Friend or foe? Resolving the status of the submerged macrophyte *Myriophyllum spicatum* L. (Haloragaceae) in southern Africa.

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Abstract

Myriophyllum spicatum L. (Haloragaceae), a submerged macrophyte, has been recorded in southern Africa since 1829, but only considered problematic as recently as 2005. In light of this, water resource managers are looking to control *M. spicatum* in southern African water bodies where it is problematic. Amongst control options available in South Africa, biological control is potentially the most cost effective and sustainable option for *M. spicatum*. However, there is a debate over the status of this plant in southern Africa with several authors reporting it as a native component of the aquatic ecosystem, while others argue that it has been introduced from Europe or Asia. The aim of this thesis is to use a multifaceted approach to resolve the status of *M. spicatum*, by studying aspects of its history, distribution, mechanisms of its adaptations, biotic interactions and genetic relationships in southern Africa. By resolving the status of this plant as either native or exotic, appropriate management strategies can be initiated for its control in situations where it is considered a problem.

A review of the evidence collected from this thesis does not provide convincing evidence for the anthropogenic introduction of *M. spicatum* into southern Africa, and it is probably native to the region. The disjunct distribution as well as regular local extinctions of populations is relatively common for species that are at the edge of their range. The populations in southern Africa could thus be relics from a much wider distribution in the past. The development of local adaptations in southern Africa provides evidence for this and suggests that the populations have been isolated for a substantial period of time and have had a long evolutionary history in the region. The lack of specialist herbivores should suggest that *M. spicatum* has been introduced, but the complete lack of herbivores, including generalists, may weaken that argument. The lack of herbivores could be a result of something inherent in

the plant, irrespective of a lack of evolutionary history in the region. The genetic evidence suggests a European origin, but is characteristic of a population (southern Africa as a whole) that has been isolated for a considerable time.

Despite the findings of this research, *M. spicatum* is considered problematic in southern Africa and warrants control in certain systems. Whether or not biological control should be a component of the management strategy is open to further debate. The benefits in a southern African context may outweigh the risks, based on the specificity of the biological control agent proposed. However, the perceived negative impacts of *M. spicatum* are likely to be a symptom of a more serious underlying cause, such as nutrient loading and changes in land use patterns. Therefore the control of this native species is a water resource management issue and not a biological control issue.

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Chapter 1: General introduction

The geographical distribution of species has long held the interest of ecologists and placing species into measureable geographic units is central in biogeographic research (Mackey *et al.* 2008; Escalante 2009). The current distribution of species is linked to the past and present interactions with biotic, as well as abiotic features of the environment where those species exist (Kreft & Jetz 2010). In recent years, global range expansions of many species have increased dramatically and are mainly linked to the increase in human travel and trade in the last 200 years (di Castri 1989). These introductions, both deliberate and accidental, have been recorded as far back as the 16th century when the Europeans transported Old World species to new locations for agricultural and aesthetic purposes during the Age of Discovery (Crosby 1986). Today, these nonindigenous species that become invasive have significant ecological as well as socio-economic impacts (Wilcove *et al.* 1998), and are rivalled only by habitat destruction as one of the biggest threats to biodiversity (Vitousek *et al.* 1996; Walker & Steffen 1997; Richardson *et al.* 2000).

Fortunately, the majority of these introduced species do not survive in the novel environment and very few become naturalised (Williamson & Fitter 1996). The low survival of new propagules is often linked to the severe conditions they face during the travel period, and upon arrival (Mack *et al.* 2000). Several attributes of both the individual and the community contribute to their success as invaders (Crawley 1987; Rejmanek & Richardson 1996) or their susceptibility to invasion (Simberloff 1986; Crawley 1987) respectively. Aquatic ecosystems are among the most vulnerable to biological invasions, which often have severe consequences for the ecosystem and the services they provide (Sala *et al.* 2000). Notorious invasive aquatic plants such as *Eichhornia crassipes* (Mart.) Solms-Laub.

(Pontederiaceae) (water hyacinth), *Pistia stratiotes* L. (Araceae) (water lettuce) and *Salvinia molesta* D.S. Mitch. (Salviniaceae) (salvinia) have had a long history of invasion and their success is often linked to the high levels of disturbance typical of aquatic systems, including eutrophication from point and nonpoint source pollution, the creation of impoundments, and changes in land use patterns (Carpenter *et al.* 1998; Camargo & Alonso 2006).

Research into the ecology and invasion biology of submerged macrophytes in southern Africa has trailed behind that of floating macrophytes, despite their damaging status (Coetzee *et al.* 2011a). Three species of submerged macrophyte are considered to have important ecological and socio-economic impacts in South Africa. These include *Hydrilla verticillata* (L.f.) Royle (Hydrocharitaceae), *Egeria densa* Planch. (Hydrocharitaceae) and *Myriophyllum spicatum* L. (Haloragaceae) (Coetzee *et al.* 2011a). These species vary in the extent of their distributions in South Africa; *H. verticillata* is confined to one water body, the Pongolapoort Dam, while *E. densa* is mainly confined to the coastal provinces of South Africa, including the Western Cape, Eastern Cape and KwaZulu-Natal but also occurs in the Limpopo Province (Coetzee *et al.* 2011a). *Myriophyllum spicatum* on the other hand, has the widest distribution of all the submerged species and has been recorded in all nine provinces of South Africa, as well as several other countries in southern Africa (Coetzee *et al.* 2011a; Weyl & Coetzee 2014). Many species of exotic submerged macrophyte have been recorded in South Africa but are not yet considered problematic, including *Cabomba caroliniana* A. Gray (Cabombaceae) and *Elodea canadensis* Mitch. (Hydrocharitaceae) (Coetzee *et al.* 2011a). Many of these are serious weeds in other regions of the world and control of these species in South Africa needs to be initiated to curb any negative effects that they cause in the region. Of the submerged species present in southern Africa, *M. spicatum* is one of the priority species for control, which has led to further investigations into its distribution,

competitive interactions, potential control options and management strategies (Fordham 2012; Martin 2013; Weyl & Coetzee 2014)

1.1 *Myriophyllum spicatum*

Within the Haloragaceae, the genus *Myriophyllum* L. is the most speciose and has a cosmopolitan distribution, with species recorded on all the continents except Antarctica (Moody & Les 2010). The genus has its centre of diversity in Australia with 42 species, of which 37 are endemic; Asia has 16 species, of which eight are endemic; and North America has 14 species, of which seven are endemic (Moody & Les 2010). Several species within the genus are recognised as invasive worldwide, including *M. spicatum*. There is general consensus that *M. spicatum* has a Eurasian distribution and is native to Europe, some parts of Asia and North Africa (Reed 1977; Cook 1985; Couch & Nelson 1985). However, some authors consider *M. spicatum* to be naturally distributed over a wider range in Africa to include the highlands of the tropical east and the south (van der Meijden & Caspers 1971; Cook 2004).

1.2 Description and life history of *Myriophyllum spicatum*

Myriophyllum spicatum is a rooted, submerged aquatic, perennial herb with a branching leafy shoot ranging from 0.4 to 7 m in length (Aiken *et al.* 1979; Cook 2004). The leaves grow in whorls of (3) 4 (5) and are pinnately divided with 4-24 pairs of threadlike segments (Figure 1.1) (Aiken *et al.* 1979; Cook 2004). The inflorescence is on a terminal spike which is between 5 and 20 cm long. The stem below the spike is often almost double the width of the rest of the stem, and usually very ridged (Aiken *et al.* 1979). The bracts are scale-like and found at the base of the inflorescence and are equal to, or slightly longer than, the fruits or flowers, lanceolate with the margins either toothed or entire; the upper bracts are shorter than the flowers, entire and elongate to rhomboid; the bracteoles are almost circular to

kidney shaped in outline (Cook 2004). The flowers are in whorls of 4 (Figure 1.1), the lowest are female, then bisexual with only male flowers found at the top of the flower spike (Cook 2004). The fruits are almost spherical, about 3mm long and wide with eight longitudinal warty ribs and at maturity, separate into 4 nutlets (Cook 2004).

Sexual reproduction is considered relatively insignificant by several authors, with auto-fragmentation as the main mode of reproduction which usually occurs after the flowering period, resulting in a reduction in the standing biomass at the end of the growing season (Patten 1956; Aiken *et al.* 1979; Bates *et al.* 1985; Smith & Barko 1990). However, Adams & McCraken (1974) suggest that if flowering occurred early enough in the season, two peaks in plant abundance and biomass may be achieved. The flowering season in southern Africa is between the months of September and February (Chapter 2) which is early in the growing season, considering that temperatures only begin to drop in April. This may lead to a double peak in biomass and abundance due to the early flowering season as Adams & McCraken (1974) suggested. In the northern hemisphere, the flowering season is much later in summer and peaks between the months of July and September (Patten 1956), which corresponds with February to April in southern Africa. However the flowering season can be extremely variable depending on geographic location, climate, water quality and hydrodynamics of the water body (Madsen *et al.* 1988; Smith & Barko 1990).

The plants follow a characteristic growth pattern where standing biomass increases in spring, the lower leaves usually senesce due to shading (Adams *et al.* 1974), and the stems branch profusely when they reach the surface (Smith & Barko 1990). In southern Africa, three morphological varieties of *M. spicatum* occur in specific biogeographic regions (see Chapter 3). The varieties are so different morphologically that it was thought that there was a possibility of at least two species in southern Africa (Jacot Guillarmod 1979). However, once pollen grains from the southern African plants were collected and analysed using a scanning

electron micrograph (SEM), it was concluded that the plants were all the same species, *M. spicatum* (Jacot Guillarmod 1979). In addition to the pollen grains, genetic analysis of the plants in southern Africa also confirms that they are *M. spicatum* (see Chapter 5).

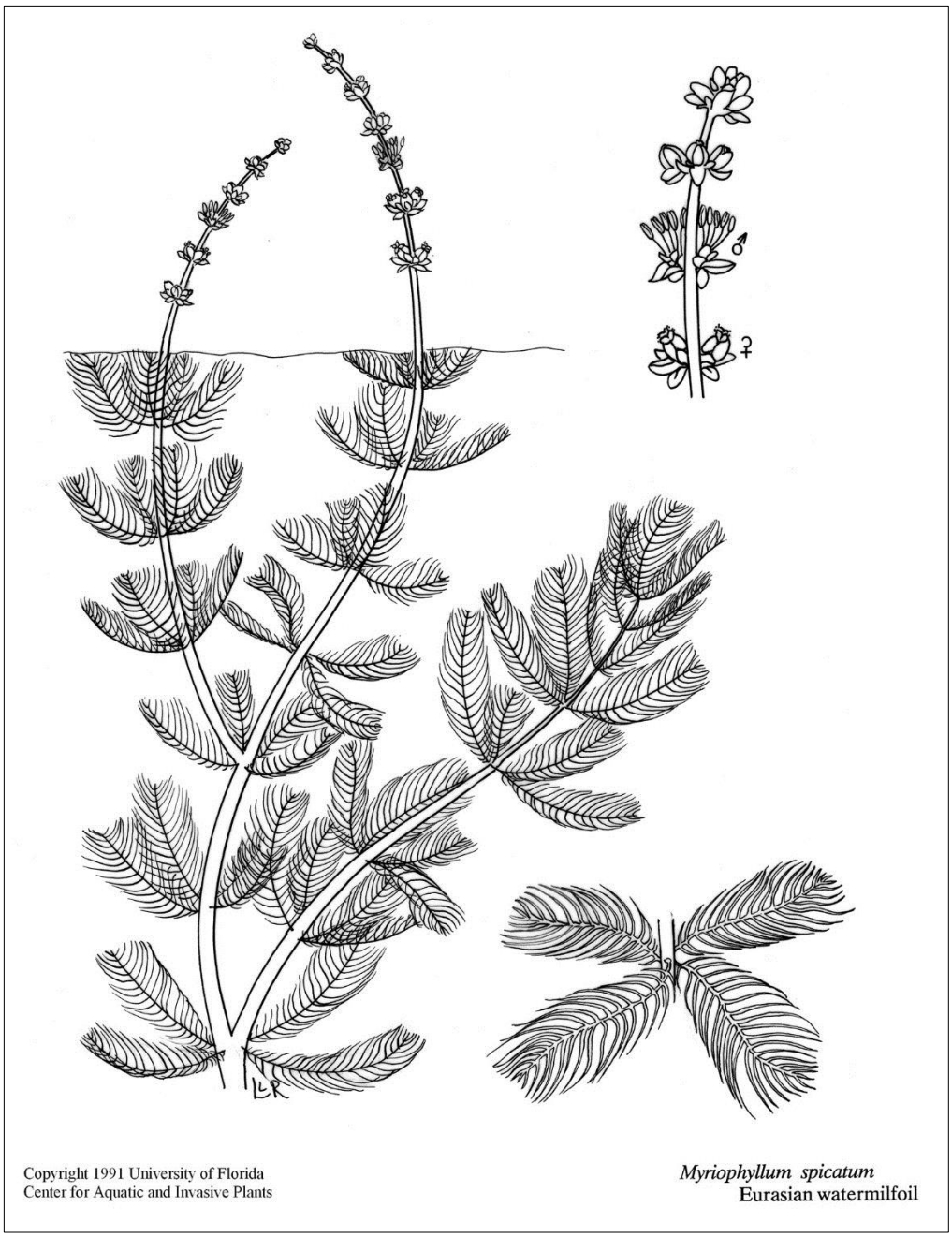

Figure 1.1 A drawing of *Myriophyllum spicatum* L. (Haloragaceae) showing the diagnostic morphological characteristics. Illustration provided by IFAS Center for Aquatic and Invasive Plants, University of Florida. Used with permission.

1.3 Impacts and control of *Myriophyllum spicatum* invasions

Myriophyllum spicatum is considered an important weed where it has been introduced (Patten 1956; Reed 1977; Couch & Nelson 1985; Smith & Barko 1990; White *et al.* 1993, Johnson & Blossey 2002). In North America, *M. spicatum* is one of the most damaging aquatic weeds, which has been implicated in having several negative effects on biodiversity, ecosystem processes, as well as socio-economic impacts (Grace & Wetzel 1978; Bates *et al.* 1985; Newroth 1985; Madsen 1997; Eiswerth *et al.* 2005). Where *M. spicatum* reaches high densities, it not only affects the physical characteristics of a water body, but also affects the chemical composition by reducing the available oxygen, resulting in changes in pH, and increasing local temperatures (Bates *et al.* 1985). *Myriophyllum spicatum* has been shown to outcompete native vegetation through overcrowding and competition for resources. This has been linked not only to the extremely high growth rates and biomass turnover (Smith & Adams 1986; Madsen *et al.* 1991), but also to the high resource use efficiency, such as nutrient uptake and photosynthesis, compared to native species (Grace & Wetzel 1978). The mono-specific beds have cascading effects on large predator species where there is less food availability and reduced feeding efficiency in largemouth bass, *Micropterus salmoides* (Lacépede) (Centrarchidae) (Engel 1987). The socio-economic impacts of this species include reduced recreational activities on an invaded water body (Newroth 1985), the blocking of irrigation canals, pipes and pumps, and increasing the costs related to hydropower production (Bates *et al.* 1985).

It costs the United States of America (U.S.A.) millions of dollars annually (between 37 000-500 000 US$ per km^2) for its removal from water bodies (White *et al.* 1993). In North America, *M. spicatum* is most frequently controlled by the use of herbicides and mechanical harvesting, which proves extremely expensive and often has non-target effects on native or beneficial organisms (Smith & Barko 1990; Nichols 1991; Cooke *et al.* 1993). In addition to

this, it has been shown that *M. spicatum* can, in some cases, respond positively to disturbance, so attempts to control the plant using mechanical methods often results in an expansion of its current range (Carpenter 1980; Einarson 1987). This led to investigations into biological control of this species in North America and the first biological control programme of *M. spicatum* began in 1965 (Sheldon & Creed 1995; Cock *et al.* 2008). This was due to the several advantages that biological control has to offer, which include a high cost-benefit ratio, with long term sustainability and little to no non-target effects (McFadyen 1998). Since then, several insects have been found that are associated with and feed primarily on *M. spicatum* (Sheldon & Creed 1995; Cock *et al.* 2008), with the most effective and commonly used species being the North American weevil, *Euhrychiopsis lecontei* (Dietz) (Curculionidae) (Creed & Sheldon 1995; Sheldon & Creed 1995; Newman *et al.* 1996). *Euhrychiopsis lecontei* is a water milfoil (*Myriophyllum*) specialist whose larvae cause the most damage by consuming the meristems and mining the stems (Sheldon & O'Bryan 1996). The biological control of *M. spicatum* using *E. lecontei* in North America has largely been successful (albeit dependent on water body and local conditions) and is implemented as a control option throughout the continent (Sheldon & Creed 1995; Newman 2001; Alwin & Cheruvelil 2009).

1.4 *Myriophyllum spicatum* in southern Africa

Myriophyllum spicatum has been recorded in South Africa since 1829 (Weyl & Coetzee 2014) but has only been reported as being problematic on one river system, the Vaal River, as recently as 2005 (Coetzee *et al.* 2011b). This has been attributed to the successful integrated control programme against *E. crassipes* on the Vaal River (Coetzee *et al.* 2011a). Massive reductions in surface cover which has allowed light to penetrate the water and the heavy nutrient loads of the system have encouraged submerged macrophyte growth and allowed *M. spicatum* to proliferate (Coetzee *et al.* 2011a). In water bodies where *M. spicatum*

is considered problematic, water resource managers are looking for control measures to be initiated (Coetzee *et al.* 2011a). There is no reference to *M. spicatum* being problematic in other countries of southern Africa where it is found, but Wild (1961) made reference to *M. spicatum* being very dense in some parts of the Kafue River, Zambia. In South Africa, *M. spicatum* is listed under the National Environment Management: Biodiversity Act (NEMBA) and Conservation of Agricultural Resources Act (CARA), 1983 as a Category 1 weed, which is the highest category an exotic weed can attain. This was done as a precautionary measure due to the plant's weedy behaviour in other parts of the world, especially the U.S.A. (Henderson, pers. comm.). The control of *M. spicatum* in South Africa is limited as there are no herbicides registered against it and manual removal is considered too expensive and requires skilled labour, so biological control is seen as the only viable option (Coetzee *et al.* 2011a). However, there is debate over whether *M. spicatum* is native to southern Africa, and this must be resolved before a biological control programme can be initiated.

1.5 Controversial status of *Myriophyllum spicatum* in southern Africa

In 1985, in a publication of the distribution of the genus *Myriophyllum*, southern Africa was not considered the native range of *M. spicatum* (Cook 1985), however in 2004, the aquatic plants guide "Aquatic wetland plants of southern Africa" considers *M. spicatum* native to southern Africa, with a native distribution in South Africa, Namibia and Botswana (Cook 2004). At the time of writing this book, Cook assumed it to be native because there was no evidence to suggest that *M. spicatum* was introduced and it was not considered problematic, so there was no reason for it to be labelled an exotic species (Cook, pers. comm.). Dr Amy Jacot Guillarmod (ex-Institute of Freshwater Studies, Rhodes University), also considered *M. spicatum* an indigenous part of the aquatic flora of southern Africa (Jacot Guillarmod 1976; 1979). In addition to this, several studies conducted in northern KwaZulu-Natal, South Africa, have not made any reference to *M. spicatum* being introduced or

problematic, and often refer to it as an important component of the aquatic flora of the region (Boltt 1969; Boltt *et al.* 1969; Breen & Jones 1971; Allanson *et al.* 1974; Howard-Williams 1979). Van der Meijden & Caspers (1971) consider *M. spicatum* a native component of the aquatic flora in the highlands of East Africa, and according to the distribution map they published, the whole of southern and east Africa is part of the native distribution. In southern Africa, the paleontological records of Haloragaceae pollen date back to 1800 – 12 000 years BP in the northern KwaZulu-Natal and Limpopo regions of South Africa (Scott 1987; Neumann *et al.* 2008). These studies suggested that the Haloragaceae in these regions was represented by one species, *M. spicatum* which was assumed to be present in the region at least 12 000 years BP (Scott 1987; Neumann *et al.* 2008). This is somewhat speculative, as the pollen was never properly identified to species, only inferences were made from the most common abundant, extant taxa at the site. Gandolfo *et al.* (2008) and Muller (1984) caution against the use of fossil pollen records for identification to species level, because of the low number of characters available, which can become ambiguous with age. On the other hand, Mendes (1978) reports in Flora Zambesiaca that the genus *Myriophyllum* is represented by two species south of the Sahara, namely *Myriophyllum aquaticum* (Vellozo Conceição) Verd. (Haloragaceae) and *M. spicatum*, both of which are probably introduced. There is thus no general consensus on the status of *M. spicatum* in southern Africa as native or exotic.

1.6 Native vs. exotic

The terms indigenous or native, and exotic or introduced, are at times used loosely by biologists and ecologists. By definition, indigenous or native refers to a species that occurs naturally in an environment or natural habitat and is consistently associated with certain other species in those habitats; while exotic or nonindigenous refers to a species that occurs in an area to which it is not native or has been introduced by humans, either intentionally or unintentionally (Lincoln *et al.* 1993). These definitions suggest that any species that has

found its way into a region through anthropogenic means would be exotic or nonindigenous. However, when the mode of introduction for a particular species is unclear, it can be difficult to determine whether a species is indigenous or not. There is no checklist or set of characters that will separate species into these definitions or terms which, in some cases, can become somewhat of a "red herring". Rather, the manner in which the species interacts with other species and the ecosystem as a whole can provide important evidence that may suggest it is or is not an indigenous component of the native flora.

When a species becomes naturalised and expands its distribution in the introduced area or region, it must interact with native species in the invaded ecosystem (Sax 2001). In many cases, the interactions can have implications for native biodiversity and can cause changes at the community, ecosystem and landscape levels (Crawley 1997; Walker & Steffen 1997; Simberloff & Stiling 1996; Midgley *et al.* 2006; Vilá *et al.* 2011; Kadye & Booth 2012; Coetzee *et al.* 2014). In this thesis, I adopt the notion that if a species is to be considered exotic, there must be evidence for an anthropogenic means of introduction. However, in the absence of conclusive evidence for anthropogenic introductions, understanding the interactions that a species has with the ecosystem may provide evidence of evolutionary history in the area. The underlying mechanisms of exotic macrophytes that affect biodiversity in freshwater systems are not fundamentally different to native macrophytes (Schultz & Dibble 2012), but exotic species with a short evolutionary history would result in fewer positive interactions and potential negative effects on the environment, similar to that of known invasive species.

1.7 A measure of evolutionary history

Several aspects of a plant's ecology can be explored for evidence supporting the native vs. exotic debate. In many cases, these interactions need to be carefully interpreted and fully understood before conclusions on evolutionary history within a region are made.

1.7.1 Phenotypic plasticity vs. local adaptations

Aquatic plants are commonly associated with variable morphology or growth forms between populations, which are usually a result of the environmental conditions experienced (Barrett *et al.* 1993; Idelstam-Almquist & Kautsky 1995). Understanding the drivers or mechanisms responsible for these variations can provide important evidence for the evolutionary history of a species in a region. Phenotypic plasticity is the ability of genetically similar individuals to exhibit different growth forms or morphology (Bradshaw 1965; Sultan 2000). Local adaptation, on the other hand, occurs when the beneficial morphological traits are selected for, resulting in a genetic shift of the population due to natural selection pressures (Kawecki & Ebert 2004; Ward *et al.* 2008). Both adaptive mechanisms have been commonly documented in aquatic plants (Santamaria *et al.* 2003; Geng *et al.* 2007; Riis *et al.* 2010). If the morphological differences between populations in a region are based on phenotypic plasticity, one would expect low genetic diversity between populations and a potentially short evolutionary history in the region (Ward *et al.* 2008). However, if local adaptations were responsible, one would expect a relatively diverse gene pool and a long evolutionary history under the current abiotic conditions (Ward *et al.* 2008).

