Insect Communities Associated with Three Submerged Aquatic Macrophytes, with Emphasis on the Phytophagous Species

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Declaration

I hereby declare that this dissertation is a result of my own, unaided work except where acknowledged. It is being submitted in partial fulfilment of the requirements for the degree Master of Science in the University of the Witwatersrand, Johannesburg. It has not been submitted for any degree or examination in any other university.

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Abstract

The implementation of classical biocontrol in managing exotic aquatic weeds, such as *Lagarosiphon major* Ridley (Hydrocharitaceae) (coarse oxygen-weed) in New Zealand (N.Z.) and Europe, and augmentation biocontrol in managing native weeds, such as *L. major, L. muscoides* Harvey (Hydrocharitaceae) (fine oxygen-weed) and *Potamogeton pectinatus* L.(Potamogetonaceae) (sago pondweed) in South Africa (S.A.) is being hampered by the dearth of knowledge surrounding submerged macrophytes and their associated insect assemblages. In addition, this knowledge gap hinders the accurate evaluation of any biocontrol initiatives that are implemented. These facts are even more sobering when considering the global number of invasive species is ever increasing, for example the recent invasion of S.A. by the submerged weed, *Hydrilla verticillata*. To begin addressing these issues, this study surveyed the invertebrates associated with three of S.A.'s indigenous submerged macrophytes, *L. major, L. muscoides* and *P. pectinatus*, while investigating the role certain environmental variables (site, plant species, nutrient levels, light penetration, water depth and distance from the shore) played in shaping these insect assemblages. In surveying these plant species on two dams within the Mooi River district of S.A., the following herbivorous insects were identified: *Athripsodes harrisoni* (Trichoptera: Leptoceridae), *Leptocerus* sp. (Trichoptera: Leptoceridae), *Parapoynx fluctuosalis* (Lepidoptera: Pyralidae), *Micronectus scutellaris* (Hemiptera: Corixidae), *M. dorothea* (Hemiptera: Corixidae) and species from the Chironominae (Diptera: Chironomidae) and Orthocladiinae (Diptera: Chironomidae). Of these, *A. harrisoni* and *Leptocerus* sp. showed the most promise of being destructive, host-specific agents to manage *L. major* in N.Z. and Europe; consequently, they require further investigation. Concerning the use of augmentation control in managing the three plant species within S.A., all the herbivorous insects have potential as future agents. However, nitrate to phosphate ratios of below ten in the sampled dams have resulted in excess nutrients being available to all three plant species; resulting in their growth and spread throughout the dams not being hampered by the feeding of the phytophagous insects. Again, this highlights the need to address eutrophication in the

management of all S.A.'s aquatic weeds. The herbivorous fauna found in this study may also have the potential to be important in the management of *H. verticillata* in S.A., given the fact that this weed and *Lagarosiphon* are from the Hydrocharitaceae family. In addition to the herbivorous insects, 18 morphospecies from 10 insect families were found associated with *L. major*, 14 morphospecies from nine families with *L. muscoides*, and 19 morphospecies from 11 families with *P. pectinatus.* The evenness of the insect communities associated with all three plant species was very similar; however, their species richness was significantly different. Consequently, the community of *P. pectinatus* was the most diverse (Fisher's alpha of 4.74), while that of *L. muscoides* was the least (Fisher's alpha of 2.95). Of the environmental variables investigated at the 10 replicate sites for each plant species, different sites, different plant species and surrounding land use were important in shaping the communities. In addition, this study highlights the feasibility of local research institutes surveying the insect assemblages associated with their indigenous macrophyte species, which are weeds or potential weeds elsewhere. This could be a very cost effective procedure of refining global biocontrol.

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1. Introduction

"One could probably remove all the larger plants and substitute glass structures of the same form and function" (Shelford 1918). This statement was made about the inability of submerged macrophytes to enter aquatic food webs through herbivory. It is damning and it is incorrect. Unfortunately, it was also the view that most ecologists adopted for the majority of the 20th century (Lodge 1991). Consequently, there is a global dearth of knowledge concerning aquatic macrophytes and their associated insects (Lodge 1991, Newman 1991), which is hampering the implementation of biocontrol programmes to manage submerged aquatic weeds. The lack of research concerning the natural enemies of aquatic macrophytes hinders the selection of control agents, while the lack of information concerning the range of plant species that the insects are known to feed on impedes host-specificity trials. Additionally, inadequate baselines concerning the insect communities associated with different macrophyte species prevent the efficient evaluation of any implemented biocontrol programmes. If biocontrol is to be used in combating the ever increasing number of aquatic invasives occurring worldwide (Charudattan 2001), e.g. *Lagarosiphon major* Ridley (Hydrocharitaceae) (coarse oxygen-weed) in New Zealand (N.Z.), Australia and Europe (McGregor and Gourlay 2002, WWF 2003, Gassmann *et al*. 2006), and *Hydrilla verticillata* (L.f.) Royle (Hydrocharitaceae) (hydrilla) in South Africa (S.A.) (Coetzee 2006), these knowledge gaps need to be filled. Therefore, this study aimed to (a) identify the phytophagous insects associated with three South African macrophytes, *L. major*, *L. muscoides* Harvey (Hydrocharitaceae) (fine oxygen-weed), and *Potamogeton pectinatus* L.(Potamogetonaceae) (sago pondweed); (b) begin creating baseline insect community lists for these plants species; and (c) investigate the environmental variables that shape these communities.

Any biocontrol programme has a number of generic steps that need to be followed (McFadyen 1998). Elaborating on these procedures provides an ideal

framework to illustrate the rationale and benefits of this study. The first step is identifying the weed and its impacts (McFadyen 1998). For example, *L. major* is a relatively new weed species in New Zealand (N.Z.), Australia and Ireland (McGregor and Gourlay 2002, WWF 2003). Surveys of this plant species in N.Z. have found it to displace and alter indigenous flora and faunal communities (Howard-Williamson *et al*. 1987, Howard-Williamson and Davies 1988). In addition to these ecological impacts, dense mats of *L. major* hinder recreational activities like swimming and angling, while broken off strands block the turbines of hydroelectric power stations, impeding the generation of electricity. In an attempt to manage *L. major*, N.Z. officials have employed chemical and mechanical control; however, these initiatives are expensive (McGregor and Gourlay 2002). The loss of recreational income, the decreased productivity of industry and expensive control methods are all negative impacts that *L. major* has had on N.Z.'s economy. These forms of economic impact are common to most invasive species (Perrings 2005), e.g. *Hydrilla verticillata* (L.f.) Royle (Hydrocharitaceae) (hydrilla) in the United States of America (U.S.A.), which results in millions of dollars being lost annually (Balciunas *et al*. 2002).

Once the impacts of a particular weed have been established, options for control need to be evaluated. In N.Z., chemical control of *L. major* is implemented by spraying the herbicide Diquat (bipyridylium), while mechanical control takes the form of suction dredging and weed matting (McGregor and Gourlay 2002). However, these control methods are expensive, require multiple replicates, ineffective at addressing large scale infestations, unsustainable, and capable of adverse environmental impacts, e.g. removing non target species (McGregor and Gourlay 2002, Coetzee 2006). In contrast, classical biocontrol has been shown to be sustainable, cost-effective and environmentally friendly. For example, the ecological and economic success of managing the invasive aquatic weed *Azolla filiculoides* Lamarck (Azollaceae) (red water fern) in S.A. using biocontrol (Hill 2003, McConnachie *et al*. 2003, 2004). It took a mere five years for the frond-feeding weevil *Stenopelmus rufinaus* Gyllenhal (Coleoptera: Curculionidae) to negate the

serious threat this weed posed to S.A.'s waters, not failing to control *A. filiculoides* at any site where it was released (McConnachie *et al*. 2004). Taking cognizance of this information, biocontrol is now being advocated as the best method of managing *L. major* in N.Z. (McGregor and Gourlay 2002). This decision is the second step of any biocontrol programme (McFadyen 1998).

The next step is agent selection (McFadyen 1998). Historically, this has involved scientists from the invaded country travelling overseas to undertake exploratory surveys in the native ranges of the plants, where the greatest diversity of natural enemies exists (Goolsby *et al*. 2003, Ding *et al*. 2004, Sun *et al*. 2006). Unfortunately, no such surveys have been conducted on *L. major* in its country of origin, S.A. (McGregor and Gourlay 2002). This absence of knowledge is cited as the reason why classical biocontrol has not been implemented to manage *L. major* in N.Z and Europe (McGregor and Gourlay 2002, Gassmann *et al*. 2006). By sampling the phytophagous insects associated with *L. major* present on Mearns Weir, KwaZulu-Natal Province (KZN), S.A., this study aimed to address this pressing issue.

In addition to *L. major,* this study surveyed the herbivorous insects associated with *L. muscoides* and *P. pectinatus*. While *L. major* is the only species to be a declared invasive overseas, all three species are problem plants within S.A. because of their ability to clog waterways (Cook 2004). One method of controlling these plants is augmentation biocontrol, a technique that involves the use of indigenous insects to manage weedy native species (McFadyen 1998). Unfortunately, surveys of the herbivorous fauna associated with South African macrophytes are essentially 'unheard off' (F. De Moor, Albany Museum, pers. comm.). Therefore, this study aimed to identify any native insects that could be used as control agents to manage *L. major*, *L. muscoides* and *P. pectinatus* within South Africa. Additionally, if *L. muscoides* and *P. pectinatus* do ever become invasive in another country, there would already be an existing catalogue of potential control agents.

In addition to identifying the natural enemies of *L. major*, *L. muscoides* and *P. pectinatus*, this survey has the potential to benefit the biocontrol programme that is being implemented to manage hydrilla in South Africa. South Africa is fortunate that this ecologically and economically destructive weed already has four identified control agents: *Hydrellia pakistanae* (Diptera: Ephydridae) (Asian hydrilla leaf mining fly); *H. balciunasi* (Diptera: Ephydridae) (Australian leaf mining fly); *Bagous affinis* (Coleoptera: Curculionidae) (Hydrilla tuber weevil), and *B. hydrillae* (Coleoptera: Curculionidae) (Hydrilla stem weevil) (Schmitz and Osborne 1984, Smart and Barko 1988, Dibble *et al*. 1996b, Balciunas *et al*. 2002). However, before these agents are released to manage the infestation on Pongolapoort Dam, KZN, S.A., they need to undergo host-specificity testing, which usually takes a number of years to complete.

This lag period need not be a waste as it allows for the invertebrates associated with the invasive and indigenous species from the same family to be surveyed, e.g. the sampling of *Azolla* species in S.A. before releasing control agents for *A. filiculoides* (Hill 1998). Such surveys are important as they can identify native insects that can contribute to the management of a weed, e.g. the native North American weevil *Euhrychiopsis lecontei* (Dietz) (Coleoptera: Curculionidae) was used in the control of the exotic weed *Myriophyllum spicatum* L. (Haloragaceae) (Eurasian watermilfoil) in the U.S.A. (Sheldon and Creed JR 1995). Unfortunately, flooding has prevented such studies being performed on the hydrilla and indigenous flora of Pongolapoort Dam. However, given the fact that *Lagarosiphon* sp. and hydrilla are both in the Hydrocharitaceae, this study aimed to determine if any indigenous fauna are likely to move onto and damage the invasive hydrilla before its control agents are released.

After agent selection, the next crucial step in any biocontrol programme is host-specificity testing (McFadyen 1998). Classical biocontrol involves the use of one exotic species to control another; therefore, there is the potential for non-target

impacts, i.e. the agent feeding on indigenous flora (McFadyen 1998, Coetzee 2006). Consequently, before an agent is imported and released, it needs to undergo host-specificity tests. The purpose of these trials is to determine the range of indigenous plants that are at risk of being feed upon once the control agent is released. As a rule, host-specificity testing is greatly improved when there are existing records detailing the host ranges of the insects and plants in question (McFadyen 1998). Therefore, for this study to help identify a potential control agent for *L. major,* it also needed to gather information on the natural enemies of other indigenous macrophytes, i.e. *L. muscoides* and *P. pectinatus.* The logic being that a herbivorous insect found on *Lagarosiphon* sp. and not *P. pectinatus* would indicate a potentially oligophagous natural enemy of *L. major*, which is the ideal classical biocontrol agent as it less likely to have non target impacts (McFadyen 1998).

In addition to manual sampling, this study also set out to observe how the herbivorous insects fed on all three plant species. Observations on feeding manner are important as they have the potential to identify different guilds of control agents, the presence of which increases the chance of successfully controlling the weed. For example, different guilds of insects are being employed in the management of hydrilla: the flies mine the leaves, while the weevils eat the tubers and stems (Balciunas and Minno 1985, Balciunas *et al*. 2002, Bennett and Buckingham 2000). In addition, quantifying the rate of feeding and indirect damage, i.e. damage not related to feeding, has the potential to identify the most destructive control agent. Such observations will also influence host-specificity as they will add to the knowledge concerning the host plant range for any particular phytophagous species. While these observations are valuable to the process of agent selection and determination of host-specificity in biocontrol programme of *L. major*, they have another intrinsic benefit. They will add to the growing body of evidence that is eroding the perception that macrophytes only enter the food chain as detritus; thereby, generating a greater appreciation of the roles these plants play in sustaining aquatic ecosystems (Lodge 1991, Newman 1991).

Once control agents have been selected, tested for host-specificity, reared and released; their impacts on the weed need to be evaluated. This is the final step in the biocontrol programme and is important as it justifies the expenses incurred by the programme, e.g. agent selection alone can cost anywhere from $150 000 - $460 000 (McFadyen 1998, McGregor and Gourlay 2002); gathers data that can be used to motivate for further funding; and determines if the agent has been successful (McFadyen 1998, McGregor and Gourlay 2002). Traditionally, evaluation has focused on pre-release surveys of the weed in the target country. While this is important, it does not represent the whole picture. For example, if correct conclusions are going to be made about the effects aquatic weeds have on insect diversity, whether it be *L. major* in N.Z. or hydrilla in S.A., there needs to be some knowledge of the communities associated with the indigenous flora with which to make the comparisons. Therefore, while this study set out to identify the herbivorous insects on *L. major*, *L. muscoides* and *P. pectinatus*, it also sampled the carnivorous community. For the sake of simplicity, the herbivores and carnivores will hereafter be referred to as the entire insect community/assemblage. Surveying the entire insect assemblage is vital in providing baseline estimates of richness, evenness and diversity for the insect assemblages associated with *L. major*, *L. muscoides* and *P. pectinatus*.

In addition to being indicators of biodiversity at the species level (Noss 1990), aquatic macroinvertebrates provide a surrogate measure of the health of the environment (Kellert 1993, Lagadic and Caquet 1998, Kotze 2005). The South African Scoring System, version five (SASS5) is one such biomonitoring protocol and is based on two simple but important concepts. First, the insects residing in the water body reflect the health of the aquatic environment; second, different insect families are tolerant of varying levels of pollutants. The SASS output is a scale of 1-15: families with a rating of 1-5 are highly tolerant of pollution; 6-10 are moderately tolerant; and 11-15 have a very low tolerance (Dickens and Graham 2001, Gerber and Gabriel 2002). Consequently, surveying the entire insect assemblage will give a more

accurate representation of the aquatic invertebrate families that reside in the sampling sites; therefore, allowing for inferences to be made about the water quality in both of the surveyed dams.

