatowitsch & Richardson 2004). Downstream reaches of rivers sampled in the present study have been invaded by alien vegetation.

2.2 STUDY SITES

The study region is the portion of Western Cape between the Cape Peninsula and Ceres. Rivers sampled in this study include the Wit, Wolwekloof, Rooielskloof, Eerste, Elandspad and Molenaars (Fig 2.1). They were chosen based on ease of accessibility and the relative ecosystem similarity among them. River sites are described based on information provided by Dallas and Rivers-Moore (2010).

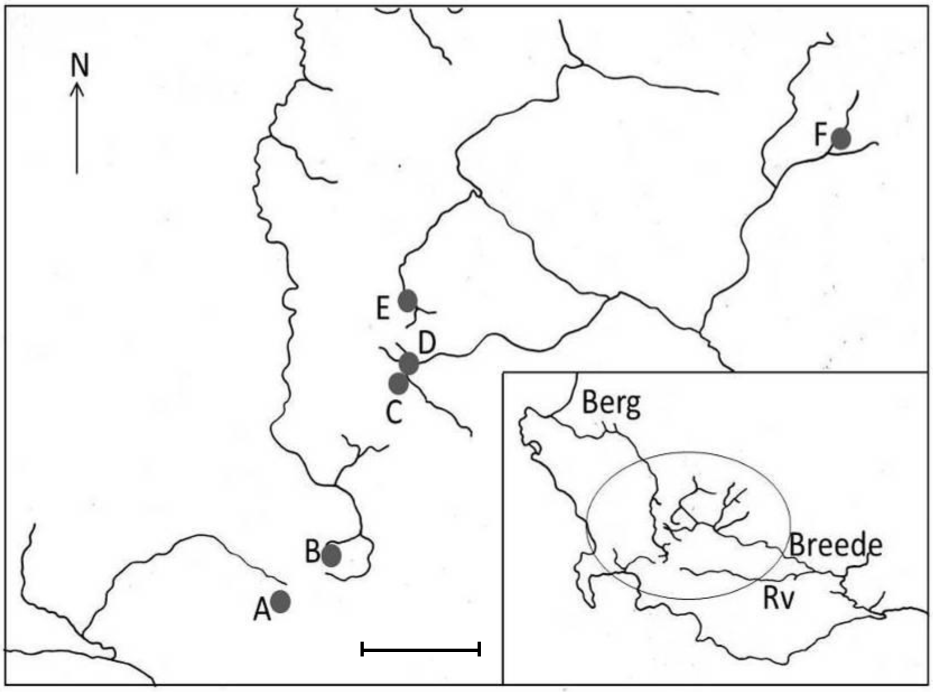

Figure 2.1. Map showing location of river sites in the Western Cape Province, South Africa: Eerste (A), Wolwekloof (B), Elandspad (C), Molenaars (D), Wit (E) and Rooielskloof (F). Abbreviation: Rv, Riviersonderend. Scale bar = 30 km.

The Wit River is a tributary of the Breede River and is located in the Western Folded Mountain eco-region. It is a single-thread sinuous river meandering through riparian vegetation consisting of undisturbed fynbos vegetation with no canopy cover. It is located in the winter rainfall region of the Western Cape, and it flows throughout the year. Its river zone is classified as transitional with reach types consisting of plane bed, pool-rapid and pool riffles. The river bed is a mixture of fluvial sediments, sandstones and predominantly boulders. The study site was not impacted by human activity.

The Wolwekloof River, located in the Southern Folded Mountain eco-region, is a perennial river which flows into the Berg River. It is a mountain stream dominated by bedrock, boulders and coarse sandstone and quartzite pebbles. Its reach types comprise cascades, bedrock fall and step-pool. Riparian vegetation consists of alien trees which were felled leaving the river system with no canopy cover. River flow is regulated by a dam and a weir. The study site was above the dam, and at some stage was surrounded by some pine plantation.

The Rooielskloof River is a perennial river flowing into the Hex River. It is located in the Western Folded Mountains eco-region and classified as a mountain stream with fluvial deposits mostly consisting of well-rounded boulders and cobbles of sandstone and quartzite. Cascades, bedrock fall and step-pool are the various reach types characterising this river system. Its river banks are lined by agricultural crops and intermittent open patches. River flow is regulated by a weir. The study site was located in an area without any impoundment or anthropogenic influence.

The Eerste River, originates in the Jonkershoek Mountains of the Southern-Folded Mountains eco-region. It is classified as a rocky mountain stream and has three reaches: cascade, bedrock fall and step-pool. Along the course of the Eerste River lies the Kleinplaas Dam (also called the Jonkershoek Dam) which is situated in the mountain stream zone and was constructed to divert water for residential and agricultural use for the town of Stellenbosch (DWAF 1993). It also serves as a trout farm supported by Department of Genetics (Aquaculture) of the University of Stellenbosch. Although the water quality upstream from the dam remains relatively undisturbed, the downstream reach below the dam has been negatively impacted by anthropogenic activities including water extraction for town use and the trout farming (Brown & Dallas 1995). Once the river leaves the protected natural area, its banks become lined by alien vegetation which is currently being removed. The Eerste River was sampled again in greater detail at six sites to determine the effect of water pollution on epibiont and macroinvertebrate communities (Table 2.1). The study sites above the dam had a natural tree canopy, but site 6 has a canopy of alien trees. The Elandspad River, a tributary of the Molenaars River, is a perennial river located in the

Western Folded Mountains eco-region. It is classified as transitional and has three reach types: plane bed, pool-rapid and pool riffles with its river bed having fluvial sediments of sandstones and boulders. The river channel is surrounded by wilderness area. A weir erected along the river course serves to regulate flow. The study site was above the weir.

The Molenaars River is located in the Western Folded Mountains eco-region. It is a perennial river which flows in to the Smallblaar River. Its reach types include plane bed, pool-rapid and pool riffles. Its river bed is characterised by fluvial sediments of sandstones and boulders. Its zone is transitional. Though the upper reaches run through an area of undisturbed fynbos vegetation, the lower reaches are lined by alien vegetation and serve as preferred fishing sites for anglers. The study site was not impacted by human activity.

2.2.1 Survey of preserved samples

Samples of benthic arthropods collected in a previous study (V. Ross-Gillespie, University of Cape Town) from these rivers and that had been preserved in 70% alcohol were microscopically examined for the presence of epibionts. These samples were collected between April 2009 and April 2010 at monthly intervals (Table 2.1) using kick-sampling method (Mackey *et al.* 1984, Bradley & Ormerod 2002) described below. Arthropods were sorted and identified to family level using dichotomous keys prepared by various authors for the Water Research Commission (WRC) (de Moor 2002, Harrison 2002, Harrison *et al*. 2002a,b, Barber & Lugo-Oritz 2003, de Moor & Scott 2003, Mansell 2003, Reavell 2003, Samways & Wilmot 2003, Stevens & Picker 2003, Biström 2007, de Moor 2007, Endrödy-Younga 2007, Endrödy-Younga & Stals 2007a-d, Nelson 2007a,b, Perkins 2007, Ribera & Bilton 2007, Stals 2007a,b, Villet & Endrödy-Younga 2007). The following arthropod taxa were examined using a Nikon SMZ800 stereomicroscope (Nikon Coorporation, Tokyo, Japan): Ephemeroptera (larvae), Plecoptera (larvae), Trichoptera (larvae) and Coleoptera (adults and larvae). The total number of individuals infested was recorded and total number of epibiont colonies per individual was counted. A colony consisted of between one and twenty zooids.

2.2.2 Additional sample collection

Further samples of benthic arthropods were collected on various occasions depending on research need (Table 2.1) using the kick-sampling method (Mackey *et al*. 1984, Bradley & Ormerod 2002). A soft net of fine mesh (pore size of 0.08mm) with dimensions 30cm x 30cm x 60cm was positioned a few metres downstream from the point of disturbance. Rocks and the substrate were dislodged by kicking and rubbing rocks by hand, and drift samples collected into a small cup at the end of the net. At each site, sampling was conducted twice for 3 minutes at each at two adjacent locations. The contents of the cup of the drift net were preserved separately for each site in 96% ethanol and refrigerated until further analysis. Arthropods were sorted in the laboratory and identified to family level using dichotomous keys (de Moor 2002, Harrison 2002, Harrison *et al*. 2002a,b, Barber & Lugo-Oritz 2003, de Moor & Scott 2003, Mansell 2003, Reavell 2003, Samways & Wilmot 2003, Stevens & Picker 2003, Biström 2007, de Moor 2007, Endrödy-Younga 2007, Endrödy-Younga & Stals 2007a-d, Nelson 2007a,b, Perkins 2007, Ribera & Bilton 2007, Stals 2007a,b, Villet & Endrödy-Younga 2007). Basibiont hosts for sessile ciliate epibionts adult beetles (families Elmidae, Hydraenidae), stonefly larvae (*Aphanicercella*, family Notonemouridae), and beetle larvae (family Dryopidae, Ptylodactylidae) were isolated from benthic macroinvertebrate samples and identified to family level. Subsets of these sessile ciliate epibionts were used for identification purposes using microscopy and DNA phylogenetic analyses. Materials for molecular analyses were preserved in 96% ethanol until DNA extraction.

Table 2.1. Samples collected in different sampling periods from the different river sites.

SITE (ALTITUDE)	COORDINATES	SAMPLING DATE	SAMPLE COLLECTED	ANALYSIS
Wit (660m)	S33° 63.7090 E19° 10.7890	Monthly sample from April, 2009 to April, 2010	Benthic samples	Epibiont occurrence and attachment site preference
		14th June, 2012		Gene sequencing
Wolwekloof (330m)	S33° 94.4167 E19° 02.6389	Monthly sample from April, 2009 to April, 2010	Benthic samples	Epibiont occurrence and attachment site preference
RooielsKloof (481m)	S33° 46.1100 E19° 61.7860	Monthly sample from April, 2009 to April, 2010	Benthic samples	Epibiont occurrence and attachment site preference
		14th June, 2012		Gene sequencing
Eerste (380m)	S33° 99.3776 E18° 97.5550	Monthly sample from April, 2009 to April, 2010	Benthic samples	Epibiont occurrence and attachment site preference
		1st July, 2011 and 7th October, 2011	Benthic samples	Changes in macro invertebrate community structure in response to pollution, light and scanning electron microscopy
			Water samples	Physicochemical water variables
		14th June, 2012	Benthic samples	Gene sequencing
Elandspad (450m)	S33° 73.6667 E19° 11.4722	Monthly sample from April, 2009 to April, 2010	Benthic samples	Epibiont occurrence and attachment site preference
		15th and 27th March, 2012		Gene sequencing
Molenaars (440m)	S33° 73.1390 E19° 11.5000	Monthly sample from April, 2009 to April, 2011	Benthic samples	Epibiont occurrence and attachment site preference

2.2.3 Infestation prevalence, host preferences and attachment site preferences

For each basibiont family, individuals were scored as either ‘infested’ or ‘uninfested’. Infestation prevalence, defined as the percent of host individuals infested with any ciliate epibiont (Willey *et al.* 1990, Bush *et al.* 1997), was calculated for each macroinvertebrate taxon from each river and for every sampling date respectively. Beetles were the most abundant hosts for the ciliates collected in this study and therefore were used to investigate attachment site preferences. Infestation density (colonies/mm^2 of host area) was determined for elmid beetles (n = 177) and hydraenid beetles (n = 48) separately. For this, the positions of colonies on different ventral body parts of these hosts (head, thorax, legs, and abdomen) were recorded and number of colonies per host body part was determined. Infestations only occurred on the ventral body surface. To estimate the relative surface areas of the different body parts, a grid (created using Microsoft Word 2007), with each cell representing an area of 0.0069 mm^2 was superimposed on images taken of the ventral surface of 36 elmid beetles and 30 hydraenid beetles with a Leica EZ4D stereomicroscope (Leica Microsystems, Taiwan) at x 350 magnification. The number of cells covered by each body part (squares per body part, sq/b.p) was counted and the total area of that body part determined by multiplying the area of each square (0.0069 mm^2) by the number of squares per body part. It was thus possible to obtain the relative surface area of each of the body parts. Dividing the number of colonies found in each body part by the area of that body part provided the number of colonies per host unit area. These areas were then standardised so that all body parts were represented by the same area allowing for epibiont density comparisons between body parts that were originally of very different surface area. In this way, it was possible to accurately compare body parts preferences, without the confounding effect of differing surface areas. The results were expressed as number of colonies/mm^2.

2.2.3.1 Data analyses

Data was analysed using Statistica Analytical Package, version 10 (StatSoft company). Prevalence (percent of individuals infested with any ciliate epibiont) were calculated separately for each monthly sample per river studied. Host abundances were quite low during the winter months when flow rates were high and sampling difficult. Consequently, months in which total number of individuals sampled per taxon was less than five (less than 10 for correlation analyses) were omitted from analyses. A test for normality (Shapirp=Wilk's test) showed non-normal distribution of prevalence data and non-parametric tests were therefore used to determine differences in median prevalence between the six rivers. Prevalence levels of ciliates epibionts on the two most abundant hosts (elmid and hydraenid beetles) were compared between the six rivers using Kruskal-Wallis ANOVA and multiple comparisons of mean ranks (post hoc test). To determine if epibiont prevalence may be dependent on host abundance (elmid and hydraenid beetles only), Spearman's rank correlations were conducted between total host numbers and the number of infested hosts collected/month/river. A Kruskal-Wallis ANOVA was also performed to determine if there were significant differences in epibiont density (colonies/mm^2) between the different attachment sites, with multiple comparisons of means. Significant differences are reported at $P < 0.05$. Graphs were drawn using Microsoft Excel for Windows (2007 version).

2.2.4 Physicochemical water variables

Physicochemical variables were collected for pollution studies from six sites in the Eerste River only (details are given in Chapter 5). Water samples were collected in sterile 300ml honey jars for chemical analysis of nutrients. Nutrients of interest were nitrate/nitrite, phosphate and ammonium ions because they are good indicators of water pollution (Pegram & Gorgens, 2001) and are also limiting nutrients (Dallas and Day, 2004). Physical parameters measured included: flow rate, depth, pH, conductivity, dissolved oxygen, turbidity and temperature. The variables measured are detailed in Table 2.2. Water samples were frozen and taken to the Council for Scientific and Industrial Research (CSIR), water laboratory in Stellenbosch, Western Cape, for determination of nutrient levels. Details of data analyses performed are given in Chapter 5.

Table 2.2. Equipment used in measurement of physical water variables.

VARIABLE	**EQUIPMENT**
Flow ($m.s^{-1}$)	FP101 Flow probe (Global Water, California, USA)
pH	PH25 pH meter (Crison Instruments, S.A., Barcelona, Spain)
Conductivity ($\mu S.cm^{-1}$)	CM35 conductivity meter (Crison Instruments, S.A., Barcelona, Spain)
Oxygen ($mg.l^{-1}$)	OXI45P oxymeter (Crison Instruments, S.A., Barcelona, Spain)
Temperature (°C)	thermometer
Turbidity (NTU)	TN – 100 turbidimeter (Eutech Instruments Pte. Ltd., Singapore)

2.2.5 South African Scoring System (SASS)

The SASS is a widely used rapid bioassessment for evaluating river health and water quality in southern Africa (Chutter 1994, Dickens & Graham 2002). In addition, it is used in the location of pollution sources, assessment of accidental spills, and as a biomonitoring tool, e.g., in monitoring the effects of road construction and the effectiveness of river rehabilitation (Dallas *et al.* 1995). Since its introduction in 1994, SASS has been revised over the years to meet with emerging research needs and standard, giving rise to its fifth version which is currently in use in river biomonitoring programmes. Presently, SASS is best suited for rivers with low/moderate flow hydrology and not for lentic systems, e.g., dams, wetlands, estuaries etc. SASS bases its assessment of river systems on the presence or absence of benthic macroinvertebrate groups (Dallas *et al.* 1995). To ensure the collection of true representatives of biota at study sites, flooded rivers should not be sampled and sample collection should be conducted over a wide area to make sure that the full variability of biotopes is sampled. SASS biotopes include: stone (stones in current, stones out of current), vegetation (both in and out of current) and gravel, sand and mud (GSM) biotopes (Dickens & Graham 2002). Depending on their sensitivity to water conditions, tolerance scores, from which total SASS and ASPT scores are calculated, are assigned to invertebrate taxa (Dallas *et al.* 1995, Dickens & Graham 2002). SASS5 score is the sum of the taxon scores for all the taxa recorded at a site while the ASPT (average score per taxon) score is SASS5 score divided by the number of taxa (Dallas *et al.* 1995). Normally, SASS scoring is done in the field but in certain cases where further examination is required; samples can be preserved and taken back to the laboratory (Dickens & Graham 2002). But in this case, it is recommended that the results should not, in the strict sense, be called SASS results. Habitat diversity, availability, quality, eco-region in which sampling was carried out and season of collection should be considered in the interpretation of SASS results (Dickens & Graham 2002).

2.2.5.1 SASS5 sampling (Dickens & Graham 2002)

Sampling was conducted for 2 minutes from the different biotopes (stones and vegetation) at each of the six sites at the Eerste River using the kick-sampling method described above (which differed from the method used in section 2.2.2). These samples were preserved in 96% ethanol and taken back to the laboratory for SASS scoring as well as for examination of levels of ciliate infestation.

2.3 CILIATE IDENTIFICATION

2.3.1 Light and scanning electron microscopy

Epibiont ciliates were identified to genus using light and scanning electron microscopy. Ciliates were tentatively categorised into morphospecies according to body shape. Representative samples of each morphospecies were used for comparisons of nuclear structure and shape. Attachment sites with their attached ciliates were dissected from substrate organisms and placed in small holding baskets. They were then fixed in Bouin's fluid and rinsed in distilled water before staining with Mayer's hematoxylin (Humason 1979). Slides were viewed with the aid of a Leica DM500 compound microscope (Leica Microsystems, Taiwan) at 400X and 1000X magnification. Line drawings were initially made directly from the microscope image and final drawings were prepared using a light box.

For scanning electron microscopy, the method of Goldstein *et al.* (1992) was followed. Infested hosts were rehydrated and fixed in 2.5% gluteraldehyde for eight hours, rinsed with

0.1M phosphate buffer and fixed with 1% osmium tetroxide for one hour. A second rinse was done with 0.1M phosphate buffer. To ensure that all fixatives were removed, the infested individuals were rinsed again with distilled water. Samples were dehydrated through a series of rising ethanol concentrations (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%), critical-point dried using a CPD 020 critical point dryer (Balzers Union, Balzers, Germany) and mounted on stubs. The stubs were sputter-coated with gold-pallidium alloy using a Polaron vacuum evaporator (Quorum Technologies, East Sussex, UK) and scanned in a NOVA NANOSEM 230 scanning electron microscope (FEI, Oregon, USA) visualisation of images.

2.3.2 Molecular identification of ciliate isolates

2.3.2.1 Sample collection

Zooids (epibiont cells) which were used for molecular studies were obtained from hosts stored in 96% ethanol (Table 2.1). Approximately fifty zooids of each isolate (Utz *et al.* 2010) were detached from their substrate organisms under a Leica EZ4 stereomicroscope (Leica Microsystems, Taiwan) using forceps and minuten needles. They were then placed into eppendorf tubes and centrifuged for ten minutes at 10,000g. The supernatant was decanted and the residue (containing the zooids) was placed in an Eppendorf Thermomixer (Merck Chemicals (Pty) Ltd, South Africa) at 60°C until alcohol had completely evaporated, after which DNA was extracted.

2.3.2.2 DNA extraction, amplification and sequencing

DNA was extracted using a REDExtract-N-Amp™ Plant PCR kit (Sigma-Aldrich Inc., Missouri, USA) comprising DNA extraction and dilution solutions, and REDExtract-N-Amp PCR ReadyMix. 25µl of DNA extraction solution was added to eppendorf tubes containing the harvested zooids and placed in the heat block at 95°C for 10 minutes after which 25µl of DNA dilution solution was added. The tubes were vortexed to mix their contents properly and stored at -20°C until the DNA amplification.

Both the 18SSU rRNA genes (Miao *et al.* 2001, Clamp & Williams 2006) and the internal transcribed spacer (ITS1-5.8S-1TS2) (Goggin & Murphy 2000, Sun *et al.* 2010) regions of each isolate identified were used for comparisons of isolates. 18SSU rRNA genes were amplified using a primer pair, F2-R1, designed by Utz *et al.* (2010): Peri18S-F2 (5'-CCGCGGTAATTCCAGCTC-3'), Peri18S-R1 (5'-TGCAGGTTCACCTACGGAAA-3'). The ITS1-5.8S-ITS2 region was amplified using the ITS-F: 5'-GTTCCCCTTGAACGAGGAATTC-3' and ITS-R: 5'-TACTGATATGCTTAAGTTCAGCGG-3' primers which were complementary to these regions: the 3'end of 18SSU rRNA, the entire ITS1-5.8S-ITS2 region and the 5'end of the 28SSU rRNA (Goggin & Murphy 2000). Polymerase Chain Reaction (PCR) amplification was performed in a 2720 thermal cycler (Applied Biosystems, Singapore) using a REDExtract-N-Amp PCR ReadyMix containing buffer, salts, dNTPs, Taq polymerase and Jumpstart Taq antibody. Cycling parameters were: initial stage of denaturation (95°C, 2 minutes), 40 cycles (95°C, 30 seconds; 55°C, 30 seconds; 72°C, 1 minute) and a final stage of extension (72°C, 8 minutes). PCR products were visualised using 1% agarose gel stained with ethidium bromide to determine DNA quality and quantity.