Introduced populations are often characterised by low levels of genetic variation, and morphological changes experienced between populations are often linked to phenotypic plasticity (Geng *et al.* 2007; Riis *et al.* 2010). In a study of the adaptive mechanisms of *Alternanthera philoxeroides* (Mart.) Griseb. (Amaranthaceae) in China, Geng *et al.* (2007) concluded that phenotypic plasticity was responsible, based on the relatively low genetic diversity, despite the morphological differences experienced between populations. A similar

study in New Zealand found that differences in the morphology of three invasive species of Hydrocharitaceae including *E. densa*, *E. canadensis* and *Lagarosiphon major* (Ridl.) Moss (Hydrocharitaceae), were also based primarily on phenotypic plasticity (Riis *et al.* 2010). Again these species exhibited low genetic differentiation between populations in New Zealand (Riis *et al.* 2010).

Studies have identified local adaptation as an important adaptive mechanism for several aquatic species, but in most cases these studies are conducted with native populations (Koch & Seelinger 1988; Barrett *et al.* 1993; Idelstam-Almquist & Kautsky 1995). The genetic diversity among native populations is inherently higher than in introduced populations and the evolutionary history is longer, so local adaptations are not surprising. In a study by Santamaria *et al.* (2003), the adaptive mechanisms accounting for the differences in morphology of *Stuckenia pectinata* (L.) (Syn. *Potamogeton pectinatus*) (Potamogetonaceae) populations around the Baltic Sea were tested using a large-scale transplanting experiment where individuals from source populations were swapped. The results suggested that plants grew better at the source population than at the novel locations (Santamaria *et al.* 2003). They concluded that local adaptations were responsible for the morphological differences, probably based on the relatively high genetic diversity of the populations (Santamaria *et al.* 2003).

High genetic diversity is characteristic not only of native populations; introduced populations have also been shown to have relatively high genetic diversity usually linked to their invasion history (Ward *et al.* 2008; Thum *et al.* 2011). Introduced populations of a species can have high genetic diversity if a large number of propagules are introduced from a genetically diverse source population or if multiple introductions from either a geographically wide area in the native range or a genetically diverse source population are involved (Ward *et al.* 2008). In such cases it is possible for introduced populations to undergo evolution of local

adaptations based on novel environmental conditions, but this would still involve a relatively long evolutionary history (Lavergne *et al.* 2010).

Southern Africa has three morphological varieties of *M. spicatum* which grow in different biogeographic regions (Jacot Guillarmod 1979). The varieties have been in southern Africa since the first herbarium records of *M. spicatum* in these regions. The Vaal River variety was first recorded in 1877, the Mooi River variety in 1885 and the Lake Sibaya variety in 1965 (see Chapters 2 & 3). Identifying the adaptive strategy of these plants as either phenotypic plasticity or local adaptations will provide evidence for genetic diversity and potential evolutionary history within the southern African populations.

1.7.2 Plant – herbivore interactions

The number of herbivorous species associated with a plant in a region or habitat can provide evidence of its evolutionary history in the region (Strong 1977; Strong *et al.* 1984; Brändle *et al.* 2008). Species with a long evolutionary history in a region are usually characterised by a rich diversity of herbivores associated with them, which can be explained by the 'host-age hypothesis' or the 'species-time hypothesis' (Fischer 1960; Brändle *et al.* 2008). However, several studies have argued that other factors play a role in the colonisation by herbivores of host plants in a region (Strong 1977; Strong *et al*. 1984; Soldaat & Auge 1998; Agrawal & Kotanen 2003; Agrawal *et al.* 2005; Schoonhoven *et al.* 2005). The ultimate number of phytophagous insect species using a particular species of plant has been shown to be dependent on the initial pool of phytophagous insects, the distributional range of the host species and the genetic similarities between the introduced species and other plants in the region (Strong 1977; Strong *et al*. 1984; Soldaat & Auge 1998; Agrawal & Kotanen 2003; Agrawal *et al.* 2005; Schoonhoven *et al.* 2005). Evidence from invasion biology suggests that introduced plants have a depauperate fauna of insect herbivores associated with them, but there is a possible link between time since introduction and the number of insect

herbivores (Brändle *et al.* 2008). In addition to fewer herbivores, the species usually comprise mainly generalist or polyphagous herbivores that have a wide host range (Strong *et al*. 1984; Jobin *et al*. 1996). Generalist herbivores are likely to have pre-adapted mechanisms to overcome the wide variety of defences that plants have to offer, which makes them more likely to colonise a novel host first (Ando *et al.* 2010; Brändle *et al.* 2008). Wapshere (1974) argues that the best source of host specific insects is the plant's centre of origin, which is likely to be the region with the longest evolutionary history. This is due to the intricate relationship between specialised insects and their host plant and the many physical and chemical defences that the plant has developed (Frenzel & Brandl 2003). In addition to the lack of specialist herbivores on exotic plant species, the community composition is often dominated by the abundance of one or a few herbivore species (Ando *et al.* 2010).

The herbivores associated with two species of *Solidago* (Asteraceae) in Japan are a classic example of differences in the herbivore abundance and community structure (Ando *et al.* 2010). There is a reduced diversity of native herbivores on the introduced species, *Solidago altissima* L. (Asteraceae), compared to the native *Solidago virgaurea* L. (Asteraceae) (Ando *et al.* 2010). The abundance of herbivores on the exotic *S. altissima* was largely dominated by two species, the aphid, *Uroleucon nigrotuberculatum* (Olive) (Aphididae) and the scale insect, *Parasaissetia nigra* (Neit.) (Coccidae), both of which are also introduced to Japan (Ando *et al.* 2010). In addition to this, in other introduced ranges, the majority of herbivores of *S. altissima* are polyphagous. In Switzerland this consists of 97% of all insect herbivores (Jobin *et al.* 1996). This is in contrast to the native range, where in North America several specialised herbivores are oligophagous and even monophagous (Abrahamson & Weiss 1997). These studies highlight the importance of not only understanding the community richness of herbivores, but also the interactions between

community composition and abundance of species, as well as the biology of the herbivores associated with a study species.

A meta-analysis of phytophagous insect species associated with 103 woody exotic species in Europe found that, in addition to time, in the introduced region, range size is important in determining the herbivore species richness (Brändle *et al.* 2008). As host plant range size increases, there is a general trend of increasing herbivore species richness, especially with the lepidopterans (Brändle *et al.* 2008). Herbivores of British trees tend to show similar patterns, where introduced trees have higher numbers of insect herbivore species as range size increases (Strong 1974; Southwood *et al.* 1982).

Not only range size, but also the taxonomic isolation of the exotic species is important in determining the species richness where the more isolated the plant, the fewer the species of herbivore associated with it (Connor *et al.* 1980; Brändle *et al.* 2008). Several studies demonstrate the importance of relatedness of the host species to natives in the introduced region. The herbivores associated with exotic species in Australia show a clear trend between species richness and phylogenetic relatedness, with a decrease in herbivores as phylogenetic distance of the host increases (Harvey *et al.* 2012), and is a characteristic of several introduced species in Australia. Hill & Kotanen (2010) found that herbivory on exotic Asteraceae was, on average, lower than that on native species and was generally lower as phylogenetic relationships between species decreased. Brändle *et al.* (2008) showed that the number of leafhopper species associated with exotic woody species in Europe also declined on distantly related species, with the highest number of species on plants with native con-specifics.

Assessing the diversity of phytophagous insects is a critical step towards inferring the evolutionary history of *M. spicatum* in southern Africa. The longer the time spent in the

region, the greater the number of phytophagous insects species expected to be associated with it.

1.7.3 Plant – plant interactions

Competitive interactions between species, especially in plant communities, are an important driver in the community structure, as well as the distribution of species in the ecosystem (McCreary *et al.* 1983). The ability of exotic species to outcompete native species in the introduced region is one attribute that has been used to explain the success of many invader species worldwide (Richardson & Pysek 2006; Pysek & Richardson 2007). Competitive interactions between species may be geographically different and species may have stronger competitive effects on species in the introduced range than on species from the native range (Ni *et al.* 2010; Callaway *et al.* 2011). Spotted knapweed, *Centaurea stoebe* L. ssp. *micranthos* (Gugler) Hayek (Asteraceae) in North America, the introduced range, exhibits much lower inhibitory effects by neighbouring vegetation when compared to European grasslands (Callaway *et al.* 2011). Callaway *et al.* (2011) performed a neighbour removal experiment in both the introduced range and the native range, where potential competitors of *C. stoebe* plants were effectively removed and the plants were allowed to grow freely. The response of *C. stoebe* was significantly different between the native and introduced range, with a significant increase in growth parameters in the native range and no difference in the introduced range (Callaway *et al.* 2011). This suggests that the plants in the native range experience significantly more competition and their growth and potential range expansions are kept in check by native competitors (Callaway *et al.* 2011). The mechanisms underlying this difference in competitive ability between geographically distinct populations were not studied in this case, and in many ways it raises questions of invasive populations of the same species. Different populations of the same species may exhibit differences in invasive characteristics, where some remain at low population levels with limited impacts in

the introduced region, while others obtain a high biomass and are considered problematic (Richardson & Pysek 2012).

Understanding the interactions between species, in terms of competitive ability may give insights into potential evolutionary history between the species. The competitive effects of *Acroptilon repens* (L.) DC. (Asteraceae) on plants that share a native range (i.e. shared evolutionary history) were lower than on naïve plants from the introduced range (Ni *et al.* 2010). In this case, the differences were thought to be driven by the 'Novel Weapons Hypothesis' whereby *A. repens* produces allelochemicals that could potentially outcompete other species (Ni *et al.* 2010). The results from this study suggest that species originating within the native range of *A. repens* were less affected than species from the introduced range because these species coevolved with *A. repens* to cope with the allelochemicals (Ni *et al.* 2010). Sun *et al.* (2013) showed that in competitive experiments between *C. stoebe* and species from both the native range and the introduced range, the species that suffered the most were species originating from the introduced range. This was largely attributed to species in the native range competing for limited resources while in the introduced range, it is possible that *C. stoebe* is better equipped to exploit resources than native species (Sun *et al.* 2013). Competition for resources, or resource use efficiency, has been used in the past to explain the success of introduced species (Funk & Vitousek 2007; Ren & Zang 2009).

Investigating the population dynamics and interactions of *M. spicatum* with other submerged macrophytes will provide important insights into the evolutionary history of this species in southern Africa, where positive interactions would suggest a long evolutionary history.

1.7.4 Genetic relationships between populations

Genetic techniques have been used extensively in invasion biology to determine the source population and invasion histories of several introduced species. An introduced population will carry a genetic footprint from its native range or population into the introduced range, and irrespective of which molecular markers are used, often this footprint can be traced to a region of origin (Madeira *et al.* 1997; Novack & Mack 2001; Gaskin *et al.* 2005; Goolsby *et al.* 2006; Taylor & Keller 2007; Paterson *et al.* 2009; Gaskin *et al.* 2011; Thum *et al.* 2011; Paterson & Zachariades 2013).

Paterson *et al.* (2009) investigated the geographic origins of the South African populations of *Pereskia aculeata* Mill. (Cactaceae), an invasive climbing cactus. The results demonstrated that these populations are closely related to the southern populations in the native range, with origins likely to be from Rio de Janeiro Province, Brazil (Paterson *et al.* 2009). But the genetic distance between the South African populations and the native populations suggests that although the plants possibly originated from the southern populations, the plants in South Africa were distinct (Paterson *et al.* 2009). In this case, the South African *P. aculeata* populations are characterised by low levels of genetic diversity and are monophyletic, suggesting that they are probably the result of a single introduction (Paterson *et al.* 2009).

The level of genetic diversity within and between populations in a given region can provide evidence for the history of a species, where many introduced populations are characterised by low genetic variation (Geng *et al.* 2007; Lambertini *et al.* 2010; Riis *et al.* 2010). This is usually linked to the introduction of low numbers of propagules from a single source population, population bottlenecks upon establishment and clonality within populations (Gornall *et al.* 1998; Parker *et al.* 2003; Thum *et al.* 2011).

Comparing molecular markers from southern African populations of *M. spicatum* to populations in the Eurasian range will therefore provide useful insights into the relatedness of the populations, potential source populations and genetic diversity within southern Africa.

1.8 Thesis aims

This thesis aims to resolve the controversial question of whether *M. spicatum* is native or exotic to southern Africa using a multifaceted approach, by studying several aspects of the biology, ecology and history. An informed decision will allow appropriate management strategies to be put in place, with or without biological control as part of the programme, depending on the plant's status.

1.9 Thesis outline

The first step in determining the status of a species as either native or exotic to a region or area is to link the presence of that species to a recent anthropogenic means of introduction. Chapter 2 investigates the history and distribution of *M. spicatum* in southern Africa. The history and distribution of a species in a region as described by historical records from herbaria, field notes and published articles, as well as books, holds a wealth of information. This information can be extremely important in tracing the history, potential mode of introduction, spread and the potential origins of a plant.

In the absence of conclusive evidence for the introduction of a species, attributes of its ecology can provide evidence of an evolutionary history in an area, assuming that a long evolutionary history in a region suggests that the species is native, while a short history suggests that the species is introduced. Chapter 3 investigates the morphological differences between three populations of *M. spicatum* in southern Africa. The drivers of these morphological differences, phenotypic plasticity vs. local adaptations, will give an indication

of the evolutionary history of each population, possible genetic diversity and the potential or amount of gene flow between these populations (i.e. the isolation of each population).

Introduced macrophytes are known to have negative effects on native biodiversity (Wilson & Ricciardi 2009), outcompete native macrophytes (Stiers *et al.* 2011) and have depauperate herbivore associations (Dray *et al.* 1993). To understand the interactions of *M. spicatum* with other species in aquatic ecosystems of southern Africa, Chapter 4 investigated the herbivore associations, macroinvertebrate diversity and the interactions between *M. spicatum* and other species of macrophyte in two major water bodies in South Africa, the Vaal River and Lake Sibaya.

Thereafter, a global genetic study is discussed in Chapter 5, where the relatedness of the southern African populations to the rest of the native range is investigated. Genetic diversity and relatedness is extremely important and molecular markers can provide invaluable clues, not only to the mode of introduction, but also to the region of the source population. Molecular genetic markers have been used by several studies to determine the origins of particular plants or populations of plants in an introduced range (Hollingsworth *et al.* 1996; Milne & Abbot 2004; Goolsby *et al.* 2006; Madeira *et al.* 2007)

The general discussion, Chapter 6, discusses the aspects that have been investigated in Chapters 2 through 5 in an attempt to determine whether *M. spicatum* has been introduced into southern Africa, with the aim of making recommendations for the control of this species in a southern African context.

Chapter 2: Does the history and distribution of *Myriophyllum spicatum* L. (Haloragaceae) in southern Africa suggest an anthropogenic origin?

2.1 Introduction

Investigations into the history and distribution of a species that may have been anthropogenically introduced provides fundamental information of their possible region of origin and spread in the invaded range. Studies on species distributions that use information collected from herbarium specimens and careful observations by biologists have provided a wealth of information for many species (Jacot Guillarmod 1979; Stuckey 1980; Couch & Nelson 1985; Novack & Mack 2001). For example, the origin of the invasive cheatgrass, *Bromus tectorum* L. (Poaceae), in North America, has been identified through a careful examination of the historical records, which have been corroborated through genetic techniques (Novack & Mack 2001). Not only the region of origin, but also the invasion dynamics of a species can be understood through the examination of historical records. The invasion by *Lythrum salicaria* L. (Lythraceae), a notorious wetland invader in North America, has been synthesised by Stuckey (1980) who noted that this species spreads relatively easily to new localities, but there is a long lag phase before it becomes abundant in the novel location. This lag phase typically ranges from 20 – 40 years, which provides important information for management strategies (Stuckey 1980).

The initial historical and distribution work on *Myriophyllum spicatum* L. (Haloragaceae) in North America by Reed (1977) reports the first recorded specimen in 1881 from the Potomac River, Virginia. Aiken *et al.* (1979) support this and assume the mode of introduction to be from ship ballast water into Chesapeake Bay, Virginia. This work was complicated by the presence of another very similar species, *Myriophyllum sibiricum*

The data from this chapter are published.
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Komarov. (ex. *Myriophyllum exalbesens* Fern.), which, in the absence of turions, is extremely difficult to separate from *M. spicatum* (Aiken 1981). Couch & Nelson (1985) re-analysed the distribution of the species where they annotated and photographed over 25 000 specimens in the genus *Myriophyllum* and found that the earliest confirmed record of *M. spicatum* was in a pond in Washington D.C., in 1942. According to these dates and vicinity to the United States Department of Agriculture (USDA) laboratory, Couch & Nelson (1985) assumed that the introduction of *M. spicatum* into North America was either through an intentional introduction by the Plant Introduction Branch of the USDA or through the aquarium trade, or potentially both. The mode of introduction, distribution and spread of *M. spicatum* in North America suggests that this species was introduced and spread through the country anthropogenically. Long distance dispersal has been linked to the aquarium and aquatic nursery trade (Reed 1977; Couch & Nelson 1985), while the rapid dispersal over shorter distances has been linked to the transport of boats and trailers between lakes (Johnson *et al.* 2001).

Similarly, the introduction and spread of its congeneric, *Myriophyllum aquaticum* (Vell.) Verd. (Haloragaceae), a declared invasive in South Africa, has been carefully documented through herbarium specimens by Jacot Guillarmod (1979). *Myriophyllum aquaticum* was first recorded in South Africa in the Western Cape in 1919. It was introduced from South America and rapidly spread throughout southern Africa (Jacot Guillarmod 1979), and is considered one of South Africa's worst aquatic weeds (Hill 2003; Coetzee *et al.* 2011b). It had a lag period of about 40 years before it began to be reported as a nuisance. The first to publish this plant as being seriously problematic were Piaget & Schliemann (1973) in the Western Cape, South Africa. The initial mode of introduction was unclear, however, the range expansion and distribution of this plant closely follows the introductions of fish fry from the Jonkershoek hatchery since 1929, when it was reported to have infested portions of

the hatchery system (Jacot Guillarmod 1979). The Amalinda fish farm in the Eastern Cape has also been implicated in the spread of *M. aquaticum* in South Africa, particularly the Eastern Cape, again through the introduction of fish fry into various water bodies, including the Assegaai River (Jacot Guillarmod 1979). These fish farms are not the only modes of spread for this species, as it is a popular pond and aquarium plant and many introductions and spread of aquatic species have been linked to the aquarium trade (Martin & Coetzee 2011). While the introduction of this plant is unclear, the timing and spread from source populations (Jonkershoek and Amalinda) is clearly anthropogenic.

In the family Haloragaceae, only two species within the genus *Myriophyllum* occur in southern Africa, *M. spicatum* and *M. aquaticum* (Cook 2004). *Myriophyllum spicatum* was first recorded in South Africa in 1829 from the Swartkops River, Eastern Cape. It has a wide geographical distribution in southern Africa, covering several climatic zones (Weyl & Coetzee 2014), but has rarely been considered problematic, until recently on the Vaal River (Coetzee *et al.* 2011b). The aim of this chapter is to review the history and distribution of *M. spicatum* through herbarium records to determine a potential mode or pathway of introduction and sources of spread through southern Africa.

2.2 Materials and Methods

In order to describe the distribution of *M. spicatum* in southern Africa, historical records were collected from herbaria in the southern African region. Each specimen was carefully examined and photographed for correct identifications and to assess the reproductive state of the plants (i.e. presence of flowers and seeds). All relevant information was extracted from the specimen label, including the collector, collector number, location or location description, GPS coordinates where possible, and any field notes about the specimen. In addition to this, all the southern African records of *M. spicatum* were

downloaded from the GBIF database (Global Biodiversity Information Facility; http//.www.gbif.org) as supplementary or additional records. The records between the different herbaria and GBIF were then cross referenced and any duplicates were removed from further analysis. Observational records were also removed due to the possibility of misidentifications. A qualitative analysis and description of the historical records was given, while the locality data (coordinates) from the herbarium samples were analysed in ArcView V.9. (ESRI 2012) and the points were plotted on a map of southern Africa.

The populations of *M. spicatum* in each river system were allocated a status as either established in the system or casual. A species is considered established in a habitat when a population persists for several generations, at least 10 years for plants (Richardson *et al.* 2011), however if a species cannot persist for whatever reason in the habitat, it is considered casual (Blackburn *et al.* 2011). To assess establishment of *M. spicatum* in river/catchment systems, the plant was considered established when herbarium records spanned at least 10 years, and in some cases, several localities within the system. When there were only one or a few records from a locality over less than 10 years, it was assumed that the populations failed to establish and persist, and were considered casual.

To determine the extent of current establishment and find living populations of *M. spicatum* in southern Africa, existing known localities as well as new water bodies were surveyed. In South Africa, the Biological Control Research Group, based at Rhodes University, has conducted extensive field surveys every year since 2008 to assess the distribution and populations of all known aquatic weeds, as well as survey for the early detection of new emerging aquatic weeds. *Myriophyllum spicatum* is included in these surveys as a priority species because it is listed under the Conservation of Agricultural Resources Act, 1983 as a Category 1 weed, albeit as a precautionary measure. In addition to this, between 2012 and 2013 several surveys to 17 independent river systems where *M.*

spicatum has been recorded in South Africa, Botswana and Namibia were conducted. During these surveys, as much shore line as possible was scoured for any floating sprigs or signs of the presence of *M. spicatum* in the system. In most cases, especially in the larger systems, a boat was used and the maximum area of suitable habitat was searched for the presence of *M. spicatum*. Where the water was stained or discoloured, a rake was used to probe the bottom and snag any submerged macrophyte to be brought to the surface for identification. The information was used to determine whether populations were still persisting in these localities and/or had spread to new areas within and between systems.

2.3 Results

2.3.1 Herbarium specimens

Twenty-one herbaria were either visited or contacted with regard to *M. spicatum* records. This yielded a total of 109 confirmed specimens from 12 herbaria (Table 2.1). In addition to this, the southern African records of *M. spicatum* were downloaded from the GBIF database (Global Biodiversity Information Facility; http//.www.gbif.org) which added an extra 53 records. Comparing the data from herbaria and GBIF, the former yielded more than double the number (109 compared to 53) of specimens for southern Africa. This is probably due to smaller herbaria and herbaria in underdeveloped countries not having the capabilities to upload data to a global database such as GBIF. When the data were combined from GBIF and the herbaria, with all replicates removed, a total of 80 independent verified records of *M. spicatum* have been collected from southern Africa since the first collection in 1829.

Table 2.1 A list of the herbaria contacted, the total number of southern African *Myriophyllum spicatum* specimens housed in each herbarium, and the dates of collection from the earliest to the most recent specimen.

Country	Herbarium	Number of *Myriophyllum spicatum* records
South Africa	National Herbarium (PRE)	40, between 1877 - 1999
	Schonland Herbarium (GRA)	21, between 1897 - 2012
	University of Cape Town Bolus Herbarium (BOL)	10, between 1884 - 1976
	South African National Biodiversity Institute KwaZulu-Natal Herbarium (NH)	9, between 1885 - 1976
	South African Museum Herbarium (SAM)	5, between 1829 - 1938
	South African National Biodiversity Institute Compton Herbarium (NBG)	5, between 1885 - 1977
	McGregor Museum Herbarium (KMG)	2, from 1919 and 1936
	University of Durban-Westville Ward Herbarium (UDW)	2, from 1967 and 1981
	University of Natal Bews Herbarium (NU)	1, from 1967
	C.E. Moss Herbarium (J)	1, from 1930
	University of Port Elizabeth Ria Olivier Herbarium (PEU)	0
	Jonkershoek Herbarium (JONK)	0
Zimbabwe	National Herbarium (SRGH)	11, between 1949 - 1982
Botswana	University of Botswana Peter Smith Herbarium (PSUB)	2, from 1974 to 1982
	National Museum, Monuments, and Art Gallery National Herbarium (GAB)	0
	Department of Agricultural Research Herbarium (MAH)	0
	University of Botswana Herbarium (UCBG)	0
Zambia	Division of Forest Research Herbarium (NDO)	0
	University of Zambia Herbarium (UZL)	0
Namibia	National Herbarium of Namibia (WIND)	0
Malawi	Botanic Gardens of Malawi National Herbarium (MAL)	0

2.3.2 Flowering season

After examining all the records housed in the southern African herbaria, flowering was recorded from specimens of *M. spicatum* that were collected between the months of September and May, but there was a peak in the number of records with flowers between the months of October and January (Figure 2.1). Of the 109 specimens found in herbaria, only 24 were flowering and of these 24 flowering specimens, 23 were from South Africa (8 from the Western Cape, 13 from the Vaal River and tributaries and 2 from the Eastern Cape) and one was found outside of South Africa by Mary Pocock in the Zambezi River, just above Victoria Falls, in 1925.