The use of invertebrates as indicators of water quality raises another important issue: the effect that different environmental variables have on insect communities. An accepted view of the biophysical environment is that of a dynamic interaction between its biological, physical and chemical elements (Kotze 2005) (Figure 1). Studies on *Eichhornia crassipes* (Martinus) Solms - Laubach (Pontederiaceae) (water hyacinth), the worst aquatic weed in S.A. (Hill 2003), further illustrates this point. Despite the establishment of its control agents on the infested Hammarsdale Dam, KwaZulu-Natal (KZN), S.A., the high nutrient levels in the water allow for rapid leaf production; thereby, negating any damage inflicted by the agents (Hill and Olckers 2001). In contrast, the increased wave and wind action associated with large waterbodies helps break down mats of water hyacinth that have been damaged by its control agents (Hill and Olckers 2001). Additionally, the chemical and physical properties of a water body have been found to be important in shaping invertebrate communities (Collier 1995, Aguiar and Ferreira 2002). For these reasons, this study also investigated the role that environmental variables play in shaping the insect assemblages found on *L. major*, *L. muscoides* and *P. pectinatus*. Given the constraints of this project, only the roles of different sites and plant species, nutrient levels, light penetration, water depth and distance to shore could be quantified.

A study that simultaneously addresses aquatic invertebrate communities, submerged macrophytes and environmental variables is generally 'unheard of' in S.A. (F. De Moor, personal communication). Therefore, it is useful to provide a conceptual diagram that highlights the main aspects of this study (Figure 2).This framework is also useful in providing the context for this project's three aims:

- Firstly, to determine the phytophagous insect species associated with *L. muscoides, L. major* and *P. pectinatus* of the Mooi River area.

- Secondly, to begin establishing lists of the baseline insect communities that are found on these three South African macrophytes.

- Thirdly, to investigate the roles of biological, physical and chemical variables in shaping the aquatic insect assemblages found in Mearns Weir and the neighbouring farm dam.

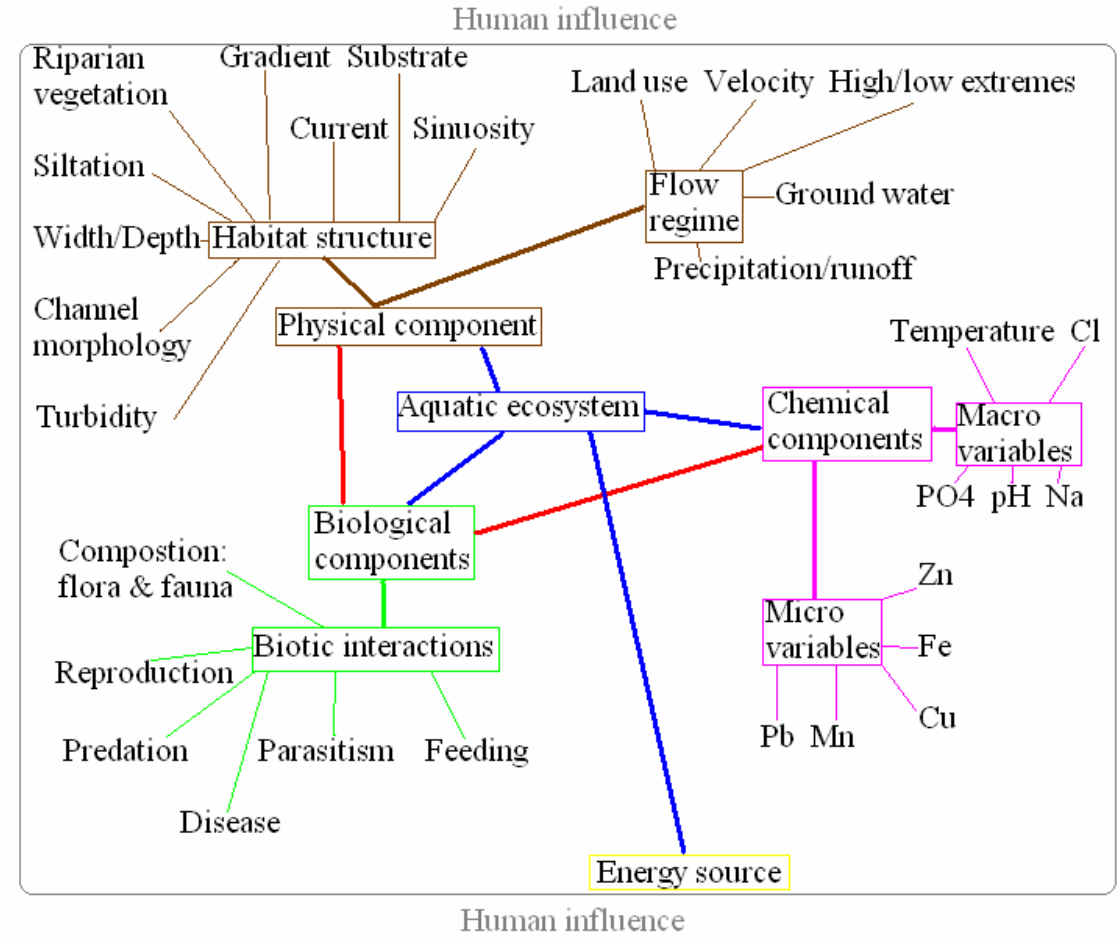


Figure 1. The aquatic environment and its linked physical, chemical and biological components, modified from (Kotze 2005).

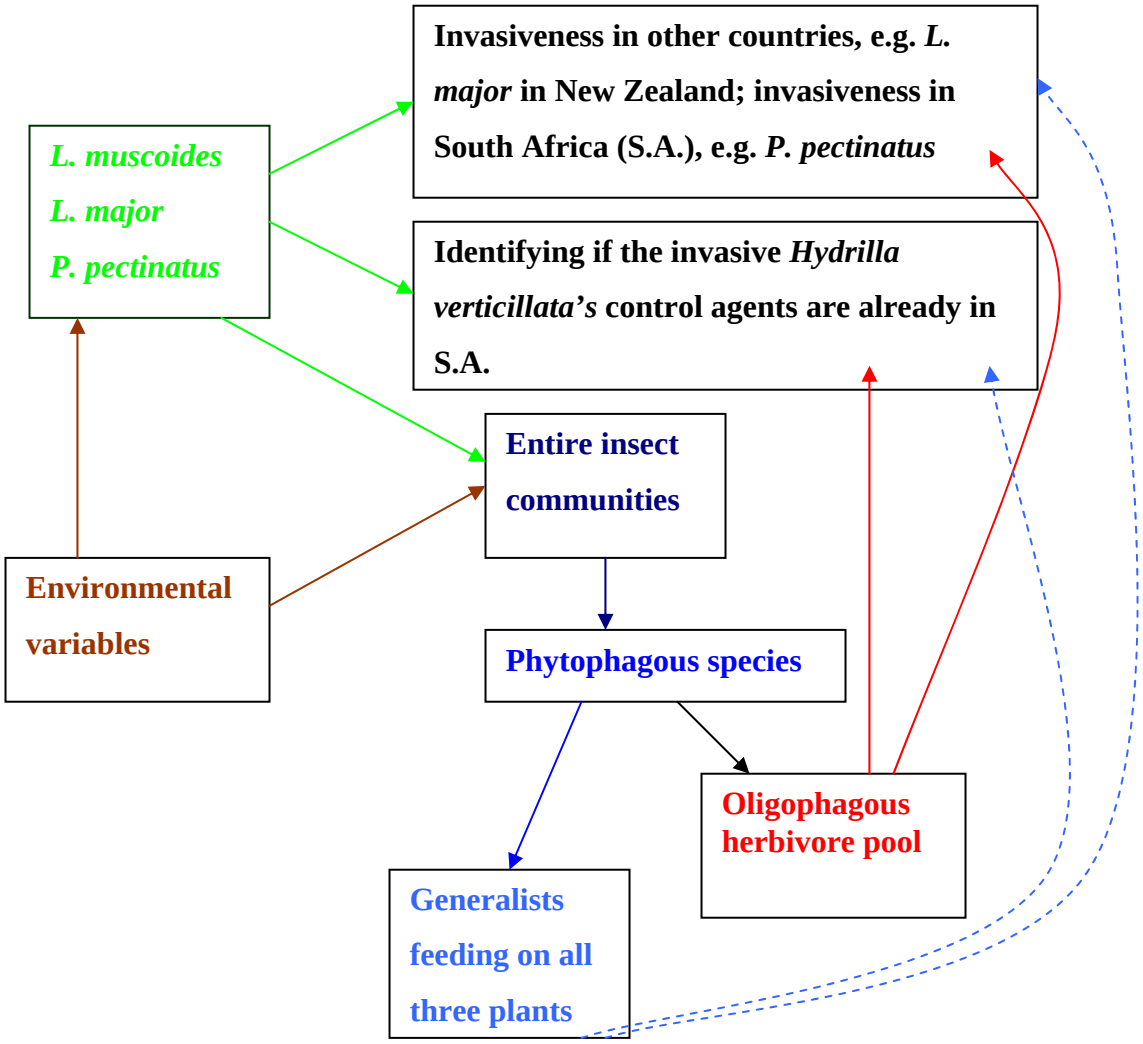


Figure 2. The conceptual framework of this study that sets out to determine the herbivorous insects associated with *Lagarosiphon major*, *L. muscoides* and *Potamogeton pectinatus* of Mooi River, KwaZulu-Natal, South Africa, while simultaneously establishing baseline insect communities for these three plant species and investigating the role biological, physical and chemical components play in shaping these communities.

2. Materials and methods

2.1. Study sites

This study took place on two dams near the small town of Mooi River, KwaZulu-Natal Province (Figure 3).

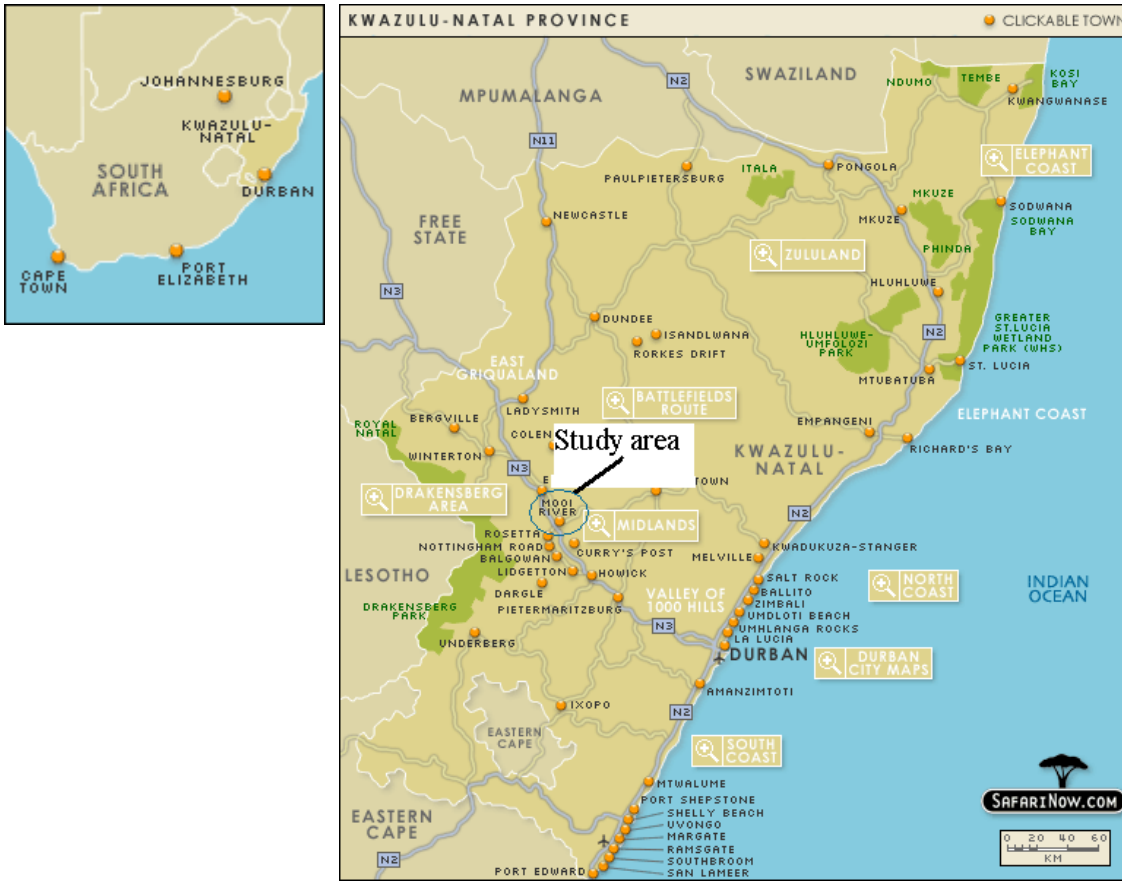


Figure 3. The location of the two dams within KwaZulu-Natal Province, South Africa, where the insects associated with the macrophytes *Lagarosiphon major*, *L. muscoides* and *Potamogeton pectinatus* were sampled (http://www.safarinow.com/).

The first site was Mearns Weir, located at the confluence of the Mooi and Little Mooi Rivers (29°14′45″S, 29°58′8″E) (Figure 4). This 6m high, 300m long weir was constructed as part of the Mooi-Mgeni Transfer Scheme, a system designed to increase the annual transfer between these two rivers; thereby, augmenting the well developed water supplies on the Mgeni (Midmar, Albert Falls, Nagle and Inanda Dams) and so enabling them to continue supplying water to the province. Along the Mooi, Little Mooi and at their confluence, the aquatic plant *Lagarosiphon muscoides* is dominant; however, smaller patches of *L. major* are also found. This site was used to sample the insects found on *L. major* and *L. muscoides*.

In terms of the rest of the Mooi River Catchment, livestock and dairy farming take up much of the land. To support the cattle, many of these farms have extensive maize fields. Most of this crop is milled into feed but some is sold to retailers. Both the cattle and maize require vast amounts of water, a need that is met by pumping water directly from the Mooi River or by constructing farm dams that are supported by the smaller tributaries of the Mooi River and/or surface run-off. One such dam is found on the farm adjacent to Mearns Weir (Figure 4). This dam is at the bottom of a valley, the hills of which have been turned into maize and grazing fields. *Lagarosiphon muscoides* and *Potamogeton pectinatus* dominate this dam, forming continuous belts around its perimeter, with *P. pectinatus* being found in a ring further away from the shore. Within these belts, *L. major* forms a few, small isolated patches. This site was chosen to sample the insects found on *P. pectinatus*.