DNA sequences were analysed on an ABI PRISM™ 3100 Genetic Analyser (Applied Biosystems, Foster City, CA, USA) using the BIG DYE™ Terminator v3.0 cycle sequencing premix kit in the DNA Sequencing facility, University of Stellenbosch, Western Cape. Sequencing was done in one direction for all amplifiable fragments using the 18SF2 and ITSF primers for the 18SSU rDNA and ITS regions respectively.

All phylogenetic analyses were conducted in Molecular Evolutionary Genetic Analysis (MEGA) software version 4.0.2 (Tamura *et al.* 2007). Both 18SSU and ITS sequence datasets were analysed separately. Each set was aligned to appropriate sequences of other ciliates obtained from GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi) using CLUSTALW algorithm in Evolutionary MEGA v4.0.2 (Tamura *et al.* 2007). Alignments were edited manually and only unambiguously aligned positions were used to construct phylogenetic trees with MEGA4. Missing data were discarded from datasets (Complete Deletion Option). The resulting 18SSU data matrix contained 767 characters with 198 parsimony-informative characters; and the ITS data matrix had 124 characters with 43 parsimony-informative characters.

Phylogenetic reconstructions were inferred using Neighbour-joining (NJ) (Saitou & Nei 1987) and Maximum Parsimony (MP) (Eck & Dayhoff 1966) methods. For NJ analyses, evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.* 2004) while computations for MP analyses followed the Close-Neighbour Interchange algorithm (Nei & Kumar 2000). Support values for branch nodes on strict consensus trees (Felsentein 1985) were inferred using 1000 bootstrap replicates. Branches supported by less than 50% bootstrap values were collapsed.

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CHAPTER 3. CILIATE EPIBIONTS ON BENTHIC MACROINVERTEBRATES FROM RIVERS OF THE WESTERN CAPE PROVINCE OF SOUTH AFRICA

3.1 INTRODUCTION

The phylum Ciliophora (ciliated protozoa) is a monophyletic assemblage (Lynn 2003) of over 8000 species (Bernhard *et al.* 2001). They occur abundantly in aquatic habitats, e.g., freshwater and marine systems (Fernández-Leborans & Tato-Porto 2000a, b), salt lakes (Pedrós-Alio *et al.* 2000, Elloumi *et al.* 2006), hot springs (Aguilera *et al.* 2010), sea ice (Garrison 1991) and soil (Foissner 1997). Most species are free-living, although some groups are symbiotic, living as ectocommensals (Fernández-Leborans & Tato-Porto 2000a, 2000b) and parasites on aquatic hosts (Nigrelli *et al.* 1976, Morado & Small 1995) and even man (Lynn 2008). Specialised life forms, such as sessile stages, swarmers or free-living stages (telotrochs) and cysts, enable ciliate dispersal and survival during unfavourable environmental conditions (e.g., desiccation and starvation) (Gutiérrez et al. 2001, Corliss 2008, Lynn 2012).

A number of sessile ciliates are epibionts of aquatic macroinvertebrates (Fernández-Leborans & Tato-Porto 2000a, 2000b). Macroinvertebrate basibionts include bivalves (Antipa 1977), gastropods (Diaz *et al.* 2008), decapods (Fernández-Leborans *et al.* 1997), amphipods (Clamp 1993), isopods (Clamp 1987) and aquatic insects (Scott 1910, Diaz *et al* 2007). Most studies have been conducted in the Northern Hemisphere with only scattered information on the Australian, Asian and African associations (Fernández-Leborans & Tato-Porto 2000a, 2000b).

Traditionally, sessile ciliate epibionts have been identified by examination of living specimens and stained slides using a light microscope (Fauré-Fremiet 1931, Noland & Finley 1931, Laird 1959, Herman & Mihursky 1964, Henebry & Ridgeway 1979, Viljoen & van As, 1983, Fernández-Leborans *et al*. 1997, Clamp & Kane 2003, Gilbert & Schröder 2003). Species identification largely depends on morphological characters such as buccal structure, body size and shape, structure and arrangement of stalks and organelles (cilia, macronucleus, contractile vacuole, and pellicle) (Bick 1972). Differences in ecology are often also useful in ciliate identification (Zagon & Small 1970). Certain light microscopy techniques have been developed to aid visualisation of important details for genera/species identification and differentiation. For example, live observations of colonies using phase contrast microscopy provide information on body shape, size and colonial morphology (Gilbert & Schröder 2003, Diaz *et al.* 2008). Using bright field and differential interference contrast (DIC) microscopy, nuclear- and stalk morphology can be studied (Diaz *et al.* 2007). In addition, special cytological techniques such as silver staining (e.g., protargol staining) are valuable tools for visualisation of cortical characters such as infraciliature, kinetosomes, pattern of pellicular striation, nuclear apparatus and body shape (Zagon & Small 1970, Nagasawa & Warren 1996).

Elucidation of surface features important for sessile ciliate identification has been restricted by problems associated with light microscopy such as narrow magnification range, inadequate resolution, limited depth of focus and difficulties in obtaining photomicrographs (Zagon 1971). Hence, scanning electron microscopy (SEM) is often used as an additional identification tool in studies involving ciliates (Evans *et al.* 1979, Turner *et al.* 1979, Evans *et al.* 1981, Kankaala & Eloranta 1987, Willey & Threlkeld 1993, Cook *et al.* 1998, Mayén-estrada & Aladro-Lubel 2007). Detailed structural descriptions of diagnostic features such as cell topography, peristomial morphology, buccal structure, pellicullar striations and variations on pores, scopula and stalks have been obtained by using SEM. This makes SEM micrographs helpful to differentiate between closely related taxa (Nagasawa and Warren 1996). SEM can also be used in comparative study of life history stages (Zagon 1971). However, these studies can be very difficult because of the contractile nature of zooids and the alteration of surface features associated with preparing samples for SEM study (Carey & Warren 1983).

Recently, molecular studies have been incorporated in taxonomic and phylogenetic studies of ciliates. They have been successfully used to infer relationships among species resulting in a reclassification of sessile ciliates (Lynn 2003, Adl *et al.* 2005). In addition to its use in genera/species identification, molecular gene sequences of 18S small subunit rRNA has raised questions concerning their monophyly with suggestions for a taxonomic review or reclassification of all ciliate groups (Miao *et al.* 2001, Miao *et al.* 2004, Clamp & Williams 2006, Utz & Eizirik 2007, Williams & Clamp 2007, Li *et al.* 2008). For example, Gong *et al.* (2006) showed that the mobiline and sessiline ciliates, traditionally regarded as sister groups in the subclass Peritrichia (Class Oligohymenophorea) based on morphological characters, are not monophyletic. Also, phylogenetic analyses using molecular data indicated that the large genus *Epistylis* is polyphyletic (Clamp & Williams 2006, Miao *et al.* 2004, Utz & Eizirik 2007). Hence, predictions of taxonomic and phylogenetic relatedness of peritrich species cannot be solely based on gross morphology (Clamp & Williams 2006).

No single method of identification is adequate to accurately describe ciliates, as different characters are revealed using different techniques. However, every technique has its own limitations. In many instances, congeners in large genera share similar morphological characters rendering traditional descriptions incomplete and necessitating taxonomic redescriptions (Nagasawa & Warren 1996, Ji *et al.* 2011).

Ciliate epibiont diversity has been studied in only a few aquatic systems in South Africa (Basson *et al.* 1983; Van As & Viljoen 1984; Viljoen & Van As 1983, 1985; Van As *et al.* 1998; Botes *et al.* 2001). Most of these were taxonomic works on ciliophoran epibionts of fish and their crustacean ectoparasites that include sessile peritrichs in lakes, rivers, streams, dams and fish ponds in the Northern and Eastern parts of the country. Currently, there are no records of sessile ciliate epibionts from rivers in the Western Cape Province of South Africa. In the present study, benthic macroinvertebrates were collected from various rivers in the Western Cape Province of South Africa to document their associated sessile ciliate taxa. An understanding of the taxonomic status of peritrich species involved is an essential basis for subsequent ecological work on basibiont and epibiont relationships. Ciliate epibionts were identified using light microscopy, scanning electron microscopy and molecular techniques.

3.2 MATERIALS AND METHODS

Collection sites and details of collecting methods are provided in the Chapter two.

3.3 RESULTS

Sessile ciliate epibionts were mainly found on individuals of the following beetle families: Elmidae (riffle beetles), Hydraenidae (minute moss beetles), Dryopidae (long-toed water beetles) and Ptylodactilidae (toe-winged beetles). Other hosts included Corixidae (water boatmen) and Notonemouridae (southern stoneflies). Ciliate groups were tentatively identified to genus level using keys and descriptions by Bick (1972), Sládecek (1981) and Foissner and Berger (1996). Unfortunately, it was not possible to identify these ciliates to species level as live observations were not possible and the Cape peritrich fauna is poorly known. However, two ciliate taxa: subclass Peritrichia (peritrichs) and subclass Suctoria were identified on these macroinvertebrates. Though ciliates were observed in their contracted form, stained slides demonstrated general body shape and nuclear- and stalk morphology, while SEM examination revealed cortical features.

3.3.1 Light microscopy

Suctoria

The suctorian epibionts had spherical (sometimes ovoid) zooids supported by stalks of varying lengths (Fig. 3.1A – C). Tentacles replaced a true mouth and were distributed on the upper half of the body. These were usually distorted as a result of the method of preparation (Fig. 3.1A). Dark-stained spherical nuclei were located within the dense cytoplasm with the large macronucleus being more centrally located than the smaller micronucleus (Fig. 3.1A, B).

Peritrichia

Contracted zooids of the peritrichs were more elongate than those of the suctorians with an inverted bell-shape and a narrow peristomial region (buccal region) (Fig. 3.1D, F). Their colonies consisted of a variable number of individuals that were supported on non-contractile, dichotomously branched, striated stalks (Fig 3.1D). Only the horseshoe-shaped macronucleus was observed. It was located at the anterior region of the zooid, near the cytopharynx. It was positioned transversely to the longitudinal axis of the zooid (Fig. 3.1D, E, F).

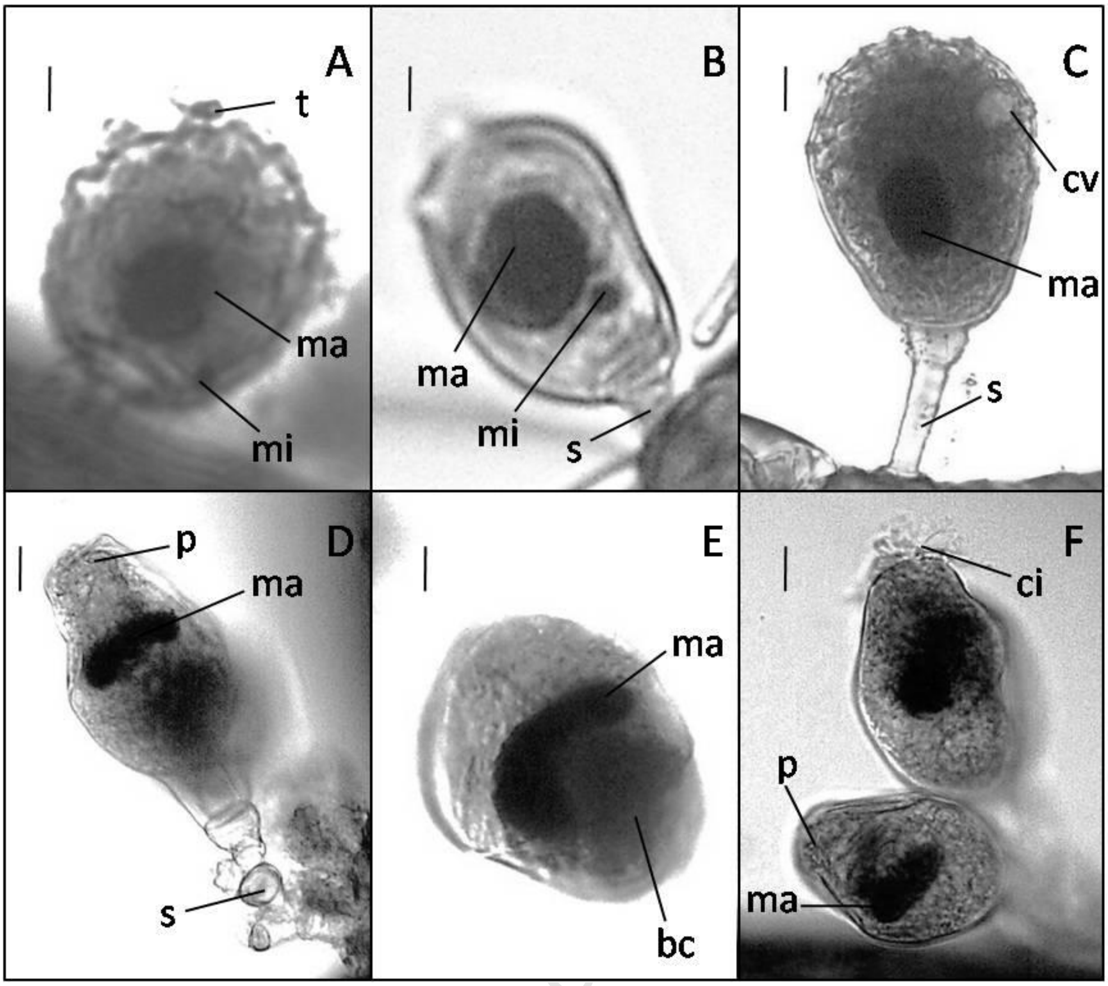

Figure 3.1. Micrographs from hematoxylin-stained materials of suctorian (A – C) and peritrich (D – F) ciliates associated with macroinvertebrates from Western Cape Province rivers. (A) spherical zooid with a centrally-located, spherical macronucleus (ma) and with micronucleus (mi) located closer to the cell membrane. Coiled and distorted tentacles (t) can also be seen, scale bar = 20µm. (B) Ovoid zooid with short stalk (s), scale bar = 20µm. (C) Zooid with longer stalk than in (A) and (B) with a dense macronucleus (ma) and a contractile vacuole (cv) located towards the anterior of the zooid, scale bar = 50µm. (D) Ovoid, elongated zooid with narrow peristomial region (p), horseshoe-shaped macronucleus and a branched stalk, scale bar = 50µm. (E) Dorsal view of zooid showing transverse orientation of the macronucleus which curves around the cytopharyngeal end of the buccal cavity (bc), scale bar = 50µm. (F) ciliary organelle (ci) associated with the buccal cavity, scale bar = 50µm.

3.3.2 SEM examination

Peritrich ultrastructure

For the peritrichs, it was not possible to demonstrate buccal structures and peristomial ciliature using SEM as the contracted zooids had tightly closed peristomial borders (Zagon 1971) (Fig. 3.2A, D, E, J). Pellicular striations of the peritrichs were observed from the peristomial border to the stalk (Fig. 3.2D, E, F). Vertical ectoplasmic structures were perpendicular to striae, connecting neighbouring striae (Fig. 3.2I). Although not illustrated here, pellicular pores were observed in the buccal regions. The scopular region was marked by a circular ring and a raised area which surrounded the stalk (Fig. 3.2F). The stalk base flared out at the point of attachment to the substrate (Fig. 3.2B) and its width gradually increased to form a cup-like structure at the point of insertion into the scopula (Fig. 3.1D, 3.2G, 3.4a).

These SEM examinations indicated that two different taxa, presumably different genera, of peritrich ciliates were associated with the collected macroinvertebrate hosts. One of these had long stalks and zooids without a constriction separating the peristomial region from the body (Fig 3.2A, C, D). The other had shorter stalks and zooids with a distinct constriction or neck separating the peristomial region from the body (Fig. 3.1D, 3.2E, J, 3.4a).

Suctorian ultrastructure

Tentacle ultrastructure of the suctorian ciliates was well-demonstrated using SEM. Tentacles appeared to have protuberances (haptocysts) in their upper region (Fig. 3.3A, B, C), which were not detected under light microscopy (Fig. 3.4b). The pellicle appeared to be smooth and stalk surfaces had irregular longitudinal folds (Fig. 3.2D).

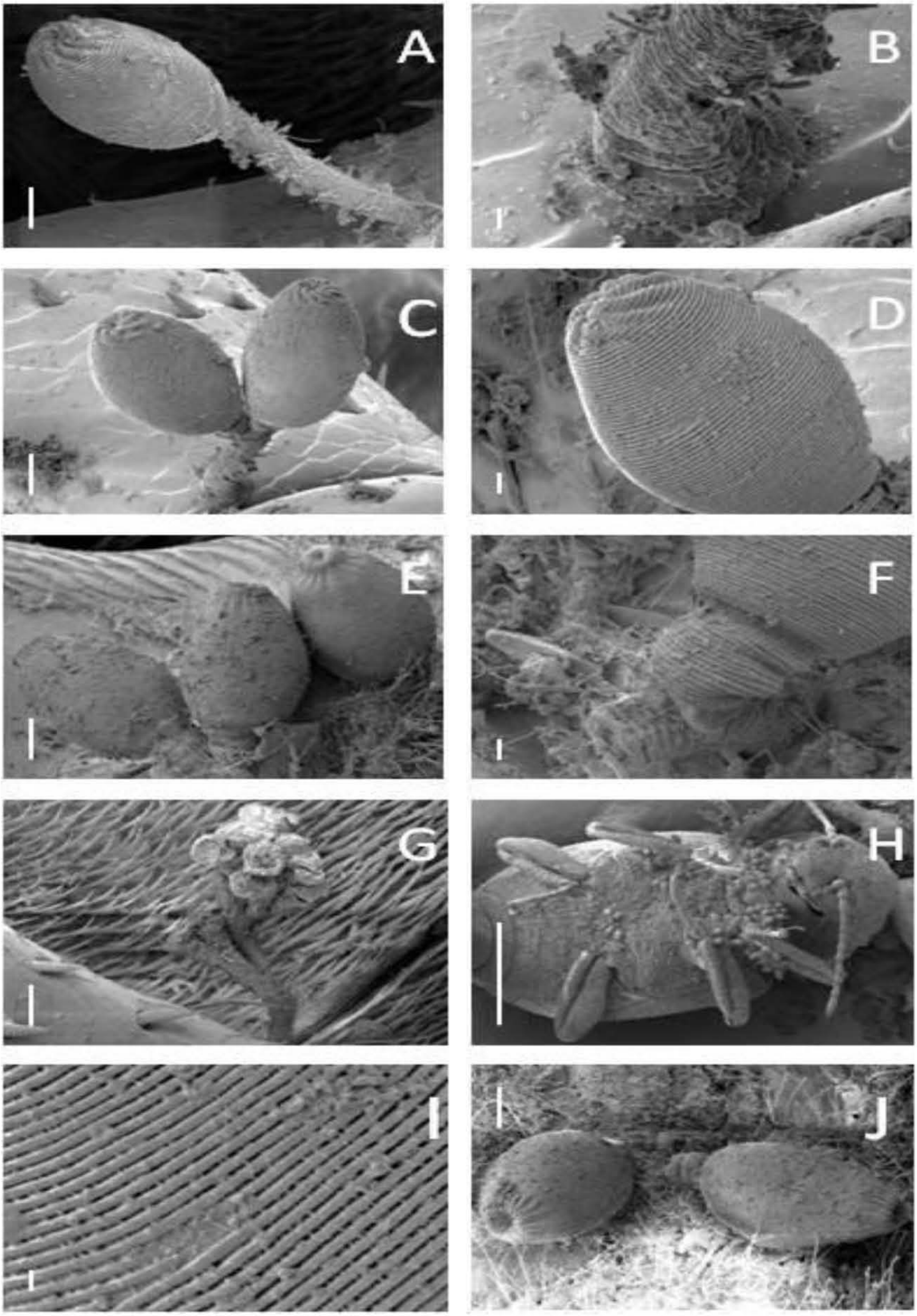
A
B
C
D
E
F
G
H
I
J

Figure 3.2. SEM images of peritrichian epibionts from rivers in the Western Cape Province of South Africa. (A) Sessile ovoid zooid with tightly contracted periostome and a long stalk, scale bar = 30µm. (B) Base of stalk with irregular striations on stalk surface, scale bar = 5µm. (C) Colony of two individuals, tightly closed peristome that is not constricted from the body, scale bar = 30µm. (D) Pellicular striations on contracted zooid, scale bar = 10µm. (E) Sessile zooids of a presumably different peritrich taxon indicating a shorter stalk and a tightly closed peristome that is separated from body by a short neck or constriction, scale bar = 40µm. (F) Posterior region of sessile zooids illustrated in (E) showing scopular region (circular depression) and with stalk embedded in debris on the host's exoskeleton, scale bar = 10µm. (G) Empty dichotomously branched stalk following cell (zooid) dispersal. The stalk is anteriorly flared at the connection point with zooids, scale bar = 40µm. (H) Peritrich ciliates on the ventral aspect of an elmid beetle, scale bar = 500µm. (I) Pellicular striations with vertical thin ectoplasmic structures connecting striae, scale bar = 4µm. (J) zooid (same as E) with closed peristomial lips separated from the body by a constricted ring (neck), scale bar = 40µm.