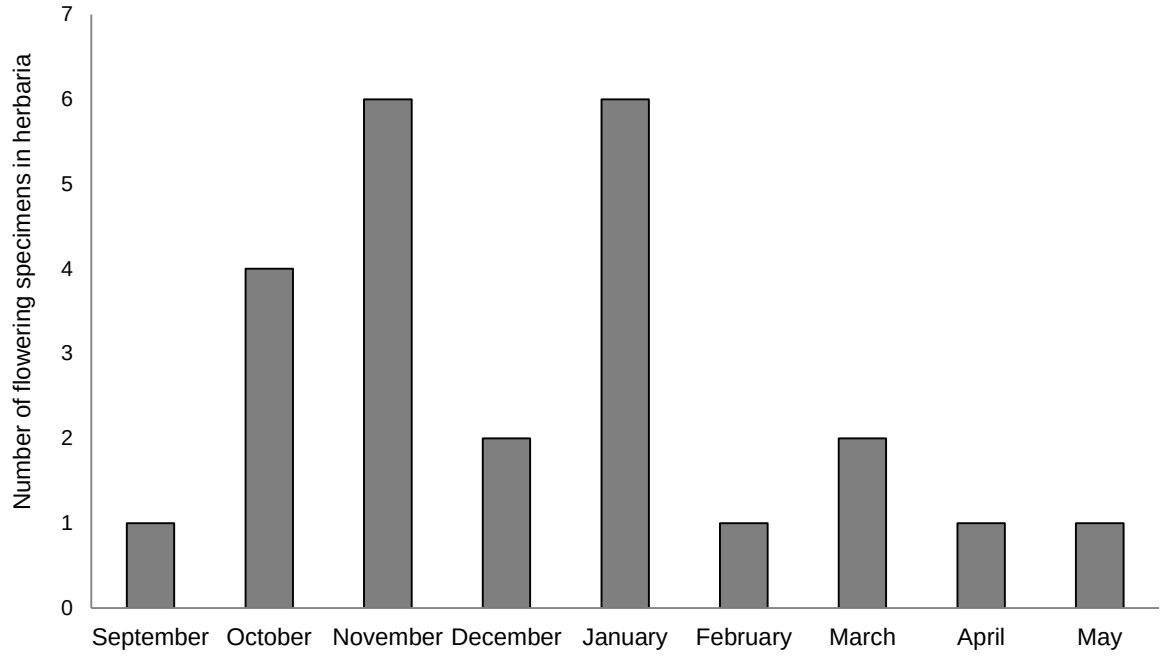

Figure 2.1 The number of flowering *Myriophyllum spicatum* specimens in herbaria, separated into each month.

2.3.3 Distribution in southern Africa from herbarium specimens

Myriophyllum spicatum has a wide recorded distribution in southern Africa, ranging from the Agulhas Plain in the Western Cape, to the highlands of the Amathola and Drakensberg mountains, to the sub-tropics of northern KwaZulu-Natal, Botswana, Namibia,

Zimbabwe, Zambia, Tanzania and Malawi (Figure 2.2). The first published record and lodged specimen of *M. spicatum* in South Africa was by the plant collectors Christian Friedrich Ecklon and Karl Ludwig Philipp Zeyher, when they surveyed the Uitenhage area in the Eastern Cape, South Africa, between July 1829 and February 1830 (Ecklon 1830). There are two specimens from this collecting period and both are lodged in the SAM herbarium in Cape Town (Table 2.2).

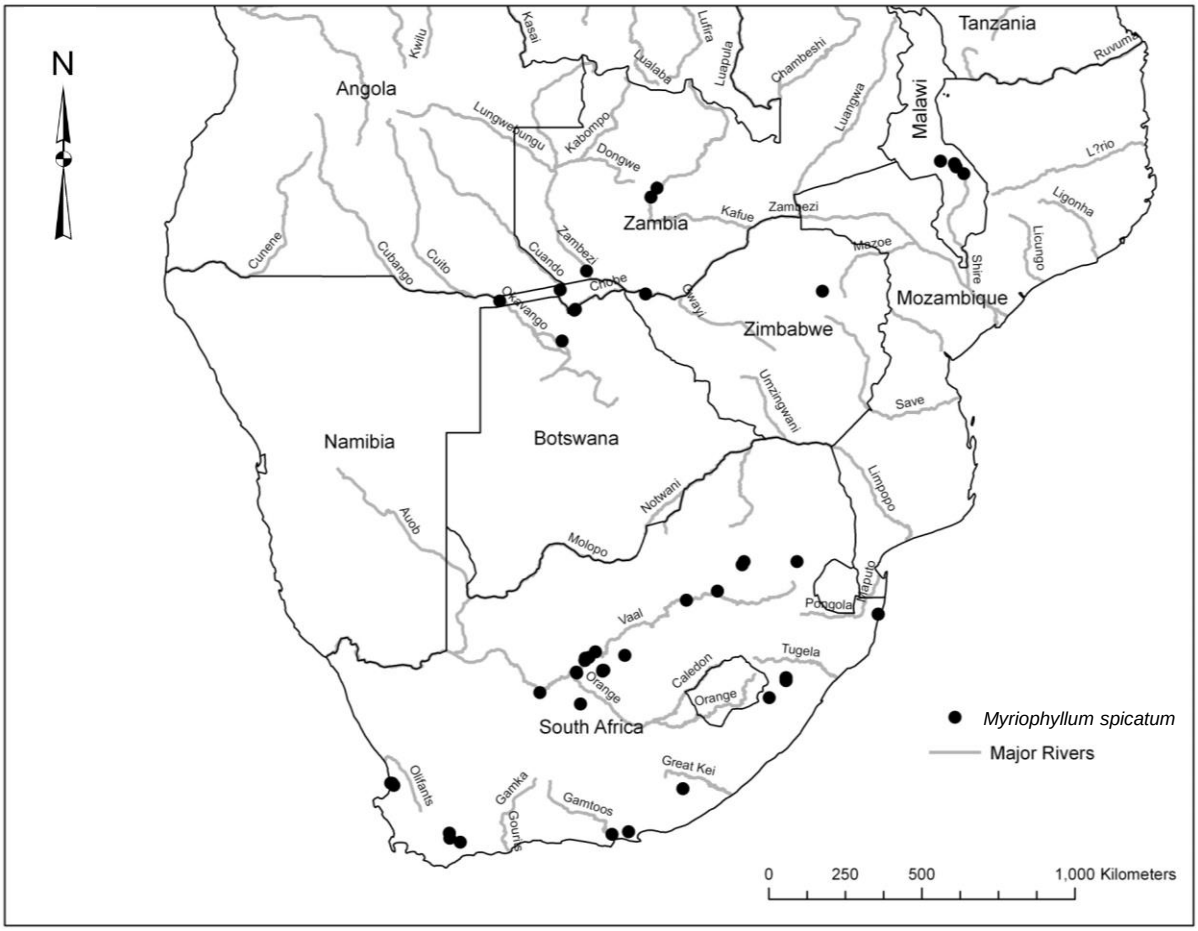

Figure 2.2 The distribution of *Myriophyllum spicatum* in southern Africa from verified herbarium specimens housed in southern African herbaria.

Soon after in the 1830s, *M. spicatum* was found, not only in the Swartkops River but also in the Gariep River, more commonly known as the Orange River, and the Kraai River, a tributary of the Orange River, however there were no lodged specimens (Harvey & Sonder 1894). There was a lag period until 1875, when Dr. A. Rehman made a collection in the

Transvaal, then several botanists such as R. Marloth, J. Mendely Wood and K. Orpen, to name a few, made a number of collections, mainly from various locations on the Vaal river and the Mooi River in the KwaZulu-Natal Midlands (Table 2.2). The Vaal River and its tributaries (Riet and Modder River) hold the most specimens and cover the widest time frame of collections, spanning from 1875 to 2012 (Table 2.2). The KwaZulu-Natal Midlands, on the other hand, has had several early collections; however, the last lodged herbarium specimen was in 1967 by R.G. Strey (Table 2.2). It was first recorded in the Western Cape in the Cogmans Kloof River in 1922, then in the Breede River, at Bonnievale in 1923 by R. Marloth, and then in Verlorenvlei, Elands Bay on the west coast in 1938. There have been several collections in Verlorenvlei since then, with the most recent being in 1994 by R.P. Glen (Table 2.2). Relatively few collections of this plant have been made in the Eastern Cape, despite having the first record of *M. spicatum* in South Africa. It has never been recorded in the Swartkops River since the 1830s. It was recorded once in a marsh in Hankey near Humansdorp in 1927, and then three times in the Hogsback area in the Klipplaat River on Rocklands farm, in 1968 by M.J. Wells and again in 1994 by C.J. Cillers and finally in 2012 by P.S.R. Weyl during this study (Table 2.2).

The first recorded specimen outside of the South African borders was that by Rev. F.A. Rodgers, at Victoria Falls, Zimbabwe in December 1913. It was collected in the vicinity of Victoria falls again by Mary Pocock in 1925; by A.O.D Mogg in 1937; and by H. Wild in 1949 (Table 2.2). It was first found in the Zambian section of Lake Tanganyika in 1947, and again in 1961 (Table 2.2). It has also been found in the Kafue River in 1959 and 1960, a tributary of the Zambezi River, and on the Zambian side of the Zambezi River (Table 2.2). There have been several collections from Malawi, where *M. spicatum* has been found in the shallow waters of the south of Lake Malawi. It was first recorded in Monkey Bay by C. Howard-Williams in 1969; it was then recorded by G.E. Gibbs Russell in 1972 also in

Monkey Bay; then in 1976 at Samama beach in the south of the Lake; and finally in Namaso Bay in 1986, also in the south of the Lake (Table 2.2). Northern Botswana and the Caprivi Strip, Namibia, also hold several specimens, the first of which was collected by C.E. Moss in 1930 in the Chobe River between Kasane and Kabulabula, Northern Botswana. The next collection was by G.E. Gibbs Russell in 1972 in the Linyanti River; and then by P.A. Smith in 1974 also in the Linyanti River (Table 2.2). The first record of *M. spicatum* in the Caprivi Strip, Namibia, was in the Singalamwe District by D. Edwards in 1975 (Table 2.2). Since then, there have been several collections of this plant in the Quando and Okavango River systems, with the most recent being from the Caprivi Strip in 1999 by collectors E. Klassen & S. Austaller (Table 2.2).

Table 2.2 A list of the *Myriophyllum spicatum* specimens and the relevant herbaria where they are housed. For several of the older collections, the latitude and longitude were estimates from the notes provided with the specimens.

Country	Collector	Collector #	Date	Locality	Lat.	Long.	Herbarium
South Africa	Zeyher	2470	1829	Swartkops River, Uitenhage	-33.7587	25.3554	SAM
	Ecklon & Zeyher	1767	1830	Swartkops River, Uitenhage	-33.7587	25.3554	SAM
	W. Nelson	67	1877	Vaal River	-26.9374	27.0559	PRE
	H. Dolier	6834	1884	Vaal River, Barkley West	-28.46	24.38	BOL
	J. Mendely Wood	3565	1885	Mooi River	-29.22	29.98	BOL
	R. Marloth	832	1885	Vaal	-28.46	24.38	NBG, PRE
	J. Mendely Wood	3357	1885	Mooi River	-29.22	29.98	NH
	Anon.	s.n.	1885	Vaal River	-28.62	24.19	PRE
	M. Wood	5481	1894	Mooi River	-29.22	29.98	PRE
	K. Orpen	189	1897	St. Clair, Douglas	-29.054	23.817	SAM
	K. Orpen	s.n.	1897	St. Clair, Douglas	-29.081	23.829	GRA
	H.G. Hanagan	s.n.	1899	Vaal river near Hebson	-28.46	24.38	BOL
	Dr. Potts	1756	1908	Modder River	-29.03	24.59	SAM
	E. Wager	10453	1910	Vereeniging	-26.672	27.963	PRE
	J. Burth-Davey	10755	1911	Smitskraal, Boshoff	-28.5604	25.2416	PRE
	J. Burth-Davey	10901	1911	Smitskraal, Boshoff	-28.5604	25.2416	PRE
	J. Burth-Davey	10067	1911	Smitskraal, Boshoff	-28.5604	25.2416	PRE
	J. Burth-Davey	11335	1911	Smitskraal, Boshoff	-28.5604	25.2416	PRE
	J.C. Moran	16248	1919	Modder River	-29.03	24.59	BOL
	J.C. Moran	s.n.	1919	Modder River	-29.03	24.59	PRE
	A. M. Cronin	815	1919	Modder River	-29	24.63	KMG
	Unknown	29	1922	Cogmans Kloof	-33.8	20.09	BOL

M.R. Buchell	s.n.	1922	Cogmans Kloof	-33.8	20.09	PRE
R. Marloth	s.n.	1923	Bonnievale, Robertson	-33.95	20.1	PRE
H.G. Fourcade	s.n.	1927	Hankey, Humansdorp	-33.83	24.87	PRE
E.G. Byanks	734	1933	Eastern? Preiska	-29.6563	22.74602	NBG
R.D. Bradfield	134	1934	Bronkhorstspruit	-25.897	28.692	PRE
J.P.H. Acocks	s.n.	1936	Riet River at Blaauwkrantz, Kimberly	-29.9952	23.9443	PRE
J.P.H. Acocks	3785	1936	Blaukrantz, Riet River, Kimberly	-29.9952	23.9443	KMG
N.D. Pillans	8597	1938	Piquetberg, Verlorenvlei, Opp. Muis Hoek	-32.33	18.41	BOL, NBG, GRA, PRE
D.K.H. Barnard	52779	1938	Breeder River, Swellendam	-34.067	20.4135	SAM
K. Tinley	120	1958	Ntumbane Inlet, Zululand Umzimkulu River.	-27.3504	32.6862	PRE
V. A. Wager	s.n.	1958	Underberg.	-29.81	29.49	PRE
O.A. Leistner	s.n.	1963	Riet River at Richie.	-29	24.59	PRE
S.C. Seagrief	s.n.	1964	Rooipoort, Vaal river, Near Kimberly	-28.6441	24.0835	GRA
J. Vahrmeijer and H.R. Tolken	884	1965	Lake Sibaya	-27.3504	32.6862	PRE
C.M. Breen	28	1966	Lake Sibaya	-27.3504	32.6862	GRA
Sibaya Project	s.n.	1966	Lake Sibaya	-27.3504	32.6862	GRA
R.G. Strey	7702	1967	Underberg "Springvale farm"	-29.3	29.98	NU, NH, UDW, PRE
M.J. Wells	3926	1968	Klipplaat River, Rockford.	-32.49	26.95	GRA
E.C.H. Oliver	s.n.	1970	Verlorenvlei, Elandsbaai.	-32.326	18.369	PRE
R.E. Haynes & F.C. Viljoen	s.n.	1971	Vaal river above the Barrage. Vanderbylpark.	-26.672	27.963	PRE
P. Linder	Unknown	1976	Piquetberg, Verlorenvlei	-32.33	18.41	BOL
C.F. Musil	443	1976	Lake Sibaya	-27.3504	32.6862	NH
C.F. Musil	451	1976	Lake Sibaya	-27.3504	32.6862	NH
C.F. Musil	452	1976	Lake Sibaya	-27.3504	32.6862	NH
C.F. Musil	459	1976	Lake Sibaya	-27.3504	32.6862	NH
A. Bruton ex. A. Jacot Guillarmod	s.n.	1976	Lake Sibaya	-27.3504	32.6862	GRA
A. Jacot Guillarmod	s.n.	1976	Vereeniging, Downstream of No. 2 inlet on vaal river	-26.672	27.963	GRA
P. Linder ex A. Jacot Guillarmod	s.n.	1976	Verlorenvlei, Piquetberg	-32.326	18.369	GRA,PRE
M.F. Thompson	3548	1977	Verorenvlei, at bridge near Bobbejaanberg	-32.326	18.369	NBG
C.J. Ward	9418	1981	Vaal River, Schmidtsdrif	-28.71	24.07	UDW
C.J. Ward	C 1025	1981	Schmidtsdrift	-28.71	24.07	PRE
L.Henderson	1079	1990	Bronkhorstspruit River at bridge near Groblersdal	-25.7976	28.7482	PRE
C.J. Cilliers	CJC 51	1994	Kromdraai, Vaal River	-26.9374	27.0559	PRE
C.J. Cilliers	CJC 75	1994	Rocklands Farm, Hogsback	-32.49	26.95	PRE
R. P. Glen	s.n.	1994	Verlorenvlei, near farm Bobbejaan berg	-32.326	18.369	PRE
E.L. Stephens	s.n.	-	Cogmans Kloof, Montagu	-33.8	20.09	UDW

	P. Weyl	PW 1	2012	Lake Sibaya	-27.2796	32.68426	GRA
	P. Weyl	PW 2	2012	Lake Sibaya	-27.2796	32.68426	GRA
	P. Weyl	PW 20	2012	Vaalhaarts Weir	-28.0922	24.9686	GRA
	P. Weyl	PW 24	2012	Klipplaat River	-32.485º	26.947º	GRA
Zimbabwe	F.A. Rodgers	s.n.	1913	Victoria Falls, Zimbabwe	-17.911	25.854	GRA
	F. Eyles	1571	1919	Salsbury, Zimbabwe	-17.83	31.05	PRE
	Pocock	129	1925	Ngambue, Zambezi River	-17.2353	24.11975	BOL
	A.O.D. Mogg	14598	1937	Zambezi River, 1 mile above the Victroia Falls, Zimbabwe	-17.911	25.854	PRE
	H. Wild	26416	1949	Zambezi River, Victroia Falls, Zimbabwe	-17.911	25.854	SRGH
Botswana	C.E. Moss	18572	1930	Chobe River, between Kasane and Kabulabula, Northern Botswana			J
	G. E. Gibbs Russell	2196	1972	Northern Botswana	-18.37	23.79	PRE, SRGH
	P. A. Smith	1168	1974	Linyanti River, Northern Botswana	-18.37	23.79	PRE, SRGH, PSUB
	D. Edwards	4388	1975	Singalamwe District	-17.7923	23.3468	PRE
Namibia	P.A. Smith	3959	1982	Linyanti River	-18.393	23.765	SRGH, PSUB
	C. Schlettwein	s.n.	1983	Singalamwe	-17.7923	23.3468	PRE
	Klaassen, E. & Austaller, S.	s.n.	1999	Caprivi strip, Namibia	-18.122	21.572	PRE
Malawi	C. Howard Williams	17	1969	Fort Johnston, Monkey Bay, Lake Malawi	-14.074	34.929	SRGH
	G. E. Gibbs Russell	2106	1972	Lake Malawi, Monkey Bay	-14.081	34.931	GRA, PRE, SRGH
	J.H. Seyani	282	1975	Lake Malawi, near Samama beach, Mangochi District.	-14.373	35.208	SRGH
	R.K. Brummitt	18295	1986	Namaso Bay, Malawi.	-14.17	34.98	PRE
Zambia	P.J. Greenway & J.P.M. Brenan	8245	1947	Lake Tanganyika, Mpulungu, Abercorn Dist.	-8.77	31.11	PRE
	R.B. Drummond & A.J. Cookson	6743	1959	Mumbwa District, Kafue Hoek Pontoon, Kafue River	-15.06	26.01	PRE, SRGH
	M. Gillhem	27	1960	Kafue River, Kafwala District, Kafue National Park.	-14.798	26.193	SRGH
	J.B. Phipps and L. Fitzgerald	3038	1961	Lake Tanganyika, Mpulungu, Abercorn Dist.	-8.69	31.19	SRGH

Myriophyllum spicatum has been recorded in 21 independent river systems or catchments in southern Africa since the first record in 1829. According to the herbarium records, this species has established and was able to persist in at least 13 of these systems, where records spanned at least 10 years (Table 2.3). Interestingly, in most cases, the records

came from the same locality in the system over the years. Only for the Vaal River, Mooi River, Breede River, Riet River and the Linyanti River were there records from multiple localities (Table 2.3). In eight of the river systems or catchments, there is no evidence of establishment because the herbarium records did not span at least 10 years (Table 2.3).

Table 2.3 The records and localities of *Myriophyllum spicatum* in southern Africa, including the status of each population as established or casual in the river system based on the herbarium specimens. If the records from the same river system or locality spanned at least 10 years, the population was considered established.

Country	Province	River system	Years of records	# of records	# of localities	Status
South Africa	Eastern Cape	Swartkops River*****	1829-1830	2	1	Casual
		Wetland near Hankey	1927	1	1	Casual
		Klipplaat River^{+}	1968-2013	3	1	Established
	KwaZulu-Natal	Mooi River	1885-1967	5	2	Established
		Umzimkulu River	1958	1	1	Casual
		Lake Sibaya^{+}	1965-2012	9	1	Established
	Western Cape	Cogmans Kloof River*****	1922	2	1	Casual
		Breede River*****	1923-1938	2	2	Established
		Verlorenvlei*****	1938-1994	6	1	Established
	Northern Cape	Vaal and Orange River below the confluence*****$^{+}$	1877-2012	20	Several	Established
		Modder River*****	1908-1919	4	1	Established
		Riet River*****	1936-1963	3	2	Established
	Gauteng	Bronkhorstspruit River	1934-1990	2	1	Established
Zimbabwe		Zambezi River, vicinity of Victoria Falls*****	1913-1949	4	1	Established
		Harare	1919	1	1	Casual
Botswana		Chobe River	1930	1	1	Casual
Botswana and Namibia		Linyanti River	1972-1999	4	4	Established
		Singalamwe District	1975-1983	2	2	Casual
Malawi		Lake Malawi	1969-1986	4	3	Established
Zambia		Lake Tanganyika	1947-1961	2	1	Established
		Kafue River^{+}	1959-1960	2	1	Established

***** Indicates flowering/fruiting individuals in the herbarium records and $^{+}$ indicates the river systems where populations of *Myriophyllum spicatum* have been found during field surveys and are currently surviving.

2.3.4 Field surveys

Despite this wide distribution and with numerous records at the same localities spanning several decades, there are only four confirmed living populations of *M. spicatum* to date – the Vaal River (-26.9374^{o}, 27.0559^{o}), Lake Sibaya (-27.3504^{o}, 32.6862^{o}) and the Klipplaat River (-32.49387^{o}, 26.94959^{o}) in South Africa; and one in the Kafue River (-14.913846^{o}, 25.928701^{o}), Zambia (Table 2.3 & 2.4). This is despite several field surveys,

through South Africa, Botswana and Namibia, to a total of 17 independent localities or river systems (Table 2.4). Many of these systems were extensively surveyed with no success at finding living populations (Table 2.4). For example, the population of *M. spicatum* in Verlorenvlei (-32.32495 º, 18.40315 º), Elands Bay on the West Coast of South Africa has been referred to as the dominant submerged aquatic species in the Vlei (Sinclair *et al.* 1986) and several specimens were collected there between 1938 and 1994. However, no *M. spicatum* plants were found during two field surveys of this study: the first in January 2012, which was a shore survey during the low water period, while the second in October 2012, during the high water period, was done by boat as well as a shore survey (Table 2.4). Approximately 15km of shoreline was scoured, revealing that the most abundant submerged aquatic plant in Verlorenvlei was *Stuckenia pectinata* (L.) Syn. *Potamogeton pectinatus* L. (Potamogetonaceae), while *Aponogeton distachyos* L.f. (Aponogetonaceae) was dominant in the river flowing into the Verlorenvlei.