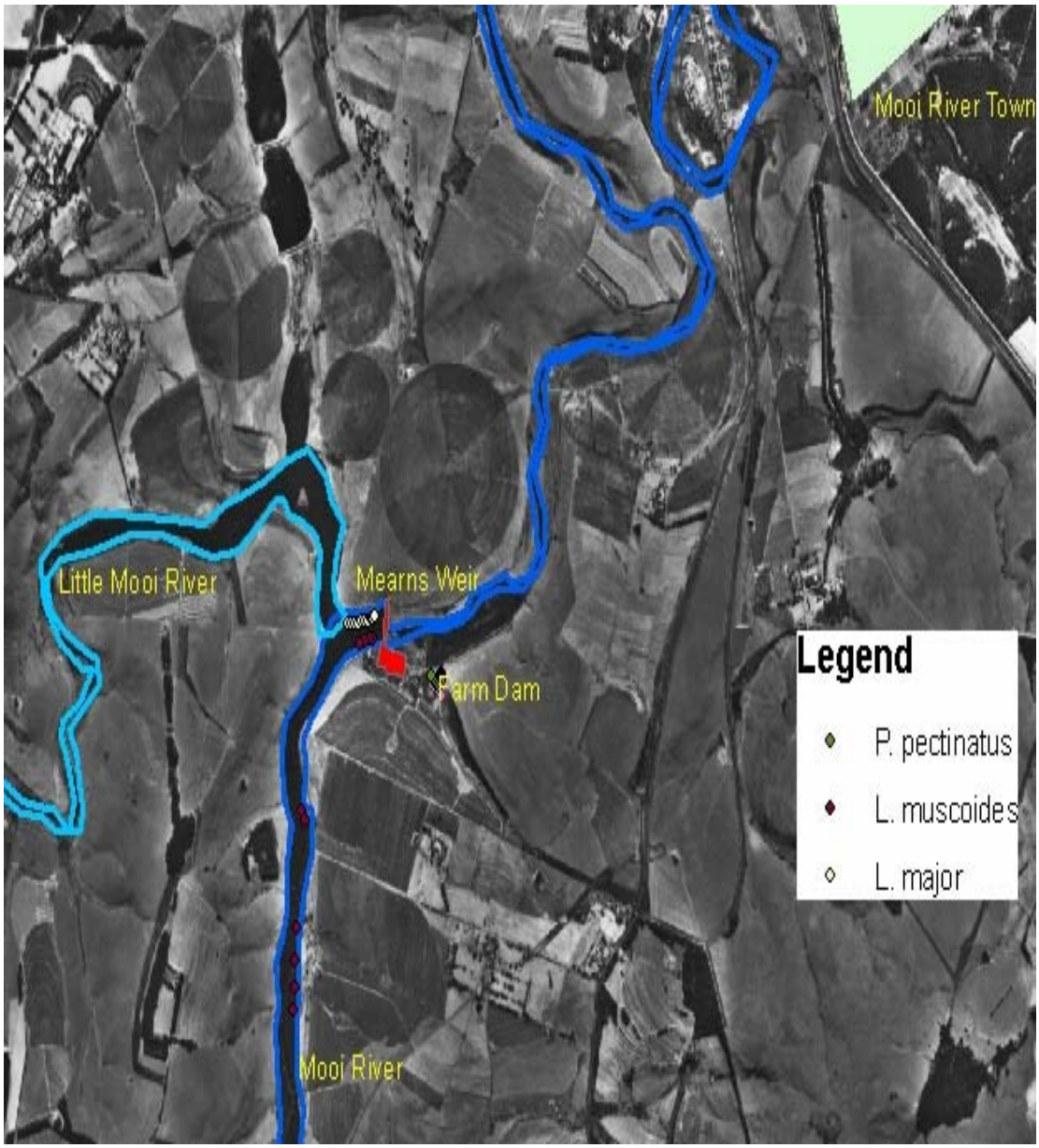


Figure 4. The confluence of the Mooi and Little Mooi River, as well as the farm dam found adjacent to Mearns Weir. Insects were collected from patches of *Lagarosiphon major* and *L. muscoides* found along the Mooi River, while the community found on *Potamogeton pectinatus* came from the farm dam. Scale 1: 30000.

2.2. Study species

Lagarosiphon major, *L. muscoides* and *P. pectinatus* are perennial, submerged, rooted, vascular plants, all of which are capable of sexual and asexual reproduction (Cook 2004). These species are indigenous to southern Africa; however, given their ability to clog up water ways, they are also considered weeds within their native ranges (Cook 2004). In addition, *L. major* is a declared invasive in N.Z., Australia and Europe (McGregor and Gourlay 2002, WWF 2003, Cook 2004, Gassmann *et al*. 2006)

Both species of *Lagarosiphon* are very similar in appearance, but close inspection of their leaves and stems allows them to be distinguished from each other. The stems of *Lagarosiphon major* (3 mm in diameter) are more robust than those of *L. muscoides* (0.5-1.5 mm in diameter) (Cook 2004). The leaves of *L. major* are 6.5-30 mm long, 2.5 mm wide and arranged alternately. They also possess the diagnostic characteristic of having translucent leaf margins consisting of two rows of fibre-like cells with more than 50-100 short blunt teeth on each side (Cook 2004). On the other hand, the leaves of *L. muscoides* are 4.8-20 mm long, 0.5-1.4 mm wide and have 28-86 sharp teeth emerging from a translucent leaf margin consisting of 3-6 rows of fibre-like cells (Cook 2004). Generally the leaves are arranged in an alternate manner; however, they also form locally consecutive whorls, which is its diagnostic feature (Cook 2004).

Potamogeton pectinatus has a very different appearance to *Lagarosiphon* sp. It has thread-like stems which are ca.1 mm in diameter and are repeatedly branched (Cook 2004). Its leaves are 10-70 mm long, 8-65 mm wide and taper to a fine point at the tip (Cook 2004). The leaf sheaths have stipules arising from them, which are

wrapped around the stem. This results in the leaves having a jointed appearance, a feature which helps in their identification (Cook 2004).

2.3. Broad overview of the experimental design

For the sake of clarity, the methods section is presented in the chronological order in which the data were gathered. Consequently, the procedures that addressed the collection of the baseline insect communities are described before those concerning the identification of the phytophagous insect species.

To achieve the aims of this study, two field trips were required. The first focused on collecting and preserving all the insects found on the three plant species. These insects were then classified into morphospecies and used in biodiversity analyses. In addition, these trips gathered environmental data for statistical and multivariate analyses to determine their roles in shaping the insect communities found on the different plant species. Finally, the first field trip also identified the herbivorous families that needed to be recollected (i.e. during the second visit), reared, observed and identified (Figure 5).

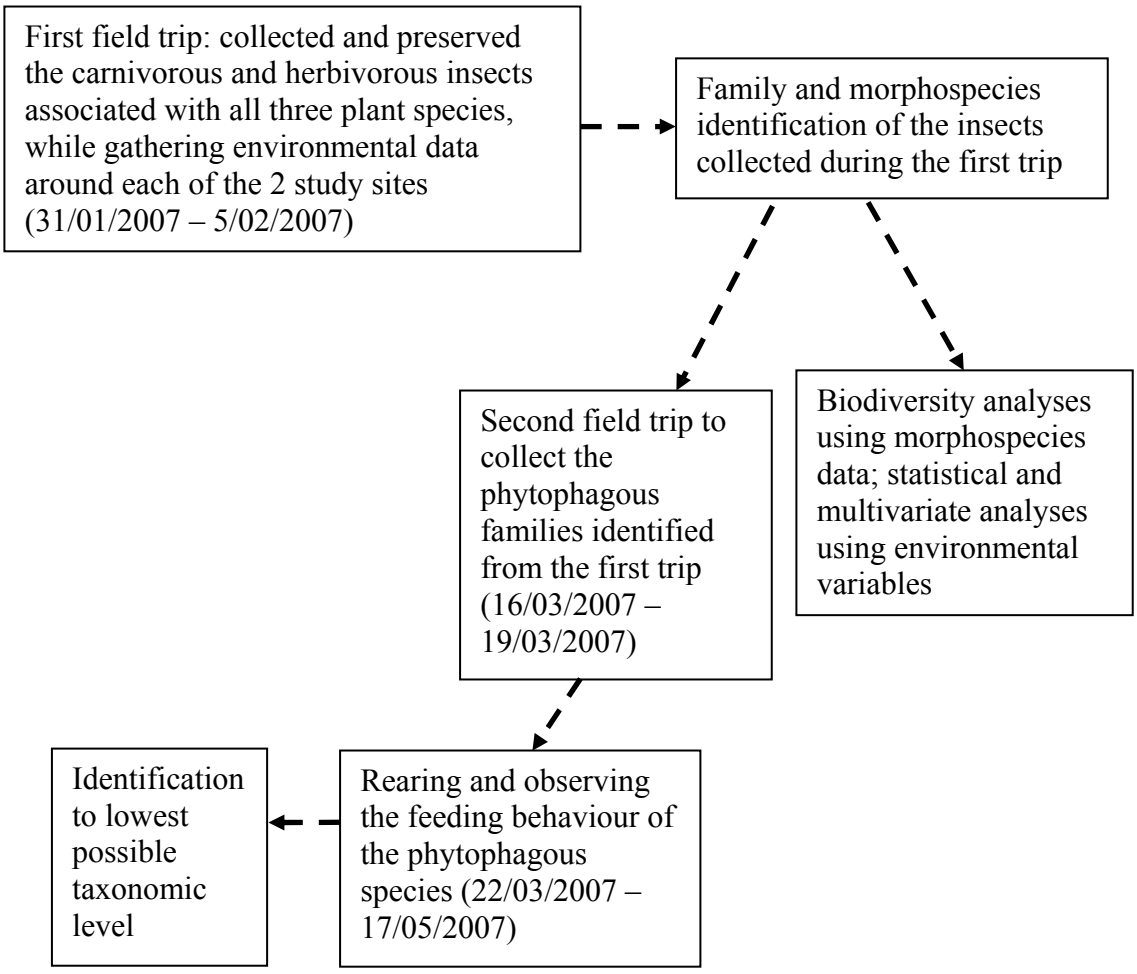


Figure 5. An overview of the study undertaken to sample the insects associated with *Lagarosiphon major, L. muscoides* and *Potamogeton pectinatus* found on two dams within the town of Mooi River, KwaZulu-Natal, South Africa.

2.4. Sampling the carnivorous and herbivorous insect assemblages

The purpose of the first field trip was to collect both carnivorous and herbivorous insects, which required different sampling techniques. The plankton net method, using a net with a ring diameter of 30cm and a mesh size of 0.01 mm, sampled the carnivorous and herbivorous communities, while manual sampling and Berlese funnels were specifically used to find the phytophagous families.

The plankton net method involved wading into the middle of the mat of plants, waiting for any substrate disturbance to settle, sweeping the net through the plants and then lifting it out of the water. The plant sample was then rinsed with four litres of dam water and placed into a two litre rectangular plastic tub. The insects that were washed off the plant and down into the collecting vial were then emptied into a gauze pouch, of 0.01 mm mesh**,** and placed in a tub of 80% ethanol. Eighty percent ethanol was used because it was expected that many insects would be found per sample; therefore, a higher concentration than the standard 70% was needed to preserve the specimens (Balciunas and Minno 1985). The same steps were followed for the ten replicates performed on each plant species, keeping a buffering distance of five meters between each replicate site. The replicates for each plant species were collected over three consecutive days (01/02/2007 - 03/02/2007).

Once back at shore, the plant samples were inspected and any insects that were seen on the plants or in the water were placed into the corresponding pouch. The next step involved salt-water extraction (Lattke 2000), which was needed because broken plant material, mud and algae would often end up in the collection pouches; thereby, making it difficult to see the insects. To solve this problem, the contents of each pouch were emptied into a two litre rectangular tub, which was then filled with a concentrated sodium chloride solution, and the insects were collected as they rose to

the top of the solution. In order to prevent decay, the insects were then rinsed in alcohol and placed in vials containing 80% ethanol.

To determine if there were any phytophagous families on the plants, another collection technique was used. Here, the major concern was transporting the plant material ca. 500 km back to the University of the Witwatersrand (Wits) before it rotted. To overcome this problem, the samples were collected and transported on the same day. To ensure that all sampling locations were visited in one day (05/02/2007), an abridged version of the plankton net method was used, whereby the plants were collected in the plankton net and then immediately placed into tubs. Once back at Wits, 500 ml (approximately 500 g) of each sample were placed into 30 Berlese funnels, i.e. a funnel for each replicate (Hill 1998). The remaining 1.5 litres (approximately 1.5 kg) of each sample were then placed into a fridge at 5 °C and manually sorted to find any herbivorous insects (Balciunas and Minno 1985). All the insects that were found were preserved in 80% alcohol.

2.5. Morphospecies identification of the insect assemblages

To identify aquatic insects to family level is relatively easy (Gerber and Gabriel 2002, Kotze 2005); however, identification to genus and species level requires specialist knowledge. In S.A., there are only a handful of taxonomists with this knowledge, each focusing on different orders. For this reason, it was not practical to identify all the insects to species level. Additionally, such an in-depth identification is not always needed to get valuable information. The use of morphospecies for assessing invertebrate (e.g. ants, beetles and spiders) diversity has been found to be cost effective, accurate and timely (Oliver and Beattie 1996a, Oliver and Beattie 1996b, Oliver *et al*. 2000).

Taking cognizance of these facts, this project employed the following identification method: all the insects were identified to family level and then sorted into morphospecies. The morphospecies data were then used in biodiversity analyses, while the information on which families were present showed which phytophagous insects needed to be re-collected. In addition, the qualification of which families were present was valuable as it created more detailed community baselines, allowing for comparisons to other studies which have identified the insect communities to family level, e.g. (Biggs and Malthus 1982); while the families SASS 5 ratings also provided an indication of the water quality in both dams.

2.6. Environmental variables

While collecting the plant material at each site, the following environmental data were recorded: species of plant being sampled; dam where the sample was being taken; distance to shore; water depth; light penetration; nitrate and phosphate concentrations; and any miscellaneous information that could prove useful. A Secchi disk was used to calculate the light penetration, while the nitrate and phosphate levels were obtained by taking a water sample, preserving it with mercury chloride, and then analyzing it using a Hach DR/890 Colourimeter.

2.7. Data analysis

2.7.1. Biological diversity

The morphospecies data were used to calculate the biological diversity of the insect communities found on the three different plant species. These analyses required three computer programs: *Estimate S,* version 7.52; *Species Diversity and Richness*, version 3.02; and *SAS Enterprise Guide,* version 9.

Estimate S was used to plot species accumulation curves to show how effective the sampling protocol had been. These curves are also useful because their extrapolation calculates two Michaels - Manton richness estimators (Williams 2007). In addition, rarefaction curves, i.e. the statistical expectation for a corresponding species accumulation curve, were plotted to show how heterogeneous the datasets were (Williams 2007).

To complement these richness estimators, the programme also calculated the Shannon Wiener and Fisher's alpha indices, both of which are diversity measures that incorporate richness and evenness into a single value. To complete the suite of community estimators, the Shannon J evenness measure was also computed. For all these indices, their values were calculated 50 different times, each time changing the order in which the samples were entered into the respective equations of each estimator. This process is known as randomization and has been shown to yield a more reliable value for any particular biodiversity estimator (Oliver *et al*. 2000).