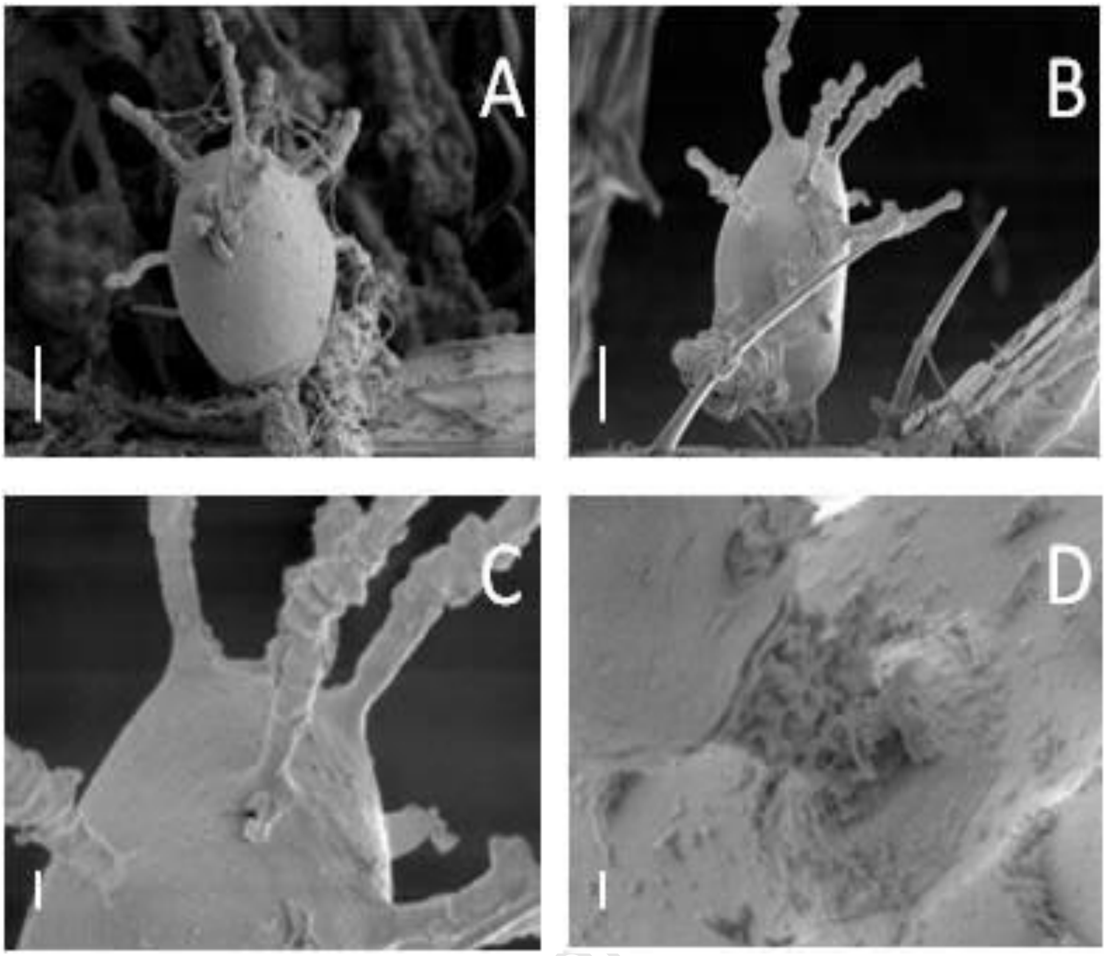

Figure 3.3. SEM images of suctorian epibionts from rivers in the Western Cape Province of South Africa. (A) Suctorian ciliate entangled in debris showing spherical body shape, scale bar = 20µm. (B) Suctorian ciliate nestled in between two setae with ovoid body shape and close-up of tentacle ultrastructure. Initial budding process can also be seen, scale bar = 20µm. (C) Suctorian zooid showing details of tentacle ultrastructure and smooth pellicule (cell membrane), scale bar = 5µm. (D) Suctorian ciliate showing stalk with longitudinal folds and adhering debris, scale bar = 4µm.

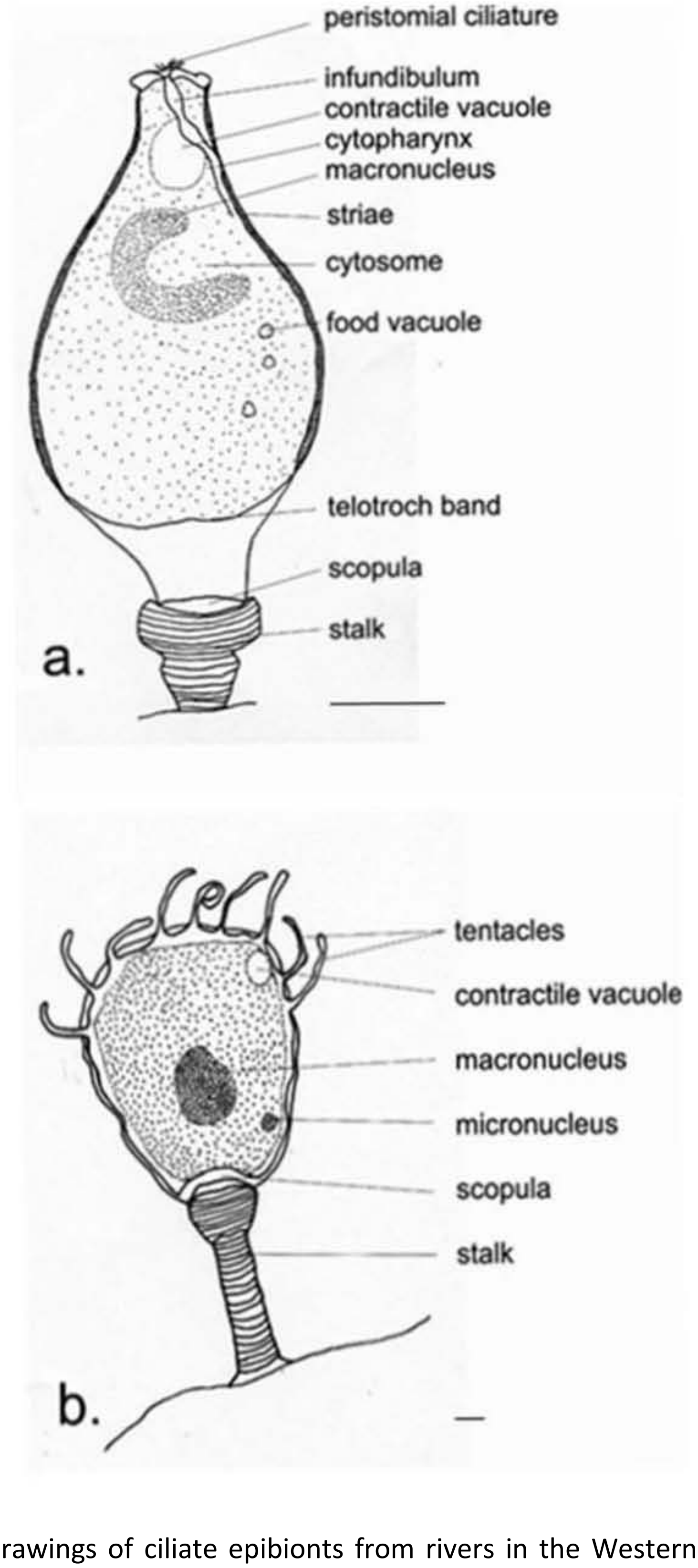

Figure 3.4. Line drawings of ciliate epibionts from rivers in the Western Cape Province of South Africa. (a) Peritrich ciliate drawn from stained slides, scale bar = 50µm. (b) Suctorian ciliate drawn from stained slides, scale bar = 20µm**.**

3.3.3 Phylogenetic analyses

Representative samples of peritrich ciliates included in phylogenetic analyses were collected from macroinvertebrate hosts from Elandspad, Eerste, Wit and Rooielskloof. Amplification of DNA from samples from the Wit River could not be included in analysis due to contamination of extracted DNA. It was only possible to include peritrich and not suctorian epibionts in phylogenetic analyses due to limited numbers of hosts collected and DNA amplification problems associated with contamination by the host's DNA. Phylogenetic trees generated using the NJ and MP methods using the 18SSU rRNA marker had the same topology, hence only the NJ tree is presented in the results. For both analyses, the trees were unrooted. Taxa grouped into two major clades with the isolates collected in this study clustering into one of these clades (Fig. 3.5). Analyses indicated the presence of three distinct taxa. The 'A' isolates includes all samples that represented the short-stalked peritrichs while the 'B' isolates were represented by the peritrichs with the longer stalks. Apart from EEA (2), collected from the Eerste River and which resolved at the base of the major clade, all other isolates grouped with *Opercularia* spp. obtained from GenBank. Sequences of the remaining 'A' isolates, ELA (s), ELA (b); and EEA (1) from the Elandspad (EL) and Eerste (EE) Rivers were identical and formed a well-supported clade (Fig. 3.5). The 'B' isolate (ELB) from Elandspad was distinct from the 'A' isolates (ELA) from the same river (Fig. 3.5). In the Elandspad River, the 'A' isolates, ELA (s) and ELA (b), collected from beetle hosts of different species had identical sequences. However, the 'A' isolates, EEA (1) and EEA (2), collected from the same beetle host species in the Eerste River differed from each other.

Similar to the results of analyses using the 18SSU rRNA marker, NJ and MP analyses using the ITS marker resulted in trees with identical topology. Taxa were grouped into two major clades in the unrooted tree. All isolates from this study clustered in one of these major clades (Fig. 3.6). Three different groups were identified: 'A', 'B' and 'C'. The 'A' isolates, ELA(s) and ELA (b) from the Elandspad River were identical to one other and to the 'A' isolate, EEA (1), from the Eerste River (Fig. 3.6). The 'B' isolate, ELB, from the Elandspad River was again shown to be different from the 'A' isolates collected from the same river (Fig. 3.6). In addition to these, another taxon (REC) from the Rooielskloof River was identified. It grouped separate from other taxa collected in this study (Fig. 3.6).

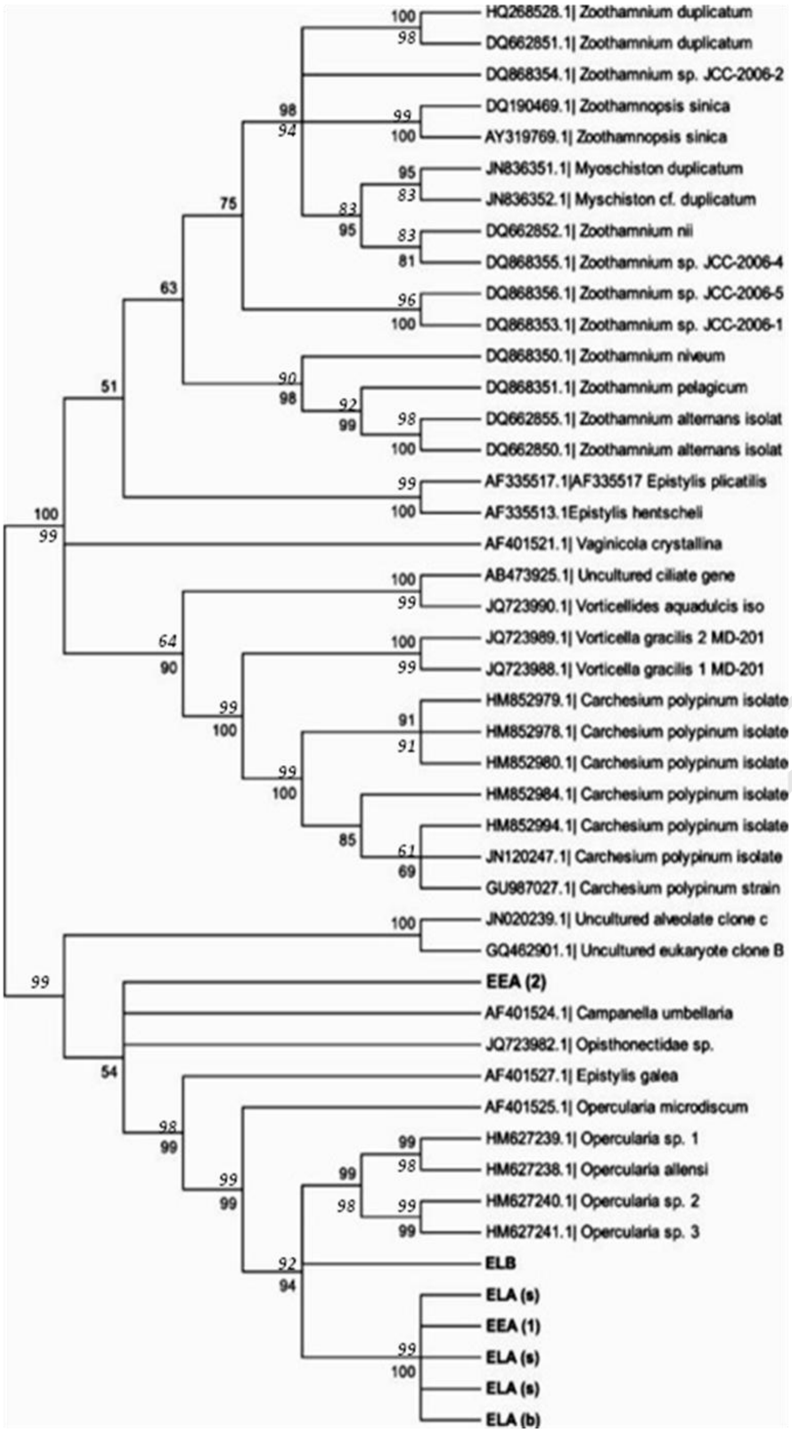

Figure 3.5. Consensus tree based on analyses of 18SSU DNA sequence data of peritrich ciliates. Numbers on branches represent neighbour-joining bootstrap values with maximum parsimony values represented in italics. GenBank accession numbers and taxon names are indicated at branch tips. Isolates collected in the present study are shown in bold type. Short-stalked taxon (SST): ELA (s), ELA (b), EEA (1) and EEA (2). Long-stalked taxon: ELB.

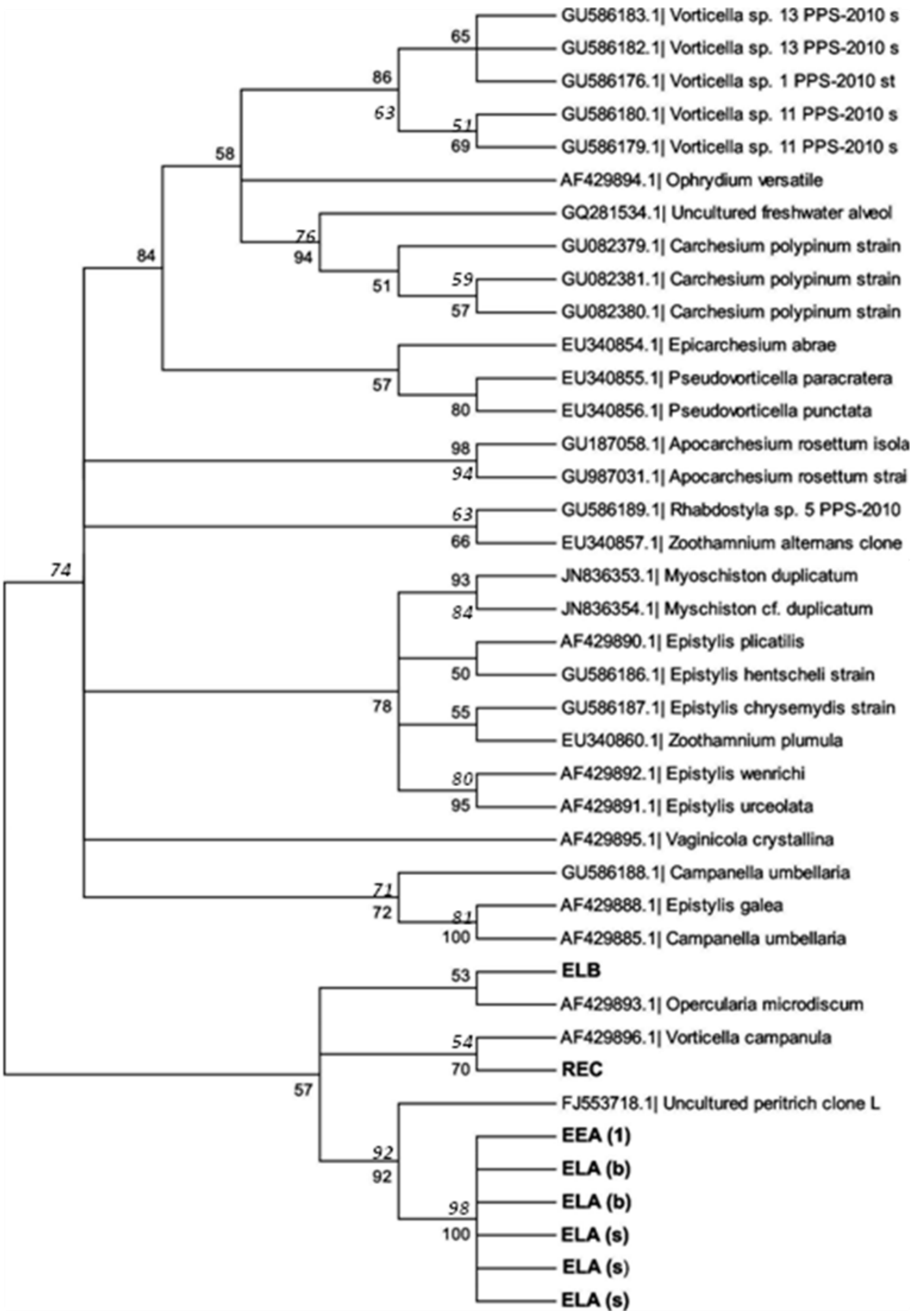

Figure 3.6. Consensus tree based on ITS DNA sequence data of peritrich ciliates. Numbers on branches represent neighbour-joining bootstrap values with maximum parsimony values represented in italics. GenBank accession numbers and taxon names are indicated at branch tips. Isolates collected in the present study are shown in bold type. Short-stalked taxon (SST): ELA (s), ELA (b), EEA (1) and EEA (2). Long-stalked taxon: ELB, REC.

3.4 DISCUSSION

Epibiotic ciliates, including peritrichs and suctorians, are important components of aquatic ecosystems and are found on various substrates, both living and non-living (Fernández-Leborans & Tato-Porto, 2000a, 2000b). Even though the majority of previous studies have focused on crustaceans, a few have also recorded the occurrence of these on aquatic insects. These include: mosquito larvae (Laird 1959), aquatic beetles (Scott 1910, Brown 1987) and water boatmen (Mariño-Perez & Mayén-Estrada, 2009). In the present study, ciliates were found on aquatic beetles (adult elmids and hydraenids, larval dryopids and ptylodactylids), water boatmen and stonefly larvae. Other studies also found peritrichs on adult aquatic beetles (Scott 1910, Brown 1987) but not on beetle and stonefly larvae. Suctorians have been reported to colonise water boatmen (Mariño-Pérez & Mayén-Estrada, 2009). In the present study, they colonised adult hydraenid beetles.

Nuclear morphology, body shape and to some extent, the stalk morphology of both groups of ciliates were well-illustrated using light microscopy with finer details such as pellicular striations, tentacle ultrastructure, variations in stalk morphology elucidated by SEM. These features have been useful in species identification of ciliates (Zagon 1971, Bick 1972, Clamp & Williams 2006). SEM studies supported the general structure of sessile zooids which were described using light microscopy, although detailed structural descriptions of relaxed buccal organelles were lacking. In this study, based on light and scanning electron microscopy, collected ciliates were grouped into three taxa: one taxon of Suctoria and two morpho-groups of peritrichs: the short-stalked taxon (SST) and the long-stalked taxon (LST).

Peritrich ciliates are a subclass within the class Oligohymenophorea (Adl *et al*. 2005, Lynn 2008). They are characterised by possession of an expanded oral or peristomial region which is surrounded by two prominent bands of cilia that run in anticlockwise direction and a non-contractile, dichotomously branched stalk or an adhesive disc at the posterior region (Miao *et al.* 2001, Gong *et al.* 2006). Both characters are effective morphological markers in the identification of peritrichs (Maio *et al.* 2001) and were observed on those collected in the present study. The macronucleus varies in shape from horse-shoe to long vermiform with the micronucleus lying close to it (Bick 1972). In the present study, peritrichs were found attached to basibionts as both colonials and solitary individuals (a phenomenon previously known; Maio *et al.* 2001), but mainly as the former. The macronucleus observed here also had a horseshoe shape in both short- and long-stalked forms. However, the micronuclei could not be demonstrated in the present study because of its small size. The abovementioned characters are consistent with those for the genus *Opercularia* as described by Bick (1972), Sládecek (1981) and Foissner and Berger (1996).