In August and September of 2012, an extensive survey of rivers, pans and Lake Liambezi in the Caprivi Strip, Namibia and northern Botswana were conducted. In Namibia, approximately 80km of shoreline on Lake Liambezi, as well as 15km of the Bukalo channel, 20km of the Linyanti channel and 30km of the Chobe channel which all feed into Lake Liambezi were searched with no sign of *M. spicatum.* In addition to this, 60km of the Quando River and 40km of the Okavango River (10km in Namibia and 30km in northern Botswana) were surveyed, again with no sign of *M. spicatum* (Table 2.4). The only recorded population of *M. spicatum* outside of South Africa that has been found to date is the population in the Kafue River. During a boat survey just south of the Kafue Hoek Pontoon Bridge, the population was found where it had been recorded in 1959 by R.B. Drummond & A.J. Cookson. No other living populations have been found in the Kafue River.

Table 2.4 Summary of the *Myriophyllum spicatum* field surveys that have been conducted in southern Africa, with detailed field notes and summary of the findings.

Country	Region/Province	River system	Coordinates	Dates surveyed	Records present	Established populations	Notes
South Africa	Western Cape	Verlorenvlei	-32.336º, 18.425 º	Jan. 2012 Oct. 2012	Herbarium records	No individuals found	The lagoon, wetland and relevant river system were extensively surveyed from the shore line during a low water period in January and during high water period from a boat when the rivers were flowing in October
		Agulhas Plain	-34.336º, 19.904º	Jan. 2012 Oct. 2012	Observational and a collection records	No individuals found	The Nuwejaars River and tributaries including natural and manmade water bodies in the area were extensively surveyed during low water periods in January and again during high water periods in October. Both surveys were shore.
		Cogmans Kloof River	-33.799º, 20.104º	Jan. 2012	Herbarium records	No individuals found	The river was surveyed in the vicinity of the town of Montagu. This was done from shore as well as snorkelling in the larger pools.
		Breede River	-33.823 º, 19.865 º	Jan. 2012 Oct. 2012	Herbarium records	No individuals found	The river was surveyed around the town of Robertson during low water periods and again during high water periods. This was done from shore.
	Eastern Cape	**Klipplaat River**	-32.485º, 26.947º	Feb. 2013	Herbarium records	Healthy population was found	The river was surveyed and plants were found to be growing in all areas where the substrate allowed. The plants were relatively abundant but did not reach a density that they could be considered problematic.
	Gauteng	Bronkhorstspruit River	-25.856º, 28.702º	April 2013	Herbarium records	No individuals found	The river was surveyed from bridges in the Bronkhorstspruit area.
	Northern Cape	**Vaal River**	-26.886º, 27.473º to -29.069º, 23.636º	2008 – April 2013	Herbarium records	Healthy population was found	A 400km extent of the Vaal River has been surveyed annually since 2008 and *M. spicatum* has been found at many localities along the length of the river.

		Riet River	-29.141º, 24.769º	April 2012	Herbarium records	No individuals found	The Riet River, a tributary of the Vaal River, was surveyed from the shore and bridges.
		Modder River	-28.992º, 25.080º	April 2012	Herbarium records	No individuals found	The Modder River, a tributary of the Riet River, was surveyed from the shore and bridges.
		Orange River	-29.057º, 23.691º	April 2013	Herbarium records	A few individuals were found	The Orange river around the town of Prieska was surveyed from the shore and bridges, with a few individuals found in an irrigation canal coming off the main river.
	KwaZulu-Natal	**Lake Sibaya**	-27.359º, 32.683º	April 2012	Herbarium records	Healthy population was found	The lake was surveyed by boat and a healthy population was found and covers majority of the lake where water depth and substrate allows.
		Muzi Pan, and surrounds	-27.646º, 32.403º	April 2012 Nov. 2012 April 2013	Observational records	No individuals found	The streams, rivers and pans in the surrounds of Muzi Pan, Northern KZN were surveyed from the shore.
Namibia	Caprivi Strip	Linyanti River	-17.938º, 24.340º	Aug. 2012	Herbarium records	No individuals found	20 km of the Linyanti channel that feeds into Lake Liambezi was surveyed by boat.
		Chobe River	-17.949º, 24.378º		Herbarium records	No individuals found	30 km of the Chobe River that feeds into Lake Liambezi was surveyed by boat.
		Bukalo Channel	-17.892º, 24.389º		None	No individuals found	10 km of the Bukalo Channel that feeds into Lake Liambezi was surveyed by boat.
		Quando River	-17.791º, 23.344º		Herbarium records	No individuals found	60 km of the Quando River was surveyed by boat.
Namibia and Botswana		Kavango River	-18.256º, 21.778º		Herbarium records	No individuals found	10km of the Kavango River was surveyed on the Namibian side and 20km surveyed on the Botswana side by boat
Zambia		**Kafue River**	-14.798º, 26.193º		Herbarium records	Healthy population was found.	The population just south of the Kafue Hoek pontoon bridge was found.

2.4 Discussion

Myriophyllum spicatum has been recorded in 21 independent river systems or catchments throughout southern Africa, from as far south as the Agulhas Plain all the way up to Lake Tanganyika. Populations of *M. spicatum* have been found in a multitude of habitats from cool mountain streams in the Amathola Mountains, Eastern Cape, and the Natal Midlands, through to the tropical swamps of the Okavango River and natural lakes such as Lake Sibaya, Lake Malawi and Lake Tanganyika. Ecklon (1830) stated that "*a most striking feature of the Uitenhage district (Eastern Cape, South Africa) is the abundance of many of the European water plants, viz:* Chara, Typha, Potamogeton *and* Myriophyllum *spp. which were found, but rarely in the other districts*"(pp 360). Examining the specimens in the SAM herbarium, the *Myriophyllum* spp. that is referred to is indeed *M. spicatum* collected in November 1829 from the Swartkops River. Of the 21 river systems where *M. spicatum* has been recorded in the past, it has established in only 13 of these where records spanned at least 10 years. However, during the field surveys, only four living populations have been confirmed. Most of the populations that have been recorded and considered established were not found during the field surveys, which suggest that some populations may have undergone local extinctions or were at such low population levels at the time of the survey that they went undetected. Interestingly, the majority of the populations that had flowering specimens recorded in the herbarium specimens were not recorded during the surveys and are assumed to either be at such low densisties that it was overlooked or to have undergone local extinctions. Flowering in *M. spicatum* has not been considered an important aspect of their life history (Aiken *et al.* 1979; Smith & Barko 1990), but suggests that these populations should be able to persist in a system through the germination of seeds after a potential stochastic event. The seed banks of several macrophytes have been identified as an important

factor of their life history, and persistence in a system, especially when found in aquatic habitats that are prone to stochastic events (Brock *et al.* 2003; Cacho *et al*. 2006).

The morphological varieties identified by Jacot Guillarmod (1979) through the original herbarium specimens and living material are still present in the populations identified in this study. The variety that was originally described from the Vaal River still had similar characteristics to those of the original population recorded in the 1880s. The same is true for the populations recorded from Lake Sibaya and Hogsback. The original description of the high altitude variety was from the Mooi River in the KwaZulu-Natal Midlands, however the Hogsback population exhibits similar characteristics probably related to the cool high altitude environment to which it is exposed (Chapter 3). The drivers of these morphological characters of the different varieties are discussed in the following Chapter, in the context of evolutionary history in the southern African region.

The population dynamics of many submerged macrophytes and interactions with other macrophytes can be extremely variable. Howard-Williams (1979) studied the distribution and biomass of aquatic macrophytes in Lake Sibaya. His results suggest that *M. spicatum* was widespread throughout the Lake, however it was not the most abundant submerged aquatic plant at that time (Howard-Williams 1979). Comparing data on the biomass and relative abundance of *M. spicatum* in Lake Sibaya collected in 2012 and 2013 (Chapter 4), suggests that *M. spicatum* is the dominant submerged aquatic plant species at present and the biomass has increased by at least an order of magnitude since the last study by Howard-Williams (1979). These increases in biomass and dominance in systems could also be linked to the anthropogenic impacts, such as eutrophication, which is known to alter species interactions and dominance (Jeppesen *et al*. 2005; Scheffer & van Nes 2007; Sayer *et al*. 2010). The nutrient levels (especially ammonia) of Lake Sibaya have increased since the 1970s which may suggest an anthropogenic impact (Hart & Hart 1977; Chapter 4). The

majority of the populations of *M. spicatum* in southern Africa are considered benign and very few have been reported as being problematic. The first record of this species as being noticeably abundant was in the late 1950s in the Kafue River, where Wild (1961) reported the dense growth of this species just south of the Kafue Hoek pontoon bridge, but this population has never been considered problematic since. The population in the Vaal River was considered highly problematic from 2005 and has significant socio-economic impacts on the riparian community (Coetzee *et al.* 2011a). This is probably attributable to the successful control of water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laub. (Pontederiaceae) in the River, allowing light through for the submerged macrophytes and the high levels of nutrient pollution in the system (Coetzee *et al.* 2011a).

The overall distribution of this species is very wide in southern Africa, however, the populations are disjunct in nature, where populations of *M. spicatum* occur at great distances from one another, between major catchments and over mountains (in some cases approx. 1000 km). It is possible for natural dispersal mechanisms such as waterfowl to transport seeds and viable fragments of *M. spicatum* between catchments, however, waterfowl dispersal does not seem to be an important vector in other regions of the world where *M. spicatum* is present (Boylen *et al.* 2006). In addition to this, waterfowl are usually only responsible for dispersal of viable propagules of aquatic macrophytes over relatively short distances 10 – 100 km (Mader *et al.* 1998). The current disjunct distribution in southern Africa and apparent dispersal over the considerable distances between and across major catchments is likely to be linked to an anthropogenic pathway and suggests anthropogenic point source introductions as is the case in North America (Smith *et al.* 2002). The most common mode of spread in the introduced range (North America) is the movement of viable vegetative fragments between catchments by recreational water users, such as boaters and fishermen (Aiken *et al*. 1979; Smith & Barko 1990; Smith *et al.* 2002). In South Africa, boats have been identified as a

potential mechanism for the dispersal of invasive submerged macrophytes (Coetzee *et al.* 2009), however, it is unlikely that boat traffic was responsible for the potential spread of *M. spicatum* in the early 1900s.

Exotic plants have been moved around the globe for hundreds of years, and for many species this was done intentionally, while for several others there is no record of the introduction. For example, the first major spread of water hyacinth, *E. crassipes*, globally, has been clearly linked to the Cotton States Exposition held in New Orleans in 1884, where the Japanese offered a water hyacinth plant as a gift to delegates (Julien 2001). It was subsequently spread to new localities for ornamental purposes and its attractive flowers, where it escapes cultivation and invades water courses (Center 1994).

When the mode of introduction is unknown, it can complicate management strategies, for example *Pistia stratiotes* L. (Araceae) in Florida (Stuckey & Les 1984). Similar to *M. spicatum* in southern Africa, there was debate over the status of *P. stratiotes* in the U.S.A. where paleobotanical evidence and fossil records were found that dated back to the Eocene (Stoddard 1989). However, *P. stratiotes* is thought to have been driven to local extinction in these regions due to changes in the climate and the advent of the Ice-Age, and then subsequently re-introduced to Florida by the early European settlers (Stuckey & Les 1984). Therefore this species is clearly anthropogenically introduced to Florida and considered an exotic species.

There are no known or recorded pathways of introduction for *M. spicatum* into southern Africa and there is no record of this particular species having a beneficial use. In more recent years, the aquarium trade has been implicated in many aquatic macrophyte introductions into South Africa (Martin & Coetzee 2011), however this is unlikely to have been the case as long ago as the 1820s. The inadvertent introduction of this species could be

linked to the introduction of common carp, *Cyprinus carpio* L. (Cyprinidae) from Europe or goldfish, *Carassius auratus* (L.) (Cyprinidae) from Asia (de Moor & Bruton 1988). The time of introduction of both these fish species would be early enough for the associated with the accidental introduction of *M. spicatum*, early 1800s for European common carp and 1726 for Asian goldfish (Bruton & Merron 1985). The associated transport of *M. spicatum* with these fish could be explained by its use as a spawning medium. Aquatic vegetation is commonly used for several fish, including carp and goldfish (Battle 1940), however, there is no record of the specific use of *M. spicatum* for this purpose. In a similar case, the inadvertent spread of *M. aquaticum* throughout South Africa has been strongly linked to the movement of fish from fish farms in South Africa, however, the initial mode of introduction of this species is unknown (Jacot Guillarmod 1979).

In conclusion, *M. spicatum* has a wide distribution in southern Africa, however the disjunct nature of the populations suggests anthropogenic point source introductions into independent catchments. There is no record of the intentional introduction or potential pathways of introduction of this species into southern Africa and in addition to this, it is rarely considered problematic in water bodies, and when it is, it may be linked to anthropogenic disturbances such as eutrophication. The evidence presented in this chapter is suggestive of an anthropogenic introduction and spread through the region, but is by no means conclusive. The following chapter discusses the morphological variations found in southern African populations in the context of phenotypic plasticity or local adaptations to gain an understanding of the potential genetic variation and evolutionary history of the southern African populations of *M. spicatum.*

Chapter 3: Morphological variations in the southern African populations of *Myriophyllum spicatum* L. (Haloragaceae): phenotypic plasticity or local adaptation?

3.1 Introduction

It is widely accepted that aquatic plants are plastic in their responses to environmental variables, and their morphology can be extremely variable between populations and/or between seasons (Barko & Smart 1981; Barko & Smart 1986; Koch & Seelinger 1988; Barrett *et al.* 1993; Idelstam-Almquist & Kautsky 1995). Changes or responses in plant morphology and/or physiology between populations of the same species are often linked to both physiological stresses, such as limited resources (de Kroons & Hutchings 1995; Hutchings & John 2004), and to physical/mechanical stresses such as wave action or current (Strand & Weisner 2001; Boeger & Poulson 2003; Arshid & Wani 2013). These responses of species are usually driven by adaptive mechanisms, such as phenotypic plasticity (Grace 1993; Barrett *et al.* 1993; Hofstra *et al*. 1995) and/or local adaptations (Sultan 2000; Kawecki & Ebert 2004; Ward *et al.* 2008) that allow them to adapt to the different climatic and environmental stresses to which they are exposed.

Local adaptation is a genetic change, primarily driven by natural selection on a local scale, where specific characters of a plant that enhance its fitness are selected for in a novel environment (Kawecki & Ebert 2004), while phenotypic plasticity is the ability of a single genotype to respond with changes in phenotypic characters that will better suit them to the prevailing habitat conditions (Bradshaw 1965). The wide distributional range of many aquatic species is often coupled with relatively low genetic variation of individuals within populations, but high variation between populations, probably linked to clonal or vegetative

reproduction (Grace 1993; Barrett *et al.* 1993). In many cases, aquatic plants are thought to have a "general purpose genotype" usually characterised by low levels of genetic variability but capable of adapting to a diverse range of environmental conditions through phenotypic plasticity (Baker 1965; Barrett *et al.* 1993). There are two forms of phenotypic plasticity that can be classed as either physiological plasticity, where the responses have a physiological end point, such as changes in photosynthetic capabilities; or as morphological plasticity where the responses are manifested as a change in morphology (Bradshaw 1965). These plastic responses, both physiological and morphological, are important for the survival of a species in a multitude of different environments over the wide geographical ranges in which they are found (Bradshaw 1965; Barrett *et al.* 1993).

Understanding the mechanisms that drive changes in the phenotype of aquatic plants can prove to be useful in gaining insights into the genetic diversity and evolutionary history of the species in a region. Morphological differences in introduced populations of aquatic plants are thought to be primarily driven by phenotypic plasticity because of the relatively low levels of genetic diversity and short time spent in the region (Riis *et al.* 2010). In a study of three invasive submerged macrophytes in New Zealand, Riis *et al.* (2010) concluded that the primary adaptive strategy of all three species was phenotypic plasticity due to the low levels of genetic diversity, coupled with the relatively short time period since the first introduction of any of the species. The oldest introduction was *Elodea canadensis* Mitch. (Hydrocharitaceae), and at just over 100 years, is considered too young for the development of local adaptations, especially given the lack of genetic diversity within and between populations in New Zealand (Riis *et al.* 2010). Local adaptations are driven by the process of natural selection, which result in different genotypes adapted to local conditions and are likely to express differences in morphological characters over much longer time scales (Kawecki & Ebert 2004). A prerequisite for local adaptations to take place between

populations is a relatively diverse gene pool within populations for natural selection to act upon (Ward *et al.* 2008). In the case of introduced species, this can be achieved through multiple introductions from different source populations, and local adaptation can be considered an important adaptive mechanism for the successful invasion of a species (Parker *et al.* 2003).

In southern Africa, there are three distinct varieties or growth forms of *Myriophyllum spicatum* L. (Haloragaceae) (Jacot Guillarmod 1979) which are found in different regions that have very different climatic conditions. The differences in the morphology are so great that it was initially thought that there were at least two species of *Myriophyllum* in southern Africa (Jacot Guillarmod 1979). These varieties in southern Africa can be identified in the earliest herbarium specimens from the regions where they originate, for example the Vaal river specimen was collected in 1897, the Lake Sibaya specimen 1966 and the Mooi River population in 1894 (Figure 3.1). These morphological characteristics are still present in the populations found in the biogeographic regions today (Figure 3.1). The first variety is characterised as large and robust with large leaves and relatively thick stems (Figure 3.1 A and D) and is found in the Vaal River, Northern Cape, South Africa. The second growth form is characterised by a delicate small plant, with small leaves, thin stems and the plant is usually highly branched (Figure 3.1 B and E). It is found growing in the subtropical environment in Lake Sibaya, KwaZulu-Natal, South Africa. The third growth form is large, and similar to the first growth form, but the internode length is very short so the leaves become tightly packed to form a bottlebrush type appearance (Figure 3.1 C and F), and found in the high altitude regions, including the Amathola Mountains, Eastern Cape and the KwaZulu-Natal Midlands, South Africa. The drivers of the morphological differences, phenotypic plasticity or local adaptation, in these populations may give an indication of the

genetic diversity of the plants and potential evolutionary history in the southern African region.

Figure 3.1 The three morphological variations of *Myriophyllum spicatum* found in southern Africa as recorded by herbarium specimens (A-C) and present day photographic representations of living specimens (D-F). The first A & D: robust large leaf form collected from the Vaal River, Northern Cape, the second B & E: delicate branched form collected in Lake Sibaya, KwaZulu-Natal and the third C & F: the large growth form with very short internode lengths giving it a bottlebrush appearance, the herbarium specimen collected in the Mooi River, Natal Midlands and the living specimen collected in Hogsback, Eastern Cape.

The aim of this study was to determine whether the morphological differences between the populations are driven by phenotypic plasticity or local adaptations through underlying genetic variation. If the morphological differences between the populations are

driven primarily by plastic responses to environmental conditions, then plants grown under the same conditions would respond in a similar way and their morphologies would converge (Santamaria *et al.* 2003; Riis *et al.* 2010). However, if the differences are local adaptations within the species, then the morphology of the plants from the different populations would not converge and the varieties would remain distinct from each other.

3.2 Materials and Methods

3.2.1 Experimental setup

The initial stock plants from the three populations of *M. spicatum* used in this experiment were collected from wild populations within a two week period during the beginning of April 2013. The three populations collected were 1) Vaal, collected from Vaalharts Weir, Vaal River (28°06'55.5"S 24°56'19.1"E); 2) Sibaya, collected from Lake Sibaya (27°25'02.9"S 32°41'47.4"E) and 3) Hogsback, collected from the low water bridge on the Klipplaat River (32°29'06.4"S 26°56'48.5"E). The plants were acclimated to the greenhouse conditions by floating sprigs freely in borehole water for a period of at least four days prior to any experimental treatments in a common garden experiment. A total of 40 activily growing sprigs from each population were cut to 10 cm growth tips with no branches, and initial morphological measurements were taken from each sprig. The morphological measurements that were taken included the stem diameter, internode length, leaf length and number of leaflet pairs on each leaf. All measurements were taken at the 5th internode to standardize the position from where the measurements were taken on each individual and between varieties.

In comparison to the experiments of Santamaria *et al.* (2003) and Riis *et al.* (2010), the environmental conditions that may have played a role in determining the morphological

characters were all kept constant: sediment type, temperature, light, photoperiod and water chemistry. The sprigs were then exposed to two growing conditions, a low nutrient (pond sediment only) and a high nutrient (pond sediment plus 30mg N/kg pond sediment, introduced in the form of a slow release fertilizer that has N:P:K of 15:8:12, Multicote® 8 (Haifa)) growth medium for a period of eight weeks. The sprigs were randomly planted into seedling trays which held approximately 100 ml of sediment in each pot, and then placed into a pond which contained borehole water to a depth of 1 m. The seedling trays were arranged in a random block design to rule out any possible location effects on light for the plants in the tank. The same morphological measurements were then taken again after a period of eight weeks and compared between populations at both nutrient levels.

3.2.2 Statistical analysis

The morphological measurements, including stem diameter, internode length, leaf length and number of leaflet pairs, between the three populations of *M. spicatum* under each growing condition, low or high nutrients, were compared using a Repeated-Measures ANOVA in the general linear model (GLM) followed by a Tukey Post-Hoc test to identify homogenous groups. To determine the similarity between the three populations of *M. spicatum* at the start of the experiment and 8 weeks later grown under the same conditions, a Principal Component Analysis (PCA) was performed using the morphological characters measured (stem diameter, internode length, leaf length and number of leaflets) and plotted to visualise the results.

3.4 Results

There was a significant increase in most of the morphological measures taken when grown under high nutrients, except for the number of leaflets, however, the growth form of

each variety remained consistent with significant differences between varieties in many of the morphological characters (stem diameter: $F_{(5,86)}= 18.435$, $P < 0.001$; internode length: $F_{(5,86)}= 5.0747$, $P < 0.001$; leaf length: $F_{(5,86)}= 19.692$, $P < 0.001$; number of leaflets: $F_{(5,86)}= 0.4126$, $P = 0.838$) (Figure 3.2-3.5).

There was no difference between the stem diameter of the Vaal and Hogsback plants, at either nutrient level, but Sibaya was always significantly smaller under both nutrient treatments ($F_{(5,86)}= 18.435$, $P < 0.001$) (Figure 3.2). The differences in the stem diameter within each population were significantly larger for both Vaal and Hogsback populations but not different for the Sibaya population (Figure 3.2).