While *Estimate S* is able to give overall indices for the insect communities found on each plant species, it does not calculate a value for each replicate. Therefore, *Species Diversity and Richness* was used to compute the Shannon Wiener, Fisher's alpha and Shannon J values for each replicate. The mean and standard errors of these values were then calculated; thereby, allowing the different estimators from the different plant species to be compared to one another using a one-way ANOVA. These analyses were performed in *SAS* and were followed by post hoc Tukey tests to show which insect communities were significantly different from one another. This approach was also used to compare the actual number of morphospecies that were found in each of the study species replicates.

While it is important to know how communities differ from one another, it is also useful to have information on how similar they are. For this reason, *Estimate S*

was used to calculate the Bray-Curtis and Morisita-Horn similarity measures, both of which are suitable for the quantitative data gathered in this study (Williams 2007).

2.7.2 Multivariate and statistical analyses of environmental variables

The environmental variables that were collected at each site were analyzed to determine their importance in shaping the insect communities. Using *Canoco,* version 4.5, the following tests were performed: Principal Components Analysis (PCA) to determine how similar the replicates were to one another; and a ReDundancy Analysis (RDA) to determine how the environmental variables differed between each replicate. In addition to the RDA, a one-way ANOVA was run in *SAS* to determine how the environmental variables differed between the replicates performed on all three plant species.

2.8 Collecting, rearing, observing and identifying the phytophagous species

Using the information the first trip provided on which herbivorous families were present, another field trip was undertaken with the specific goal of bringing the insects back to Wits for rearing. Over a two-day period (17/03/2007 – 18/03/2007), the plant material was collected using the abridged version of the plankton net method, transported back to the laboratory and placed in a cold room at 5°C (Balciunas and Minno 1985). The samples were then sorted to find individuals of phytophagous families, which were then placed into two litre rectangular tubs within different emergence boxes, each containing live plant material rooted in a sand substrate, and left in a controlled growth room at 25°C, with a photoperiod of 12 hours.

Once set up, the rearing tubs required maintenance to prevent fungal contaminantion that could affect the development of the insects. Clean de-chlorinated water was placed in the tubs daily. Every fourth day, the entire contents of the tubs (i.e. sand, plants and insects) were removed, the tubs wiped clean, fresh sand laid down, and the plants and insects were returned. Fresh plant material was also added and decaying material removed as needed. During the rearing stage, as many different life stages as possible were collected. These larvae, pupae and adults were then identified using taxonomic keys and consultations with various experts.

Besides rearing insects for identification, behavioural observations were made on insect feeding. In doing so, insect species were separately given healthy, live strands of *L. major, L. muscoides* and *P. pectinatus*; then the individuals' feeding behaviours would be observed every day for a week, paying particular attention to the manner in which they fed. After the week was over, the plants were removed and the numbers of leaves that had been damaged were counted, classifying the damage into three categories, namely: healthy, eaten, and stripped, i.e. the leaf was so damaged it had fallen off. These strands were then returned to their tubs and the daily observations began again; ending when the plants were taken out after another week, i.e. the second week, and the damage to strands had again been measured.

3. Results

3.1. Insect communities associated with *Lagarosiphon major, L. muscoides* and *Potamogeton pectinatus*

Seven insect orders and 17 families (with their respective SASS 5 value) were collected at both sites using the plankton method, Berlese funnels and manual sorting (Table 1). A broad overview of the communities collected from the three plant species using the plankton net method is given in Table 2.

Table 1. Insect orders and families collected at Mearns Weir and the adjacent farm dam. The South African Scoring System, Version 5 (SASS 5), value for each family is given in parenthesis (Gerber and Gabriel 2002).

Insect order	**Insect family and SASS 5 rating**
Ephemeroptera (mayflies)	Baetidae (5) Caenidae (6)
Odonata (damselflies and dragonflies)	Aeshnidae (8) Coenagrionidae (4)
Hemiptera (bugs)	Belostomatidae (3) Corixidae (3) Hydrometridae (6) Notonectidae (3) Pleidae (4)
Coleoptera (beetles)	Dytiscidae (5) Hydrophilidae (5)
Diptera (flies and mosquitoes)	Chironomidae (subfamilies: Chironominae and Orthocladiinae) (2) Culicidae (1) Muscidae (1)
Trichoptera (caddisflies)	Ecnomidae (8) Leptoceridae (6)
Lepidoptera (moths and butterflies)	Pyralidae (12)

Table 2. Insect families, their percentage frequency of occurrence in ten plankton net replicates, and the number of morphospecies found on *Lagarosiphon major, L. muscoides* and *Potamogeton pectinatus.* The phytophagous insect species are shown in bold text and with an asterix

	L. major		***L. muscoides***		***P. pectinatus***	
Family	Frequency of occurrence	Number of morphospecies	Frequency of occurrence	Number of morphospecies	Frequency of occurrence	Number of morphospecies
Baetidae	60	2	40	2	100	2
Caenidae	90	2	80	2	-	-
Aeshnidae	-	-	-	-	60	2
Coenagrionidae	20	1	-	-	90	1
Belostomatidae	30	1	-	-	40	3
Corixidae*	**90**	**2**	**30**	**1**	**50**	**2**
Pleidae	100	2	100	2	100	2
Dytiscidae	60	3	60	2	60	2
Hydrophilidae	-	-	10	1	-	-
Chironomidae*	**90**	**3**	**90**	**2**	**80**	**3**
Culicidae	-	-	-	-	40	2
Muscidae	-	-	-	-	10	1
Leptoceridae*	**20**	**1**	**70**	**1**	**-**	**-**
Pyralidae*	**60**	**1**	**60**	**1**	**90**	**1**

3.2. Biological diversity estimators

From the ten plankton net replicates performed on each plant species during the first sampling trip, 345 individual insects (112 herbivores and 233 carnivores) from 18 morphospecies were collected within *L. major*, 338 individuals (105 herbivores and 233 carnivores) from 14 morphospecies within *L. muscoides*, and 394 (131 herbivores and 263 carnivores) individuals from 19 morphospecies within *P. pectinatus*. The species accumulation and rarefaction curves for the ten plankton net replicates on each plant species are shown in Figure 6.

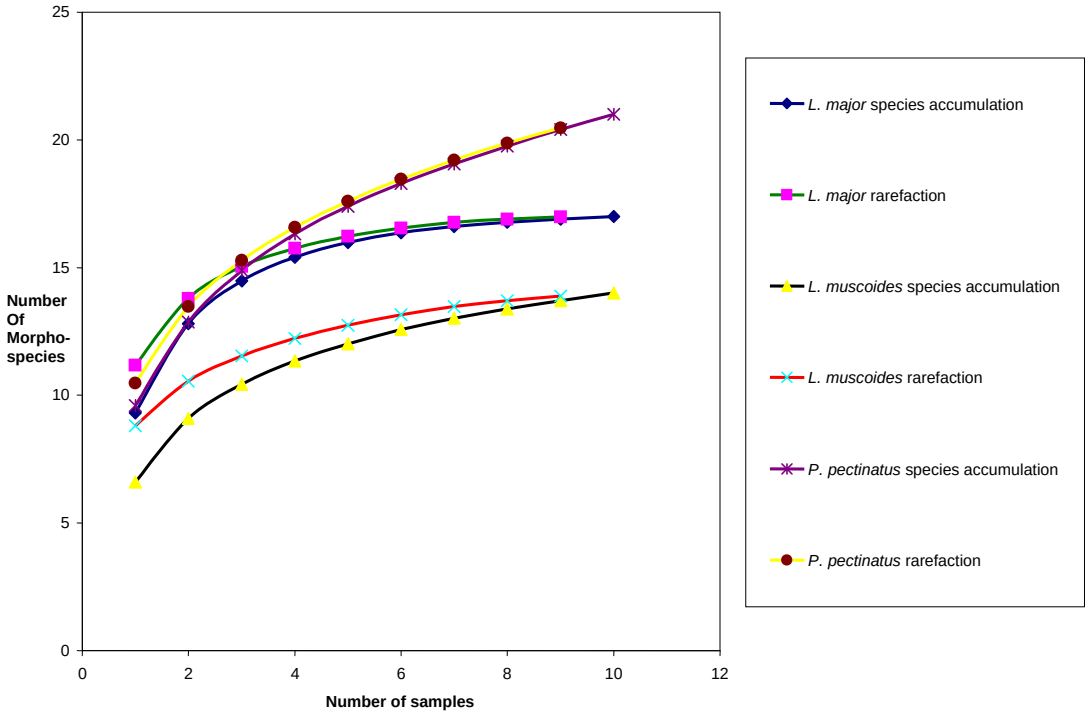


Figure 6. Species accumulation and rarefaction curves for the insects collected off *Lagarosiphon major, L. muscoides* and *Potamogeton pectinatus* in the Mooi River district of South Africa using the plankton net method.

The levelling off of the species accumulation curves for *L. major* and *L. muscoides* (Figure 6) shows that the sampling protocol was efficient and collected almost all the insect species that were expected to be associated with the plants at the time of sampling. However, the same cannot be said for *P. pectinatus*; as its curve is still increasing (Figure 6) it is likely that additional insect species would have been found if more replicates had been conducted. Despite this fact, the ten replicates of the plankton net method appeared to be an efficient sampling protocol for this study, a fact that can probably be attributed to the homogeneous nature of the communities associated with each plant species, shown by the close proximity of the rarefaction curves to their corresponding species accumulation curve (Williams 2007).

By extrapolating the species accumulation curves, the Michaelis - Menton richness estimators were calculated (Table 3). In addition to these values, other community estimates (number of morphospecies, Shannon Wiener, Fisher's alpha and Shannon J) and other statistics were calculated (Table 3).

Table 3. The richness, diversity and evenness indices for the insect communities collected off *Lagarosiphon major*, *L. muscoides* and *Potamogeton pectinatus* using the plankton net method. Values within rows with no or the same superscript letters are not significantly different (Tukey HSD, $P < 0.05$)

Index	***L. major***	***L. muscoides***	***P. pectinatus***
Michaelis – Menton averaged over 50 randomizations	18.92	15.84	23.59
Michaelis – Menton calculated once for the mean species accumulation curve	19.02	15.64	23.24
Mean and standard error for the number of morphospecies per replicate	9.30 ± 0.99^{a}	6.60 ± 0.62^{b}	9.60 ± 0.69^{a}
Shannon Wiener Cumulative Mean and Standard error per replicate	2.34 1.87 ± 0.11^{a}	2.06 1.52 ± 0.08^{b}	2.13 1.87 ± 0.08^{a}
Fisher's alpha cumulative Mean and standard error per replicate	3.75 4.81 ± 0.51	2.95 3.23 ± 0.28	4.74 5.46 ± 1.75
Shannon J cumulative Mean and standard error per replicate	0.83 0.66 ± 0.04	0.78 0.58 ± 0.03	0.70 0.61 ± 0.03

The insect community found on *L. muscoides* is significantly less diverse than that found on the other two plant species. While the evenness of its assemblage is similar to those of *L. major* and *P. pectinatus*, it has significantly fewer species, which is the reason for a lower overall diversity. The diversities of the insects associated with *L. major* and *P. pectinatus* are comparable. Although not statistically significant, the community on *L. major* is slightly more even than that on *P. pectinatus* and this is probably the reason why the community associated with *L. major* has a higher Shannon Wiener index. In contrast, the richness estimators for *P. pectinatus* show that this plant supports the greatest number of insect species. The fact that this is not statistically significant can be attributed to the fact that the species accumulation curve for *P. pectinatus* curve had not levelled off after ten replicates, while that of *L. major* had. Taking cognizance of this fact and realizing that Fisher's alpha is being advocated as the best diversity measure (Williams 2007), the assemblage found on *P. pectinatus* is probably more diverse than that on *L. major*, due to the fact that they have similar evenness but *P. pectinatus* supported a greater number of species.

The Bray-Curtis and Morisita-Horn similarity measures for the insect assemblages collected off the different plant species using the plankton net method are presented in Table 4.

Table 4. A similarity matrix comparing the insect communities collected within *Lagarosiphon major, L. muscoides* and *Potamogeton pectinatus* using the plankton net method.

Insect communities from the plants compared	**Bray-Curtis**	**Percentage similarity (Bray-Curtis X 100)**	**Morisita-Horn**	**Percentage similarity (Morisita-Horn X 100)**
L. major and *L. muscoides*	0.61	61	0.76	76
L. major and *P. pectinatus*	0.52	52	0.56	56
L. muscoides and *P. pectinatus*	0.5	50	0.54	54

The assemblages found on *L. major* and *L. muscoides* are approximately 70% similar to one another in the number of species they support and their relative abundances. The community found on *P. pectinatus* is 50% similar to those associated with *L. major* and *L. muscoides.*

3.3. Multivariate and univariate statistical analyses of the environmental data

3.3.1 Principal Components Analysis (PCA)

The results of the PCA, which compared the invertebrate community found in each replicate to the communities in every other replicate, are presented in Figure 7.

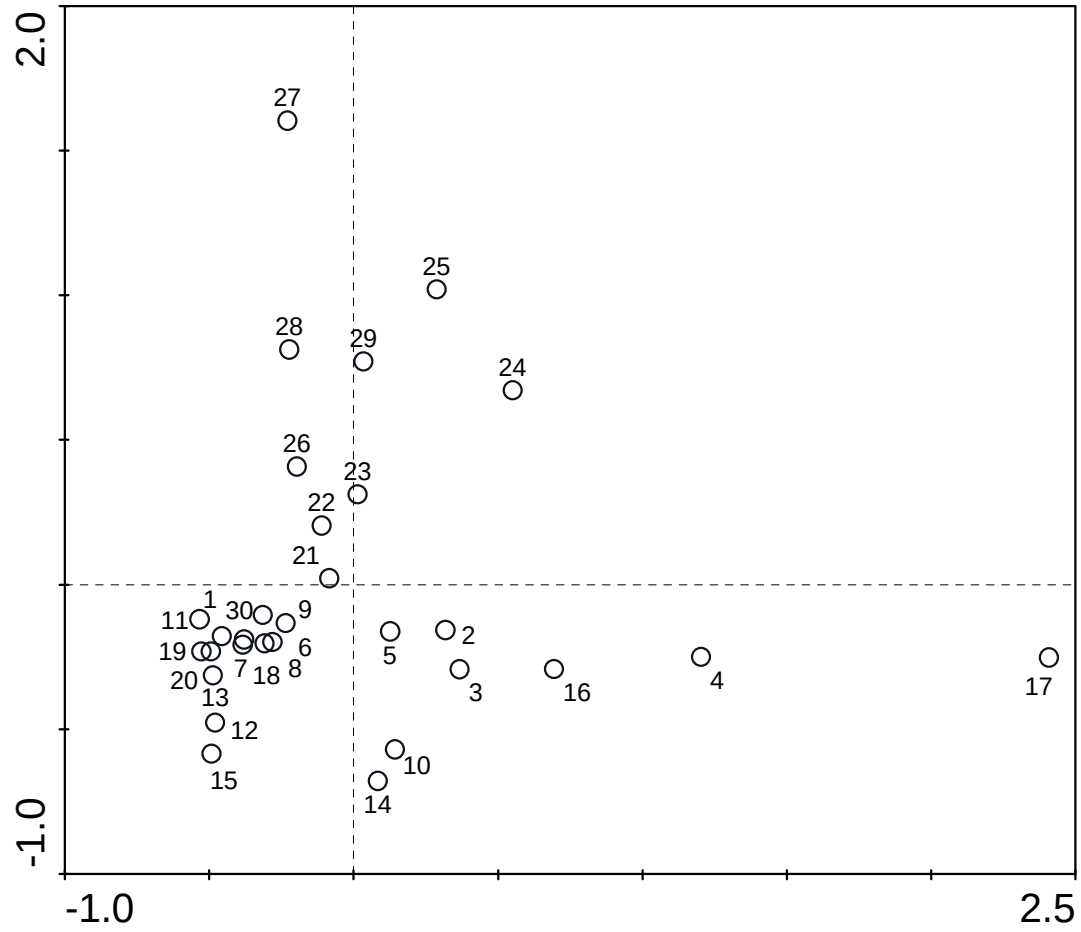


Figure 7. The principal components analysis for the insect species found in the different replicates: 1- 10 are for the *Lagarosiphon major* replicates, while 11-20 and 21-30 are for *L. muscoides* and *Potamogeton pectinatus* replicates, respectively.