Suctorians are a subclass of the class Phyllopharyngea (Adl *et al*. 2005, Lynn 2008) and are characterised by possession of numerous tentacles instead of a mouth, ciliated larvae and a non-ciliated adult stage (Dovgal 2002, Adl *et al.* 2005). They may exhibit variable body shapes (Batisse 1994 cited by Dovgal 2002). Suctorians exist only as solitary individuals with the macronucleus varying in shape from spherical to ribbon-like and with numerous micronuclei scattered around the macronucleus (Dovgal 2002). In the present study, suctorians were found on hosts only as solitary individuals, with spherical macronuclei and with a single micronucleus lying close to it as previously described by Bick (1972) and Dovgal (2002).

In this study, it was only possible to successfully generate DNA sequence data for some of the peritrichs. Unfortunately, the ITS and 18SSU sequences for all taxa included in analyses could not be generated. However, phylogenetic analysis did confirm that more than one species of peritrich were collected. The short-stalked morpho-group was found to actually represent at least two different taxa as illustrated by results from analyses of 18SSU rRNA data. All isolates, except EEA (2), grouped in a clade that contained various *Opercularia* isolates, suggesting that these isolates represent a species of *Opercularia*.

Analysis of ITS data supported the monophyletic grouping of these taxa. The placement of the short stalked taxon, EEA (2) was far removed from these and may even represent another, as yet unknown, genus. The long-stalked taxon, ELB was unresolved in the opercularid clade using 18SSU data, and was weakly grouped with *Opercularia microdiscum* using ITS data suggesting that it is a second species of *Opercularia*. However, an expanded molecular investigation and additional morphological character representation (buccal and somatic morphology using special stains such as protargol) are needed for species delineation.

The two peritrich species (ELA and ELB) were collected consistently from different hosts in a single river, the Elandspad River. ELA was only collected from elmid beetles and ELB was only collected from dryopid larvae. It is thus likely that these have coevolved with their hosts and may be host-specific. Previous studies have also reported that some ciliate epibionts of aquatic hosts can be host-specific (Herman & Mihursky 1964, Willey & Threlkeld 1993, Gilbert & Schröder 2003). However, host specificity can also vary due to other factors as is illustrated in the case of *Vorticella microstoma* which can prefer different hosts in different aquatic systems. It is specific to the cladoceran *Scapholeberis kingi* in a lake in Illinois (Henebry & Ridgeway 1979) but is non-specific in a different lake in Michigan (Pratt & Rosen 1983).

One of the peritrich species (the short-stalked taxon) was collected from two rivers, the Eerste (EEA (1)) and the Elandspad (ELA), but had identical 18SSU and ITS sequences respectively. Interestingly, these rivers are separated by a distance of approximately 31km. It is thus likely that these ciliates can be transported to different aquatic systems via infested elmid adults during dispersal flights (Scott 1910, Brown 1987). The two different short-stalked peritrich taxa represented by the codes, EEA (1) and EEA (2) were both found on elmid beetles in the Eerste River. It is unknown whether both taxa can coexist on the same host organism. Multispecies colonisation of substrate has been observed among ciliate epibionts and could affect epibiont host and body part preferences as well as occurrence in any given system through interspecific competition and intra-epibiont predation (Cook *et al.* 1998). As EEA (2) was found only once, it is likely it is rare in this system. Interspecific competition for host colonisation between the two taxa may thus favour taxon EEA (1) above its congener EEA (2).

In conclusion, results suggest that peritrich ciliates collected from rivers in the Western Cape Province are closely related to operculariad ciliates. Host associations and species richness of epibiotic ciliates in the Western Cape Province warrant further detailed investigation using combined molecular and detailed histological approaches.

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CHAPTER 4. EPIBIOTIC PREVALENCE OF CILIATES ACROSS SIX RIVERS IN THE WESTERN CAPE PROVINCE OF SOUTH AFRICA

4.1 INTRODUCTION

Epibiosis is a symbiotic interaction in which sessile epibionts attach to the surface of various substrata; a phenomenon common in aquatic environments (Fernández-Leborans & Tato-Porto 2000a, 2000b). Epibiotic prevalence is dependent on complex interactions between biotic and abiotic factors and has received a great deal of attention in the case of ciliate protozoan epibionts of aquatic invertebrates (Evans *et al.* 1979, Turner et al. 1979, Evans et al. 1981, Xu 1992, Weissman *et al.* 1993, Cook *et al.* 1998, Utz & Coats 2005, Diaz *et al.* 2008). Infestation prevalence is defined as the proportion of individuals that are infested by epibionts at any given time (Willey *et al.* 1990). Numerous aquatic invertebrate taxa have been evaluated for the presence of these organisms, but in freshwater systems, most studies have focused on crustacean basibionts including decapods (Fernández-Leborans *et al*. 1997, Mayén-Estrada & Aladro-Lubel 2000), copepods (Weissman *et al.* 1993, Utz & Coats 2005) and cladocerans (Xu 1992, Willey & Threlkeld 1993). Only a few studies have included aquatic insects: beetles (Scott 1910, Brown 1987), flies (Laird 1959, Diaz *et al.* 2007) and water boatmen (Mariño-Perez & Mayén-Estrada 2009).

Observed seasonal patterns in epibiotic prevalence in aquatic systems could relate to seasonality in host abundances (Nagasawa 1986, 1988, Weissman *et al.* 1993) or seasonal influences on water parameters (Chiavelli *et al.* 1993, López *et al.* 1998). Abiotic conditions, such as conductivity, salinity, turbidity, temperature, pH, dissolved oxygen concentration and nutrient levels of carbon and nitrogen, in the aquatic systems can influence epibiotic prevalence and often change according to season (Chiavelli *et al.* 1993, Ebert *et al.* 2001, Elloumi *et al.* 2006). These water variables also indicate water quality and determine the environmental conditions for aquatic life (Dallas *et al.* 1999). Ciliates have been reported to respond to deterioration in water quality (Antipa 1977, Henebry & Ridgeway 1979) with certain species such as *Vorticella microstoma* responding positively to water pollution (Bick 1972).

Availability of attachment surface is an important factor in the ciliate-host association (Wahl 1989). Substrata serve as habitat patches which are subject to fragmentation and collapse during host physiological events such as moulting and predation. Therefore, moulting frequency (Henebry & Ridgeway 1979, Xu 1992, Willey & Threlkeld 1993, Hanamura 2000) as well as rate of predation of hosts (Green 1974, Willey *et al.* 1990, Chiavelli *et al.* 1993, López *et al.* 1998) often affects epibiont prevalence. Prevalence level of epibionts may often increase with increased intermoult period (substrate instar duration) (Allen *et al.* 1993, Al-Dhaheri & Willey 1996).

The relationship between the intermoult period and epibiont life cycle on a host is described by the 'instar duration: doubling time ratio' with epibiont doubling time defined as the time needed for epibiont attachment, growth and reproduction (Threlkeld *et al.* 1993). Ratios which are more than 1.0 imply that rate of epibiont growth and reproduction surpasses the rate of mortality in the habitat patch, while ratios below 1.0 signify that the rate of epibiont growth and reproduction is less than the rate of mortality (Threlkeld *et al.* 1993). The habitat patches which have instar duration: doubling time ratios exceeding 1.0 are called 'source' habitats, while habitats with ratios less than 1.0 are the ephemeral 'sink' habitats (Pulliam 1988). This implies that large populations of epibionts are found on source habitats indicating a preference by epibionts for these habitats. However, Pulliam (1988) predicted that in the absence of good breeding sites in overpopulated source habitats and with the resultant continual immigration to the sink habitats, large epibiont populations may still be sustained on the latter. Observed variations in epibiont seasonal prevalence and zooplankton infestation levels may thus also be a result of the inter- and intraspecies variations in intermoult period (Bottrell *et al.* 1976) as well as seasonal changes in the instar duration-epibiont doubling time ratio (Threlkeld *et al.* 1993).

High epibiont prevalence is observed when the substrate zooplankton species are most abundant (Xu 1992, Chiavelli *et al.* 1993). This is especially expected when the epibionts are highly host specific (Threlkeld *et al.* 1993). However, the association between epibiont prevalence and substrate density are not always explicit (López *et al.* 1998, Hanamura 2000, Utz & Coats 2005). Increases in host population numbers without a resultant increase in the epibiont prevelance have also been recorded (Henebry & Ridgeway 1979). In many cases, other factors such as pollution, variability in phytoplankton and bacteria populations, and influx of water into the system in addition to host abundance may be more important in determining epibiont prevalence (Utz & Coats 2005). High epibiont burdens can also alter the abundance of hosts and affect epibiotic prevalence (Weissman *et al.* 1993).

Epibionts do not only show strong host preferences (Herman & Mihursky 1964, Henebry & Ridgeway 1979, Willey & Threlkeld 1993, Gilbert & Schröder 2003), but also attachment site preference on their hosts (Willey & Threlkeld 1993, Cook *et al.* 1998, Mayén-Estrada & Aladro-Lubel 2001). For example, the epibiont *Colacium calvum* prefers the anal region of cladoceran hosts where it feeds off the nutrient-rich host excreta (Chiavelli *et al.* 1993). Peritrichian ciliates have often been observed to prefer certain crustacean body parts such as setae, antennal segments and scales, likely because they are able to gain extra protection or easier access to food (Mayén-Estrada & Aladro-Lubel 2001). Therefore, host feeding habits and behavioural patterns contribute to attachment site preferences (Green 1974, Clamp 1987, Fernández-Leborans *et al.* 1997). While some ciliates avoid certain attachment sites (Green 1974), intraspecific morphological variations of certain peritrich taxa aid attachment to sites on the host. These include features such as the production of shorter stalks (Laird 1959, Gilbert & Schröder 2003), the reduction or flattening of lorica or the secretion of more robust stalks (Cook *et al.* 1998).

The degree of host specificity found in epibionts varies not only between different epibiont species, but sometimes between populations of the same species in different systems. In these instances, interaction between different epibiont species may influence their prevalence and distribution (Cook *et al.* 1998, Ebert *et al.* 2001, Utz & Coats 2005). An epibiont can enhance the colonisation of a substrate by another, by making the substrate easier to attach to, e.g., by slowing down the movement of the host (Wahl 1989). Conversely, it can inhibit the colonisation by another epibiont, by attaching to preferred sites or by preying on others (Cook *et al.* 1998). However, highly specialised epibionts still show a fixed site choice, regardless of the presence of other species (Cook *et al.* 1998). The nature of interactions between epibiont taxa and their substrate organisms; and the range of interactions between the epibiont, basibiont and the rest of the aquatic community determines the persistence of epibiont communities in an aquatic system (Threlkeld *et al.* 1993).

Information on these interactions is largely confined to the European and American ciliate fauna. Currently, there is no published information regarding patterns in epibiotic ciliate prevalence on aquatic insects in South African rivers. In this chapter, a survey of benthic arthropods in six rivers in the Western Cape Province of South Africa was conducted to investigate epibiotic prevalence (including seasonal effects), host preferences and attachment sites preferences. It will form a baseline data set for future studies on the use of these as indicators of environmental change in freshwater aquatic systems in South Africa.

4.2 MATERIALS AND METHODS

Details of materials used, study sites (see Fig. 2.1) and the methods employed in the study are given in Chapter 2.

4.3. RESULTS

4.3.1 Host preferences

Ciliate-infested individuals were only found amongst Elmidae (*Stenelmis* sp., *Pachyelmis* sp.), Hydraenidae (*Hydraena* sp.) and Notonemouridae (*Aphanicercella* sp.). Two main groups of peritrichs were identified based on their morphology (see Chapter 3 on ciliate identification): long-stalked taxon (LST) and short-stalked taxon (SST). The LST was found on adult hydraenid beetles and stonefly larvae while the SST was found only on adult elmid beetles. Suctorians were not commonly found in samples and were excluded from further analyses. The few suctorian individuals that were found always occurred on Hydraenidae. Epibiotic prevalence was analysed separately for the different basibionts (Elmidae, Hydraenidae, Notonemouridae).

4.3.2 Infestation prevalence on elmid beetles

Peritrichs were found on elmid beetles in all rivers but at varying prevalence levels. Three rivers (Molenaars, Elandspad and Eerste) had high prevalence of ciliate epibionts on these beetles, while the rest (Wolwekloof, Wit and Rooielskloof) had significantly lower levels (Kruskal-Wallis ANOVA, $n = 6$, $df = 5$, $P < 0.05$) (Table 4.1). Median prevalence was zero in the Wolwekloof, Wit and Rooielskloof Rivers; and highest in the Eerste River (Table. 4.1). Except for the Rooielskloof river, that had very low numbers of elmid beetles, no significant difference in host abundance was detected between the rivers (Kruskal-Wallis ANOVA, $n = 6$, $df = 5$, $P < 0.05$).

Table 4.1. Host abundance and prevalence of ciliate epibionts (SST) on elmid beetles collected from rivers in the Western Cape Province of South Africa during the sampling period (April 2009 to April 2010, 13 sampling events). Data are presented as medians (interquartile range) with different superscript letters within a variable indicating significant differences based on Kruskal-Wallis ANOVA with multiple comparisons of mean ranks. n, number of monthly samples; SST, short-stalked taxon.

RIVER	PREVALENCE (%)	N	HOST ABUNDANCE	TOTAL NUMBER OF HOSTS EXAMINED
Wolwekloof	0.0 ^{a}	10	111 (14 – 296)^{a}	1671
Wit	0.0 (0.0 – 1.42)^{a}	11	171 (27.0 – 319)^{a}	2649
Rooielskloof	0.0 ^{a}	12	24 (11 – 35)^{b}	304
Molenaars	48 (29.17 – 72.33)^{b}	9	52 (28 – 118)^{a}	740
Elandspad	61.60 (46.64 – 67.89)^{b}	8	105 (49 – 156.50)^{a}	884
Eerste	78.87 (62.53 – 82.34)^{b}	8	131 (42 – 290)^{a}	1330

Correlation analyses using monthly sampling data showed a significant positive relationship between elmid beetle abundance and number of infested individuals in three rivers:

Molenaars, Elandspad and Eerste (Table 4.2). No significant correlation was found between elmid abundance and epibiotic prevalence in the other rivers (Table 4.2).

Table 4.2. Spearman's rank correlation coefficient (r_s) for relationship between host abundance and number of infested elmid beetles collected from rivers in the Western Cape Province of South Africa during the sampling period (April 2009 to April 2010). n, number of sampling events (months) used in analyses. N.S, non-significant; S, significant; SST, short-stalked taxon.

RIVER	**SST**	**P < 0.05**
Wolwekloof	r = -0.58 (n = 8)	N.S
Wit	r = -0.52 (n = 9)	N.S
Rooielskloof	r = 0.55 (n = 9)	N.S
Molenaars	r = 0.90 (n = 9)	S
Elandspad	r = 0.97 (n = 9)	S
Eerste	r = 0.98 (n = 8)	S

Marked seasonal fluctuations in epibiont prevalence on elmid beetles were recorded in all rivers but no general pattern emerged (Fig. 4.1). Prevalence was observed as peaks only in certain months of the year in Wolwekoof, Wit and Rooielskloof Rivers, unlike in Molenaars, Elandspad and Eerste Rivers that recorded ciliate prevalence for most part of the year. Seasonal prevalence was not clearly related to host abundance in all rivers (Table 4.3), for example, high epibiotic prevalence was observed when host density was high (84.02%, n = 338; March, Eerste) as well as in cases of low host density (85.71%, n = 7; October, Eerste). Likewise, low prevalence was recorded when host density was either high (1.42%, n = 141; December, Wit) or low (11.11%, n = 9, October, Wit). In the Wolwekloof River, no ciliate epibionts were recorded, except during November (7.14%, n = 14), independent of high host abundance (Fig. 4.1).

Table 4.3. Spearman's rank correlation coefficient (r_s) for relationship between host abundance and seasonal prevalence of SST on elmid beetles collected from rivers in the Western Cape Province of South Africa during the sampling period (April 2009 to April 2010). n, number of months within each season used in analyses; N.S, non-significant; S, significant; SST, short-stalked taxon.

RIVER	**SUMMER**		**WINTER**	
	r_s (n = 6)	**P < 0.05**	**r_s (n = 6)**	**P < 0.05**
Wolwekloof	-0.39	N.S	-	-
Wit	-0.74	N.S	-	-
Rooielskloof	0.65	N.S	-0.39	N.S
Molenaars	0.77	N.S	0.94	S
Elandspad	0.93	S	0.96	S
Eerste	1.00	N.S	0.87	S

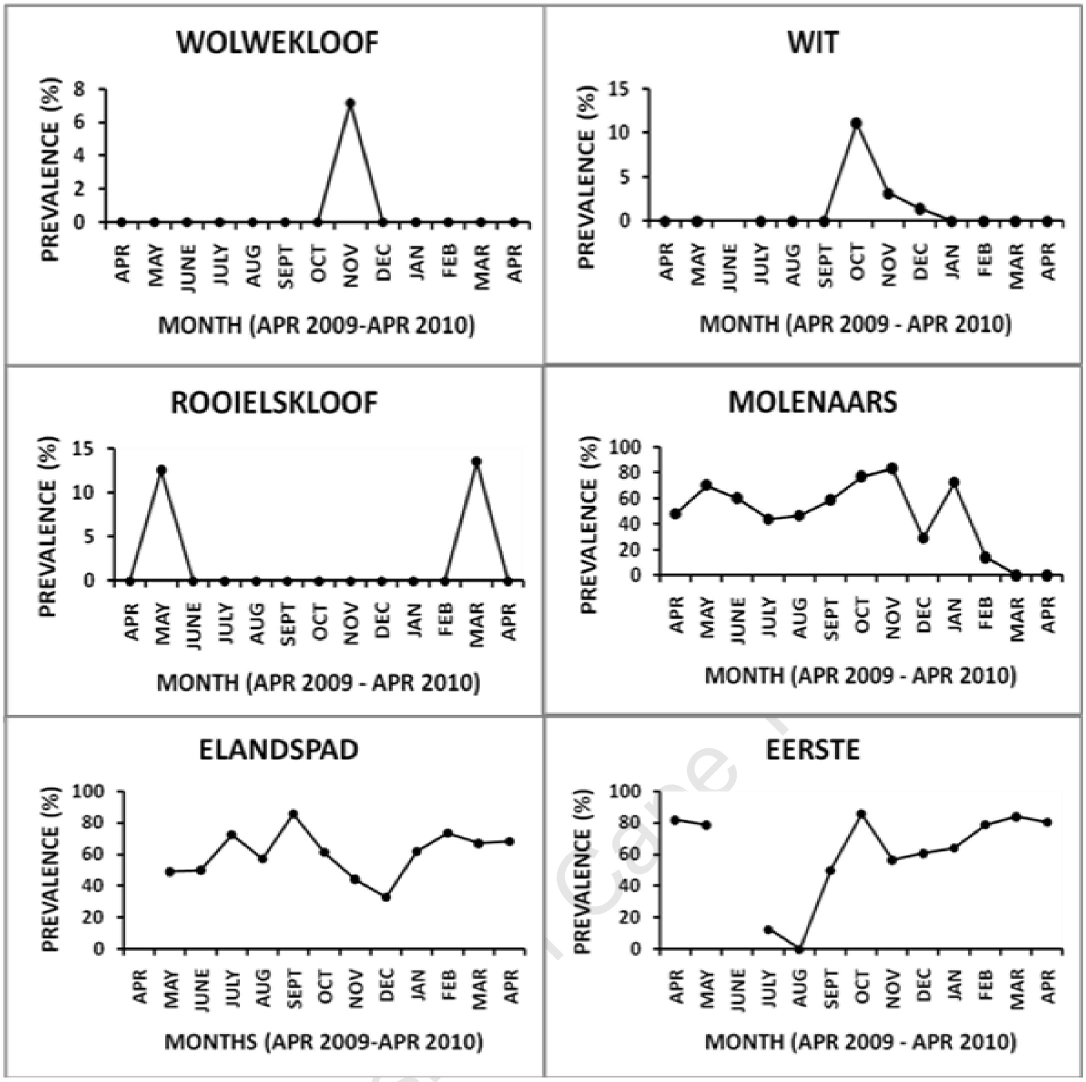

Figure 4.1. Seasonal prevalence of short-stalked ciliate taxa (SST) on elmid beetles (% of beetles infested with ciliates) collected from six rivers in Western Cape Province of South Africa during the sampling period (April 2009 – April 2010). Breaks in line graphs are months in which elmid beetles were not present in benthic samples**.**

4.3.3 Infestation prevalence level on hydraenid beetles

On adult hydraenid beetles, median total prevalence ranged from 3.18% (1.15% – 6.38%) in the Wolwekloof River to 24.24% (20.00% – 40.00%) in the Molenaars River (Table 4.4). Ciliates on elimids exhibited similar prevalence levels in all rivers, except for the Molenaars River that had the highest prevalence of infested beetles (Kruskal-Wallis ANOVA, n = 6, df = 5, P < 0.05) (Table 4.3). Although variations in host abundance were observed for all rivers, overall abundances in Wolwekloof, Wit, Elandspad and Eerste Rivers were significantly different (Kruskal-Wallis ANOVA, n = 6, df = 5, $P < 0.05$) (Table 4.4).