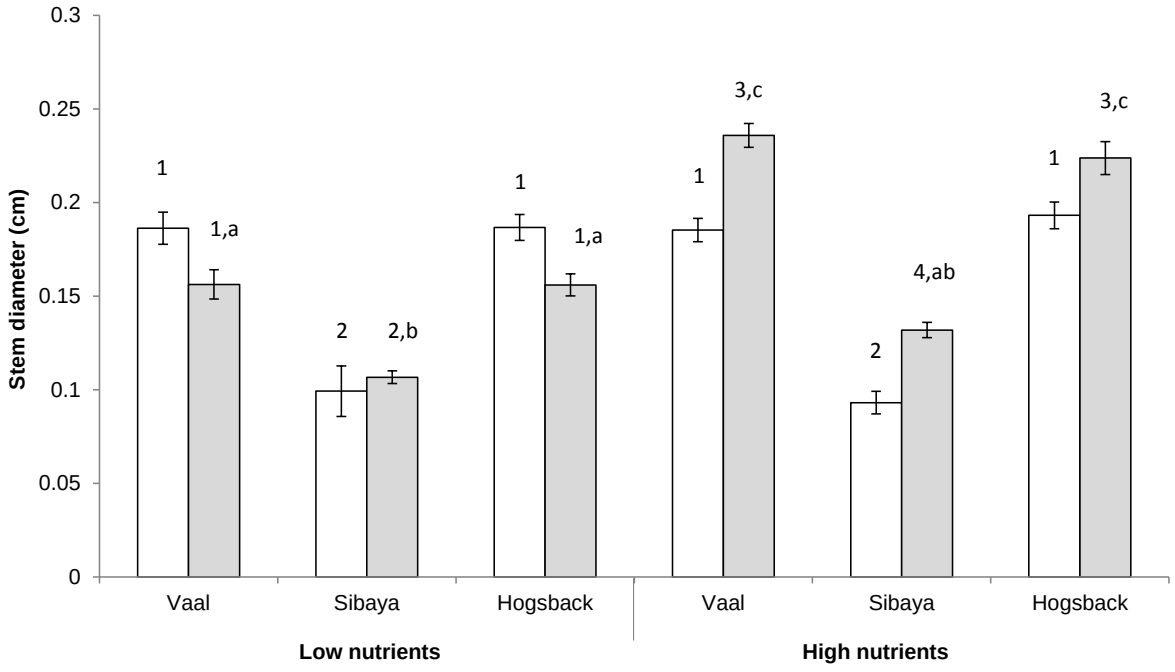

Figure 3.2 The start (white bars) and end (grey bars) stem diameter of each population of *Myriophyllum spicatum* grown under the two nutrient conditions, high nutrients (30mg N /kg pond sediment) and low nutrients (pond sediment only). The numbers above the means indicate significant differences between the start and end values, while the letters indicate significant differences between treatments at the end of the experiment ($P < 0.001$). Error bars indicate standard error of the mean.

The internode length for Hogsback was always significantly lower under both high and low nutrients than the Vaal and Sibaya populations which ranged between 1.05 cm and 1.49 cm depending on nutrient level ($F_{(5,86)}$= 5.0747, $P < 0.001$) (Figure 3.3). The significant differences within populations was that of the Vaal and Sibaya populations which had a significantly smaller internode length under lower nutrient conditions (Figure 3.3).

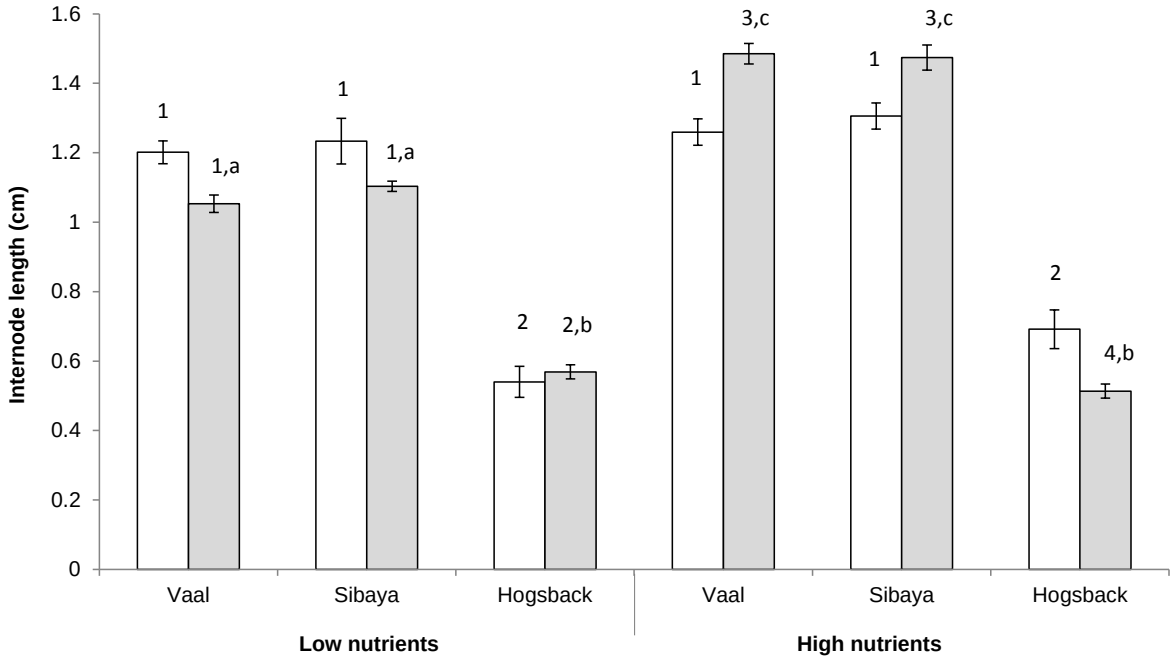

Figure 3.3 The The start (white bars) and end (grey bars) internode length of each population of *Myriophyllum spicatum* grown under the two nutrient conditions, high nutrients (30mg N /kg pond sediment) and low nutrients (pond sediment only). The numbers above the means indicate significant differences between the start and end values, while the letters indicate significant differences between treatments at the end of the experiment ($P < 0.001$) and error bars indicate standard error of the mean.

Irrespective of nutrient level, the Sibaya population had significantly smaller leaf lengths (1.21±0.027 cm high nutrients and 0.9±0.028 cm low nutrients) than both the Vaal and Hogsback populations which ranged between 2.22 cm and 2.76 cm depending on nutrient

level ($F_{(5,86)}$= 19.692, $P < 0.001$) (Figure 3.4). There was no difference between the leaf lengths of each variety, except for Sibaya population which had a significantly longer leaf under high nutrients (Figure 3.4).

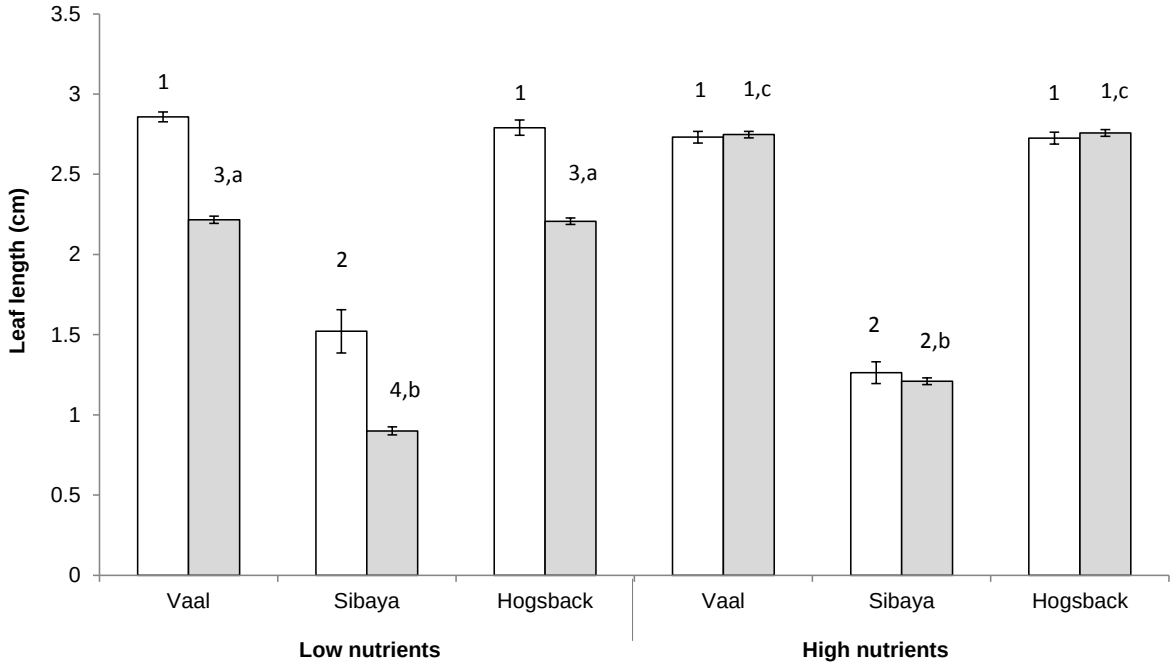

Figure 3.4 The The start (white bars) and end (grey bars) leaf length of each population of *Myriophyllum spicatum* grown under the two nutrient conditions, high nutrients (30mg N /kg pond sediment) and low nutrients (pond sediment only). The numbers above the means indicate significant differences between the start and end values, while the letters indicate significant differences between treatments at the end of the experiment ($P < 0.001$) and error bars indicate standard error of the mean.

The Vaal population, under both nutrient conditions, had significantly fewer leaflet pairs (11.75±0.437 for high and 11.38±0.317 for low nutrients) than both the Sibaya and Hogsback population which ranged between 12.47 and 13.71 leaflet pairs, except for Hogsback under low nutrients ($F_{(5,86)}$= 0.4126, P = 0.838) (Figure 3.5).

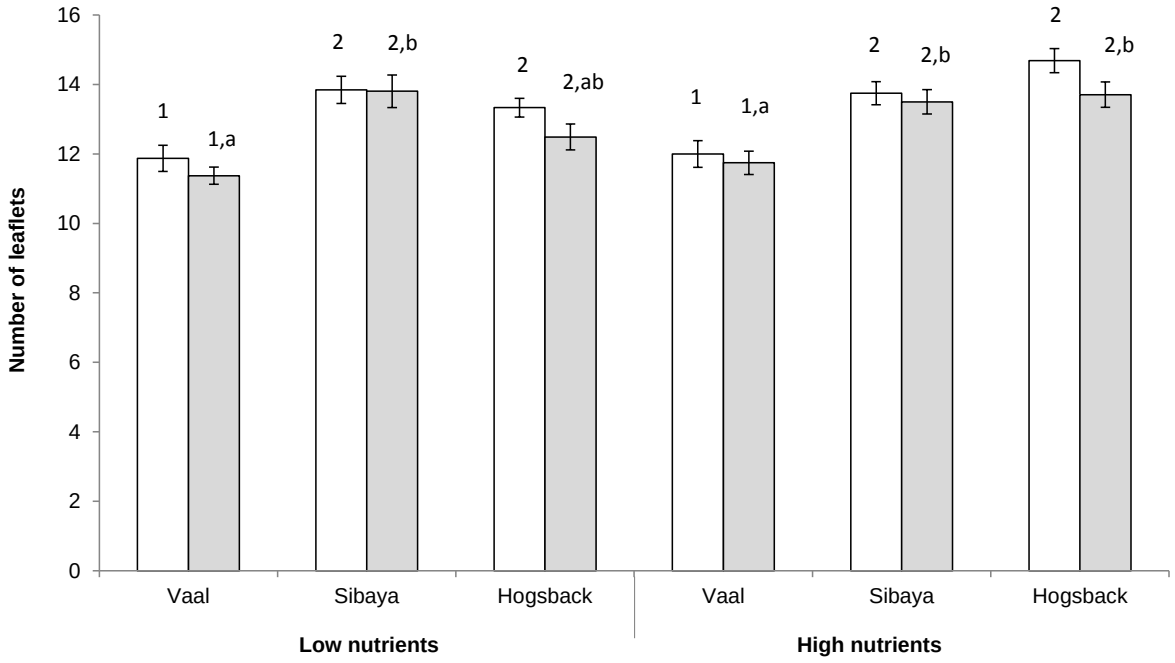

Figure 3.5 The start (white bars) and end (grey bars) number of leaflets of each population of *Myriophyllum spicatum* grown under the two nutrient conditions, high nutrients (30mg N /kg pond sediment) and low nutrients (pond sediment only). The numbers above the means indicate significant differences between the start and end values, while the letters indicate significant differences between treatments at the end of the experiment ($P < 0.001$) and error bars indicate standard error of the mean.

The PCA shows no overlap in the groupings between the populations both at the start of the experiment when the plants were first collected from the field populations and after the eight week experimental period, grown under the two nutrient treatments high (pond sediment plus 30mg N/kg sediment) and low (pond sediment only) (Figure 3.6). The PC1 accounts for 59.7% of the variation (Eigenvalue = $1.75x10^{-2}$) and the PC2 accounts for 27.3% of the variation (Eigenvalue = $8.02X10^{-3}$).

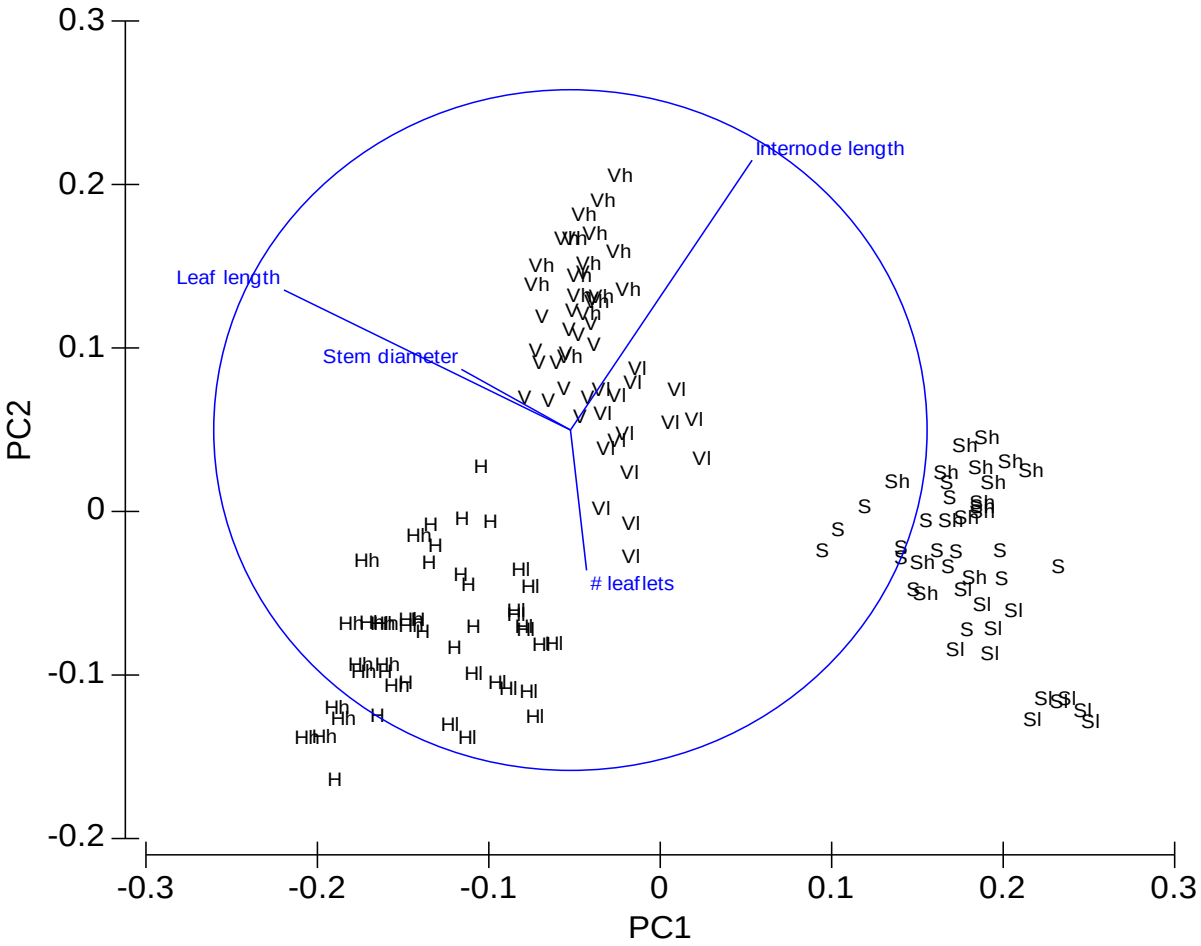

Figure 3.6 PCA showing the similarity between the populations of *Myriophyllum spicatum* at the start of the experiment and 8 weeks later, based on the four morphological characters measured (stem diameter, internode length, leaf length and number of leaflets). PC1 accounts for 59.7% of the variation (Eigenvalue = 1.75×10^{-2}) while PC2 accounts for 27.3% of the variation (Eigenvalue = 8.02×10^{-3}). The letters correspond to the source population and growing condition for an 8 week period; V = Vaal population at start, Vl = Vaal low nutrients, Vh = Vaal high nutrients, S = Sibaya population at start, Sl = Sibaya low nutrients, Sh = Sibaya high nutrients, H = Hogsback population at start, Hl = Hogsback low nutrients, Hh = Hogsback high nutrients

The plants from each population showed a response to the different growing conditions for every morphological measurement taken, except for the leaf length, where plants grown under high nutrients were always larger. The growth pattern of each population,

however, did not change based on nutrient condition and the plants from each population were in all cases more similar to each other than to another population.

3.4 Discussion

Despite the same growing conditions, sediment type, nutrient availability, water depth and light, the three different populations of *M. spicatum* did not converge in their morphology. This suggests that the differences in the morphological varieties are driven by local adaptations and southern Africa has different ecotypes of *M. spicatum* that have adapted to their current environmental conditions. This is fairly common in terrestrial plants with wide distributions, which are often characterised by large genetic variation (Bradshaw 1965) and often specialised to local environmental conditions which allows them to cover such wide geographic and climatic regions (Van Tienderen 1990). Often aquatic plants are characterised by low levels of genetic variation which makes phenotypic plasticity extremely important and a common occurrence among aquatic spices is the development of a "general purpose genotype" capable of surviving in a wide range of environments (Baker 1965; Barrett *et al.* 1993). However, this is not always the case, studies on aquatic plants have shown in several cases that although phenotypic plasticity is important, local adaptations are possible and do play a role in the survival and fitness of some species across a multitude of environmental conditions (Barrett *et al.* 1993; Santamaria *et al.* 2003).

The different *M. spicatum* populations that were grown under the two nutrient treatments did show some degree of plasticity and a response to the growing conditions. All three ecotypes that were grown under the lower nutrient condition (pond sediment only) adopted a significantly smaller size in most of the morphological characters measured. This was not surprising as several aquatic plant species have been shown to respond under limiting nutrient conditions, Riis *et al.* (2010) reported that *Lagarosiphon major* (Ridl.) Moss

(Hydrocharitaceae) and *E. canadensis* had reduced shoot diameter and leaf width under low nitrogen and phosphorous conditions. Individuals of *Ranunculus peltatus* Schrank (Ranunculaceae) were smaller, when grown under low nutrients, than individuals grown under high nutrients, the latter tending to have long branching shoots (Garbey *et al.* 2004). Barko (1983) suggested that not only nutrient composition, but the interaction with nutrients and sediment type, play a role in the growth form of *M. spicatum,* while Barko & Smart (1981) suggest that light may play a role in the morphology of aquatic macrophytes. Strand & Weisner (2001) also suggest that light and water depth play a role in the morphological characteristics of *M. spicatum* where plants that are light limited (periphyton growth or depth) are usually longer and more branched. The drivers of the subtle morphological changes in this study suggest that levels of nutrients were the most important, as these were what varied across treatments, however, the potential effect of light and water depth on the morphology of *M. spicatum* was not tested in the current study.

The findings from the current study were in contrast to the situation in North America (Aiken *et al.* 1979) where *M. spicatum* was introduced in the 1940s (Couch & Nelson 1985). Plants from different regions in Canada and USA were grown side by side in a greenhouse. The responses to the similar growing conditions were the same, which resulted in the morphology of the different populations converging, which Aiken *et al.* (1979) attributed to a common clonal origin of the North American material. This is despite the recent discovery of two genotypes on *M. spicatum* in North America (Zuellig & Thum 2012), however, it is possible that during the study by Aiken *et al.* (1979), plants from the same genotype were inadvertently selected. Riis *et al.* (2010) had similar findings when three introduced submerged aquatic plants, *L. major, Egeria densa* Planch. (Hydrocharitaceae) and *E. canadensis* were grown under similar conditions. Initially the plants had different growth forms or morphologies, and physiologies (photosynthetic rates) that were presumably adapted

to the conditions that they were exposed to in the wild populations. The morphologies and photosynthetic rates of all the species reverted to a similar point or growth form after a seven-week growing period (Riis *et al.* 2010). In addition to this, they tested the genetic diversity between the populations using amplified fragment length polymorphisms (AFLPs), which resulted in very little difference between the populations, suggesting that each species originated form a single introduction (Riis *et al.* 2010). This suggests that for introduced species that lack genetic diversity, phenotypic plasticity may be the most important factor driving the differences between populations of the same species growing in different climatic or environmental conditions (Riis *et al.* 2010).

The three ecotypes of *M. spicatum* identified in southern Africa are similar to *M. spicatum* populations from the native range in England and the Netherlands, which showed slightly different morphologies when grown under the same conditions (Aiken *et al.* 1979). This suggests that these populations also exhibit subtle local adaptations. The different populations of *M. spicatum* in southern Africa are locally adapted presumably to the conditions where they are found, however this does not rule out the importance of phenotypic plasticity for these populations to adapt to changing conditions. In a transplanting experiment, Santamaria *et al.* (2003) transplanted *S. pectinata* populations from different regions of Europe. Their results suggest that there were strong local adaptations and the performance of transplanted individuals was much lower in the novel environment than when grown at the source location. However despite the local adaptations, the different populations of *S. pectinata* also showed a certain degree of phenotypic plasticity (Santamaria *et al.* 2003), suggesting that local adaptation and phenotypic plasticity may work synergistically. The study by Santamaria *et al.* (2003) was within the native range of *S. pectinata* which suggests a long evolutionary history in the region and local adaptations are not surprising due to the relatively higher genetic diversity in native populations compared to introduced populations

(Ward *et al.* 2008). In many introduced aquatic species, including *Eichhornia crassipes* (Mart.) Solms-Laub. (Pontederiaceae), *E. densa* and *Alternanthera philoxeroides* (Mart.) Griseb. (Amaranthaceae), genetic variation is low between populations, likely linked to their clonal reproduction (Ward *et al.* 2008) and the adaptive mechanisms are probably linked to phenotypic plasticity rather than local adaptations (Parker *et al.* 2003; Geng *et al.* 2007).

The evolution of locally adapted populations requires an interaction between divergent selection and other evolutionary forces such as natural selection and gene flow (Kawecki & Ebert 2004). The development of locally adapted populations of *M. spicatum* in southern Africa suggests that the populations are sufficiently isolated that there is little or no gene flow between them. This isolation could be geographic as there are significant distances, over major catchments, between the populations (Chapter 2), or it could be reproductive, as sexual reproduction is not considered very important for *M. spicatum* (Patten 1956; Aiken 1981), or a combination of both, which would further isolate the populations. This is a similar situation to North America, where *M. spicatum* is characterised by two genotypes with overlapping distributions, however, there is little evidence of sexual reproduction between them in the field populations (Zuellig & Thum 2012). The development of these locally adapted ecotypes also suggests that there could be a relatively high genetic diversity of the populations in southern Africa. What is unclear is whether this diversity has resulted from multiple introductions (Ward *et al.*2008; Lavergne *et al.* 2010) or a long enough evolutionary history in the region for genetic mutations to occur. It is possible for genetic differentiation to occur quite rapidly, for example, despite the low levels of genetic variation, *E. densa* in New Zealand is showing signs of genetic differentiation between populations in less than 100 years since it was first introduced (Lambertini *et al.* 2010). This could suggest that the evolution of locally adapted populations in *E. densa* could occur quite rapidly in New Zealand, under the right conditions (Kawecki & Ebert 2004; Ward *et al.* 2008) despite the

low genetic variation inherent in so many species (Barrett *et al.* 1993; 2008; Lambertini *et al.* 2010).

Local adaptation and not phenotypic plasticity is the more likely driver of the different morphological variations of the *M. spicatum* populations from southern Africa that were tested in this study. This does not rule out the importance of phenotypic plasticity in shaping the morphology of these species in the environments where they occur, and probably explains why they exist in a wide variety of habitats in southern Africa (Chapter 2). What is unclear from this study is whether the potential genetic diversity is a result of multiple introductions from a genetically diverse source population/s or a long evolutionary history in the region. The development of the local adaptations in southern Africa suggests that the populations are isolated and it is unlikely that there is much genetic mixing between the systems where these populations are found. This is not surprising as the populations are separated by major catchments, geographical barriers such as mountains and climatic zones, all of which make dispersal between the populations extremely difficult. The next chapter investigates the insect herbivore associations and faunal and floral community interactions of two of the ecotypes of *M. spicatum* in southern Africa in an attempt to estimate the evolutionary history of this species in the region.