Effectively, this is a graphical combination of the trends shown in the rarefaction curves with the Bray-Curtis and Morisita Horn similarity measures. The clustering of 1-10, 11-20 and 21-30 around each other shows that the intra-species replicates are fairly homogeneous. Additionally, replicates 1-20 overlap each other quite extensively, while occupying a distinct area of the graph compared to replicates 21-30. This supports the finding that the entire assemblages found on *L. major* and *L.*

muscoides are approximately 70% similar to one another and 50% similar to the community associated with *P. pectinatus.*

3.3.2 One-way ANOVA and ReDundancy Analysis (RDA)

Concerning the environmental data collected at each of the replicate sites on Mearns Weir and the adjacent farm dam, Table 5 presents the results of the ANOVA, while Table 6 and Figure 8 present the results of the RDA.

Table 5. The environmental data (mean ± S.E.) collected from the sites on Mearns Weir and an adjacent farm dam where the insect communities found on *Lagarosiphon major*, *L. muscoides* and *Potamogeton pectinatus* were sampled. Values within rows with no or the same superscript letters are not significantly different (Tukey HSD, $P < 0.05$).

Environmental variable	***L. major* on Mearns Weir**	***L. muscoides* on Mearns Weir**	***P. pectinatus* on farm dam**
Distancefrom shore (m)	4.1±0.28 [a]	2.02±0.48 [b]	6.5±0.37 [c]
Water depth (m)	0.93±0.05	1.04±0.08	0.97±0.03
Light penetration (m)	0.93±0.05	1.04±0.08	0.97±0.03
Nitrate concentration (mg/l)	1.05±0.05 [a]	0.95±0.13 [a]	0.45±0.08 [b]
Phosphate concentration (mg/l)	0.33±0.01	0.49±0.17	0.25±0.02

The 10 replicates for each plant species were taken at significantly different distances away from the shore, with the replicates of *L. muscoides* taken closest to the bank, and those on *P. pectinatus* furthest away. Despite this fact, the mean water depth was not significantly different between the plant species. Concerning the light penetration at each replicate site, the results are also not significantly different between plant species the same as those of water depth. This is because the Secchi disk was still visible after reaching the dams' floor at all the sites.

The nitrate levels from water samples within *L. major* and *L. muscoides* were not different to one another. This is to be expected as the plants are found in close proximity to one another on the same water body, i.e. Mearns Weir. However, the replicates from *P. pectinatus* came from another water body, i.e. the farm dam, which has significantly lower nitrate levels than that of Mearns Weir. The farm dam also tended to have lower phosphate levels than Mearns Weir, but this was not significantly different.

Table 6. Summary table of the ReDundancy Analysis (RDA) concerning the relationships between the environmental variables and the insect assemblages found on *Lagarosiphon major*, *L. muscoides* and *Potamogeton pectinatus* in the Mooi River district of South Africa.

Axes	**1**	**2**	**3**	**4**
Eigenvalues	0.203	0.180	0.080	0.012
Species-environment correlations	0.772	0.809	0.791	0.443
Cumulative percentage variance of species data	20.3	38.4	46.4	47.6
Cumulative percentage variance of species-environment relation	42.2	79.7	96.4	99.0

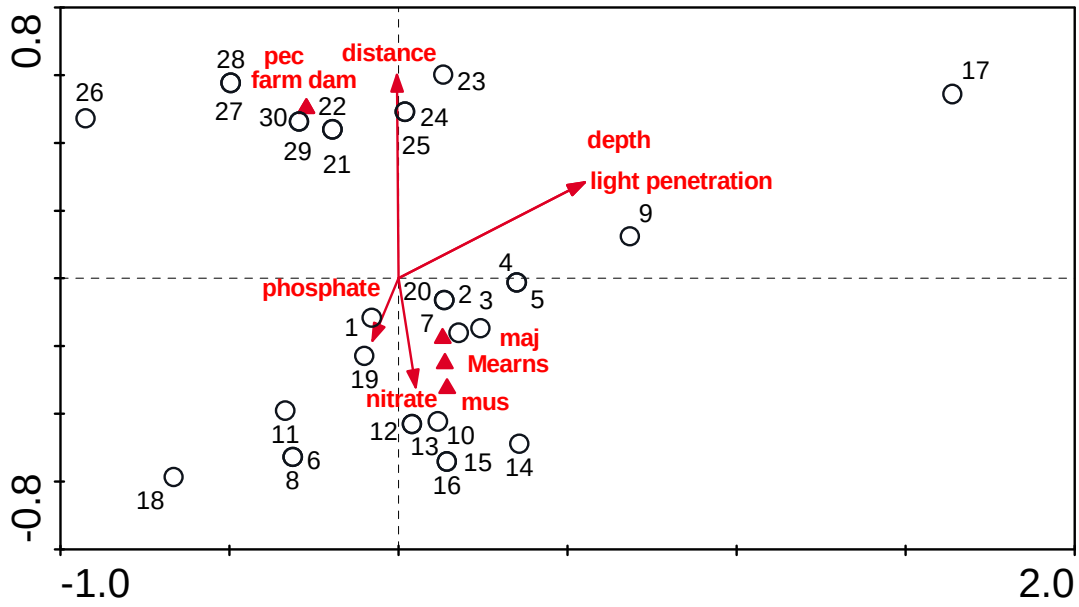


Figure 8. ReDundancy Analysis (RDA) of the relationships between the environmental variables and the insect communities found on *Lagarosiphon major* (1-10), *L. muscoides* (11-20) and *Potamogeton pectinatus* (21-30). The different variables in this RDA are plant species ('maj' for *L. major*, 'mus' for *L. muscoides*, and 'pec' for *P. pectinatus*); sites (Mearns Weir and the farm dam); distance to shore (distance); depth; light penetration; nitrate and phosphate levels.

The environmental variables in the RDA explain almost 100% of the variation between the different insect communities found (Table 6). The insect communities collected off both species of *Lagarosiphon* came from the same site, i.e. Mearns Weir; therefore, the environmental variables were not found to differ substantially between their replicates. Consequently, the replicates for *L. major* (1-10) and *L. muscoides* (11-20) cluster together in the RDA. Another potential reason for this

clustering is that the limited number of replicate sites may not have been able to discern any physical gradients that did exist at smaller spatial scales.

When comparing the replicates done on *Lagarosiphon* sp. (1-20) to those on *P. pectinatus* (21-30), site, plant species, distance to shore and nutrient levels, especially nitrate, are orientated 180° away from each other. This means that these variables between the replicates taken from *Lagarosiphon* sp. and those taken from *P. pectinatus* are negatively correlated to one another. Consequently, these variables could explain most of the differences between the insect assemblages on *Lagarosiphon* sp. and those on *P. pectinatus*. Lastly, for all replicates, the light penetrated to the bottom of the water body; therefore, light penetration and depth values are exactly the same, and hence represented by a single line. However, the graph shows them to be at right angels to the other variables, indicating that they do not play a major role in shaping the insect assemblages found in this study. However, this is also because there is little variation between the three plant species in terms of these variables within the collection sites (the replicates) used in this study. At broader scales, light and depth conditions are likely to be key variables in the distribution of each of these three plant species within water bodies across their distributional ranges.

3.4. Phytophagous species: identification and behavioural observations

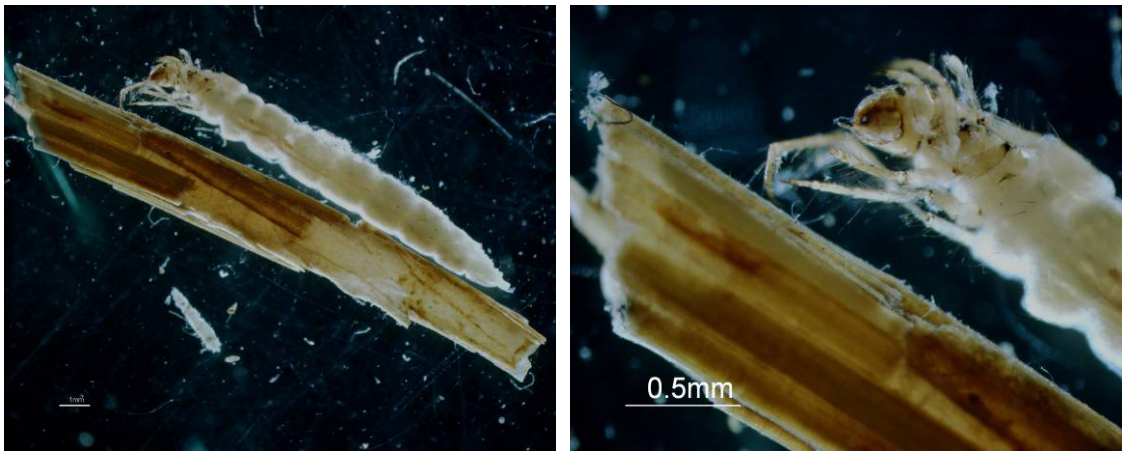
This section gives the lowest taxonomic level identifications for the phytophagous species found in this study. Additionally, it reports on the observations of the feeding behaviour of these insects.

3.4.1. *Athripsodes harrisoni* (Trichoptera: Leptoceridae)

Athripsodes harrisoni (n=2) (Figure 9) actively fed on living *L. major* and *L. muscoides*. Their bites had an oval shape and were generally located between the leaves margins (Figure 10). They moved up and down the lengths of the stems while feeding on most of the leaves (Figure 11). Often, they would take numerous bites along the entire length of a leaf; this damage would eventually break through one leaf margin, leaving the leaf hanging on a by a thread that would often break off the stem (Figure 11).

Although the feeding behaviour of *A. harrisoni* was the same on both species of oxygen weed, the rate of damage differed between the species. After one week of feeding on *L. major*, 8% of leaves were completely healthy, 59% of the leaves showed feeding damage, and 33% had been fed on so extensively that they had fallen off, i.e. stripped (these observations were made on a single 5 cm strand). After two weeks, 8% of leaves were still undamaged, only 44% of the leaves exhibited feeding damage, but the number of leaves that had been stripped had increased to 48%. Although, almost half of the leaves had been stripped, the strand appeared to be quite healthy.

The feeding on *L. muscoides* was much more destructive. None of the three strands (mean length of 11 cm) placed in the emergence boxes lasted more than a week, i.e. in seven days, all the leaves from one strand were stripped from the stem, leaving the strand looking extremely unhealthy. While the leaves were eaten, the stems were not. However, this species of caddisfly occupies a portable case made of vegetation and silk and on one occasion, an individual was observed to severely damage a stem of *L. muscoide* while constructing a new case.



a **b**

Figure 9. *Athripsodes harrisoni* (Trichoptera: Leptoceridae): its entire body (a), case (a) and mouthparts (b).

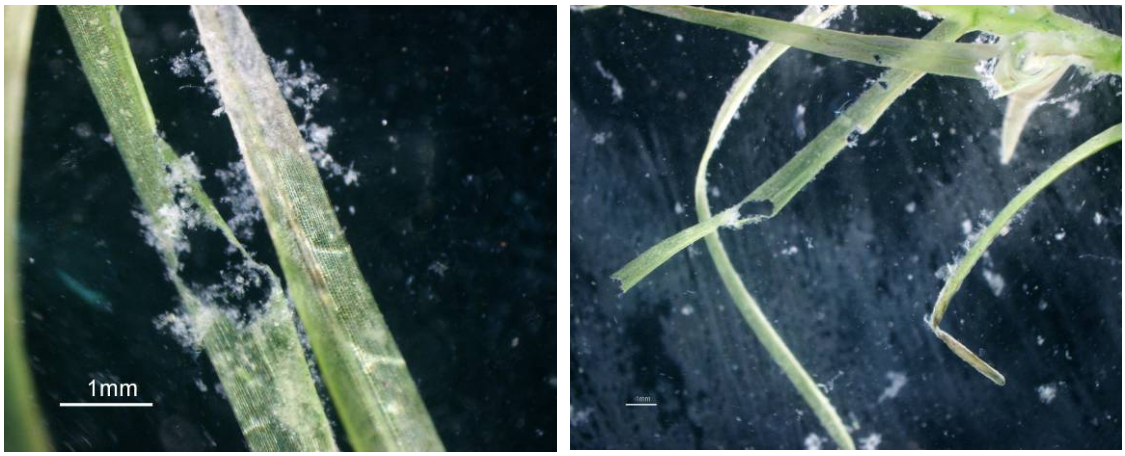
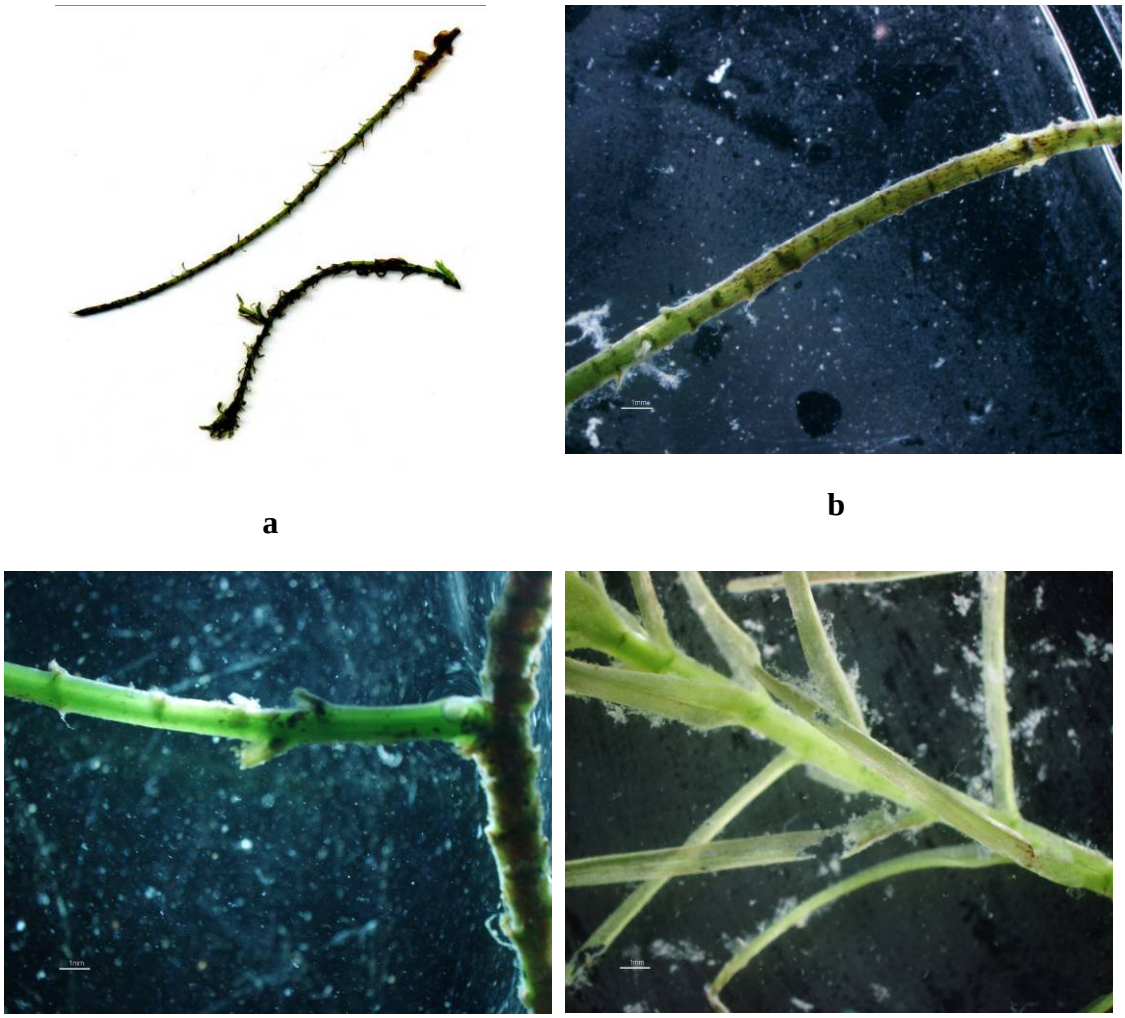