Table 4.4. Host abundance and prevalence of ciliate epibionts (LST) on hydraenid beetles collected from rivers in the Western Cape Province of South Africa during the sampling period (April 2009 to April 2010). Data are presented as medians (interquartile range) with different superscript letters within a variable indicating significant differences based on Kruskal-Wallis ANOVA with multiple comparisons of mean ranks. n, number of monthly samples; LST, long-stalked taxon.

RIVER	PREVALENCE (%)	n	HOST ABUNDANCE	TOTAL NUMBER OF HOSTS EXAMINED
Wolwekloof	3.18 (1.15 – 6.38)^{a}	10	54.50 (28 – 64)^{a}	597
Wit	7.31 (3.96 – 14.23)^{a}	11	120 (53 – 304)a	1759
Rooielskloof	15.77 (12.7 – 25.00)^{a}	12	32 (18 – 43)^{ab}	388
Molenaars	24.24 (20 – 40)^{b}	9	15 (14 – 31)^{ab}	384
Elandspad	13.45 (6.02 – 21.79)^{a}	8	17.5 (13.5 – 22.5)^{ab}	142
Eerste	13.97 (0.00 – 33.64)^{a}	8	11 (6 – 17)^{b}	95

Correlation analysis using monthly collection data showed a significant positive relationship between total host abundance and number of infested beetles only in Wit and Molenaars Rivers (Table 4.5).

Table 4.5. Spearman's rank correlation coefficient (r_s) for relationship between host abundance and number of infested hydraenid beetles collected from rivers in the Western Cape Province of South Africa during the sampling period (April 2009 to April 2010). n, number of sampling events (months) used in analyses. N.S, non-significant; S, significant; LST, long-stalked taxon.

RIVERS	**LST**	**P < 0.05**
Wolwekloof	r = 0.19 (n = 9)	N.S
Wit	r = 0.66 (n = 10)	S
Rooielskloof	r = 0.48 (n = 10)	N.S
Molenaars	r = 0.75 (n = 8)	S
Elandspad	r = 0.33 (n = 7)	N.S
Eerste	r = 0.15 (n = 5)	N.S

Similar to elmids, no clear pattern of seasonal ciliate prevalence was observed (Fig. 4.2). Correlation analysis failed to show a clear relationship between seasonal prevalence and host abundance (Table 4.6). High prevalence levels were recorded in cases of both high (54.29%, n = 175; May, Molenaars) and low host densities (100%, n = 1; July, Molenaars).

Table 4.6. Spearman's rank correlation coefficient (r_s) for relationship between host abundance and seasonal prevalence of LST on hydraenid beetles collected from rivers in the Western Cape Province of South Africa during the sampling period (April 2009 to April 2010). n, number of months within each season used in analyses; N.S, non-significant; S, significant; LST, long-stalked taxa.

RIVER	**SUMMER**		**WINTER**	
	r_s (n = 6)	**P < 0.05**	**r_s (n = 6)**	**P < 0.05**
Wolwekloof	-0.35	N.S	0.69	N.S
Wit	-0.84	S	0.90	S
Rooielskloof	0.87	S	0.84	S
Molenaars	0.76	N.S	0.99	S
Elandspad	0.06	N.S	0.93	S
Eerste	0.56	N.S	0.77	N.S

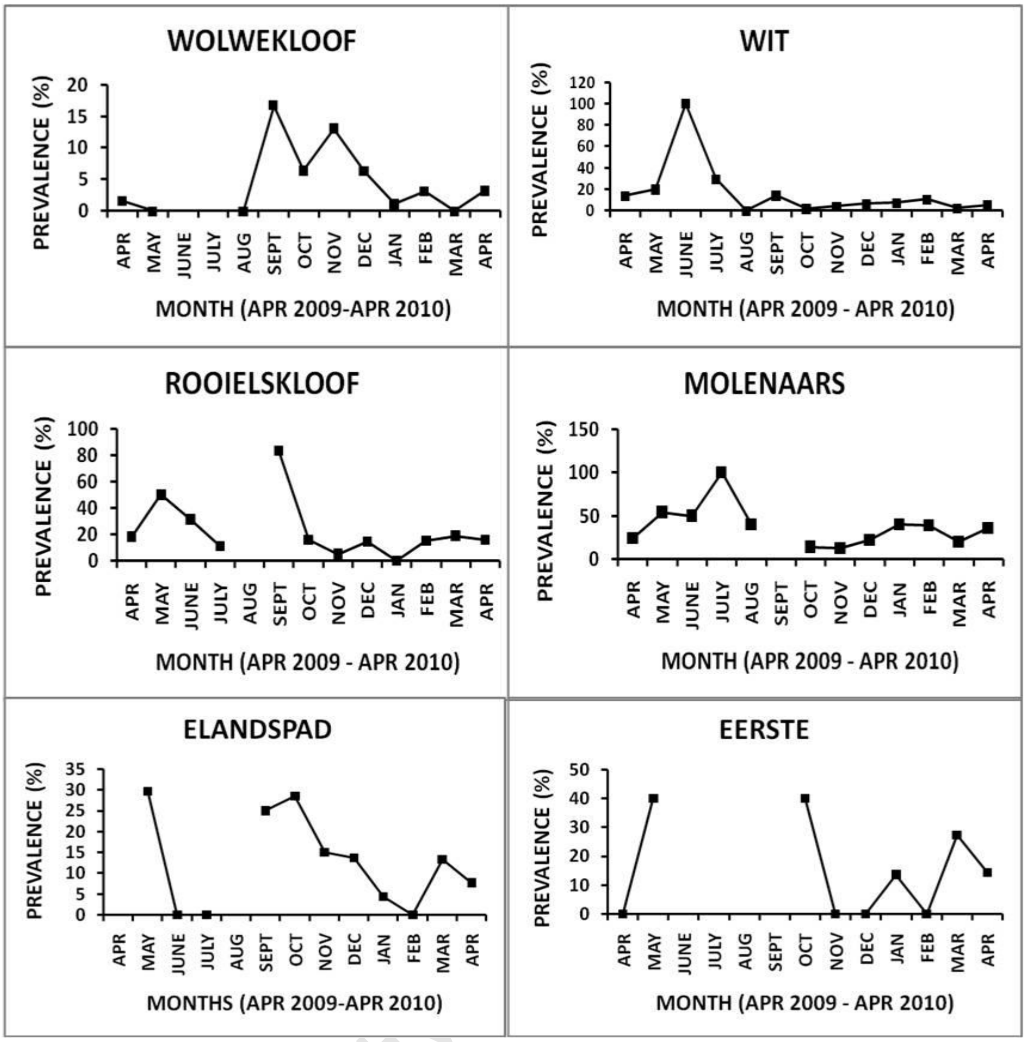

Figure 4.2. Seasonal prevalence of long-stalked ciliate taxa (LST) on hydraenid beetles (% of beetles infested with ciliates) from six Western Cape Province rivers (April 2009 – April 2010). Breaks in line graphs are months in which hydraenid beetles were absent. 100% prevalence values of LST on hydraenid beetles in Wit (June) and Molenaars (July) were obtained from samples containing only one individual.

4.3.4 Infestation prevalence on stonefly (Aphanicercella spp.) larva

For stonefly larvae, ciliate epibionts occurred on these insects in all rivers, except the Wolwekloof River (Table 4.7). Median prevalence was zero in the Wolwekloof, Wit and Elandspad Rivers while Molenaars River had the highest prevalence (Table 4.7). No significant differences in prevalence or host abundance were observed in the six rivers (Kruskal-Wallis ANOVA, n = 6, df = 5, P < 0.05) (Table 4.7). Because of the variations in prevalence, correlation analyses were performed on data sets from only three rivers: Wit, Rooielskloof and Eerste (other river had too few data points for correlation analyses). There were no significant relationship between infestation prevalence and larval stonefly abundance in these rivers (Spearman's rank correlation: Wit, $r_S = -0.31$, n = 13; Rooielskloof, $r_S = 0.23$, n = 12; Eerste, $r_S = 0.18$, n = 8; $P > 0.05$).

Table 4.7. Host abundance and prevalence of ciliate epibionts (LST) on stonefly larvae collected from rivers in the Western Cape Province of South Africa during the sampling period (April 2009 to April 2010). Data are presented as medians (interquartile range) with different superscript letters within a variable indicating significant differences based on Kruskal-Wallis ANOVA with multiple comparisons of mean ranks. n, number of monthly samples; LST, long-stalked taxon.

RIVER	PREVALENCE (%)	n	HOST ABUNDANCE	TOTAL NUMBER OF HOSTS EXAMINED
Wolwekloof	0.0 (0.0 – 0.0)^a	10	41 (9 – 33)^a	190
Wit	0.0 (0.0 – 0.0)^a	13	23.5(25 – 90)^a	81
Rooielskloof	2.18 (0 – 12.06)^a	12	23.5 (15.5– 32.5)^a	156
Molenaars	6.25 (0 – 12.5)^a	2	15 (8 – 22)^a	30
Elandspad	0.00 (0.0 – 21.43)^a	4	11.5 (7 – 32)^a	14
Eerste	3.14 (0.0 – 16.67)^a	8	19 (16.5 – 41)^a	160

4.3.5 Body part preferences of epibionts

Estimates of number of epibiont colonies per host unit area (colonies/mm^2) were obtained for elmid (n = 177) and hydraenid (n = 48) beetles. For the hydraenid beetles, ciliates were observed to colonise only the ventral part of the body. On elmid hosts, ciliates were found attached to the ventral part of 176 individuals while dorsal attachment was observed on only one beetle. Higher epibiont density was observed on the thorax and leg bases of hosts than on the head and abdomen for elmids (Kruskal-Wallis ANOVA, n = 4, df = 3, $P < 0.05$) (Table 4.8). A significantly higher epibiont density was observed on the thorax when compared to the legs (Table 4.8). On hydraenid hosts, epibionts only occurred on the head (Table 4.8).

Table 4.8. Epibiont density of ciliates on different body parts of elmid beetles and hydraenid beetles from rivers in the Western Cape Province of South Africa. Data are presented as medians (interquartile range) with different superscript letters within a host taxon indicating significant differences based on Kruskal-Wallis ANOVA with multiple comparisons of mean ranks.

BODY PARTS	DENSITY OF SST ON ELMID BEETLES/MM^2	DENSITY OF LST ON HYDRAENID BEETLES/MM^2
Abdomen	0.00 (0.00 -0.00)^a	0.00 (0.00 – 0.00)^a
Head	0.00 (0.00 – 0.00)^a	8.89 (8.89 – 17.78)^b
Leg	5.68 (0.00 – 11.36)^b	0.00 (0.00 – 0.00)^a
Thorax	10.56 (5.28 – 21.12)^c	0.00 (0.00 – 0.00)^a

4.4 DISCUSSION

Ciliate epibionts are known to colonise aquatic insects such as beetles (Scott 1910), chironomids (Diaz *et al.* 2007), dragonfly (Laird 1960), and mosquitoe larvae (Laird 1959, Medlock & Snow 2008), as well as aquatic bugs: corixids (Mariño-Pérez & Mayén-Estrada 2009) and notonectids (Mariño-Pérez *et al*. 2010). In the present study, epibionts in the Western Cape rivers colonised adult elmid and hydraenid beetles as well as stonefly larvae. These are the first host-association records for peritrichs in Western Cape rivers. However, sessile peritrich ciliates have been reported from other aquatic systems in South Africa. Viljoen and Van As (1983) found ciliates on dragon fly larvae, in addition to other substrates in a Johannesburg dam. They also (Viljoen & Van As 1985) reported the occurrence of peritrich ciliates on freshwater fishes in Mpumalanga, and Botes *et al.* (2001) reported the occurrence of ciliate epibionts on the gills of a marine mollusc on the south coast of South Africa. In the present study, prevalence of peritrichs on elmid beetles ranged from 0% to 78.87%; on hydraenid beetles 3.18% to 24.24%; and on stonefly larvae and 0% to 6.25%.

These values are higher than was reported in other aquatic systems elsewhere. Ciliate prevalence ranged from 0.01% to 0.49% on cladocerans and copepods in the Deer Creek ponds, Colorado (Willey & Threlkeld 1993). Also, in the Chesapeake Bay, USA, prevalence on calanoid copepods ranged from 0.1% to 1.7% (Utz & Coats 2005). Furthermore, ciliate prevalence on copepods and cladocerans (11.6%) in the Ashmore Lake, Illinois (Henebry & Ridgeway 1979) was lower than the prevalence levels on elmids and hydraenids but higher than the prevalence on the stonefly larvae observed in this study.

Marked seasonal fluctuations in ciliate prevalence were observed in the present study; however, no clear pattern of seasonality was shown. On the elmid hosts, ciliates occurred on these hosts in almost all the monthly samples collected from Molenaars, Elandspad and Eerste Rivers. In contrast, ciliate prevalence in the Wolwekloof, Wit and Rooielskloof were zero for most of the year except in November (for Wolwekloof), October to December (for Wit), and May and March (for Rooielskloof). The effect of seasonal fluctuations on ciliate prevalence was more pronounced on the hydraenids and stonefly larvae confounding any underlying seasonal trend. It is likely that host availability may have influenced these results as hosts appeared and disappeared from samples at various times (as shown by the breaks in the line graphs). This disappearance and reappearance were not linked to seasonal fluctuations. Seasonality in ciliate prevalence has however often been reported for other systems (Nagasawa 1986, 1988; Weissman *et al.* 1993, Chiavelli *et al.* 1993, Lopez *et al.* 1998). Nagasawa (1986, 1988) and Weissman *et al.* (1993) each reported the disappearance of infested copepods in certain periods within the study years which affected data collection in their study and this is similar to what was observed in the present study.

Preferential colonisation of hosts is a common phenomenon in epibiont-host interactions in freshwater systems and to a large extent determines the distribution of epibiotic ciliates. In the present study, two morpho-groups of ciliate peritrichs were commonly observed in all rivers: the short-stalked group (SST) on elmid beetles and long-stalked group (LST) on hydraenid beetles and stonefly larvae. They were not found on any other aquatic insect species (Ephemeroptera and Trichoptera) present in the samples, suggesting the possibility of host specificity or high preference for certain hosts. This is a well known phenomenon

(Herman & Mihursky 1964, Willey & Threlkeld 1993, Gilbert & Schröder 2003). However, in rivers such as Wolwekloof and Wit, which had a low prevalence of ciliates (particularly the short-stalked taxa), the identified hosts may not represent the preferred substrate, but rather represent sink habitats (Pulliam 1988). Alternatively, some physical water quality differences (e.g., temperature, pH, etc.) between the different rivers could account for the low numbers of peritrichs observed (Chiavelli *et al.* 1993, Ebert *et al.* 2001, Elloumi *et al.* 2006). We found no evidence of switching between different hosts as preferred taxa as was found in other systems (Henebry & Rigdeway 1979, Pratt & Rosen 1983, Garcia *et al.* 1989), and the host specificity observed for the two taxa of peritrich was constant across season and river.

High epibiont prevalence was found on elmid beetles in three rivers: Molenaars, Elandspad and Eerste. For hydraenids, high prevalence levels were seen in the Molenaars River. While no river had significantly higher infestation levels than any other for the stonefly larvae, prevalences were highest in the Molenaars. When samples of hydraenid beetle hosts from the different rivers were pooled, host density correlated positively with ciliate prevalence as found in a few other studies, e.g., Chiavelli *et al.* (1993) and Xu (1992). Conversely, no link could be found between host density of elmid beetles and ciliate prevalence as ciliate prevalence was high at both high and low host densities. In this sense, the results thus agree with other studies (Lopez *et al.* 1998, Hanamura 2000, Utz & Coats 2005). However, in a few instances, there was a strong positive relationship between these two parameters when focusing on single rivers. This suggests that site factors other than host abundance may play a more important role in ciliate prevalence and that studies should investigate these factors (biotic and abiotic) on a per-river basis (as has been done for the Eerste River in Chapter 5).

Colonisation and occurrence of epibionts in any given system can be influenced by factors such as phytoplankton availability, bacterial populations, influx of water into the system, pollution and host abundance (Utz & Coats 2005). Prevalence is expected to be higher in systems which provide better conditions for growth and multiplication. It is likely that the Molenaars river (for both hydraenid and elmid beetle epibionts) and the Molenaars, Elandspad and Eerste rivers (for elmid beetle epibionts) provided such conditions.

On stonefly larvae, prevalence was highly variable with ciliates being absent during certain months of the year across the six rivers, even in the presence of hosts. Ciliate colonies were never found on the 190 stonefly larvae collected from the Wolwekloof River. The observed lower prevalence may be related to the repeated moulting (approximately every two weeks) of notonemourid stoneflies (V. Ross-Gillespie, pers. comm.) which would negatively affect epibiont prevalence (Xu 1992, Willey & Threlkeld 1993, Hanamura 2000). Thus, they are not as stable a substrate for epibiont colonisation as offered by adult beetles. It is also possible that the stonefly larvae act as a sink habitat for LST and that these colonise stonefly larvae when the preferred hosts are heavily colonised. In the case of the LST ciliates, the hydraenid beetles seemed to be the preferred hosts as these had much higher epibiont prevalence levels. Pulliam (1988) predicted that when source habitats are overpopulated, organisms would migrate to a less-preferred sink habitat in search of attachment space. There was some evidence in support of this phenomenon on the stonefly larvae as, with an increase in prevalence of the LST ciliates on the hydraenid beetles in the different rivers, so too did the prevalence of the LST ciliates generally increase.

In the present study, colonies of ciliate epibionts were only found on the ventral surface of host beetles. Attachment on the dorsal part of the host's exoskeleton places epibionts at the risk of predation and possibly abrasion by objects carried in the current. Ciliates have also been reported to prefer the less mobile body parts of their hosts (Utz & Coats 2005). For elmid beetles, this would be the abdomen, thorax and head. On elmid beetles, although legs carried approximately half of the ciliate load found on the thorax, a significantly higher epibiont density was observed on the thorax. For hydraenids, ciliate colonies were only encountered on the head. This agrees with the results of Utz and Coats (2005) who found higher ciliate density on the cephalothorax and abdomen of copepods in comparison to the swimming legs and antennae. The absence of the epibionts from the abdomen is more difficult to explain, but may relate to the positioning required for the peritrich to maximize food capture. For example, Evans *et al.* (1979) reported that the suctorian epibiont *Tokophrya quadripartita* preferentially attached to the head of its host to increase its contact with prey organisms; the LST ciliates on the hydraenids in this study may have enjoyed a similar benefit by their placement on the ventral parts of the head. However, for the elmids, no SST ciliates were found associated with the head. The significantly higher infestation density by SST ciliates on the thorax and legs of their elmid hosts may suggest that this position brings the ciliates closer to their preferred food source on the substrate and that they may have different food requirements than the LST ciliates. It is also conceivable that their placement occurs in areas where the beetle body part causes localised vortices, creating areas of slower flowing water where particulate food may be trapped.

In this study, ciliates were never found on other notonemourid genera. *Aphanicerca*, *Desmonenoura* and *Aphanicercopis* are typically syntopic with *Aphanicercella* in most of the six rivers sampled, but are smooth-bodied compared to *Aphanicercella*. In contrast, *Aphanicercella* larvae that hosted LST ciliates are highly setose (Stevens & Picker 2003). These setae may thus provide a favourable environment for ciliate colonisation. Ciliates have been observed to prefer certain crustacean body parts such as setae, antennal segments and scales, possibly because they were able to provide an attachment site, shelter from predation or trap bacteria and debris (Mayen-Estrada & Aladro-Lubel 2001).

This study is the first documentation of the occurrence of ciliate epibionts on aquatic insects in the rivers in the Western Cape Province of South Africa with the different ciliate taxa exhibiting varying prevalence levels, preference for aquatic hosts and for attachment sites on hosts across the rivers. Pattern of epibiotic prevalence is decided, not by a single factor, but by the outcome of the interactions between host species, seasonal influence and abiotic conditions in aquatic systems. The study focused only on the ciliate epibionts of aquatic macroinvertebrates. Nevertheless, it does not exclude the possibility of the occurrence of other ciliate epibionts on other substrates that was sampled in the rivers. Although detailed taxonomy of ciliate epibionts was not carried out in this study (where the emphasis was on peritrich ecology), the ciliates were identified to a level which permitted ecological studies on ciliate-host relationship. Further studies on the identification and taxonomic description of the ciliate fauna and if possible, protozoan communities in these rivers is recommended for better understanding of the epibiotic relationships. In addition to being a major component of the aquatic food chain, ciliates have been used as ecosystem study tools for studies on population dynamics of their hosts since ciliate epibionts strongly affect host populations. They have also been employed in ecological and environmental studies especially water quality studies as they respond rapidly to environmental changes and can be collected for experimentation. Thus, an understanding of the patterns of ciliate prevalence in aquatic systems will enhance the understanding of their responses to deteriorating water quality in aquatic systems in South Africa.