Chapter 4: Do aquatic macroinvertebrate faunal and floral associations with *Myriophyllum spicatum* L. (Haloragaceae) infer a long evolutionary history in southern Africa?

4.1 Introduction

4.1.1 Phytophagous insect associations

Phytophagous insect diversity has been shown, in many cases, to be linked to the evolutionary history of a plant in a particular region (Strong 1977; Strong *et al.* 1984; Brändle *et al.* 2008). A long evolutionary history in a region usually results in a rich diversity of phytophagous insects and can be explained by two hypotheses, the 'host-age hypothesis' or the 'species-time hypothesis' (Fischer 1960; Brändle *et al.* 2008). The ultimate diversity of the phytophagous insects on a particular species is closely linked to several factors which include the initial pool of phytophagous species, geographic size or the distributional range of the species, and the morphological, physiological, and genetic similarities between the host and other plants in the region (Strong 1977; Strong *et al*. 1984; Soldaat & Auge 1998; Agrawal & Kotanen 2003; Agrawal *et al.* 2005; Schoonhoven *et al.* 2005). As a plant increases its range, phytophagous insects will attempt to transfer to the novel host, which is typically slow, but given time, a species usually attains an asymptotic number of herbivores (Strong *et al.* 1984). The number of phytophagous insects using a plant as a food source can be used as a measure of the plant's evolutionary history the longer the plant has been in an area, usually the higher the number of herbivores (Strong 1977; Strong *et al*. 1984). Therefore, the best source of host-specific phytophagous insects is often the plant's centre of origin (Wapshere 1974).

The number of phytophagous invertebrates associated with a plant species has been used to test the evolutionary history of the floating macrophyte *Pistia stratiotes* L. (Araceae), in Florida (Dray *et al.* 1993). There was debate over the status of *P. stratiotes* in the USA as either native or introduced, mainly because the paleobotanical evidence and fossil records were dated back to the Eocene (Stoddard 1989). However, based on historical records, Stuckey & Les (1984) assume that *P. stratiotes* was driven to local extinction in the USA due to changes in the climate and the advent of the Ice-Age. It was then thought to have been subsequently re-introduced to Florida by the early European settlers (Stuckey & Les 1984). However, the status of *P. stratiotes* needed to be resolved before management strategies could be put into place, therefore Dray *et al.* (1993) investigated the herbivore diversity to gain insights into the evolutionary history of this species in the USA. During these faunal surveys, Dray *et al.* (1993) reported that *P. stratiotes* had a diverse invertebrate community (95 species) associated with it in Florida, but very few, only 7.4%, of these used the plant as a direct food source. These phytophagous insects were generalist feeders and were found on several other species of macrophyte in Florida (Dray *et al.* 1993). The number of phytophagous invertebrates recorded to use *P. stratiotes* in South America was 17, with 10 (58.8%) being considered oligophagous and relatively host specific (Dray *et al.* 1993). The stark contrast between Florida and South America in terms of these herbivore numbers suggests that Florida has a relatively short evolutionary history for *P. stratiotes* and is thus not the native range of this species (Dray *et al.* 1993). In addition to this, there were no specialist herbivores found in North America, also suggesting that North America is its adventive range.

This theory has been further tested with aquatic floating macrophytes when, during surveys of water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laub. (Pontederiacae), for natural enemies, it was noted by Bennett & Zwolfer (1968) that 40 phytophagous insects

were associated with it in South America, its native range, while Perkins (1984) noted only 20 in the USA and Gopal (1987) found only 12 in India, both the introduced ranges. This suggests that there is a depauperate phytophagous insect fauna in the introduced range, supporting the theory that the number of herbivores can be used as a measure of evolutionary history in a region.

Given that Chapter 3 suggests a long evolutionary history because the different varieties of *M. spicatum* are driven by local adaptations, the phytophagous invertebrate diversity associated with *M. spicatum* should provide evidence for the 'time spent' in southern Africa. Historically it was thought that submerged macrophytes were important only as a structural environment and as a substrate for periphyton growth, entering the aquatic food web only once dead (Shelford 1918). There is a growing body of literature to suggest that submerged macrophytes are an important food source, fed directly upon by a multitude of organisms, including several invertebrates, fish, birds and mammals (Welch 1952; Lodge 1991; Newman 1991; Wood *et al.* 2012). Despite the small proportion of insect species that use macrophytes as food (Newman 1991), they form the vast majority of aquatic plant herbivores (Gaevskaya 1969). This diverse phytophagous insect fauna feeds directly on macrophytes and these have been shown to have impacts on macrophyte productivity and growth (Soszka 1975; Wallace & O'Hop 1985; Nachtrieb *et al.* 2011; Morrison & Hay 2012; Reynolds *et al.* 2014). Newman (1991) summarised the orders of insect that form the main component of herbivore diversity for macrophytes and noted that it was dominated by Diptera, Lepidoptera, Coleoptera, Hemiptera and Trichoptera. Of these herbivorous taxa, there is a low proportion of specialist herbivores, with oligophagous invertebrates constituting approximately 58%. This is much lower than is the case in terrestrial systems where the proportion is approximately 80% (Newman 1991).

Direct herbivory has been shown to be important for several species of submerged macrophyte. In a study documenting the effect of herbivory on three species of macrophyte, American pondweed, *Potamogeton nodosus* Poir. (Potamogetonaceae), Illinois pondweed, *Potamogeton illinoensis* Morong (Potamogetonaceae), and Mexican water lily, *Nymphaea mexicana* Zucc. (Nymphaeaceae), herbivory was dominated by a few species (Nachtrieb *et al.* 2011). These species included *Rhopalosiphum* sp. (Aphidae); larval *Synclita* sp. (Crambidae) which were found on all three species of macrophyte; *Parapoynx* sp. (Crambidae) and two species of hydrellia fly, *Hydrellia discursa* Deonier (Ephydridae) and *Hydrellia bilobifera* Cresson (Ephydridae), which were found feeding only on the pondweeds, while *Donacia cincticornis* Newman (Chrysomelidae) was found only on the water lily (Nachtrieb *et al.* 2011). The impact of herbivory was most pronounced on the *Potamogeton* species, with no differences observed with the Mexican water lily (Nachtrieb *et al.* 2011). This was not necessarily attributed to less herbivory on the Mexican water lily than on the other species, but rather the ability of the plant to compensate by increasing the leaf turnover rate to a point where the biomass at any one time was not significantly different from the "no herbivory" treatment (Nachtrieb *et al.* 2011). The same result was experienced in a similar species, *Nuphar luteum* (L.) SM. (Nymphaeaceae), where the longevity of leaves was significantly decreased due to herbivory pressure by *Pyrrhalta nymphaeae* (L.) (Chrysomelidae) (Wallace & O'Hop 1985). There was, however, a significant reduction in the standing biomass due to the increased leaf turnover rate (Wallace & O'Hop 1985).

Herbivory by Lepidopterans, Trichopterans and Dipterans has also been shown to reduce leaf biomass of two species, *Potamogeton perfoliatus* L. (Potamogetonaceae) and *Potamogeton lucens* L. (Potamogetonaceae) by up to 90%, however there was less than 10% reduction in most cases from *Elodea canadensis* Rich. (Hydrocharitaceae) and *Myriophyllum spicatum* L. (Haloragaceae) (Soszka 1975). Not all species of macrophyte are equally

preferred by herbivores: in most cases *Potamogeton* species are the most preferred with both *M. spicatum* and *E. canadensis* being of intermediate to poor preference (Soszka 1975; Sheldon 1987; Sand-Jensen & Madsen 1989; Lodge 1991). This preference between species of submerged macrophyte varies among plant and herbivore groups. The biological control of macrophytes offers evidence for this, where many species of insect not only prefer a particular species of macrophyte, but are completely host specific either to the species or at least the genus (Julien & Griffiths 1998).

4.1.2 Macroinvertebrate and macrophyte community interactions

It is widely accepted that submerged macrophytes play an essential role in the aquatic environment and in many cases are considered ecosystem engineers (Jones *et al.* 1994; 1997). They provide important habitat for many aquatic species (Dibble *et al*. 1996), improve water quality and clarity by reducing the re-suspension of sediments, as well as sieving out any allocthonous material that may have been washed into the system (Duarte 2000). They are also considered important as nutrient sinks and play a role in facilitating the nutrient cycling and influencing the nutrient dynamics of many aquatic systems (Kufel & Kufel 2002; Schultz *et al.* 2003). Furthermore, it is now well known that macrophytes support high densities of organisms and are usually associated with high levels of species diversity (Cyr & Downing 1988a, 1988b; Cheruvelil *et al.* 2002; Ferreiro *et al*. 2011). This has been linked to macrophytes playing key roles in ecosystem processes of aquatic habitats, including the physical, chemical and biotic environment (Carpenter & Lodge 1986). The increase in the species diversity of fauna associated with macrophytes has been attributed to the structural complexity that macrophytes offer in the aquatic environment (Kovalenko *et al.* 2012). The underlying mechanisms are not fully understood, but have been related to macrophytes offering refuge from predators, increase in habitat (especially for macroinvertebrates) and

more foraging surface area (periphyton growth) (Hutchinson 1975; Dvořak & Best, 1982; Cyr & Downing 1988a, 1988b; Cheruvelil *et al.* 2002; Ferreiro *et al.* 2011).

Nonindigenous species have been shown to negatively impact the novel environment through a multitude of mechanisms, including increased competitive ability (Ni *et al.* 2010; Callaway *et al.* 2011), resource use efficiency (Funk & Vitousek 2007; Ren & Zhang 2009) and allelochemicals (Sun *et al.* 2013). Introduced submerged macrophytes are known to alter aquatic ecosystems and affect both the floral and faunal communities that they invade (Santos *et al.* 2011; Stiers *et al.* 2011). A study in Belgium showed that with an increase in the dominance of three invasive marginal aquatic species, *Hydrocotyle ranunculoides* L. (Umbelliferae), *Ludwigia grandiflora* (Michaux) Greuter & Burdet (Onagraceae) and *Myriophyllum aquaticum* (Vell.) Verd. (Haloragaceae), the native macrophyte and invertebrate diversity and abundance decreased (Stiers *et al.* 2011). The impact of these invasive species was clearly linked to the level of dominance (Pyšek & Pyšek 1995), where the greatest impacts were seen in the fully invaded sites which had between 90 – 100% cover (Stiers *et al.* 2011). These species were able to outcompete, and in some cases completely exclude native macrophytes, through competition for resources such as space, light and nutrients. The macroinvertebrate communities were largely affected by the change in water quality and chemistry, with sensitive species such as mayflies being completely absent from the heavily invaded sites (Stiers *et al.* 2011). Similarly, in the Sacramento-San Joaquin River Delta, California, the non-native macrophyte *Egeria densa* Planch. (Hydrocharitaceae) was the dominant macrophyte species regularly found in mono-specific beds (Santos *et al.* 2011). *Egeria densa* in the Delta has been shown to alter the physical and chemical environment to a point where it is capable of outcompeting native flora. For example, it offers anchorage substrate for *Ceratophyllum demersum* L. (Ceratophyllaceae), which in turn forms an

extremely dense surface coverage over the sediment which limits the establishment and growth of many other native species (Santos *et al.* 2011).

The abundance and community composition of macroinvertebrates is sensitive to macrophyte abundance, composition and species (Cyr & Downing 1988a, 1988b; Cheruvelil *et al.* 2002). This has been linked to the architecture of macrophytes where the higher the complexity, the more macroinvertebrates the plants can support. Cheruvelil *et al*. (2002) measured the abundance of macroinvertebrates between macrophyte species with different architecture and the results suggested that macrophytes with dissected leaves resulted in high level of complexity and thus supported higher densities and biomass of macroinvertebrates. For example, *Lagarosiphon major* (Ridl.) Moss (Hydrocharitaceae) supports much higher densities of macroinvertebrates (up to 300%) than macrophytes which had a relatively simple structure (Kelly & Hawes 2005). However, some species of macrophyte support fewer and less diverse macroinvertebrate communities despite having high structural complexity (Cattaneo *et al.* 1998; Cheruvelil *et al*. 2000, 2002; Taniguchi *et al*. 2003; Cremona *et al.* 2008). This is often linked to invasive species that create dense homogeneous macrophyte beds that can have implications for the physical and chemical environment, often creating anoxic conditions (Stiers *et al.* 2011; Teixeira *et al.* 2015). Brown *et al.* (1988) suggests that mono-specific beds generally support fewer species and lower abundances of macroinvertebrates than macrophyte beds with mixed macrophyte species.

The interactions of *M. spicatum* with species in the aquatic environment of southern Africa may shed light on the controversial status of this species in southern Africa (described in Chapter 1). Chapter 3 suggests that *M. spicatum* could have a long history in southern Africa, where local adaptations have evolved to the conditions experienced in the different geographic regions. This study aims to investigate whether the community interactions between *M. spicatum* and the fauna and flora naturally found in the environment corroborate

the potentially long evolutionary history within the southern African region. Specifically, the phytophagous insect community in the Vaal River and Lake Sibaya may provide essential information on the history of this species within these two systems. These two systems were chosen on the basis that the Vaal River currently supports the oldest extant population of *M. spicatum* known in southern Africa and Lake Sibaya is one of southern Africa's natural lakes, relatively isolated and pristine. The macroinvertebrate community composition and the interactions with other submerged macrophytes in these two systems were also studied to determine if the interactions between species also to infer a long evolutionary history in the region.

4.2 Materials and methods

4.2.1 Study area

Bi-annual (April and November) field surveys were conducted in 2012 and 2013 in two systems where *M. spicatum* occurs in South Africa, Lake Sibaya (-27.3561^{o}, 32.6915^{o}) and the Vaalharts Weir (-28.1138^{o}, 24.9275^{o}), Vaal River.

4.2.1.1 Lake Sibaya

4.2.1.1.1 Background

Lake Sibaya is one of South Africa's few natural lakes, situated in Northern KwaZulu-Natal. It is approximately 20m above sea level, separated from the sea by a series of forested sand dunes (Hill 1979). The Lake has a maximum depth of 40-43 m with an average of 12.6 -13.1 m, it holds 778-981 million m^3 of water, with a surface area of 59400-77500 ha (the values are adjusted for high and low lake levels) (Hill 1979). The climate is characterised as humid subtropical (Allanson 1979), with mean maximum and minimum

monthly temperatures of 35.3ºC and 5.5ºC (Mucina *et al.* 2006). The winters are drier than summers but the region shows weak seasonality with rainfall. Lake Sibaya receives between 900 and 1200 mm of rain annually (Allanson 1979; Mucina *et al.* 2006).

4.2.1.1.2 Associated aquatic vegetation

Lake Sibaya, being a relatively old natural lake in a tropical environment, has a wide diversity of aquatic plants (Howard-Williams 1979). The dominant emergent species at each of the sites sampled included *Typha capensis* (Rohrbach) (Typaceae), and on occasion, *Ludwigia adscendens* (L.) (Onagraceae) and *Phragmites mauritianum* Kunth (Poaceae) around the fringes and usually in the bays (Figure 4.1). The submerged plant species were dominated by *M. spicatum* and *C. demersum*. Other species present included *Stuckenia pectinata* (L.) Syn. *Potamogeton pectinatus* (Potamogetonaceae), *Potamogeton schweinfurthii* Bennett (Potamogetonaceae), *Najas horrida* Br. ex Magnus (Najadaceae), *Nymphaea nouchali* Burman (Nymphaeaceae) and *Utricularia inflexa* Forsskål (Lentibulariceae), but these were never recorded in the samples. *Myriophyllum spicatum* was first recorded in Lake Sibaya in 1965 (Chapter 2; Weyl & Coetzee 2014), and in 1977, during the study by Howard-Williams (1979) *M. spicatum* was widespread and relatively abundant in the lake but did not appear to be the dominant species. Surveys in 2010 by the Biological Control Research Group, based at Rhodes University, Grahamstown, showed that this species was present and at high abundance.

4.2.1.2 Vaalharts Weir, Vaal River

4.2.1.2.1 Background

The construction of the Weir was completed in 1938 on the confluence of the Harts and Vaal Rivers (Van Vuren 2010). The Weir has a capacity of 49 million m^3 and covers an

area 2118.9 ha (DWA Data Bank), with a mean depth of 3 m and is considered a turbid system with an average Secchi reading of 0.4 m in 1978. The weir is at approximately 1200 meters above sea level and the region is characterised by having summer and autumn rainfall, with 300 to 500 mm annually. The area frosts frequently and annual maximum and minimum temperatures of 37.4 ºC and -3.9 ºC (Rutherford *et al.* 2006).

Figure 4.1 A photographic representation of a typical sampling site on Lake Sibaya during the sampling period from April 2012 – November 2013.

4.2.1.2.2 Associated aquatic vegetation

The Vaalharts Weir was almost completely surrounded by a fringe of *P. mauritianum* reeds (Figure 4.2). Water hyacinth, *E. crassipes*, was very abundant during the surveys and an estimated percentage cover of the whole weir was usually 20-50%, and about 70% in April 2012. These infestations were usually along the edges (Figure 4.2). The only submerged macrophyte recorded in the weir during the surveys was *M. spicatum*, however, as recently as 5 years ago, *C. demersum, S. pectinata* and *Ranunculus rionii* Lagger (Ranunculaceae) have been recorded (Personal Observations). The lack of submerged macrophytes could be linked

to the recent invasion of grass carp, *Ctenopharyngodon idella* Cuvier and Valenciennes (Cyprinidae) in the Vaal River. *Myriophyllum spicatum* was first recorded in the Vaal River in 1877, and subsequently several records from the river have been lodged at various herbaria (Chapter 2; Weyl & Coetzee 2014). The plant is now widespread along the Vaal River from Parys to the confluence at Douglas (Fordham 2012) and into the Orange River as far down as Prieska (Chapter 2; Weyl & Coetzee 2014).

Figure 4.2 A photographic representation of a typical sampling site on the Vaalharts Weir during the sampling period from April 2012 – November 2013.

4.2.2 Site selection

A minimum of 12 sites (Figure 4.3 and 4.4) in each system were selected for sampling during each survey, except for the Vaalharts Weir in April 2012 when dense water hyacinth populations blocked access to five of the *M. spicatum* sampling sites. The sampling sites on Lake Sibaya were based on the previous work by Howard-Williams (1979). A total of 20 sites which were relatively evenly distributed across the Lake in each main basin were selected (Figure 4.3) and on each sampling occasion at least 12 of these sites were sampled and revisited on subsequent sampling trips. In the Vaalharts Weir, sampling sites were based on the presence of *M. spicatum* usually in water depth of 2 m or less, as there was no

macrophyte growth deeper than 2 m. It was rarely possible for the sites to be revisited during subsequent sampling occasions, but this was done where possible (Figure 4.4).

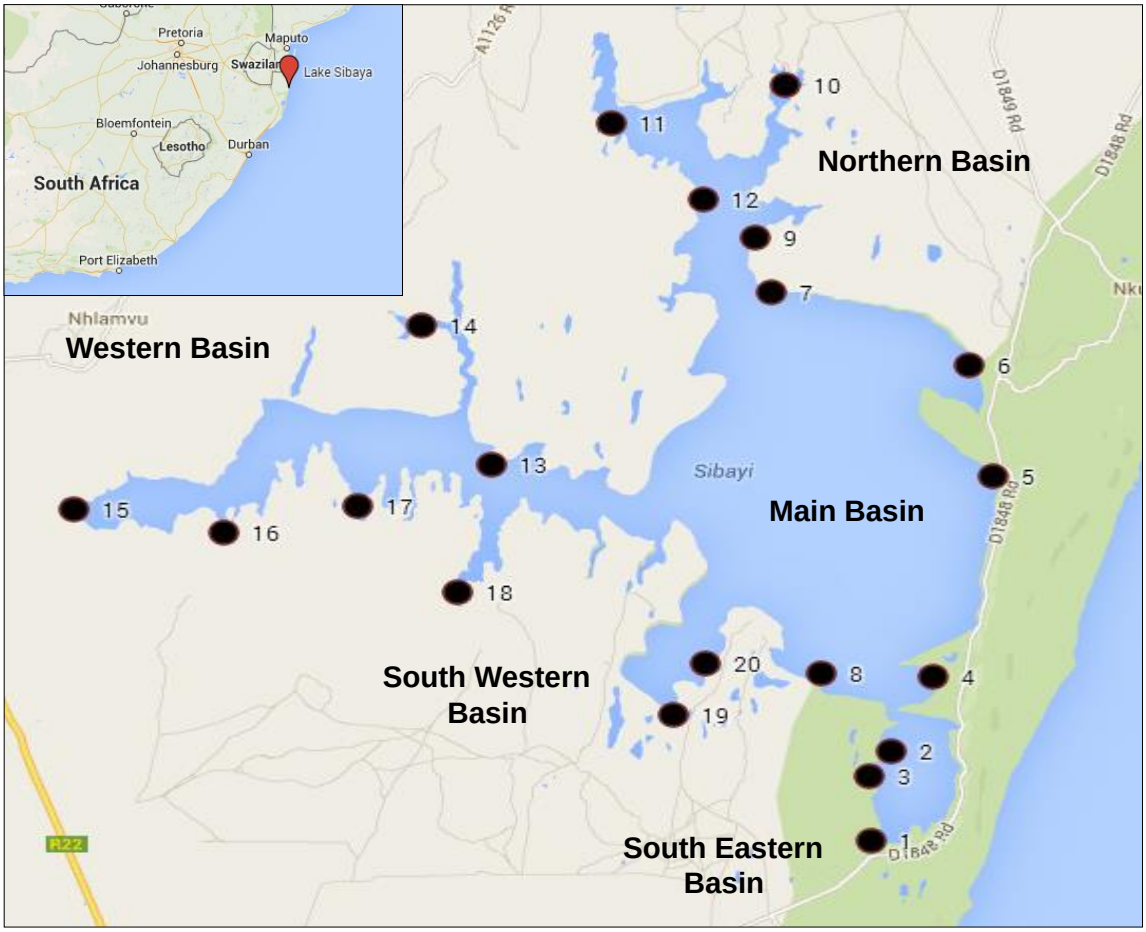

Figure 4.3 A map of Lake Sibaya showing the sites that were sampled during the surveys conducted in 2012 and 2013. The map shows the different areas or basins of Lake Sibaya, South East Basin, Main Basin, Northern Basin, Western Basin and the South Western Basin. The numbers indicate the approximate location of each sample site where macrophyte biomass and macroinvertebrate samples were taken during this study. The sample sites were revisited on subsequent sampling occasions.

4.2.3 Physicochemical parameters

Various physicochemical parameters were measured in both systems, at each site, on every sampling occasion. The hand-held probe Multi –Parameter Testr^{TM} 35 Series was used to measure pH, conductivity, TDS, salinity and temperature, while the Dissolved Oxygen

Pen Meter (Sper Scientific) was used to measure the dissolved oxygen and percent saturation. A standard Secchi disc was used to measure water clarity. The disc readings were taken at each sampling site for the Vaalharts Weir. The secchi disc reading for Lake Sibaya had to be taken in the channel adjacent to the sample site where there was sufficient depth, usually between 4-6 m. A 250 ml water sample was collected at each site for ammonia and nitrate analysis. The samples were frozen each day to ensure no breakdown of nutrients and transported back to the laboratory at Rhodes University for analysis. The analysis was done using the ion-selective electrodes (Vernier Software & Technology) specific to ammonia (NH_4^+) (NH4-BTA) and nitrates (NO_3^-) (NO3-BTA), connected to a LabQuest®2 digital interface (also Vernier, order code LABQ2). A total of 11 sediment samples were collected from the Vaal River in October 2011 and 30 from Lake Sibaya in April 2012 using a stainless steel cone dredge. The sediment samples were sent to Bemlab Laboratory (Strand, Western Cape, South Africa) for analysis of nutrient levels and soil characteristics.