Figure 10. The oval shaped bites that *Athripsodes harrisoni* (Trichoptera: Leptoceridae) took out of the leaves of *Lagarosiphon major*.



a **b**

c **d**

Figure 11. *Athripsodes harrisoni* (Trichoptera: Leptoceridae) fed along the entire lengths of *Lagarosiphon major* (c and d) and *L. muscoides* (a and b) stems and leaves. Their bites would often break the leaf margins (d); thereby, leaving them weakened and susceptible to falling off (a, b and c).

3.4.2. *Leptocerus* sp. (Trichoptera: Leptoceridae)

The feeding behaviour of *Leptocerus* sp. (n=6) (Figure 12) was very similar to that of *A. harrisoni*, the only differences being that the oval bites were smaller in size and the rate of damage was less (Figure 13 and 14). After one week of feeding on *L. major*, 76% of leaves were completely healthy, 22% of the leaves showed feeding damage and 2% of the leaves had been stripped (these observations were made on four strands with a mean length of 3 cm). After two weeks, 34% of leaves were still undamaged, the number of leaves showing feeding damaged increased to 50% and the number of leaves that had been stripped increased to 16%. Despite this feeding damage, the plants were still quite healthy.

With regards to *L. muscoides,* the rate of damage was higher than that on *L. major.* After one week of feeding on *L. muscoides*, 34% of leaves were completely healthy, 53% of the leaves showed feeding damage and 13% of the leaves had been stripped (these observations were made on five strands with a mean length of 6.5 cm). After two weeks, 30% of leaves were still undamaged, the number of leaves showing feeding damaged increased to 47% and the number of leaves that had been stripped increased to 20%. Despite this feeding damage, the plants appeared to be quite healthy

Throughout the time that these caddisflies were observed, no damage was observed to the stem of any strand.

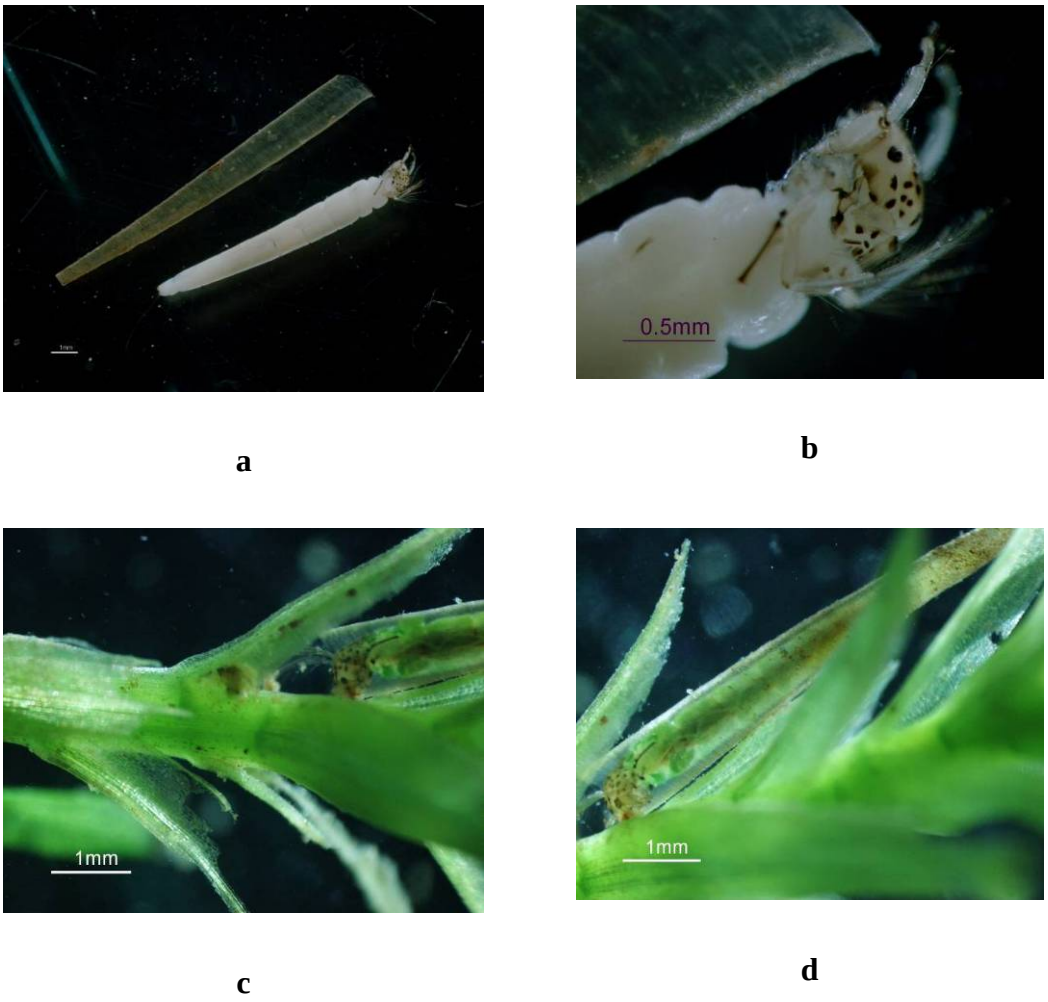


Figure 12. *Leptocerus* sp. (Trichoptera: Leptoceridae): its entire body (a), case (b) and head pigmentation (b,c and d).

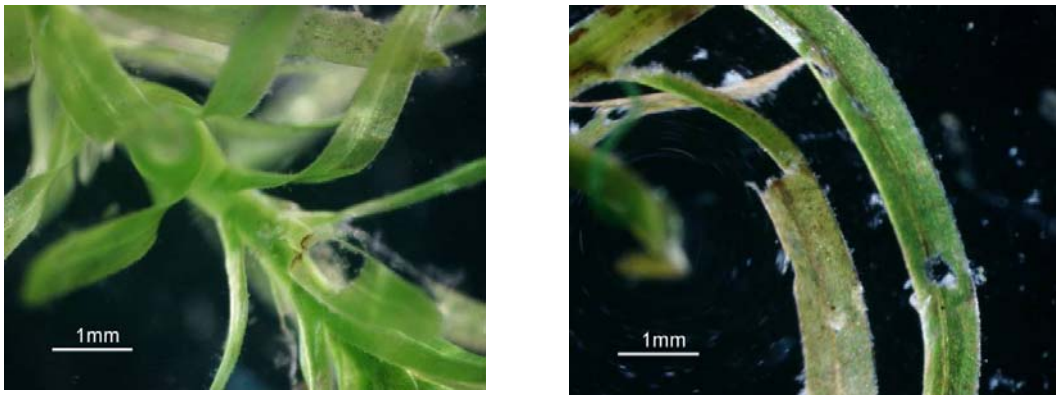


Figure 13. The oval shaped bites, located between the leaf margins, that *Leptocerus* sp. (Trichoptera: Leptoceridae) took out of *Lagarosiphon* sp.

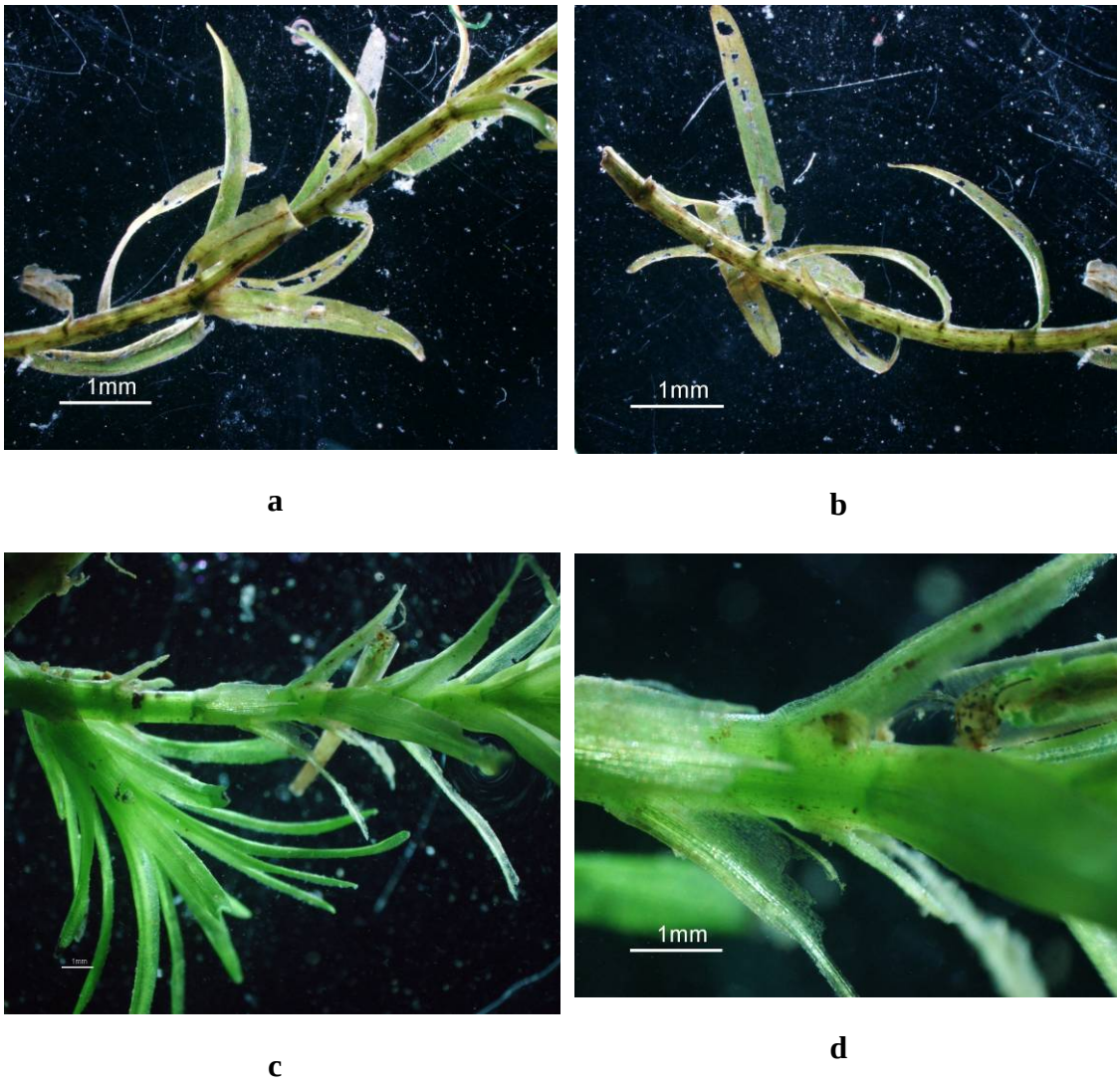


Figure 14. *Leptocerus* sp. (Trichoptera: Leptoceridae) fed on *Lagarosiphon major* (c and d) and *L. muscoides* (a and b); they fed along the entire lengths of the stems and leaves; their bites would often break the leaf margins (b); thereby, leaving them weakened and susceptible to breaking off (a, b and d).

3.4.3. *Parapoynx fluctuosalis* (Lepidoptera: Pyralidae)

Parapoynx fluctuosalis Zeller 1852 f. *circealis* Walker 1859 (n=3) (Figure 15) was observed to feed on *L. major*, *L. muscoides* and *Potamogeton pectinatus.* With

regards to both species of oxygen weed, the caterpillar damaged these plant species in the same manner. Within three days of receiving fresh plants, 100% of the leaves in the top 3cm were damaged (observations made on two strands of *L. major* with a mean length of 10cm and two strands of *L. muscoides* with a mean length of 10cm). Most of this damage was the result of the caterpillar using the leaves to make cases to occupy. These leaves showed two distinct types of damage: firstly, the leaves were cut in a smooth, diagonal line, close to the leaf base at stem, and then folded over each other to make a case; secondly, there were bites, i.e. feeding damage, taken out of the leaves comprising the case. The bites were semi-circular in shape and taken from the leaf margins (Figure 16). After one week, 71% of the leaves exhibited damage as a result of case construction, which increased to 83% after the second week. It is important to note that while making their cases the caterpillars were able to cut through the stem of both plants species.

Once the caterpillars made their cases, they remained in them most of the time. This led to very little damage being noted on the bottom half of each strands. For both plant species, the observations after the first and second week revealed less than 10% of the leaves showing feeding damage. Consequently, the tops of each strand were damaged but the lower sections were very healthy.

Unfortunately, the individual feeding on the *P. pectinatus* died before any data on the feeding rate could be obtained; however, there was evidence of it feeding on *P. pectinatus* (Figure 17).