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CHAPTER 5. RESPONSE OF CILIATE EPIBIONTS OF MACROINVERTEBRATES IN THE EERSTE RIVER TO A WATER POLLUTION GRADIENT.

5.1 INTRODUCTION

Water is vital for life as well as for human and economic development (Sullivan 2002). Unfortunately, global increases in human population numbers and increased usage have compromised water resources to such an extent that protection of this valuable resource is paramount (Roux 1999). Scarcity of clean water and water pollution are recognised as important challenges facing global economies (Simonovic 2002, Dudgeon *et al.* 2006). Consequently, river monitoring programmes and bioassessment techniques were developed to monitor water quality, detect unacceptable changes and identify factors influencing these changes (Mueller *et al.* 1997, Roux 1999).

Monitoring of water quality initially involved only the measurement of levels of physical water attributes and chemical composition (Day 2000). These methods often lack the ability to provide information on the overall condition of the aquatic ecosystem. This is largely due to sampling results that provide information only on water conditions at the exact time of sampling such that a temporary but disastrous decline in water quality might be missed (Roux 1999, Day 2000). Also, the physico-chemical methods may not always account for major anthropogenic disturbances, e.g., flow alterations and habitat degradation that negatively affect biological health (Mason 1996). Additionally, it is neither possible nor economical to measure the wide range of chemicals contained in water (Mason 1996) and some toxic substances may be found below detection levels (Day 2000). In light of this, biomonitoring techniques have gained popularity in providing critical aquatic ecosystem information (Roux *et al.* 1993).

Biomonitoring is the use of biological variables to assess the environment (Bonada *et al.* 2006). It often serves as a sensitive surveillance tool to monitor toxic substances entering water systems before and after completion of projects (Chapman & Jackson 1996). It makes use of the response of bioindicators (fish, algae, macroinvertebrates) to natural or anthropogenic-related changes in the environment (Cairns & Dickson 1973, Lamb 1985, Hohls 1996). The usefulness of bioindicators in these systems stems from their ability to give a broader view of ecosystem health, as effects on water quality will manifest in the responses of these organisms over extended periods of time and not just at a specific point in time (Roux *et al.* 1993). However, biological methods may not be able to identify the nature of the specific pollutant/disturbance factor in the aquatic ecosystems (Moore & Macmillian 1993). Hence, they should be used complementary to physical and chemical methods (Davies & Day 1998).

The most popular bioindicators used for biomonitoring purposes are benthic macroinvertebrates, especially aquatic insects, which have been used in a range of biomonitoring programs, e.g., RIVPACS in the U.K and Australia (Wright *et al.* 1984), BWMP in the U.K (Armitage *et al.* 1983), SASS in South Africa (Chutter 1994, 1998). This is because of their wide-spread abundance, sedentary nature, high level of sensitivity, relatively long life cycle and ease of identification (Rosenberg & Resh 1993). Other taxa often used include leeches, crustaceans and molluscs (Rosenberg & Resh 1993). Ideal macroinvertebrates used in biomonitoring should be easily recognised by non-specialists to avoid confusion, have cosmopolitan distribution to allow for comparative studies, have large body size and high numerical abundance for ease of sampling and sorting, have limited mobility and a relatively long lifecycle to enable integration on temporal and spatial scales, have well-known ecological characteristics and should be suitable for use in the laboratory (Rosenberg & Wiens 1976, Hellawell 1986). However, not all of these conditions are usually met because certain macroinvertebrate taxa are not well-known; and macroinvertebrates are affected by other factors, apart from water quality, which influence their abundance and distribution (Resh 2008). Also, seasonal variations may interfere with the interpretations of results (Linke *et al.* 1999).

In South Africa, water resources are also facing the global challenge of water pollution. Consequently, the amount of clean water available for use per person is limited (CSIR 2010). Freshwater resources are progressively contaminated by urban and sewage effluents, industrial waste products, seepages and discharges from mines and agricultural areas (CSIR

2010). This results in these water resources becoming moderately to highly eutrophic (Oberholster & Ashton 2008). This decline in water quality negatively affects the aquatic ecosystem, decreases yield and quality of agricultural products, increases the treatment costs of water for urban and industrial use, and exposes human beings and animals to pathogenic micro-organisms and disease conditions (Oberholster & Ashton 2008, CSIR 2010). To ensure the effective protection of rivers and maintenance of river health status for both present and future urban, industrial and agricultural uses, the South African River Health Programme was developed (Roux 1999). This programme makes use of a rapid bioassessment technique (SASS) as a monitoring tool in the assessment of river health and for the management of water resources (Day 2000).

The South African Scoring System (SASS) is a modification of a biomonitoring tool initially developed by the Biological Water Monitoring Party in the U.K (Chutter 1994, 1998). It makes use of benthic macroinvertebrates as bioindicators of water quality and has gained a wide acceptance as a standard bioassessment technique for monitoring river health in South Africa. It is currently in its fifth version (SASS5) having undergone a series of refinements (Dickens & Graham 2002). SASS is interpreted based on a SASS and ASPT (Average Score Per Taxon) score. ASPT is considered a more consistent measure of water quality than SASS. This is because in cases where habitat diversity is naturally poor, there will be lower biotic diversity, resulting in a lower SASS score. In contrast, ASPT will be less affected by pollution because it accounts for differences in the number of taxa (Dallas 1997, Chutter 1998, Dickens & Graham 2002).

Although macroinvertebrates are popular as bioindicators, microbes such as diatoms have long been used to monitor water quality (Patrick 1973, Gaiser & Bachmann 1993). In addition, protozoans present taxa whose potentials have not been fully explored as water monitoring tools. Sessile protozoan ciliates have been identified as valuable bioindicators because they may be more sensitive to water pollution than invertebrates. This is due to their unicellular nature which ensures intimate contact with their immediate environment (Laird 1959, Cairns 1974, 1978). This makes them responsive to even small environmental changes like those found in the early stages of pollution (Cairns 1974, Antipa 1977).

Prevalence of especially the ciliated protozoan epibionts on aquatic hosts has been linked to increases in pollution and the eutrophic nature of aquatic systems in various parts of the world where ciliate response to pollution have been observed or studied (Laird 1959, Bick 1972, Antipa 1977, Cairns 1978, Henebry & Ridgeway 1979, Xu & Wood 2011).

Ciliates have previously been reported as epibionts of macroinvertebrates in South African water systems (Viljoen & Van As 1983, Basson *et al*. 1983, Van As *et al.* 1998, Botes *et al.* 2001). However, there is no published information on their response to pollution stress in South African ecosystems. With increasing deterioration of both natural and artificial water resources in South Africa, it is important to include all available methods in assessments in order to make informed decisions on water management and conservation. This study investigates the response of ciliate epibionts of macroinvertebrates in the Eerste River, Western Cape province, South Africa to a pollution gradient that is imposed by the placement of a dam and a fish farm along the upper reaches of the river. In addition, the response of macroinvertebrate communities to this pollution gradient is also examined.

5.2 MATERIALS AND METHODS

5.2.1 Study area

This study was conducted in the Mountain Stream and Upper River zones of the Eerste River, Jonkershoek Nature Reserve, Stellenbosch, South Africa (see Fig. 2.1, Chapter 2). These river zones occupy an expanse of 7km and 5km respectively with a substratum of bedrock, boulders and stones. Riparian vegetation consists of small indigenous trees within the Fynbos vegetation (King 1983). At the lower end of the Mountain Stream zone is the Kleinplaas Dam (also called the Jonkershoek Dam) which was constructed to divert water for residential and agricultural use for the town of Stellenbosch (DWAF 1993). It also served as a trout farm. Water quality upstream from the dam remains relatively undisturbed while the river section below the dam has been negatively impacted by anthropogenic activities including water extraction for town use and trout farming (Brown & Dallas 1995). A further point source of water pollution is imposed by the University of Stellebosch experimental aquaculture facility, which is situated approximately 2km below the dam. Water circulated through the facility re-enters the Eerste river at its junction with the Assegaaiboschkloof river.

To study the response of ciliate epibionts to pollution in the Eerste River, three sites were selected above the Kleinplaas dam and three sites below the dam (Table 5.1). The sites were chosen based on ease of accessibility to the river and in the case of site 4, proximity to the source of disturbance (the Kleinplaas dam). The sites above the dam are oligotrophic while those below the dam were assumed to be polluted from various activities in the dam and fish farm. The location and elevation of each site were recorded using a Global Positioning System. Distances of sites from the dam were then measured using the ruler function in Google Earth.

Table 5.1. Study sites within the Eerste River. Sites 1, 2 and 3 represent near pristine sites above the Kleinplaas dam while sites 4, 5 and 6 represent anthropogenically impacted sites below the dam.

SITE	ALTITUDE	COORDINATES	LOCATION
1	392m	33° 9763S 18° 9435E	Inside reserve, at the white bridge, ca. 3.51km upstream from the dam
2	351m	S33° 9897S E18° 9694E	Inside reserve, below confluence with Jakkals River, ca. 2.85km upstream from the dam
3	285m	S33° 9789S E18° 9497E	Inside the reserve, ca. 0.65km upstream from the dam
4	176m	S33° 9736S E18° 9382E	Inside the reserve, ca. 0.44km downstream from the dam
5	148m	S33° 9658S E18° 9257E	Just below the University of Stellenbosch experimental trout farm, ca. 1.9km downstream from the dam
6	140m	S33° 9581S E18° 9158E	River Cottage Guest House, ca. 3.20km downstream from the dam

5.2.2 Sample collection

General materials and methods have been described in Chapter 2. Samples were collected on 1st July, 2011 (winter) and 7th October, 2011 (early summer) to examine the effect of seasonal variation. Benthic arthropods were collected from the six sites (Table 5.1), sorted and identified to family level using appropriate keys (Chapter 2, Table 2.1). In addition, further samples were collected following protocols outlined for the South African Scoring System (SASS) (Dickens & Graham 2002, Chapter 2). These samples were collected at each site and for both sampling periods.

Normally, SASS samples are examined in the field and macroinvertebrates found in samples are scored on site. However, in the present study these were preserved in 70% ethanol to examine macroinvertebrate individuals for ciliate infestation. Hence, the procedure will be referred to as the modified SASS (as outlined in chapter 2). Low scores were assigned to pollution-tolerant taxa and high scores to pollution-sensitive taxa following methods of Chutter (1998). Modified SASS scores were calculated by summing the scores for each taxon at each site. Dividing the total SASS score by the number of invertebrate taxa at each site provides the ASPT (Average Score Per Taxon). Interpretations of the SASS results were based on empirical interpretations provided by Chutter (1998) for SASS4 version (Table 5.2).

Table 5.2. Guidelines to interpretation of SASS4 (Chutter 1998)

SASS4 SCORE	ASPT	INTERPRETATION
>100	>6	Natural water quality, high habitat diversity
<100	>6	Natural water quality, reduced habitat diversity
>100	<6	Borderline between natural water quality and some deterioration in water quality
50 – 100	<6	Some deterioration in water quality
<50	Variable	Major deterioration in water quality

5.2.3 Physicochemical water variables

Physicochemical variables represent environmental variables that are commonly used as indicators of water quality (Dallas *et al.* 1999). In our study, these served as baseline data with which to compare the responses of biotic communities (SASS) and their ciliate epibionts in predicting water quality. Physical variables measured at each site include: flow rate, river depth, pH, conductivity, dissolved oxygen levels, turbidity and temperature. A reduction in flow rate translates to increase in concentration of certain chemicals and can disrupts the aquatic system (Malan & Day 2003) while increases in temperature, turbidity, pH levels, conductivity coupled to decreases in dissolved oxygen concentration usually indicate deterioration in water quality (Chapman & Kimstach 1992).

In this study, measurements were taken on site with the appropriate instruments following the manufacturer's instructions (see Chapter 2, Table 2.1). At each site and collecting period, each water physico-chemical variable was measured at four different points and a mean value was calculated. Water samples were collected from each site in sterile 300ml honey jars for later analyses of nutrient levels. Nutrient variables assayed included nitrate, nitrite, phosphate and ammonium ions. Water samples were analysed at the Centre for Scientific and Industrial Research (CSIR), Stellenbosch, South Africa, as well at the Oceanography Department, University of Cape Town**.**

5.2.4 Ciliate epibiont assessments

Macroinvertebrates from benthic samples (both primary samples and SASS samples) collected from the six study sites were examined with a Nikon SMZ800 stereomicroscope (Nikon Coorporation, Tokyo, Japan) for the presence of ciliate epibionts and scored as either 'infested' or 'uninfested'. Infestation prevalence and zooid abundance (Bush *et al.* 1997) was recorded for each ciliate taxon at each site.

5.2.5 Data analyses

5.2.5.1 Macroinvertebrate community structure

The percentages of macroinvertebrate taxa collected from the six study sites in the Eerste River and total abundances of individuals of each taxon for the combined abundance data for the two sampling periods were calculated. Bray-Curtis similarity scores were calculated from fourth root-transformed abundance data using the analytical package, PRIMER (Plymouth Routines in Multivariate Ecological Research) v6.1.5 (Primer-E Ltd., Plymouth, UK) (Clarke & Gorley 2006), and used to generate ordinations (MDS) and cluster diagrams using (group average linking procedure). Environmental variables were superimposed on the biotic community ordinations to visualize possible trends in biotic responses to pollutants.

5.2.5.2 Physicochemical data

Results on chemical analyses of nutrients from CSIR showed nutrient levels to be below 0.1mg.L^{-1} which was uninformative, and when repeated at Oceanography Department, University of Cape Town, the same sample gave different readings on repeated measurements which were inconsistent (Table 5.3). For this reason, results of the chemical analysis were excluded from statistical analyses.

Table 5.3. Nutrient levels at sites above- (1 – 3) and below (4 – 6) the Kleinplaas dam in Eerste River, Jonkershoek. PO_4, phosphate ion; NO_3, nitrate ion; NO_2, nitrite ion; NH_4; ammonium ion; DOC, dissolved organic carbon**.**

NUTRIENT	**SITE 1**	**SITE 2**	**SITE 3**	**SITE 4**	**SITE 5**	**SITE 6**
PO4 ± 0.16 (µmol.L^{-1})	0.81	0.39	0.42	0.32	0.29	0.17
NO3 ± 1.37 (µmol.L^{-1})	1.32	0.63	4.58	3.12	0.68	1.26
NO2 ± 0.12 (µmol.L^{-1})	1.13	0.08	0.06	0.07	0.09	0.06
NH4 ± 0.31 (µmol.L^{-1})	0.9	0.69	0.72	0.65	1.68	0.79
DOC (mg.L^{-1})	2	1	<1	<1	1	<1

Spatial (above dam versus below dam) and seasonal (winter versus summer) variations in physical water variables were compared to gain a better understanding of water quality changes. Normality tests showed these data to be non-normally distributed, thus Mann-Whitney U tests were performed in Statistica Analytical Package, version 10 (StatSoft company) to test differences for each variable between the seasons as well as above- and below the dam. Preliminary statistical analyses showed no significant difference between the different seasons (winter versus summer) except for water temperature. Therefore, data for the two sampling periods were pooled for further analyses to improve sample sizes.

Comparison of biotic and physical water variables were performed using the BIOENV (Biological and environmental variables correlation) procedure in PRIMER v6.1.5. In addition, the best combination of environmental variables responsible for observed structuring of community patterns was determined by calculating Spearman rank correlation coefficient (ρ_S). Physical data was normalised and a resemblance matrix was calculated using the Euclidean distance similarity procedure.

5.2.5.3 Modified SASS data

Differences in mean SASS5 values and ASPT scores between sites above dam (non-impacted sites) and sites below dam (impacted sites) were tested using a parametric t-test after Shapiro-Wilkes test indicated normal distribution of data. Analyses were performed in the Statistica Analytical Package, version 10 (StatSoft company).

5.2.5.4 Ciliate prevalence and zooid abundance

Both prevalence and zooid abundance data were non-normally distributed. Hence, non-parametric t-tests and Kruskal-Wallis ANOVAs with multiple comparisons of mean ranks (post-hoc tests) were performed to determine differences in median prevalence and abundance values above- and below the dam.

5.3 RESULTS

5.3.1 Macroinvertebrate community structure

A total of 12,127 invertebrate individuals belonging to 30 families and eight insect orders, Ephemeroptera (53.01%), Diptera (26.99%), Coleoptera (13.71%), Plecoptera (3.45%), Trichoptera (2.54%), Odonata (0.07%), Megaloptera (0.21%) and Hemiptera (0.02%), were collected from the six sites in the present study (Fig. 5.1, Appendix I). The macroinvertebrate community of the Eerste River is an insect-dominated community which showed a spatial decrease in abundances of some taxa in sites below the dam (See Appendix I, Fig. 5.1). Higher abundance of all macroinvertebrates was observed above the dam than below the dam. However, the decreases in abundance below the dam were not significant (Table 5.4) probably because of low sample size.

Table 5.4. Macroinvertebrate abundances at sites above- (1 – 3) and below (4 – 6) the Kleinplaas dam in Eerste River, Jonkershoek. MWU, Mann-Whitney U value; n_a, sample size above the dam; n_b, sample size below the dam; N.S, non-significant; S, significant.

TAXON	MEDIAN ABUNDANCE (INTERQUARTILE RANGE)		MWU	n ($n_a = n_b$)	P < 0.05
	ABOVE THE DAM	BELOW THE DAM			
Coleoptera	18.5 (5 – 92.5)	1.0 (0 – 6.5)	0.0	3	N.S
Diptera	15 (0 – 114)	0.5 (0 - 75)	4.0	3	N.S
Ephemeroptera	66.5 (0 - 298)	3.5 (1 -11)	2.0	3	N.S
Plecoptera	153 (93 - 168)	1.0 (1 - 2)	0.0	3	N.S
Trichoptera	11.5 (4.5 – 22.5)	1.0 (0 - 12)	3.0	3	N.S
Other taxa	1.67 (0 – 1)	0.73 (0 – 1)	4.5	3	N.S

Arthropod communities from sites above the dam differed compositionally from those below the dam and clustered together (Fig. 5.1). Pollution sensitive taxa (e.g., Notonemouridae, Leptophlebidae, Teloganodidae, Blephariciidae) were dominant in non-polluted sites but a shift in dominance to the pollution-tolerant taxa (e.g., Chironomidae and Baetidae) was observed in the polluted sites (Appendix II).

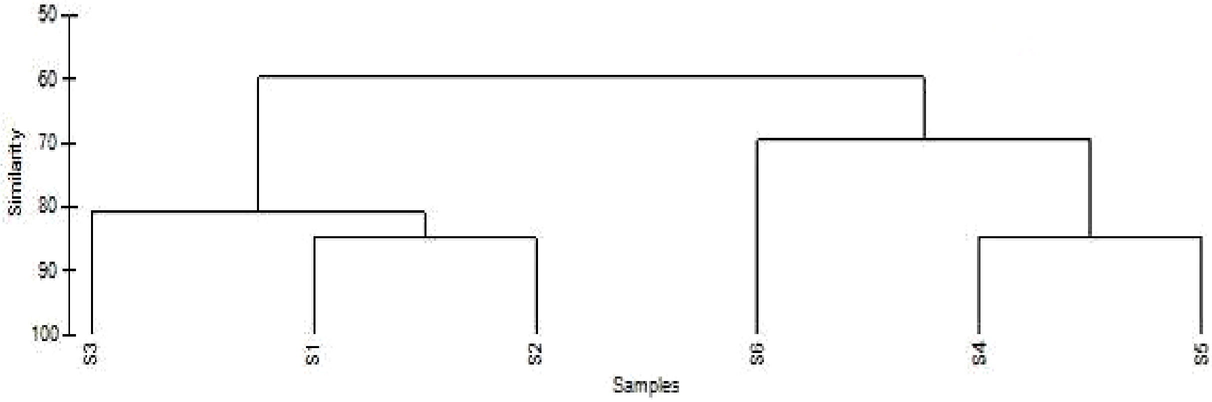

Figure 5.1. Dendrogram depicting Bray-Curtis similarity of aquatic insect communities from sites above- (1 – 3) and below (4 – 6) the Kleinplaas dam in the Eerste River, Jonkershoek.

5.3.2 Physical water variables

Mean values for the different water variables at each site are presented in Figure 5.2. Flow rates decreased downstream with less turbulent flow observed in sites where there is an increase in the width of river bed. Maximum flow rate was highest at site 1 (0.99 ± 0.24 $m.s^{-1}$) and site 2 (1.34 ± 0.16 $m.s^{-1}$). Width of the river bed increased from site 3 resulting in flow reduction (Fig. 5.2). A similar pattern was observed for average flow which reached its highest value in site 2 (0.78 ± 0.47 $m.s^{-1}$) and then showed a continuous downstream decline. Oxygen concentration decreased downstream with a slight recovery at site 6, while pH-values, temperature, conductivity and turbidity generally increased from sites 1 to 6, (Fig. 5.2). However, a decrease in turbidity was observed in site 6 suggesting that sedimentation of silt and detritus from the dam had occured. Conductivity showed a steep increase at sites after the dam.