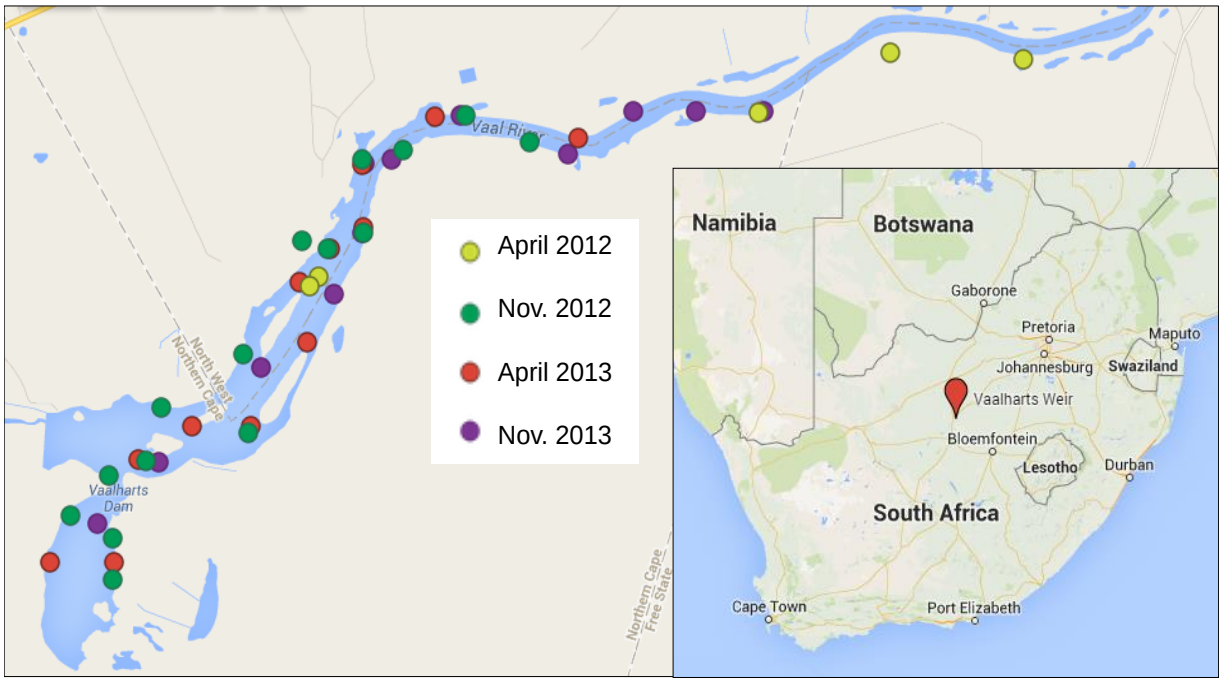

Figure 4.4 A map of the Vaalharts Weir, showing the location of the Weir on the map of southern Africa, a zoomed in map of the Weir with the different sample sites during each sampling occasion, April 2012, Nov. 2012, April 2013 and Nov 2013. The sites could not be revisited during subsequent sampling occasions due to the different levels of water hyacinth.

4.2.4 Aquatic macrophyte and macroinvertebrate sampling

The sampling regime was similar to that of Weyl & Coetzee (2013)*, and is described below. Once a site was selected, a random 30 m transect was laid across the site, this was done in a downwind direction and the boat was pulled upwind for the next sampling station along the transect. There were three sampling stations along the transect every 10 m where two (one on either side of the boat) macrophyte and associated macroinvertebrate samples, as well as water column samples to collect free-swimming invertebrates, were taken. This resulted in a total of six macrophyte samples and six water column samples for each site.

4.2.4.1 Macrophyte and associated epiphytic macroinvertebrate sample

To collect a macrophyte sample, the Howard-Williams & Longman sampler (Figure 4.5 A & B) (described in Weyl & Coetzee (2013)) was lowered down to the sediment surface and a full three revolutions were made, which in most cases was sufficient to wrap the plants around the sampler, and for the blades to sever the plants at the base. The plant material was then brought to the surface and placed in a bucket containing approximately 10 l of water. The plants were vigorously agitated to knock off any epiphytic macroinvertebrates, the remaining water was then sieved in a 0.5 mm mesh bag and the sample fixed in 10% formaldehyde. Once back in the laboratory, the samples were transferred to 70% ethanol for later processing and identification.

The macrophyte samples were taken to the field laboratory where the different species within the sample were separated. The plants were placed in a commercial salad spinner and spun dry (30 full handle revolutions) to remove any excess water. The material was then weighed to obtain a biomass (wet mass) for the sample of each species. Due to logistical issues surrounding the large size and numbers of samples taken during the field surveys, it

*To ensure a representative sample of the macroinvertebrate and macrophyte community, this sampling regime was tested in a pilot study prior to the thesis.

WEYL, P.S.R & COETZEE, J.A. 2013. An integrated remote sampling approach for aquatic invertebrates associated with submerged macrophytes. *African Journal of Aquatic Science* **38:** 337-340.

was necessary to obtain a wet mass to dry mass conversion. During the first field survey, April 2012, a representative sample of the dominant species present in both systems, were collected for wet mass to dry mass conversions. The plant material was first weighed wet after being spun dry in the salad spinner, then dried in an oven at 60^{o}C until a constant mass was obtained. A linear regression was used to model the relationship between the wet mass and dry mass. The resulting formula for each species was then used to convert the wet mass measured in the field to a dry mass for the sample.

4.2.4.2 Free-swimming macroinvertebrates

To collect a representative sample of the free-swimming invertebrates associated with macrophyte beds, a modified plankton net was employed (Figure 4.5 C) (described in Weyl & Coetzee (2013)). The net was lowered down to the bottom, shifted approximately 1 m to the side and swiftly and steadily brought to the surface. The macroinvertebrates collected in the collecting bottle were then immediately fixed in 10% formaldehyde, and transferred to 70% ethanol in the laboratory for later processing and identification. The depth of each sample was recorded so that the total volume of water sampled could be calculated and the abundance of each taxon could be standardised to per litre.

4.2.5 Feeding damage assessment of *Myriophyllum spicatum*

To assess feeding damage inflicted directly on *M. spicatum* in each system, 100 random sprigs were collected from each sampling site, from both the Vaalharts Weir and Lake Sibaya. The sprigs were then dissected under a stereo-microscope in the field laboratory. The damage inflicted was separated into three categories: tip damage, stem damage and foliar damage. Tip damage was characterised by either direct feeding or galling and manipulation of the tissue around the growth tip. Stem damage was characterised by any form of stem mining and possible egg laying of phytophagous insects. Foliar damage was

characterised as any direct removal of leaf mass due to feeding, chlorosis of the leaf or galling and manipulation of the leaf material.

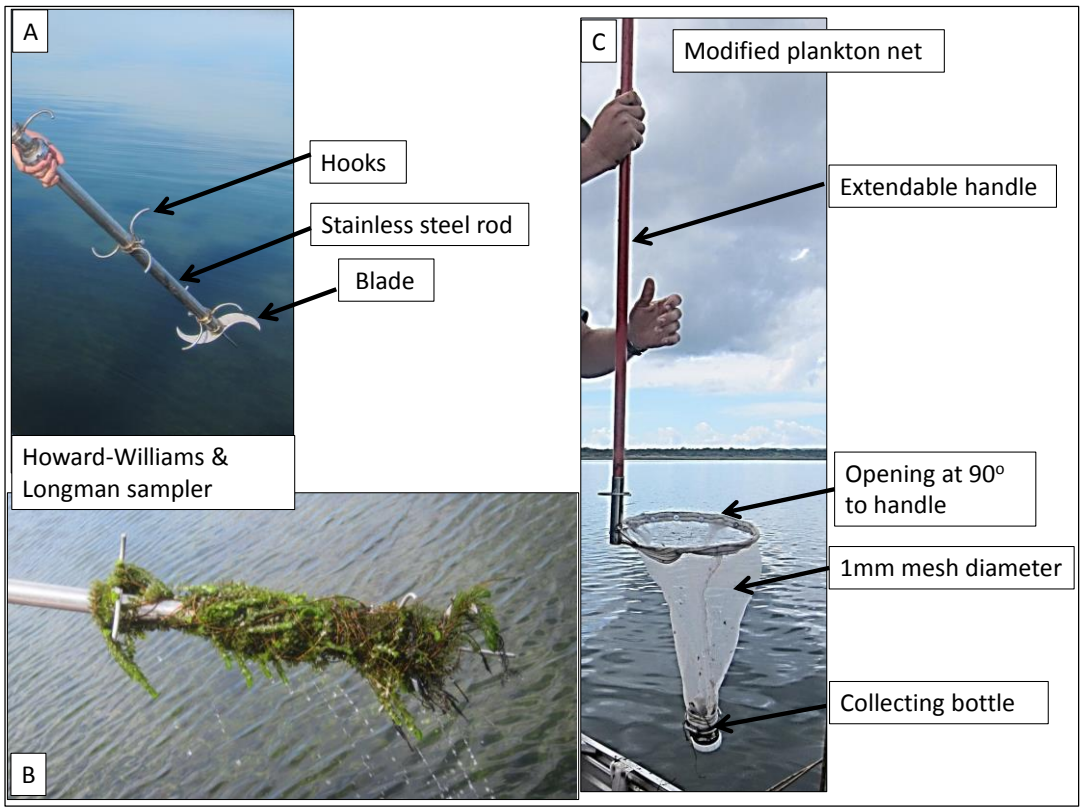

Figure 4.5 A photograph of the apparatus used to sample both epiphytic and free-swimming macroinvertebrate communities. A: the Howard-Williams and Longman sampler used for submerged macrophyte and associated epiphytic macroinvertebrate sampling; B: the Howard-Williams and Longman sampler with submerged macrophytes twisted on the hooks and severed at the base; and C: the modified plankton net used to collect the free-swimming macroinvertebrates utilizing the water column between the submerged macrophytes.

4.2.6 Statistical analyses

4.2.6.1 Physicochemical analyses

To test for differences between the water and sediment nutrient levels, specifically nitrates and ammonia for the water and total N for the sediment, the data collected from the Vaalharts Weir and Lake Sibaya were compared using a Student's *t*-test. The other water parameters (pH, DO2, O2%, conductivity, TDS, Secchi, salinity and temperature) were not

statistically compared as these systems would be inherently different due to differences in geomorphology of the different regions and climatic differences. The differences were descriptively compared between the systems.

4.2.6.2 Macroinvertebrate functional feeding group, abundance and community structure

The taxa recorded in this study were identified to the lowest possible level using the morphological keys for southern Africa in Day *et al.* (2001), Day & de Moor (2002), Day *et al*. (2003), de Moor *et al.* (2003), de Moor *et al.* (2004) and Stals & de Moor (2008). Each taxon was allocated a functional feeding group based on information collected from Merrit & Cummins (1984) and Pennak (1978). In some cases the level of identification was not specific enough to allocate one functional feeding group, so more than one was allocated. Based on the frequency of occurrence, each taxon was issued with a frequency, where 'Dominant' accounted for more than 50% occurrence in the samples, 'Frequent' was based on between 25-50% occurrence, 'Occasional' accounted for between 5-25% occurrence and 'Rare' was based on less than 5% occurrence.

For all subsequent analyses, the abundance of macroinvertebrates was standardised to a unit of dry mass, by dividing the abundance by the dry mass of macrophyte collected in that sample. This was done to remove the variability in abundance related to amount of macrophyte sampled (i.e. the higher the biomass sampled the higher the number of macroinvertebrates). The abundances of the individual macroinvertebrate taxa were pooled for each sample site to ensure a representative sample for each site (n=6 for each community sampled at each site). Because EstimateS V9.1.0 (Colwell 2013) only accepts abundance as whole numbers, the abundance was expressed as number of individuals per 10 g dry mass, as

10 g was sufficient for all taxa to be expressed as whole numbers without losing the variability between taxa.

Sample-based accumulation curves for each community (epiphytic macroinvertebrates and the free-swimming macroinvertebrates) were used to determine whether the sampling effort on each survey, at both the Vaalharts Weir and Lake Sibaya, was sufficient to collect and record the associated macroinvertebrates. The curves were compiled using the analytically calculated S_{obs}, which is the number of taxa observed, the Michaelis-Menten Mean (MMMean) estimator (Toti *et al.* 2000), and the incidence based coverage estimator (ICE), which are the number of taxa expected based on occurrence and abundance observed (Chazdon *et al.* 1998), in EstimateS V9.1.0. The richness observed is considered representative when the observed rarefaction curve (S_{obs}) converges with the estimators, in this case the ICE and MMMean, at the highest possible taxa richness (Longino *et al.* 2002). In addition to this, the Fishers α, Shannon-Weiner and Simpsons diversity indices were calculated using EstimateS V9.1.0. These diversity measures incorporate the taxa richness and evenness and report one value for each site. These diversity indices were compared using a General Linear Model (GLM) between sampling occasions for the Vaalharts Weir and Lake Sibaya separately. The Tukey Post-Hoc test was used to determine homogenous groups.

To investigate the community structure between the different sampling occasions within each system (Vaalharts Weir and Lake Sibaya separately), non-parametric multivariate analyses were used in PRIMER version 6.1.5 (Clarke & Gorley 2006). The data were fourth root transformed, and then a Bray-Curtis resemblance matrix was calculated as a measure of similarity between sampling sites at the different sampling occasions. The data were then visualised using a multi-dimensional scaling (MDS) plot. An analysis of similarities (ANOSIM) determined differences in the community structure between sampling occasions. The closer the Global R value is to 1, the more distinct the differences between the groupings

in the MDS plot. A similar analysis was performed to investigate the potential differences in the community structure between the different basins of Lake Sibaya where there were differences in the composition of the macrophyte species, *M. spicatum* and *C. demersum.* The analysis was done for each sampling occasion separately, comparing between each basin of Lake Sibaya. An MDS plot was used to visualise the similarity between the sample sites in each basin and an ANOSIM was performed to test for differences in the community structure of the macroinvertebrates at each site.

4.2.6.3 Macrophyte biomass

Following the wet mass to dry mass conversion using the equation obtained in the linear regression, the biomass for each species for each sample was expressed as g dry mass/m^2. This was done by dividing the total dry mass of each species collected per sample by the total surface area collected (in this case 625cm^2), then multiplying the result by 10 000, to obtain a per m^2 biomass estimate. See equation below.

$$\text{Biomass}/m^2 = (\text{Total dry mass in the sample}/625cm^2) \times 10\,000$$

According to Levene's Test for homogeneity and the Kolmogorov-Smirnov test for normality, the biomass measures for both the Vaalharts Weir and Lake Sibaya at each sampling occasion fulfilled the requirements for parametric analyses. To determine the change in biomass of *M. spicatum* over time and between the systems, the measures of biomass from the Vaalharts Weir and Lake Sibaya were compared. A GLM determined whether there were any significant differences between the biomass of each system and between sampling events, and a Tukey Post-Hoc test identified any homogenous groups.

The interaction between *M. spicatum* and *C. demersum* in Lake Sibaya was investigated where the biomass for each species at each sampling occasion were compared

using a GLM followed by a Tukey Post-Hoc test to determined homogenous groups. The distribution of *C. demersum* in the different basins was investigated where the biomass at sample sites within each basin were compared. These data did not fulfil the requirements of parametric analysis, therefore the nonparametric Kruskal-Wallis ANOVA was used to test for differences. A multiple comparison of mean ranks was used to determine homogenous groups.

4.3 Results

4.3.1 Physicochemical parameters

The physicochemical parameters were quite different between these systems, which is not too surprising in terms of the differences in geomorphology and region. Lake Sibaya had significantly lower concentrations of nitrates in the water than did the Vaalharts Weir ($t_{(33)}$ = 13.27, $P < 0.05$) yet there were no significant differences in the concentrations of ammonia between Lake Sibaya and the Vaalharts Weir ($t_{(33)}$ = -0.26, $P > 0.05$) (Figure 4.6). Despite the soil having the same characteristics, (sandy), there were slight differences in the levels of carbon, where surprisingly Lake Sibaya had three times more carbon than the Vaal River (3.4±1.1% and 0.8±0.1 % respectively) but this difference was not significant ($t_{(39)}$ = 1.36, $P < 0.05$) (Table 4.1). The soil nitrogen levels were significantly higher in the Vaal River than in Lake Sibaya, with a total N of 571.8±125.0 mg/kg and 82.0±39.2 mg/kg respectively ($t_{(39)}$ = -4.96, $P > 0.05$) (Table 4.1).

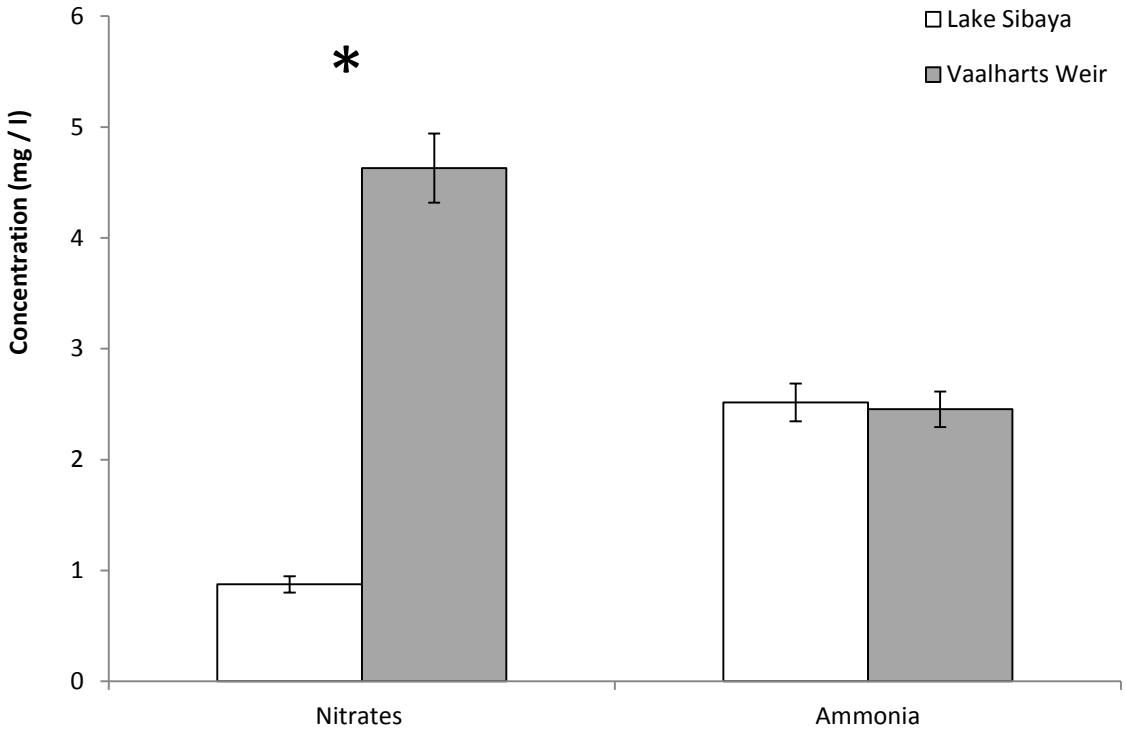

Figure 4.6 A comparison of the mean available nitrates and ammonia in the water between Lake Sibaya and the Vaalharts Weir. The "*" indicates a significant difference between the nitrate concentrations in Lake Sibaya and the Vaalharts Weir ($t_{(33)} = 13.27$, $P < 0.05$). There was no difference in the concentrations of ammonia between Lake Sibaya and the Vaalharts Weir ($t_{(33)} = -0.26$, $P > 0.05$). The error bars indicate standard error of the mean.

Lake Sibaya had relatively warm waters, with temperatures ranging from 21.9°C to 25.5°C, which is typical of a sub-tropical lake (Allanson 1979), while the Vaalharts Weir was comparatively cool, with temperatures from 20°C to 20.8°C (Table 4.2). The percent oxygen measures of Lake Sibaya were high, ranging between 98.7% and 106%, while the Vaalharts was comparatively lower, ranging between 77.8% and 97.8%, except for April 2013 which was 111.7% (Table 4.2). The Vaalharts Weir was a turbid system where the Secchi depth never exceeded 0.5m, while Lake Sibaya was relatively clear with a Secchi depth ranging between 3.8m and 5.9m (Table 4.2) and a maximum depth of 8m achieved in November 2012. The pH for both the Vaalharts Weir and Lake Sibaya was high, and ranged between 8.9

and 10.6 for the Vaalharts Weir, and from 8.6 to 9.9 for Lake Sibaya (Table 4.2). The salinity was relatively constant for both systems at 0.03 ppm for Lake Sibaya and 0.02 ppm for the Vaalharts Weir (Table 4.2). The conductivity and TDS were on most occasions higher in Lake Sibaya than the Vaalharts Weir, except for the final sampling occasion, where the Vaalharts readings were higher than Lake Sibaya (conductivity 692.3 µS and 657.1 µS respectively and TDS 491.5 ppm and 465.4 ppm respectively) (Table 4.2).

Table 4.1 The mean physicochemical parameters measured from the sediment samples collected from Lake Sibaya and the Vaal River. The '-' indicates unavailable data, where the separation between ammonia and nitrates was not taken.

Parameter	**Lake Sibaya**	**Vaal River**
Classification	Sandy	Sandy
% Carbon	3.4 ±1.1	0.8±0.1
Total N (mg/kg)	82.0±39.2	571.8±125.0
Ammonia NH4-N (mg/kg)	80.6±39.1	-
Nitrates NO3-N (mg/kg)	1.5±0.3	-

Table 4.2 The mean ± standard error, physicochemical parameters for both the Vaalharts Weir and Lake Sibaya on each sampling occasion over the two-year sampling period. The '-' indicates missing data, usually related to equipment malfunction.

	Lake Sibaya				**Vaalharts Weir**			
	Apr-12	Nov-12	Apr-13	Nov-13	Apr-12	Nov-12	Apr-13	Nov-13
pH	9.8±0.07	9.9±0.05	-	8.6±0.07	-	10.6±0.21	-	8.9±0.05
DO_2	8.2±0.38	8.1±0.34	9.2±0.32	-	7.2±0.41	6.9±0.33	9.0±0.22	8.9±0.27
O_2%	98.7±4.25	96.6±4.07	106.0±3.78	-	77.8±4.20	77.2±3.63	111.7±3.15	97.8±1.94
Conductivity (µS)	582.4±8.16	575.2±10.68	577.6±12.18	657.1±8.63	429.1±71.313	481.4±5.60	484.9±4.95	692.3±5.77
TDS (ppm)	291.1±4.12	287.8±3.58	292.8±5.58	465.4±6.28	246.1±6.54	232.6±3.80	230.8±1.68	491.5±4.00
Secchi (m)	3.8±0.12	4.0±0.06	5.3±0.13	5.9±0.09	0.5±0.03	0.5±0.03	0.5±0.03	0.2±0.00
Salinity (ppm)	0.3±0.004	0.3±0.005	0.3±0.001	0.3±0.000	0.2±0.007	0.2±0.004	0.2±0.000	0.2±0.000
Temperature (^oC)	25.5±0.23	23.5±0.17	21.9±0.08	24.3±0.16	20.0±0.48	20.6±0.22	20.8±0.11	20.5±0.21

4.3.2 Macroinvertebrate diversity and abundance

Over 420 000 individual macroinvertebrates in a total of 45 different taxa were collected in association with *M. spicatum* from both the Vaalharts Weir and Lake Sibaya over the sample period (Table 4.3). Lake Sibaya had a total of 23 taxa recorded during this study, which ranged between 6 and 23 different taxa depending on sample occasion and whether the epiphytic or free-swimming community was sampled (Figure 4.7 and 4.8). The freshwater shrimp, *Caridina nilotica* Roux (Atyidae), was collected only when sampling the free-swimming community and never collected in the epiphytic community from Lake Sibaya. The dominant species in both frequency and abundance collected from the lake was the invasive snail, *Tarebia granifera* Lam. (Thiaridae), which averaged 14.47±0.63 individuals/g macrophyte dry mass, followed by Chironomidae larvae at 4.09±0.95 individuals/g macrophyte dry mass (Table 4.3).