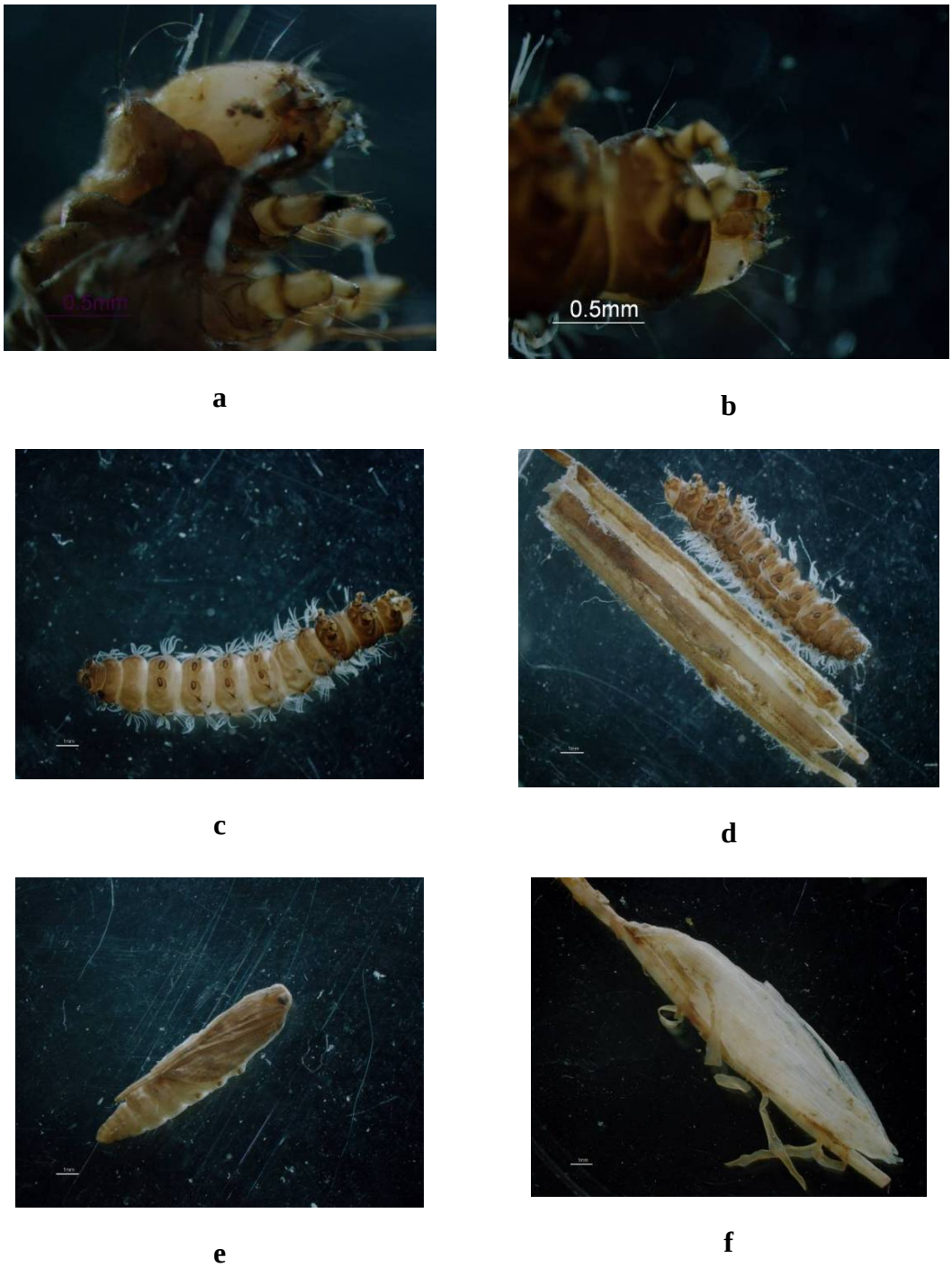
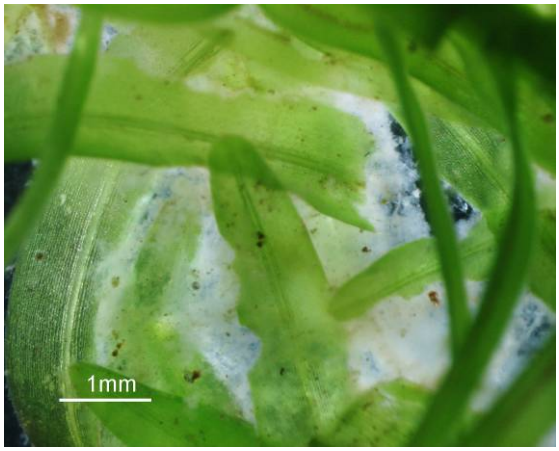


Figure 15. The larva (a and b) and pupa (e and f) of *Parapoynx fluctuosalis* (Lepidoptera: Pyralidae), as well as the cases they occupy during the larval (c and d) and pupal stages (f).

a

b



c

d

Figure 16. The cases (a and b) of *Parapoynx fluctuosalis* (Lepidoptera: Pyralidae) and the damage that results due to their construction, namely: cutting and folding the leaves into a case (a, b, c, and d), semi-circular feeding damage (c), and the slicing of the stem (b and d).

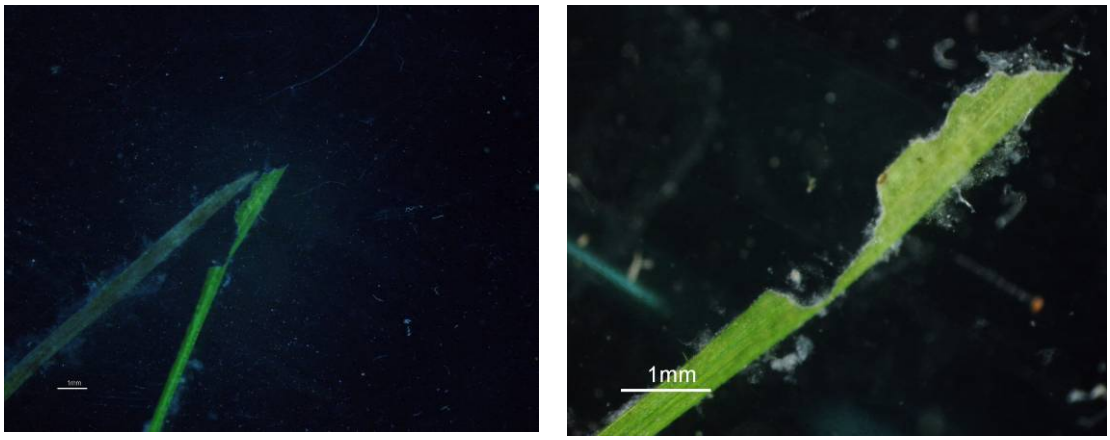


Figure 17. *Parapoynx fluctuosalis* (Lepidoptera: Pyralidae) feeding damage on *Potamogeton pectinatus.*

3.4.4. Diptera: Chironomidae

The subfamilies Chironominae (n=14) and Orthocladiinae (n=19) (Figure 18) were found at Mearns Weir and the adjacent farm dam. Unfortunately, the lack of expertise in Chironomidae taxonomy within S.A. meant that these specimens could not be identified further.

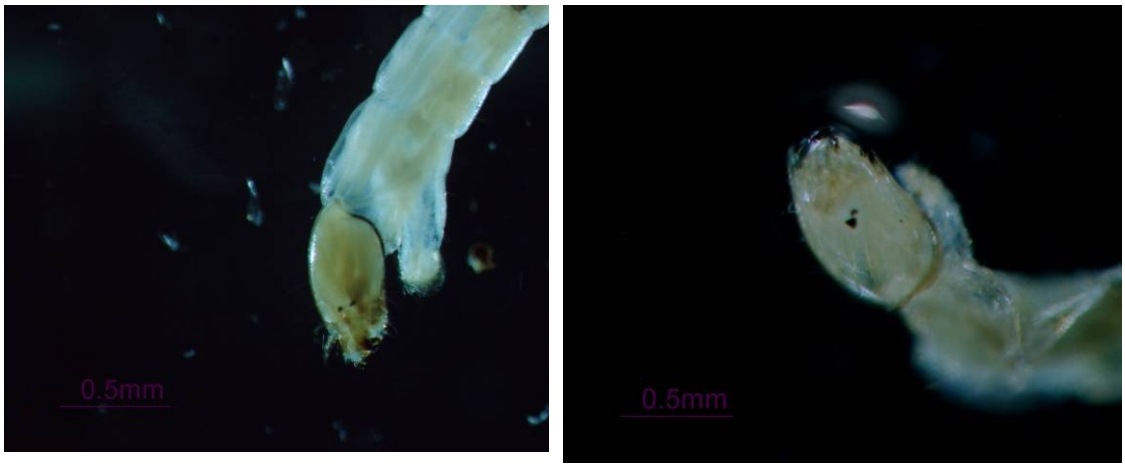


Figure 18. The heads and prolegs of Chironomidae (Diptera).

These midges damaged all three plant species and were responsible for three types of damage. Firstly, they removed minuscule amounts of leaf material to form cases for themselves on the bottom of the rearing tub, resulting in little holes forming in the leaves. Secondly, on four occasions (two on *L. major* and two on *L. muscoides*), the midges manipulated the leaves into cases which lay on the stem, which was associated with localized stem damage (Figure 19), possibly from the midges sucking the sap of the plants. Over the entire duration of observations, these midges were not particularly destructive. For all three plant species, less than 10% of the leaves were observed to be damaged at the one and two week intervals. These observations were made on five strands of *L. major* (with a mean length of 9 cm), five strands of *L. muscoides* (with a mean length of 9cm), and two strands of *P. pectinatus* (with a mean length of 6cm).

Figure 19. Leaves that were damaged in the construction of a Chironomid case (a and b). The larva would lie in the grove of the damaged leaf (c and d), positioning their heads at the petiole (c and d).

3.4.5. *Micronectus scutellaris* and *M. dorothea* (Hemiptera: Corixidae)

Two species of bugs were identified in this project, namely: *Micronectus scutellaris* (Stal) and *M. dorothea* (Hutchinson). Unfortunately, these bugs did not survive as an unidentified fungus smothered them in the rearing tubs. Consequently, no observations on feeding behaviours were recorded.

4. Discussion

The primary aim of this study was to determine the phytophagous insect species associated with *Lagarosiphon major, L. muscoides* and *Potamogeton pectinatus* on two dams within the Mooi River district of South Africa. Consequently, this aim shall be discussed first. *Parapoynx fluctuosalis, Micronectus scutellaris, M. dorothea* and two sub-families of the Chironomidae were the herbivorous insects found associated with *Lagarosiphon major, L. muscoides* and *Potamogeton pectinatus.* In addition, *Leptocerus* sp. was found on *L. muscoides,* while *Athripsodes harrisoni* resided on *L. major.*

While *Parapoynx fluctuosalis* and two representatives falling into the Chironomidae and Orthocladiinae all fed on and damaged *L. major,* they were also found to damage *L. muscoides* and *P. pectinatus.* This, combined with their broad geographical host ranges (Picker *et al*. 2004), indicates them to be more generalist than specialist feeders. Consequently, they are unlikely to be viable control agents for *L. major.* In contrast, *A. harrisoni* and *Leptocerus* sp. were only found to feed on and damaged *L. major* and *L. muscoides*. Their absence from surveys on *P. pectinatus* is important as it indicates the possibility of them being oligophagous herbivores. This fact is even more pertinent when it is considered that N.Z. has indigenous species from the *Potamogeton* genus, e.g. *P. chessemanii* (Biggs and Malthus 1982). Additionally, these caddisflies were the most destructive of all the herbivorous insects found in this survey. In summary, *Athripsodes harrisoni* and *Leptocerus* sp. are potentially destructive, host-specific agents of *L. major*, and their suitability as control agents should be investigated further.

While *A. harrisoni* and *Leptocerus sp.* caused extensive damage to the leaves, they left the stems untouched. However, this study does demonstrate that different insect species have different feeding patterns, e.g. the damage of *P. fluctuosalis* was almost exclusively limited to the leaves on the tip of the stem. Therefore, an argument

can still be made for additional exploratory surveys that aim to discover natural invertebrate enemies of *L. major* that are host-specific and that damage its stems and/or tubers.

In addition to finding classical biocontrol agents for *L. major*, this study attempted to identify augmentation biocontrol agents that could be used in the management of *L. major, L. muscoides* and *P. pectinatus* within South Africa. The abundance of herbivorous insects found associated with these plant species does demonstrate the potential of employing indigenous insect species to manage weedy native macrophyte species. However, it must be pointed out that despite this faunal abundance, *L. major*, *L. muscoides* and *P. pectinatus* are not under control in Mearns Weir or the farm dam. For example, *L. muscoides* is still forming 2km long, 15m wide patches on Mearns Weir, which make travelling by boat extremely difficult at times (personal observation).

The most likely reason for this nuisance growth is the high nutrient levels in the waters. The South African water quality guidelines (DWAF 1996) advocate the use of total nitrogen to assess eutrophication. The nitrate levels of the dams in this study represent approximately 80% of the total nitrogen (DWAF 1996); therefore, rough estimates of the total nitrogen in the water surrounding the replicate sites for each plant species are 1.31mg/l for *L. major,* 1.16mg/l for *L. muscoides,* and 0.56 mg/l for *P. pectinatus.* These are mesotrophic conditions and are associated with high species diversity (DWAF 1996), e.g. the insect diversity in this study. However, the nitrate: phosphate ratios in the water surrounding all three plant species were less than 10, indicating conditions which are associated with eutrophication and nuisance plant growth (DWAF 1996). Consequently, any damage inflicted by the phytophagous species does not effectively slow the growth and spread of the plant species. This finding is akin to those concerning water hyacinth in South Africa. For example, the high nutrient levels in Hammarsdale Dam, KZN, S.A., enable rapid growth of leaves of water hyacinth, thereby negating the damage inflicted by its control agents (Hill

and Olckers 2001). Clearly, eutrophication is a massive problem facing S.A., which if not addressed will continue to facilitate the unchecked growth of aquatic plant species, whether they are indigenous or exotic.

In identifying potential classical and augmentation biocontrol agents, this study observed numerous herbivorous insects feeding on living macrophyte tissue. This is important as it adds to the growing body of evidence that advocates the vital role live macrophytes play in sustaining aquatic food webs (Lodge 1991, Newman 1991).

Dispelling the myth that macrophytes only enter food webs as detritus highlights the scarcity of knowledge surrounding aquatic insect herbivory. For example, only one (Newman 1991) of the two major reviews of freshwater macrophyte herbivory (Lodge 1991, Newman 1991) mentions caddisfly herbivory. Even then, the author highlights the lack of knowledge concerning the manner and rate at which these insects feed. This dearth of knowledge is probably the reason why South African entomological field guides (Gerber and Gabriel 2002, Picker *et al*. 2004) give differing accounts of the feeding behaviour of the Leptoceridae. Gerber and Gabriel (2002) do not even mention that Leptoceridae are herbivorous, while Picker *et al*. (2004) state that they are omnivorous. However, this study shows these caddisflies are destructive herbivores that are potentially the most promising control agents for *L. major*, a finding that would have been very unlikely if the current literature on Leptoceridae had been the only guide in selecting control agents for *L. major*.