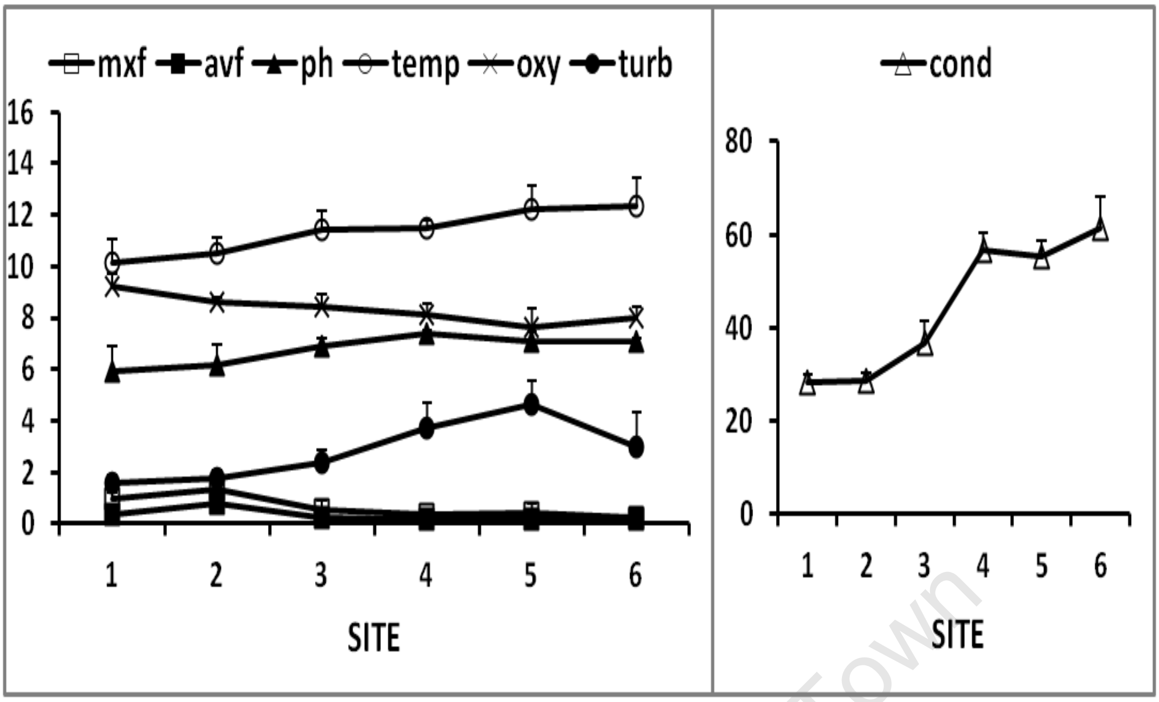

Figure 5.2. Physical water variables at the six study sites in the Eerste River, Jonkershoek. Error bars represent the standard deviation from the mean values generated from combined samples. Abbreviations: mxf, maximum flow ($m.s^{-1}$); avf, average flow ($m.s^{-1}$); ph, pH (power of hydrogen ion activity); temp, temperature (°C); oxy, oxygen concentration ($mg.l^{-1}$); turb, turbidity (NTU).

Statistical analysis of water sample variables reflected significant differences in physico-chemical water characteristics above- and below the dam. Significant increases below the dam were detected for pH, temperature, conductivity and turbidity while water flow rates (maximum flow and average flow) and oxygen concentration decreased significantly in the sites below dam (Table 5.5).

Table 5.5. Differences in physical water variables above- and below the Kleinplaas dam in the Eerste River, Jonkershoek Data are presented as medians (interquartile range). Different superscript letters indicate significantly different associations based on Mann-Whitney U (MWU) tests ($P < 0.05$). n_a, sample size above the dam; n_b, sample size below the dam.

WATER VARIABLE	**ABOVE THE DAM**	**BELOW THE DAM**	**MWU**
Maximum flow rate ($m.s^{-1}$)	0.93 (0.75 – 1.25)^a; $n^a = 24$	0.48 (0.20 – 0.40)^b; $n^b = 22$	116.0
Average flow rate ($m.s^{-1}$)	0.47 (0.18 – 0.67)^a; $n^a = 24$	0.15 (0.15 – 0.17)^b; $n^b = 21$	101.0
pH	6.30 (5.3 – 6.97)^a; $n^a = 22$	7.18 (6.95 – 7.38)^b; $n^b = 21$	52.5
Temperature (°C)	10.60 (9.8 – 11.0)^a; $n^a = 24$	12.18 (11.2 - 13)^b; $n^b = 19$	49.0
Conductivity ($\mu S.cm^{-1}$)	30.95 (26.8 – 32.0)^a; $n^a = 23$	57.68 (53.0 – 60.6)^b; $n^b = 21$	0.0
Oxygen concentration ($mg.l^{-1}$)	8.80 (8.54 – 9.09)^a; $n^a = 24$	7.91 (7.63 – 8.32)^b; $n^b = 22$	33.5
Turbidity (NTU)	1.88 (1.52 – 2.15)^a; $n^a = 23$	3.74 (2.87 – 4.77)^b; $n^b = 20$	41.0

Superimposition of environmental variables on to an MDS ordination of biotic communities (Fig. 5.3) showed consistent differences in water quality in sites above- and below dam. However, Spearman's rank correlations showed that a combination of maximum flow rate, pH, conductivity, oxygen concentration and turbidity correlated significantly with arthropod community structure included. This combination of environmental variables best explained the observed community pattern ($\rho_S = 0.83$, $P < 0.05$).

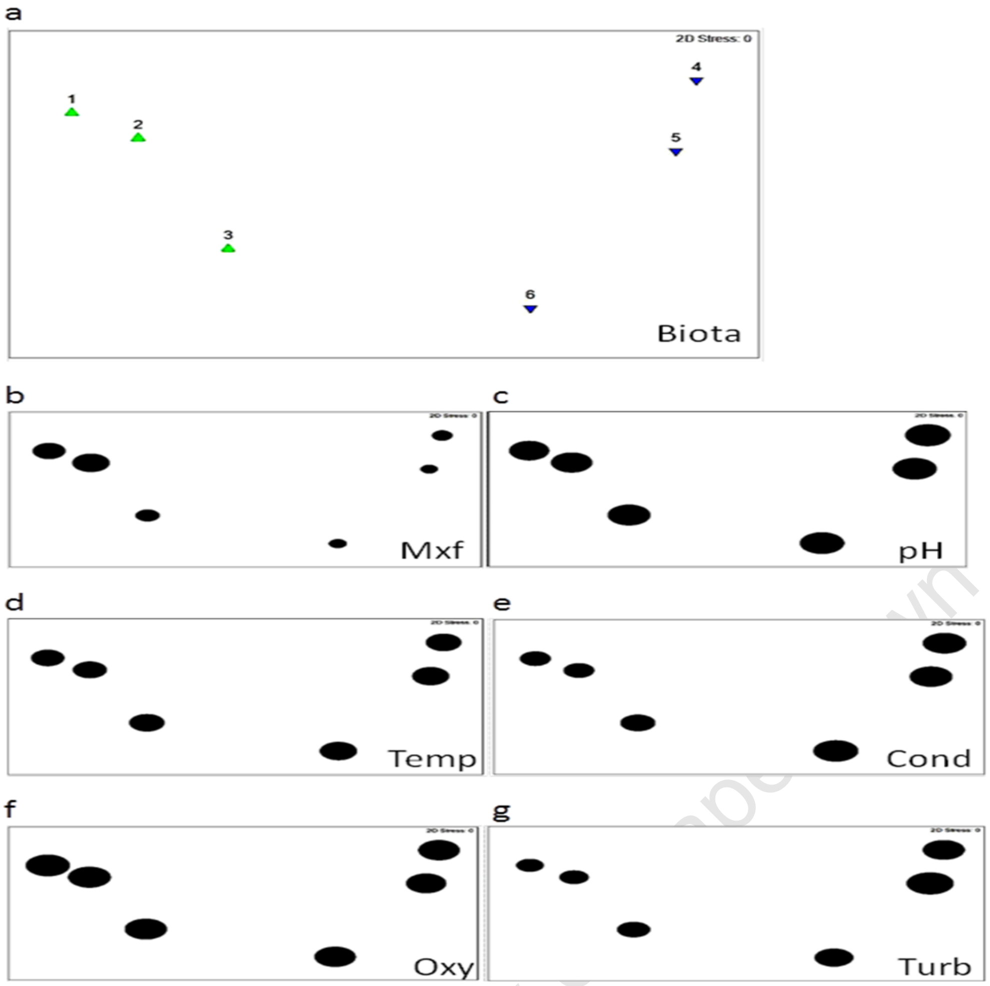

Figure 5.3. (a) MDS (Bray-Curtis similarity) from fourth root-transformed species abundance data at sites above- (1-3) and below (4-6) the Kleinplaas dam, Eerste River, Jonkershoek. (b) – (g) the same MDS but with superimposed circles of differing sizes representing differences in physical water variables (Stress = 0). Abbreviation: Mxf, maximum flow; pH, power of hydrogen ion activity; Temp, temperature; Cond, conductivity; oxy, oxygen concentration; Turb, turbidity.

5.3.3 Modified SASS values

SASS5 and ASPT scores for sites above the dam were significantly higher than for sites below the dam (For SASS; $n = 6$, $df = 5$, $t = 5.61$, $P < 0.05$ and for ASPT; $n = 6$, $df = 5$, $t = 3.87$, $P < 0.05$) (Fig. 5.4). Sites above the dam for the two sampling periods had SASS scores and ASPT which were higher than 100 and 6 respectively except for Site 1 in July 2011 which had a SASS score of 88 but an ASPT of 8.8. SASS scores and ASPT of sites below the dam indicated deterioration in water quality at these sites except for Site 6 in October, 2011 which had a high SASS score of 105 and ASPT of 8.08 (Appendix III), suggestive of some recovery.

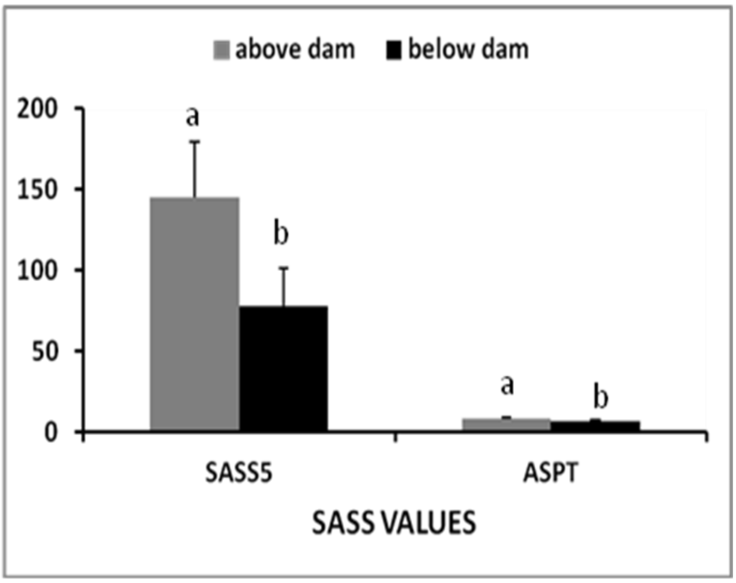

Figure 5.4. SASS and ASPT scores for sites above- and below the Kleinplaas dam in the Eerste River, Jonkershoek). Error bars represent the standard deviation from the means. Different letters above bars indicate significant differences.

5.3.4 Epibiont prevalence and zooid abundance

Ciliate epibionts were found on adult Elmidae (*Stenelmis*, *Pachyelmis*), Hydraenidae (*Hydraena*), Corixidae (*Micronecta*); and on larval Notonemouridae (*Aphanicercella*), Dryopidae and Ptylodactylidae. Ciliate epibionts found on macroinvertebrates in study sites included: peritrichs (short-stalked taxa, SST; and long-stalked taxa, LST) and suctorian ciliates (SUC). The short-stalked peritrich ciliates occurred mainly on adult elmid beetles and on adult water bugs; while the long-stalked peritrich ciliates were observed on adult hydraenid beetles, stonefly larvae, dryopid larvae and ptylodactylid larvae. Suctorian epibionts occurred mainly on hydraenid beetles.

Both peritrich taxa had similar responses to pollution with regards to prevalence (Table 5.6) and zooid abundance (Table 5.7), decreasing below the dam. However, the decrease in prevalence was not significant for the SST (Table 5.6). Unlike the peritrichs, the suctorian ciliates exhibited a significant increase in prevalence (Table 5.6) but not in zooid abundance

(Table 5.7) in sites below the dam. It is likely that these analysis were affected by small sample size and the high data variance, hence the inability of the test to detect significant differences in SST mean prevalence and suctorian zooid abundance above- and below the dam.

Table 5.6. Comparison of prevalence of ciliate epibiont taxa in sites above- and below the Kleinplaas dam in the Eerste River, Jonkershoek. Data are presented as median (interquartile range) with different letters indicating significant differences at $P < 0.05$ based on Mann-Whitney U (MWU) tests. SST, short-stalked taxon; LST, long-stalked taxon; SUC = suctoria; n_a, sample size above the dam; n_b, sample size below the dam.

TAXON	ABOVE DAM	BELOW DAM	n ($n_a = n_b$)	MWU
SST	44.31 (28.05 – 61.22)^a	0.0 (0.00 – 25.00)^a	6	9.00
LST	27.69 (13.26 – 48.00)^a	3.47 (0.00 – 8.33)^b	6	1.00
SUC	0.0 (0.00 – 9.38)^a	28.5 (22.73 – 41.67)^b	6	0.00

Table 5.7. Comparison of zooid abundance of ciliate epibiont taxa at sites above- and below the Kleinplaas dam in the Eerste River. Data are presented as median (interquartile range) with different letters indicating significant differences at $P < 0.05$ based on Mann-Whitney U (MWU) tests. SST, short-stalked taxon; LST, long-stalked taxon; SUC = suctoria; n_a, sample size above the dam; n_b, sample size below the dam.

TAXON	ABOVE DAM	BELOW DAM	n (na = nb)	MWU
SST	298 (51 – 586)^a	0.0 (0.0 – 7.0)^b	6	4.00
LST	177 (169 – 480)^a	0.0 (0.0 – 5.0)^b	6	0.00
SUC	0.0 (0 – 21)^a	53 (11.0 – 85.0)^a	6	7.00

5.4 DISCUSSION

Human manipulation of rivers, commonly in the form of dam and reservoir construction has impacted nearly all major river systems arround the world (Williams & Feltmate 1992). These impoundments alter the physical condition of rivers they are built in, affecting plant and animal survival (Allan 1995). The Eerse River is no exception. The upstream reaches of the Mountain stream zone of this river seem to be relatively pristine (Brown & Dallas 1995). In contrast, the downstream reaches from the dam show evidence of disturbance due to the construction of the dam (Brown & Dallas 1995).

All physical water variables showed significant differences between sites above- and below the dam, with the trends below the dam being indicative of water pollution. Water conductivity increases along with the concentrations of ions in water (Dallas & Day 1993) while turbidity is influenced by type and concentration of suspended solids which include silt, clay, fine particle of organic and inorganic matter, microscopic organisms etc. (Chapman & Kimstach 1992). Water pH influences both chemical and biological processes with changes in pH levels often indicating the presence of pollutants (Chapman & Kimstach 1992). In freshwater systems, increases in conductivity, turbidity and pH are associated with pollution (Chapman & Kimstach 1992). Dissolved oxygen is an important component of the aquatic environment and decreases in concentration below $5 mg.l^{-1}$ adversely affect aquatic communities (Chapman & Kimstach 1992). Water released from the dam carries silt, dissolved salts and sediments into downstream water reaches causing the observed increase in conductivity, turbidity and pH, and decrease in oxygen concentration in the present study.

Significantly higher water temperatures below the dam could be as a result of the reduction in flow rate caused by the Kleinplaas dam. Flow rate is intimately linked with water quality. A steady flow rate helps to reduce the variability in concentrations of instream chemical components and can maintainin a balanced aquatic environment. A reduction in flow rate translates to increase in concentration of certain chemicals and can disrupt the aquatic system (Malan & Day 2003). Variations in temperature may also be due to differences in elevation, extent of riparian vegetation and ground water inputs (Allan 1995). Temperature affects physico-chemical and biological processes in water with increases impacting on metabolic rate of aquatic organisms as well as on the rate of chemical reactions (Chapman & Kimstach 1992). However, sites 1 and 6 were separated by a distance of approximately 7 km, which in itself might account for some of the downstream trends. Nevertheless, the significant contrasts between above- and below dam sites for most of the water variables, and particularly the recovery noted for some variables at site 6, suggests that the dam itself is responsible for the change in water variables. This was particularly pronounced for conductivity which increased steeply at the first site below the dam. The findings in the present study is in line with the results of Bredenhand (2005) who attributed the downstream changes in water variables at sites below the Kleinplaas dam in the Eerste river to water quality deterioration caused by the dam.

Sediments and other substances released from an impounded river can affect aquatic organisms occupying downstream reaches located far away from the source of pollution (Palmer & O'Keffe 1990). King (1983) reported downstream changes in the abundance, biomass and species diversity of benthic invertebrates in the Eerste River in response to the river's deteriorating water quality caused by dam construction. In the present study, there were also changes in the macroinvertebrate community in the Eerste River as reflected in the decrease in the abundances and taxon richness below the Kleinplaas dam, indicating that the river ecosystem in sites below the dam has been impacted by the dam and its effluents. The changes in abundance and invertebrate community structure were strongly related to changes in the water quality as supported by correlation analyses with the most influential variables being maximum flow rate, pH, conductivity, oxygen concentration and turbidity.

Above the dam, the Ephemeroptera was the dominant macroinvertebrate taxon having high abundances of teloganodids, leptophlebiids and baetids. However, Ephemeroptera numbers downstream from the dam consisted mainly of Baetidae, a family that contains species known to occur in a wide variety of flow regimes as well as pollution-tolerant species (Barber-James & Lugo-Oritz 2003). Change in Ephemeroptera abundance and diversity is considered an indication of a change in the abundance and diversity of the whole fauna since it usually forms a greater propotion of the total macroinvertebrate density (King 1983). Plecoptera are known to be highly sensitive to pollution (Stevens & Picker 2003), as was found in this study. High abundance of this taxon was found at sites above the dam in contrast to the very low levels below the dam. Conversely, higher abundance of individuals of Trichoptera (mostly Hydropsychidae) and Diptera (largely Chironomidae and Simuliidae) were found at sites below the dam. This is in line with previous studies that show that moderately polluted waters are dominated by Chironomidae and Trichoptera while non-polluted waters are usually dominated by Ephemeroptera, Plecoptera and Trichoptera (Rosenberg *et al.* 1986).

The ASPT is considered the most reliable measure of river health, although SASS scores can carry great weight in polluted areas (Chutter 1998, Dickens & Graham 2002). In the present study, both SASS and ASPT scores were significantly higher upstream from the dam than for sites below the dam, indicating a change in species abundances and diversity related to changes in water quality. SASS results show a disappearance of pollution-sensitive taxa, e.g., Plecoptera from sites below the dam, supporting the occurrence of a downstream deterioriation in water quality at these sites. Following Chutter (1998), sites above the dam that had SASS scores and ASPT higher than 100 and 6 respectively can be interpreted as having natural water quality and high habitat diversity. Although Site 1 had a SASS score of 88 which is lower than 100, the ASPT (8.8) was still higher than 6 indicating that site 1 has natural water quality. The low SASS score in site 1 could be as a result of downward vertical migration of benthos into the river bed to avoid fast currents at this site. Previous studies (Bredenhand 2005) on the influence of the Kleinplaas dam in the Eerste River also reported a decrease in SASS scores and a lower macroinvertebrate diversity in sites below the dam indicating that the dam is a clearly a disturbance to Eerste river system.

Site 6 appeared to be undergoing a recovery, based on its SASS and ASPT scores. As site 6 was the furthest downstream from the dam in our study, it is likely that water quality has started to improve after siltation and other processess has taken effect. This is supported by results of analyses on physical water variables that indicated a drop in turbidity at this site. This finding is in line with results of previous studies by Camargo & De Jalon (1990) and

Camargo (1992) in a Spanish river, Río Duratón where a spatial recovery of the macroinvertebrate community at sites downstream of the disturbance point (Burgomillodo dam and industrial effluent) was observed. In addition, the joining of the Eerste and the smaller Assegaaiboschkloof river downstream from the dam and experimental fish farm would further assist in recovery.

The response of ciliates to polluted conditions of water systems varies among species. Responses are usually observed as changes in prevalence (Henebry & Ridgeway 1979) or a complete disappearance of species (Antipa 1977). Also, low species richness, abundance or diversity in response to pollution has been reported on artificial substrates (Henebry & Cairns 1980, Xu & Wood 2011). In the present study, the different taxa attached to invertebrate hosts also exhibited differing responses to pollution. A decrease in prevalence and zooid abundance of the peritrich ciliates (SST and LST) was found below the dam. The high prevalence and abundance of the peritrichs at the sites above the dam indicate that these taxa prefer pristine habitat conditions as they virtually dissapeared from sites below the dam. The suctorians showed the exact opposite pattern, with an increase in prevalence and zooid abundance at sites below the dam. Very few individuals were found at pristine sites. This suggests that they have a preference for more impacted conditions (due to possible increases in food sources) and/or that they are weak competitors with other cilliates in pristine areas. As both peritrichs and the suctorians shared the same host, and in some cases even coexisted on a single hydraenid beetle, it is likely that a combination of these two factors may explain the observed patterns. Suctorians have also been found to persist in moderately to highly polluted waters in other environments (Laird 1959, Antipa 1977, Henebry & Ridgeway 1979). Though site 6 appeared to be undergoing a recovery process, the short-stalked peritrichs never recovered (prevalence: 0%) even in the presence of their elmid hosts (n = 38). The long-stalked peritichs appeared but remained at low when prevalence and abundance (prevalence of long-stalked peritrichs: 4.17%; host abundance, n = 14) compared with the more abundant suctorians (prevalence 51.37%, host abundance, n = 14). This suggests that ciliates might be better bioindicators than their aquatic hosts. A phenomenon which has been previously reported by Antipa (1977) in the Vermilion River where the ciliate epibionts *Conchophthirus* and *Heterocinetopsis* were found be more sensitive to water quality than their molluscan hosts.

Results of the present study clearly demonstrate the potential of ciliates as bioindicators and agree with a growing number of studies that recognise ciliates as good indicators of water pollution (Bick 1972, Antipa 1977, Cairns 1978, Xu & Wood 2011). Sessile ciliates present a rapid way of detecting the ecological effect of pollution stress because they have a shorter response time-lag due to their unicellular nature compared to higher organisms (Xu & Wood 2011). They can also be easily maintained in laboratories where their rapid multiplication allows for their use in testing the effects of specific pollutants on growth, metabolism and reproduction (Cairns 1978). With an increased threat to South African water resources, there is a growing need for very rapid and sensitive water quality monitoring tools. The incorporation of ciliates as bioindicators in biomonitoring of river health would thus be of great value. The present study represents a first step in the process of identifying suitable epibiont ciliates as bioindicators. Further studies would confirm the sensitivity of these sessile organisms to water pollution by examining their responses in other South African rivers to other forms of water pollution.

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CHAPTER 6. GENERAL DISCUSSION

Ciliate epibiosis is a ubiquitous phenomenon in freshwater environments. The nature of the interactions between ciliates and their hosts vary between ciliate taxa, between water systems and is influenced by the complex interaction of biotic and abiotic components. Most of the current information on ciliate epibiosis was obtained from studies carried out in America and Europe. The present study is the first account of host associations of ciliate epibionts in the rivers of the Western Cape Province of South Africa. Sessile ciliate epibionts were found attached to the ventral side of macroinvertebrates in the Wolwekloof, Wit, Rooielskloof, Molenaars, Elandspad and Eerste Rivers. Macroinvertebrate hosts identified were aquatic insects in the families Elmidae (riffle beetles), Hydraenidae (moss beetles), Notonemouridae (southern stoneflies), Corixidae (water boatmen), Dryopidae (long-toed water beetles) and Ptylodactilidae (toe-winged beetles). Based on morphological results, the collected ciliates belonged to two classes, the Peritrichia and Suctoria. Combined morphological data (light microscopy and scanning electron microscopy) and molecular data (using the 18S and ITS rDNA markers) suggested a further division of the peritrichs into two main taxa, the short-stalked taxon (SST) and the long-stalked taxon (LST), both falling within the *Opercularia* clade.

Studies on host specificity rely heavily on accurate identification of both host and epibiont, and the greater the systematic resolution, the more accurately the relationships can be described. In this study, it was not possible to compare host specificity of peritrich and suctorian taxa across the six rivers because of systematic restrictions, largely related to the limited DNA sequencing that was carried out. Since the epibionts are unicellular, and are firmly attached to their hosts, it is difficult to remove them without contamination with host haemolymph. While it would have been useful to have been able to compare peritrich taxa across rivers (to see if local speciation had taken place in isolated catchments), this was not possible here. Where successful sequences were obtained, it was found that the same species of the short-stalked taxon appeared to occur on elmid beetles in the two rivers, the Elandspad and Eerste Rivers which have separate catchments and are approximately 31km apart. Elmid beetles have been known to transport ciliate epibionts to a different aquatic ecosystem during nocturnal migratory flights (Scott 1910, Brown 1987), a phenomenon which might explain the occurrence of the same ciliate species in the Elandspad and Eerste Rivers. While all short-stalked peritrichs from elmid beetles collected from Elandspad River had identical sequences, molecular analyses indicated the existence of a second, less abundant, taxon within the short-stalked peritrichs attached on elmid beetles collected from Eerste River. This less abundant taxa is thought to be a rare epibiont which may also use elmid beetles as hosts and might co-exists with the more abundant short-stalked peritrichs on these elmid hosts. It is possible that with a denser sampling, this and possibly additional cryptic taxa will be uncovered in the Eerste River. Such cryptic species may require very detailed morphological studies for their resolution, such as protargol staining. Further taxonomic studies aimed at resolving this taxon were not possible here since the aims of the study were largely ecological. However, both morphological and molecular studies were able to identify the different epibiont classes encountered in the present study which was adequate for subsequent ecological studies.

The survey of ecological and host preferences of the peritrich taxa was therefore limited to some extent by the taxonomic considerations, although the short- and long-stalked taxa were treated as two operational taxonomic units for the purposes of establishing prevalences. Since thorough taxonomic and systematic studies (including DNA sequencing) are the foundation for studies in fields such as ecology, there is a need for more intensive systematic work on the ciliated epibionts in the rivers of South Africa, particularly those in the Western Cape Province.

The two main peritrich ciliate taxa (short-stalked peritrichs and long-stalked peritrichs) identified in this study exhibited different patterns of host preference, attachment site preference and infestation prevalence, with no consistent and predictable seasonal trend in prevalence in the six rivers examined. The short-stalked peritrichs were consistently found on elmids and corixids while the long-stalked taxa were found only on hydraenids, notonemourids, larval dryopids and larval ptylodactylids. Suctorians were found commonly on the hydraenid beetles; and in some cases, they were found to coexist with long-stalked peritrichs on hydraenid individuals. The observed host preferences were consistent and likely represent long-term evolutionary adaptation to features of selected hosts, as has been reported elsewhere. Herman and Mihursky (1964) found that the epibiont *Zoothamnium* was specific to *Acartia tonsa* and never colonised *A. clausi* even in cases of low *A. tonsa* and high *A. clausi* densities in the Chesapeake Bay, USA. Also, Henebry & Ridgeway (1979) reported that *Vorticella microstoma* exclusively colonised the cladoceran *Scapholeberis kingi* in the Ashmore Lake, Illinois.

The results of the present study represent the first accounts of epibiont ciliate associates for these arthropod basibionts in South Africa. Viljoen and Van As (1983) found ciliates on dragonfly larvae in their survey of substrates in the Westdene dam, Johannesburg. Also, they found host-specific ciliates on fish (Viljoen & Van As 1985) and on fish ectoparasites (Van As & Viljoen 1984) in freshwater systems. In the present study, no ciliate epibiont was found on dragonfly larvae. The previous study (Viljoen & Van As 1983) was taxonomy-based and thus did not determine the prevalence of ciliates on substrates in the Westdene dam. However, it is possible that dragonfly larvae may not be a suitable host (possibly a sink habitat), in the rivers sampled in this study. The same species of ciliate has been reported to colonise different hosts in different aquatic systems, e.g., *Vorticella microstoma*, which colonised only the cladoceran *Scapholeberis kingii* in a lake in Illinois, was reported as an epibiont of the cyanobacterium *Microcystis aeruginosa* in a lake in Michigan (Pratt & Rosen 1983); and of inert surfaces in a Spanish river (Garcia *et al.* 1989). Both abiotic and biotic factors are likely responsible for such host shifts.

Attachment surfaces provide a substrate for ciliates and, perhaps more importantly, provide shelter and protection from predation by suctorians and multicellular organisms (Wahl 1989). This possibly explains the occurrence of peritrichs on the ventral aspect of their basibiont hosts in the present study (however, Suctoria and the long-stalked peritrich taxon coexisted on hydraenids). Ciliates have also been reported to more easily colonise the less active parts of their host (Utz & Coats 2005). In this study, the short-stalked taxon preferred the non-motile thoracic region. This finding agrees with the results of Scott (1910) who reported the attachment of *Epistylis* on the edge of the posterior angle of an aquatic beetle thorax. The long-stalked peritrichs were nearly always only found on the head of their hosts.

Their attachment was very close to the mouthparts and palps, suggesting that they may benefit from particles released by the feeding beetles. In the present study, it is likely that the long-stalked taxon benefit from the nutrient zone created by the feeding activities of their hosts reducing the cost of generating their own separate feeding current (Threlkeld *et al.* 1993). The preferred attachment site for the short-stalked peritrichs on the thorax, and to a lesser extent on the legs is more difficult to explain, but may relate to a combination of factors including protection and food availability due to localised current vortices generated around the legs and body which aids the trapping of particulate matter. This has also been suggested by other authors. Clamp (1987, 1993) attributed the differential distribution of ciliates on basibionts to food availability. He reported that the suspension-feeding *Lagenophrys*, attached to the pleopods of marine isopods, feeds off the food-bearing current generated by the respiratory or locomotory activities of their hosts. In addition, the differences in the distribution of *L.crutchfieldi* on the bodies of the amphipod, *Parhyle* were attributed to the differences in the respiratory current created by the amphipod (Clamp, 1993).

Epibiont prevalence of the two main peritrich taxa differed in the different rivers. The short-stalked taxon had its highest prevalences in the Molenaars, Elandspad and Eerste Rivers – possibly a function of the high density of their elmid hosts in these rivers. This strong positive host density dependence suggests that they may be host specific (see also Threlkeld *et al.* 1993), and that elmid beetles might represent source habitats. In contrast, epibiotic prevalences of the short-stalked taxon in the Wit and Wolwekloof Rivers were very low, regardless of host availability and abundance. While the long-stalked peritrich taxon was present on hydraenids in all rivers, the highest prevalence levels again occurred in the Molenaars River. On the stonefly larvae, generally lower prevalences of long-stalked ciliates were observed with Molenaars River having the highest prevalences; and Wolwekloof, Wit and Elandspad Rivers having zero prevalence. The lack of a consistent relationship between prevalence and basibiont density suggests that other factors, such as fluctuations in phytoplankton and bacteria populations, pollution, freshwater influx and other biotic and abiotic factors seem to play a major part in determining ciliate prevalence (Utz and Coats 2005). Previous studies on the relationship between host abundance and epibiotic prevalence also showed variable density relationships. For example, Xu (1992) and Chiavelli *et al.* (1993) reported that high prevalence was related to high host density while López *et al.* (1998) and Hanamura (2000) were unable to show a clear relationship between host abundance and prevalence. Determining the exact biotic or abiotic variable (or combination of variables) responsible for peritrich prevalences will require more detailed studies that include a wide range of possible explanatory variables.

The high variation in the prevalences of the long-stalked taxon on stonefly larvae suggests that the latter act as sink habitats (Pulliam 1988). There was some evidence of this as an increase in prevalence of the LST ciliates on hydraenid beetles (the preferred hosts) appeared to lead to increase in their prevalence on the stonefly larvae. Prevalence levels on hydraenids were 15.77% for Rooielskloof; 13.97% for Eerste, 24.24% for Molenaars. This was reflected in proportionate prevalence values on stonefly larvae (2.18%, Rooielskloof; 3.14%, Eerste; 6.25%, Molenaars). Generally, the majority of aquatic insects, except aquatic beetles and bugs, live in rivers and streams as juveniles and are poor hosts as a result of their frequent moulting habit. The repeated moulting of these hosts leads to subsequent reductions in epibiont population size (Xu 1992, Willey & Threlkeld 1993) as moults are washed downstream, affecting recolonisation. This is particularly true in the fast-flowing rivers sampled in the present study. This might not be the case in dams or lakes where the low flow rate ensures that contact between moults, telotrochs or larval stages and newly moulted hosts is maintained for a longer period facilitating recolonisation and reducing losses in the epibiont population. Previous studies in the Chesapeake Bay (USA) (Utz & Coats 2005) found higher epibiont levels on juveniles, than on adult copepods probably because of the higher predation pressure on infested adults. Additionally, the higher prevalence recorded on juvenile copepods could be because free-swimming epibionts face less danger of predation by the former, or perhaps epibionts prefer the soft exoskeleton exposed after moulting of their hosts.

In present study, ciliates were found to be restricted to host stonefly taxa that were setose (genus *Aphanicercella*). These setae probably make colonisation easier for telotrochs and larval stages which would otherwise be washed downstream or be exposed to predation if an attachment surface is not available. The setae may also protect these stages from predation or pathogens as suggested by previous studies on other systems (Mayen-Estrada & Aladro-Lubel 2001).

Sessile ciliate protozoans are recognised as good indicators of water quality (Bick 1972, Antipa 1977), with certain ciliates such as *Vorticella microstoma* and *Opercularia coarctata* considered excellent bioindicators (Bick 1972, Sládecek 1981). In the present study, changes in abundances of both peritrich and suctorian ciliates reflected poor water quality below the Kleinplaas dam. The two taxa showed opposite response patterns to the pollution gradient, with a decrease in prevalence and zooid abundance of all peritrichs in sites below the Kleinplaas dam while suctorians exhibited an increase in both prevalence and zooid abundance below this dam. This strongly implies that there were changes in water conditions (as measured by decreases in oxygen concentration and increases in pH, conductivity, turbidity) due to the presence of the dam. This was corroborated by observed changes in the community structure of benthic arthropods (as measured by SASS and ASPT). Also, results of the present study agree with the results of previous studies on the impact of the dam on the Eerste River (King 1983, Bredenhand 2005) which reported that the downstream deterioration in water quality and changes in macroinvertebrate communities were caused by dam effluents and activities. Changes in these water variables, as observed in the present study, have been associated with water pollution (Chapman & Kimstach 1992).

In the present study, there appeared to be evidence of stream recovery at site 6 which is 1.9km downstream from the dam. At this site, there was a decrease in turbidity and an increase in SASS score and ASPT: 105 and 8.08 respectively, indicating ‘natural water quality’ comparable to sites above the dam. This could be as a result of the combined effects of sedimentation of silt and debris, and the dilution of concentrations of dam effluent by the smaller Assegaaiboschkloof River which joins the Eerste River after the University of Stellenbosch fish farm situated close to the Assegaaibosch Nature Reserve (at site 5). Previous studies (Camargo & De Jalon 1990, Camargo 1992) have also reported a spatial recovery of the macroinvertebrate community at sites downstream of the Burgomillodo dam, Río Duratón, Spain and the point of entry of industrial effluent into the Spanish river.

Previous studies have reported both decreases (Antipa 1977) and increases (Laird 1959, Henebry & Ridgeway 1979) in ciliate prevalence on aquatic macroinvertebrates in response to pollution, with some species being pollution intolerant, and others resistant to, or even preferring polluted habitats. In this study, the short-stalked peritrichs (prevalence above dam = 44.35%) disappeared from hosts in sites below the dam (prevalence below dam = 0%) while prevalence of the long-stalked peritichs below the dam (3.47%) was approximately eight times lower than upstream prevalence (27.69%), even though beetle hosts were available for colonisation below the dam (n = 58 for elmids; 54 for hydraenids). Disappearance of ciliates from polluted waters have been reported in a previous study by Antipa (1977) where the ciliate epibiont *Heterocinetopsis* disappeared from polluted stations even when its hosts (mussels) were abundant. The decrease in zooid abundance of peritrichs observed below the dam indicated the unsuitability of environmental conditions for the establishment of populations at these polluted sites. This taxon thus seems to be very sensitive to impacted water conditions and is therefore a potentially useful bioindicator for Western Cape rivers. Conversely, the suctorians were very abundant in the polluted sites (prevalence of 28.5%) compared to near-pristine sites above the dam (prevalence of 0%) suggesting a preference of Suctoria for polluted waters as earlier reported by Laird (1959). These too may be good indicators of water quality, but their low prevalence and abundance in general probably makes them less useful than the peritrichs.

In site 6 which appeared to be undergoing recovery, prevalence of short-stalked peritrichs on elmid beetles remained zero in contrast to its hosts, elmid beetles that showed recovery as evident in the increase in host abundance at this site from 4 (in site 5) to 38. This suggests that they may be more sensitive to pollution than their hosts. Antipa (1977) reported a similar finding where ciliates epibionts where found to be more sensitive than their molluscan hosts. The long-stalked peritrichs reappeared on hydraenid beetles but at very low level (4.17%) when compared with the prevalence of pollutant-tolerant suctorians (51.37%). Both long-stalked peritrich and suctorians colonise the same host and the results show that though 14 hydraenid hosts were available for colonisation at this site, suctorians had a higher prevalence than the long-stalked peritrichs. Generally, beetle hosts were more abundant above the dam (n = 347 for elmid beetles; 155 for hydraenid beetles) than below the dam (n = 58 for elmids; 54 for hydraenids) but apparently, the decrease in host abundances did not affect the trend in the response to pollution observed in the present study.

Sessile ciliates are microscopic and difficult to work with. However, assessment of water quality based on their presence or absence has been extremely accurate (Antipa 1977) as their unicellular nature, exposed membranes and rapid life cycles make them ideal and sensitive indicators for water quality assessments. This report provides a baseline for further ecological and systematic studies on peritrichs in Western Cape rivers.

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APPENDICES

APPENDIX I

Total abundances of individuals belonging to different macroinvertebrate orders collected from sites above (1 – 3) and below (4 – 6) the Kleinplaas dam in the Eerste River.

ORDER	SITE 1	SITE 2	SITE 3	SITE 4	SITE 5	SITE 6
EPHEMEROPTERA	2940	849	1071	126	237	1206
PLECOPTERA	153	93	168	1	1	2
TRICHOPTERA	62	44	73	21	30	78
COLEOPTERA	798	369	351	59	25	61
DIPTERA	1017	258	563	484	566	384
ODONATA	0	0	1	3	3	1
MEGALOPTERA	4	6	14	0	0	2
HEMIPTERA	0	0	0	1	1	0

APPENDIX II

Total abundances of individuals belonging to different macroinvertebrate families collected from sites above (1 – 3) and below (4 – 6) the Kleinplaas dam in the Eerste River.

TAXA	SITE 1	SITE 2	SITE 3	SITE 4	SITE 5	SITE 6
TELOGANODIDAE	1044	248	122	2	2	1
BAETIDAE	603	298	802	115	220	1173
LEPTOPHLEBIIDAE	1289	292	139	4	3	3
BLEPHAROCIDAE	93	18	4	1	1	0
CORYDALIDAE	4	6	14	0	0	2
SIMULIDAE	199	61	114	0	110	75
HYDROPSYCHIDAE	30	10	20	18	28	72
CHIRONOMIDAE	683	167	444	481	456	308
ATHERICIDAE	41	12	1	0	0	1
NOTERIDAE	11	9	15	1	2	5
ELMIDAE	133	98	116	16	4	38
SCIRTIDAE	509	221	21	1	1	0
DIXIDAE	1	0	0	0	0	0
NOTONEMOURIDAE	153	93	168	1	1	2
AESHNIDAE	0	0	1	0	0	1
PHILOPOTAMIDAE	25	17	48	1	1	0
LEPTOCERIDAE	2	4	5	2	1	6
PTYLODACTILIDAE	5	10	16	8	0	2
DYTISCIDAE	3	0	73	0	0	0

HYDRAENIDAE	40	26	89	32	18	14
ASPIDYTIDAE	1	0	0	1	0	0
GLOSSOSOMATIDAE	5	13	0	0	0	0
DRYOPIDAE	96	5	21	0	0	2
CAENIDAE	0	0	7	4	4	11
PLATYCNEMIDAE	0	0	0	1	1	0
CORIXIDAE	0	0	0	1	1	0
LIBELLULIDAE	0	0	0	2	2	0
HEPTAGENIIDAE	0	0	0	1	8	18
EPHEMERYTHIDAE	4	11	1	0	0	0
TIPULIDAE	0	0	0	2	0	0

APPENDIX III

Modified SASS scores and ASPT of benthic samples collected from sites above (1 – 3) and below (4 – 6) the Kleinplaas dam in the Eerste River.

SITE	SASS (JULY 2011)	ASPT (JULY 2011)	SASS (OCTOBER 2011)	ASPT (OCTOBER 2011)
1	88	8.80	161	7.67
2	115	9.58	164	8.20
3	167	7.59	174	7.90
4	41	5.86	63	5.73
5	96	6.86	80	6.15
6	82	6.31	105	8.08