In addition to the lack of research into macrophyte-herbivory creating a dearth of knowledge concerning the feeding behaviours of aquatic invertebrates, it has also resulted in submerged macrophytes being under-studied. While numerous work has been done on the structural, digestive-inhibiting chemicals, toxic chemicals, and

nutritional content defences that land plants employ to deter herbivory (Belovsky and Schmitz 1994), similar studies on submerged plants have not received the same amount of interest. This is problematic as these defences are vital in determining the efficiency of an agent. For example, field samples and cultured strands of hydrilla have been shown to vary in their nitrogen concentration, with lower nitrogen levels increasing the mortality and developmental period of its control agents, *Hydrellia pakistanae* and *Bagous hydrillae* (Wheeler and Center 1996, 1997, Grodowitz and Mcfarland 2002). Such issues need to be considered in management of exotic *L. major* infestations as plant samples collected from New Zealand lakes with differing nitrate and phosphate levels have different nutrient profiles (Rattray *et al*. 1991).

One method of solving the lack of knowledge concerning macrophyte invertebrate herbivory would be to incorporate systematic observations during agent selection and host-specificity trials. Not only would these observations add to the information on the individual plant and insect species, e.g. how certain aquatic invertebrates feed, but they would also provide valuable information for submerged weed biocontrol programmes, e.g. how the defences of a particular weed affect the efficiency of its control agent(s).

Finally, the last aspect of surveying the herbivorous fauna found on *L. major, L. muscoides* and *P. pectinatus* was to determine if S.A.'s native fauna could control the hydrilla infestation on Pongolapoort Dam. *Parapoynx fluctuosalis*, Leptoceridae and Chironomidae have all been recorded to damage hydrilla found in North America (Balciunas and Minno 1985, Bennett and Buckingham 2000). Combining this fact with their national distribution within S.A. (Picker *et al*. 2004) means that it is likely that these insects will move onto the hydrilla found in Pongolapoort Dam. In addition, the ubiquitous presence of Corixidae on the plant species in this survey and their national distribution (Picker *et al*. 2004) means that they are also likely to move onto hydrilla. However, no insects besides hydrilla's control agents have demonstrated the potential for successfully controlling this weed. Therefore, while

herbivorous insect fauna of S.A. could move onto and feed on hydrilla, they are unlikely to affect its growth and spread. Consequently, S.A. should continue to focus on importing and testing the identified control agents of hydrilla.

The secondary aims of this study were to begin creating baseline insect community catalogues for *L. major, L. muscoides* and *P. pectinatus* in the Mooi River district of S.A., while simultaneously investigating the role that biological, physical and chemical components of the environment play in shaping these communities. Given the intertwined nature of these issues, they need to be discussed concurrently.

At first glance, the Shannon-Weiner and Fisher's Alpha values for each plant species seem low (Table 3). However, when these results are compared to insect communities found on three indigenous (*P. chessemanii, Myriophyllum propinquum* and Characeae) and three adventive macrophytes (*Elodea canadensis, L. major* and *Ranunculus fluitans)* in New Zealand (Table 6), a different picture emerges. Despite performing more replicates and shifting through more plant material, all six plants in the N. Z. survey (Biggs and Malthus 1982) had fewer insect orders and species than this study. Additionally, these plants had lower Shannon Wiener values than the macrophytes investigated from the Mooi River area. Freshwater bodies are not known for their ability to support diverse animal communities. For example, it is rare to find an abundance of families or species when sampling other aquatic phyla (e.g. Mollusca), classes [Turbellaria (flatworms), Oligochaeta (aquatic earthworms), Hirudinae (leeches) and Gastropoda (snails and limpets)] and orders [Amphipoda (scuds), Decapoda (crabs and shrimps) and Bivalvia (clams and mussels)]. Both the S.A. and the N.Z. surveys support this statement. Although they were not included in the results of this paper, two snail species and one fish (bass), flatworm, leech and aquatic earthworm species were found in the Mooi River survey. These finding are similar to the N.Z. study, which only found one species of aquatic earthworm and six

species of snails associated with the macrophytes (Biggs and Malthus 1982). In both studies, the diversity of the other fauna pales to that of the aquatic insects. In all likelihood, the communities on Mearns Dam and the adjacent farm dam represent healthy and diverse aquatic insect assemblages.

Table 6. The communities and their corresponding Shannon Wiener values of the insects associated with six macrophytes found in the lakes of the upper Clutha Valley, New Zealand (Biggs and Malthus 1982)

Insect order	**Number of species found on *Potamogeton chessemanii***	**Number of species found on *Myriophyllum propinquum***	**Number of species found on Characeae**	**Number of species found on *Elodea Canadensis***	**Number of species found on *Lagarosiphon major***	**Number of species found on *Ranunculus fluitans***
Odonata	1	1	2	2	2	2
Trichoptera	6	5	3	5	6	7
Lepidoptera	1	1	0	-	1	-
Diptera	1	1	2	1	1	1
Coleoptera	1	1	1	1	2	2
Neuroptera	-	-	-	-	-	1
Total number of species	**10**	**9**	**8**	**10**	**12**	**13**
Shannon Wiener	**1.67**	**2.07**	**1.32**	**1.43**	**1.99**	**1.99**

The next step is to compare the communities found on each plant species to one another. *Potamogeton pectinatus* supported 19 morphospecies from 11 families; *L. major* supported 18 morphospecies from ten families; and *L. muscoides* supported 14 morphospecies from nine families. Importantly, it must be remembered that the species accumulation curve of *P. pectinatus* was still increasing so it is likely that more morphospecies would have been found if more sampling had been done. In contrast, the species accumulation curves for *Lagarosiphon* sp. had already levelled off. Overall, the insect community on *P. pectinatus* was most diverse (Fisher's Alpha of 4.74) because it supported the greatest number of species, while the community on *L. muscoides* was the least diverse (2.95) because it supported the fewest number of species.

When the actual families comprising these baseline insect catalogues are examined, it is evident that there are variations in the carnivorous insect communities associated with each plant species. *Lagarosiphon major* is more diverse than *L. muscoides* as it supports Coenagrionidae (Odonata), Belostomatidae (Hemiptera) and more species of Dytiscidae (Coleoptera). *Potamogeton pectinatus* is more diverse than both other plant species as it supports Aeshnidae (Odonata), Coenagrionidae and more species of Dytiscidae and Belostomatidae.

Floral composition is a biological component of an aquatic environment that can change; therefore, one potential reason for different carnivore communities being found on *L. major, L. muscoides* and *P. pectinatus* is the fact that different plant species where being sampled. Different plants vary in their architecture, with submerged macrophytes species comprising more dissected leaves and having a higher spatial complexity providing more forage for epiphytic, periphytic and herbivorous macroinvertebrates (Cheruvelil *et al*. 2000, Cheruvelil *et al*. 2001, Cheruvelil and Soranno 2002), which in turn attracts and supports a larger carnivore pool. *Potamogeton pectinatus* is a dissected plant with a high level of spatial

complexity (Dibble *et al*. 1996a, Cheruvelil and Soranno 2002). Unfortunately, extremely few macrophytic species have had their architecture adequately described (Dibble *et al*. 1996a), and *Lagarosiphon* sp. are not included in that list. However, hydrilla, which has a similar morphology to the oxygen weed species in this survey, has been adequately described (Dibble *et al*. 1996a), thus providing a surrogate measure for the architecture of *L. major* and *L. muscoides.* Hydrilla is less spatially complex than *P. pectinatus* (Dibble *et al*. 1996a)**;** therefore, it can be inferred that the architecture of *P. pectinatus* allows it to support slightly more herbivores than *L. major* and *L. muscoides,* which could then attract more carnivorous macroinvertebrates.

In addition to different plant species, the RDA (Figure 8) shows that the different dams played a role in shaping the insect communities found on each plant species. One variable that varied between the dams was the surrounding land use (a physical component of the aquatic environment). Consequently, it may be a factor in explaining why *P. pectinatus* supported a more diverse insect community than both species of oxygen weed. Culicidae (mosquitoes) and Muscidae (houseflies) were both found in the *P. pectinatus* dominated farm dam but were absent from Mearns weir, which is surprising considering that they are two very common insects that are extremely tolerant of pollution, having a SASS 5 rating of 1 (Gerber and Gabriel 2002). The most likely reason for this finding is that the cattle on the farm are being continually brought down to the dam to feed and drink, ultimately resulting in large amounts of cow dung being present on the edge of the dam (personal observation). The close proximity to water, dung and cows seems to make the farm dam an ideal place for these two dipterans to reside. While the adult flies and mosquitoes are supported by the cows and dung, the proximity of the water's edge makes it easy for these insects to lay their eggs in the dam. It is important to emphasise that the close proximity of these factors plays a major role; for instance, the land surrounding the Weir is also used for cattle farming, but the cattle are rarely able to get to the waters' edge to drink and defecate.

Another physical variable that was found to vary between the sampling of each plant species was the distance of its replicates from the shore. However, water depth and light penetration did not vary between the replicates for each plant species. Presently, the role that the distances from shore played in shaping the communities is not clear. In fact, it may even be incidental, potentially owing its significance to these replicates being performed on *P. pectinatus* on the farm dam, two variables with much more obvious roles.

The final component of the aquatic environment that could shape the insect communities is its chemical composition. However, it does not seem to play a role in this study. Although the nitrate concentrations are significantly different between Mearns Weir and the farm dam, they both fall within the mesotrophic range (0.5-2.5mg/l of total nitrogen) (DWAF 1996). Additionally, the phosphate levels did not differ between the two dams. The nutrient profiles of the dams are comparable and unlikely to have changed the composition of the insect assemblages. However, nitrates and phosphates are not the only pollutants that degrade water quality; therefore, another estimator is needed. The SASS 5 scoring system allows for an assessment of water quality by identifying the insect families in the aquatic environment (Dickens and Graham 2001). In this study, the most sensitive family to be found was Pyralidae. This family has a SASS 5 score of 12 and is highly susceptible to pollution. Therefore, by finding Pyralidae on both dams, it can be deduced that in addition to having similar nutrient profiles, Mearns Weir and the adjacent farm dam have similar pollution levels. Thus, the chemical properties of these dams are unlikely to have caused the variations between the insects found on *L. major, L. muscoides* and *P. pectinatus* in this study.

Temperature is another macro variable that could affect the insect communities; however, it is not likely to play a role in this study. Insects, especially

the herbivorous species, are generally found near the waters' surface (Biggs and Malthus 1982, Bennett and Buckingham 2000). Consequently, in areas that receive the same amount of sunshine (as occurred in this study) the temperature within a metre of the waters surface is likely to be very similar. Temperature is more likely to affect insect communities on a large scale. For example, N.Z. has cold springs, e.g. Pupu Springs (Michaelis 1977), and glacially formed lakes, e.g. Lake Wanaka (Biggs and Malthus 1982); both of which will have colder water temperatures than the dams in the temperate to tropical KZN province. This fact is important as it highlights the need to survey insect communities in a wide variety of different locations.

Pre-release surveys are vital for efficient evaluation of implemented biocontrol programmes. While this study has begun to create baselines that will prove valuable to both the biocontrol programmes of *L. major* and hydrilla, it also illustrates the need to investigate the biological, physical and chemical components of the environment that shape these communities. Unfortunately, the time constraints of this study limited the number of these variables that it could addressed. However, future studies that are not hampered by these limitations should investigate different macrophyte species, their nutritional content and architecture; different waterbodies with different sizes, substrates, nutrient levels, pollutions levels, temperatures, light penetration, depth, water velocities, positions within the catchment and surrounding land uses. All these variables have the potential to alter the composition of the invertebrate assemblages (Collier 1995, Aguiar and Ferreira 2002, Scallenberg and Waite 2004). In addition, future studies should be done at a variety of times throughout the year as there will be seasonal variations in the biological, physical and chemical components of the environment that will affect the insect communities (Fureder *et al.* 2001)

Another benefit of this holistic approach is its potential to find new or undescribed species. For example, the member of the Ecnomidae found in this study is an undescribed larva of the *Ecnomus* genus. This specimen has been lodged at the

Albany Museum, Grahamstown, S.A., to be documented, recorded and described; thereby, making Trichopteran taxonomy a little more complete, one case at a time.

The final aspect of this study is the role that local research institutes and universities play in surveying their indigenous macrophytes. There is a large financial cost to agent selection, for example McGregor and Gourlay (2002) state that it will cost $160 000 for N.Z. scientists to travel to S.A. and search for natural enemies of *L. major*. However, to determine the invertebrates associated with it in N.Z. will only cost $35 000. Local surveys are much cheaper than overseas ones as they do not require expensive airfares, hotels, etc. Therefore, local research institutes surveying their indigenous flora, such as in this study, could identify potential classical biocontrol agents, e.g. *A. harrisoni* to manage *L. major* in N.Z., at a fraction of the current price.

Complementing this pecuniary benefit, exploratory surveys by local institutes could identify more control agents for more weeds. Despite the fact that *L. major* is a problem plant, N.Z. has not undertaken surveys in S.A. to find its natural enemies because *Ceratophyllum demersum* L. (Ceratophyla) (hornwort) is the most destructive aquatic weed within N.Z., and so the top management priority. However, that does not mean that South African scientists should avoid studying *L. major*; in fact, it should be a source of motivation for South African research institutes to undertake these exploratory surveys; thereby aiding N.Z. and Europe to manage this weed in their countries. This study shows that exploratory surveys by local research institutes can greatly benefit overseas biocontrol programs. Consequently, future studies by South African research institutes should focus on surveying its indigenous macrophytes that are invasive in other countries, for example, *P. crispus* is indigenous to S.A. but invasive in America, Canada and N.Z (http//www.issg.org/database/). The same logic applies to rest of the globe; to illustrate this point, Table 7 provides a list of a few aquatic invasive species that have no identified invertebrate control agents (http//www.issg.org/database/), which could be surveyed by the

research institutes within their host countries. If it became an international trend for countries to survey their indigenous macrophyte species, control agents for more weeds would be identified faster and more cheaply. This, combined with the existing global cooperation between countries invaded by exotic species (McFadyen 1998), could be the best way to refine the biocontrol procedure, which given the current increase in invasives worldwide, would be invaluable.

Table 7. The native ranges of a few aquatic plant species that could be surveyed by the corresponding local research institutes. The natural invertebrate enemies identified in these surveys would then be potential control agents within the areas where the plant species have been introduced. Data compiled from the Global Invasive Species Database (http://www.issg.org/database/).

Plant species	**Native range**	**Introduced range**
Potamogeton perfoliatus	Continental United States of America (U.S.A.) and Europe	Australia and New Zealand
Butomus umbellatus	Africa, Asia and Europe	North America
Hydrocharis morsus-ranae	Asia and Europe	North America
Hydrophila polysperma	Asia	North America
Azolla pinnata	Africa, Madagascar, India, Southeast Asia, Malaysia and the Phillipines	Papua New Guinea, Australia, New Zealand, Vietnam and U.S.A.
Cabomba caroliniana	Brazil, Paraguay, Uruguay and Argentina	Peru, China, Japan, Malaysia, U.S.A. and Australia

To summarise, this study identified seven phytophagous insects associate with *L. major, L. muscoides* and *P. pectinatus*. In addition, it has initiated the recording of insect communities within these macrophyte species, while demonstrating how aquatic ecosystem components shape these assemblages. In doing so, this study has

successfully reached its original aims and so should contribute significantly to any biocontrol programme implemented against *L. major* in its introduced range.

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