The Vaalharts Weir had a total of 21 taxa, the majority of which were collected in the macrophyte sample, usually ranging between 7 and 15 different taxa depending on sample occasion and the community of invertebrates sampled, epiphytic or free-swimming (Figure 4.9 and 4.10). Of these taxa, three were strictly collected from the free-swimming community collected by the water column samples; these included the freshwater shrimp, *Caridina africana* Kingsley (Atyidae), *Anisops* sp. (Notonectidae) and *Agraptocorixa* sp. (Corixidae). The macrophyte samples were largely dominated in frequency and abundance by Chironomidae larvae at 32.19±3.84 individuals/g macrophyte dry mass.

Five true macrophyte feeders were recorded in each system, but in most cases these were rarely found and in low abundance (Table 4.3). For example, *Nymphula* sp. (Crambidae) larvae that were recorded only from the Vaalharts Weir were in low numbers (0.068±0.02 individuals/g macrophyte dry mass) and rarely found in the samples (Table 4.3).

This is true also for *Leptocerus inflatus* Kimmins (Leptoceridae), recorded from Lake Sibaya, the numbers were low (0.03±0.02 individuals/g macrophyte dry mass) and only rarely found in the samples (Table 4.3). In both the systems, the most abundant functional feeding group in terms of number of taxa and abundance was the detritivores, with eight taxa for Lake Sibaya and four for the Vaalharts Weir. Herbivores specialising on microphytes and epiphytes were also high in abundance and constituted a total of nine taxa for Lake Sibaya and seven for the Vaalharts Weir. The predators constituted eight taxa for Lake Sibaya and nine for the Vaalharts Weir, but were typically low in abundance. There were comparatively fewer filter feeders and collector gatherers with only two taxa from each functional feeding group in each system (Table 4.3).

4.3.3 Feeding damage assessment of *Myriophyllum spicatum*

Despite the five different herbivore taxa collected from both systems, there was never any consistent macroinvertebrate feeding damage recorded in either Lake Sibaya or the Vaalharts Weir (Table 4.4). There was never any tip or stem damage recorded, and foliar damage, which included any type of mechanical damage (damaged leaflets and missing leaves), was in all the cases recorded on less than 5% of the sprigs, in both systems (Table 4.4).

Table 4.3 The different taxa, functional feeding groups, mean abundance and standard error (individuals/g macrophyte dry mass) at each locality, and the frequency of occurrence in the samples (Dom. = Dominant > 50%, Freq. = Frequent between 25-50%, Occ. = Occasional between 5-25% and Rare = <5%) of the macroinvertebrates collected at both Lake Sibaya and Vaalharts Weir during all of the sampling surveys, in April and November, 2012 and 2013.

Class	Order	Family	Dominant species	Functional feeding group	Lake Sibaya abundance	Vaalharts Weir abundance	Frequency
Annelida	Oligocheata (Aquatic worms)	Tubificidae	Undetermined species	Detritivore	0.101±0.027	0.615±0.200	Freq., Freq.
	Hirudinea (Leeches)	Glossiphoniidae	Undetermined species	Predator, parasite		0.789±0.168	Freq.
		Glossiphoniidae	*Placobdella unita*	Predator, parasite	0.843±0.322		Freq.
		Hirudinidae	*Limnatus fenestrata*	Predator	0.001±0.001		Rare
Mollusca	Gastropoda (Snails and Limpets)	Physidae	*Physa acuta*	Herbivore (microphytes), detritivore		0.061±0.017	Freq.
		Lymnaeidae	*Lymnaea natalensis*	Herbivore (microphytes), detritivore		0.341±0.110	Freq.
		Planorbidae	*Biomphalaria* sp.	Herbivore (microphytes), detritivore	0.077±0.032		Occ.
			Bulinus nataliensis	Herbivore (microphytes), detritivore	0.516±0.086		Occ.
		Ancylidae	Undetermined species	Herbivore (microphytes), detritivore		0.156±0.048	Occ.
			Burnupia sp.	Herbivore (microphytes), detritivore	0.163±0.055		Freq.
		Thiaridae	*Tarebia granifera*	Herbivore (microphytes), detritivore	14.468±0.631		Dom.

	Pelecypoda (Clams)	Corbiculidae	*Corbicula africana*	Filter feeder	0.076±0.020		Freq.
Crustacea	Isopoda	Sphaeromidae	*Pseudosphaeroma barnardi*	Detritivore	0.165±0.032		Occ.
	Amphipoda (Scuds or sideswimmers)	Aoridae	*Grandidierella lignorum*	Detritivore	0.505±0.088		Occ.
	Tanaidacea (Tanaids)	Apseudidae	*Halmyrapseudes digitalis*	Filter feeder	0.718±0.134		Occ.
	Decapoda (Freshwater Shrimp)	Atyidae	*Cardinia nilotica*	Detritivore, Herbivore both microphytes and macrophytes	0.056±0.010		Dom.
			Cardinia africana	Detritivore, Herbivore both microphytes and macrophytes		0.063±0.007	Dom.
	Cladocera (water fleas)	Daphniidae	*Daphnia* sp.	Filter feeder		2.753±0.882	Occ.
Arachnida	Arachniodea (water mites)	Hydrachnidae	*Hydracarnia* sp.	?		0.154±0.049	Freq.
Insecta	Ephemeroptera (Mayflies)	Beatidae	*Afroptilum* sp.	Detritivore, Herbivore (microphytes)	0.050±0.020		Occ.
			Cloeon sp.	Detritivore, Herbivore (microphytes)		0.022±0.011	Occ.
		Polymitarcyidae	*Povilla adusta*	Detritivore	0.017±0.005		Occ.
	Odonata (Dragon flies and Damselflies)	Gomphidae	*Ictinogomphus ferox*	Predator	0.004±0.001		Occ.
		Cordulidae	*Phyllomacromia picta*	Predator	0.076±0.001		Occ.

	Libellulidae	Undetermined species	Predator		0.341±0.003	Occ.
	Coenagrionidae	*Pseudagrion* sp.	Predator		0.186±0.034	Freq.
		Teinobasis sp.	Predator	0.025±0.010		Freq.
	Chlorolestidae	Undetermined species	Predator		0.003±0.002	Rare
Diptera (Flies and Midges)	Ceraptogonidae	*Bezzia* sp.	Predator	0.001±0.001		Rare
	Culicidae	*Coquillettidia* sp.	Predator, Filter feeder		0.003±0.002	Rare
	Chironomidae	Chironominae	Collector gather, predator, herbivore (macrophytes)	3.158±0.290	27.246±2.263	Dom., Dom.
		Orthocladiinae	Collector gather, predator, Herbivore (macrophytes)	0.936±0.627	4.946±1.555	Dom., Dom.
Hemiptera (True Bugs)	Corixidae	*Agraptocorixa* sp.	Predator		0.023±0.001	Freq.
	Notonectidae	*Anisops* sp.	Predator		0.022±0.001	Freq.
Trichoptera (Caddisflies)	Dipseudopsidae	*Dipseudopsis capensis*	Filter feeder	0.001±0.001		Occ.
		Dipseudopsis sp.	Filter feeder		0.007±0.003	Occ.
	Ecnomidae	*Ecnomus thomasetti*	Herbivore (microphytes)	0.015±0.010		Occ.
	Hydroptilidae	*Orthotrichia* sp.	Detritivore, Herbivore (microphytes), Herbivore (macrophytes)	0.174±0.045	0.409±0.089	Freq., Occ.
	Leptoceridae	*Leptocerus inflatus*	Detritivore, Herbivore (macrophytes), Predator	0.003±0.022		Rare
Lepidoptera (Moths and Butterflies)	Crambidae	*Nymphula* sp.	Herbivore (macrophytes)		0.068±0.021	Rare

Table 4.4 Feeding damage observed on dissected, 30cm sprigs of *Myriophyllum spicatum* from the Vaalharts weir and Lake Sibaya on each sampling event.

	Lake Sibaya				**Vaalharts Weir**			
	Apr-12	Nov-12	Apr-13	Nov-13	Apr-12	Nov-12	Apr-13	Nov-13
# of sprigs sampled	1500	1500	1200	1200	700	1500	1200	1200
% Damaged growth tips	0	0	0	0	0	0	0	0
% Stem damage	0	0	0	0	0	0	0	0
% Foliar damage	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5

4.3.4 Sampling effort

Twenty three taxa were observed (S_{obs}) from the epiphytic community for Lake Sibaya in April, which was not much lower than the estimators asymptote where the ICE = 23.81 taxa, and the MMMean = 25.87 taxa (Figure 4.7 A). In November 2012, the observed number of taxa was lower than that of April 2012, with 19 taxa recorded. The asymptote for the ICE was at 19.41 taxa and the MMMean was at 21.95 taxa (Figure 4.7 B). The lowest number of taxa was recorded in April 2013 with a total S_{obs} of 17 taxa, but the estimators reached an asymptote at ICE = 19.41 taxa and the MMMean = 21.95 taxa (Figure 4.7 C). In November 2013, a total of 19 taxa were observed, and the ICE estimator converged at 19 as well, while the MMMean estimated 20.12 taxa (Figure 4.7 D).

For the free-swimming community in Lake Sibaya the estimators for the most part did converge, except in April 2012, when the number of observed taxa was only six, the ICE estimated a total of 14.37 taxa, but the MMMean predicted only 5.85 taxa (Figure 4.8 A). The number of observed taxa for November 2012 was 8, and the estimates did converge and predicted 9.6 for the ICE and 8.5 for the MMMean (Figure 4.8 B). The observed number of taxa in April 2013 was the same as April 2012 which totalled six, but the estimators in this case did converge where the ICE = 6 taxa and the MMMean = 6.39 taxa (Figure 4.8 C). November 2013 had the lowest observed taxa for the free swimming community in Lake Sibaya, with a total of only 5 taxa recorded. The estimators did, however, reach an asymptote and converged with the S_{obs}, at ICE = 5 taxa and the MMMean = 5.45 taxa (Figure 4.8 D).

The number of taxa observed (S_{obs}) for the epiphytic community for the Vaalharts Weir in April 2012 was much lower than that of Lake Sibaya at 15 taxa, which was lower than the estimators asymptote where the ICE = 18.97 taxa and the MMMean = 18.09 taxa (Figure 4.9 A). In November 2012, the observed number of taxa was higher than that of April

2012, with 17 taxa recorded. The asymptote for the ICE was at 17.72 taxa and the MMMean was at 18.08 taxa (Figure 4.9 B). 15 taxa were recorded in April 2013, which was similar to April 2012, but the estimators reached an asymptote at ICE = 15.36 taxa and the MMMean = 16.31 taxa (Figure 4.9 C). In November 2013, the total number of taxa observed was 15, and the ICE estimator converged at 15.4, while the MMMean estimated 16.2 taxa (Figure 4.9 D).

For the free-swimming community in the Vaalharts Weir, the estimators mostly converged, except for April 2012, when the total number of observed taxa was 11, while the estimators were much higher, the ICE estimated 20.75 taxa and the MMMean predicted 18.09 taxa (Figure 4.10 A). The number of observed taxa for November 2012 was 15, and the estimates did converge with an asymptote for the ICE of 17.25 taxa and the MMMean of 16.94 taxa (Figure 4.10 B). The observed number of taxa in April 2013 was lower than that of April 2012 with eight taxa, but the estimators in this case did converge where the ICE = 8 taxa and the MMMean = 8.43 taxa (Figure 4.10 C). November 2013 had the lowest observed taxa for the free-swimming community in the Vaalharts Weir, with a total of only 7 taxa recorded. The estimators did, however, reach an asymptote with the ICE at 7 taxa and the MMMean at 7.21 taxa (Figure 4.10 D).

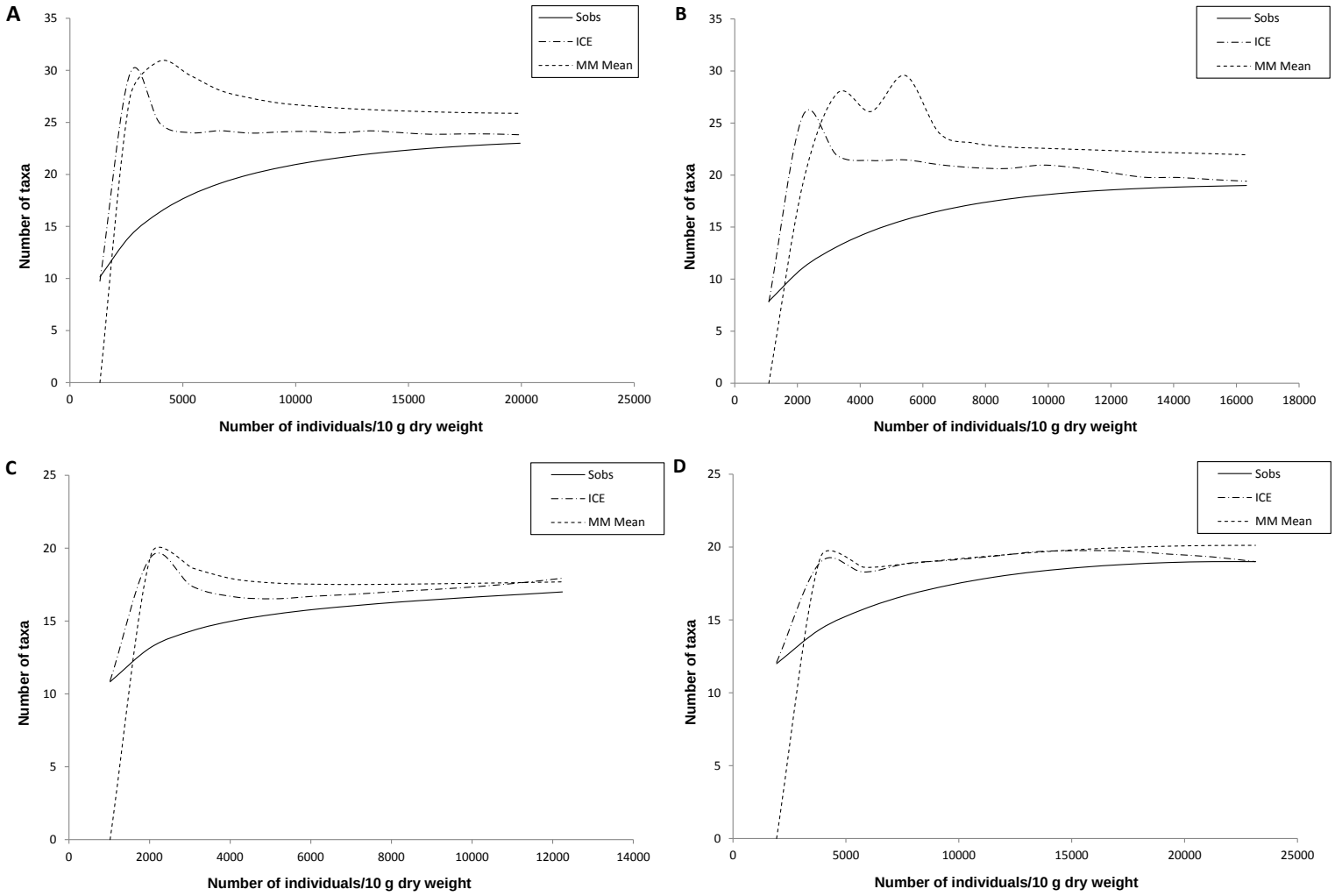

Figure 4.7 The species rarefaction curves for the epiphytic community recorded from Lake Sibaya for each sampling event (A: April 2012; B: November 2012; C: April 2013; D: November 2013), indicating the species observed (S_{obs}), Incidence-based coverage estimator (ICE) and the Michaelis-Menten Mean (MMMean) species richness estimators. Convergence of the estimators with the S_{obs} indicates adequate sampling effort.

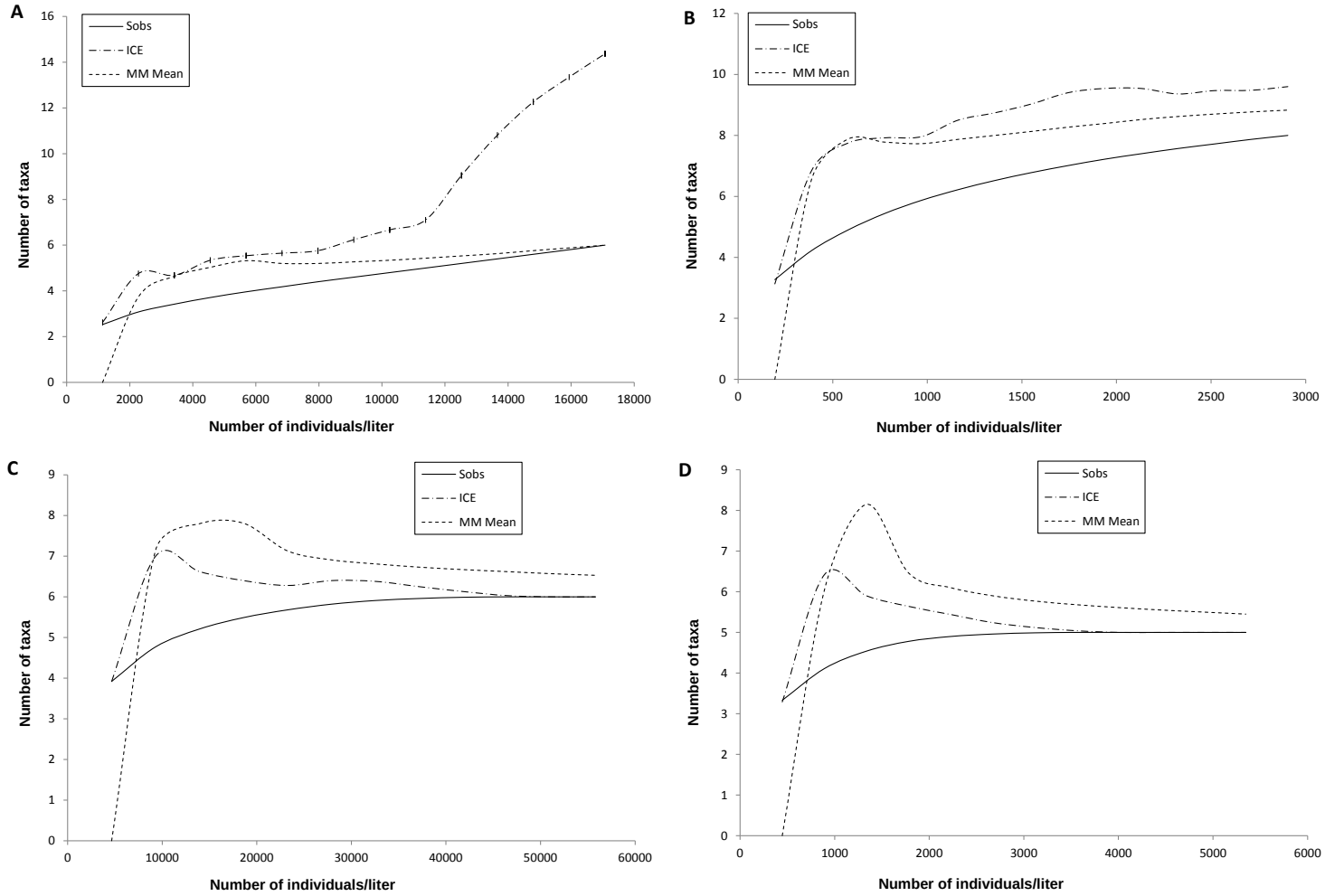

Figure 4.8 The species rarefaction curves for the free-swimming community recorded from Lake Sibaya for each sampling event (A: April 2012; B: November 2012; C: April 2013; D: November 2013), indicating the species observed (S_{obs}), Incidence-based coverage estimator (ICE) and the Michaelis-Menten Mean (MMMean) species richness estimators. Convergence of the estimators with the S_{obs} indicates adequate sampling effort.

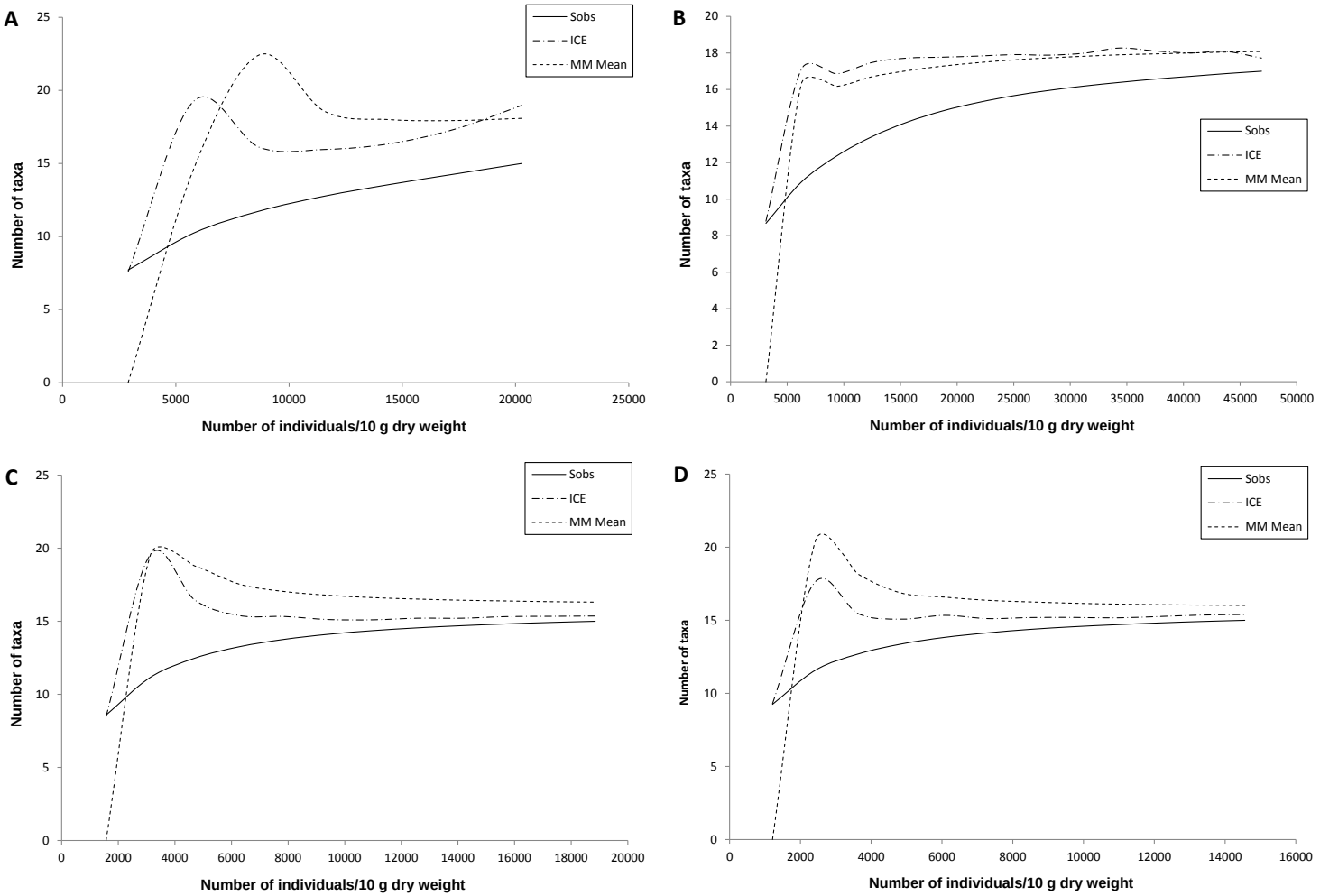

Figure 4.9 The species rarefaction curves for the epiphitic community recorded from the Vaalharts Weir, Vaal River for each sampling event (A: April 2012; B: November 2012; C: April 2013; D: November 2013), indicating the species observed (S_{obs}), Incidence-based coverage estimator (ICE) and the Michaelis-Menten Mean (MMMean) species richness estimators. Convergence of the estimators with the S_{obs} indicates adequate sampling effort.

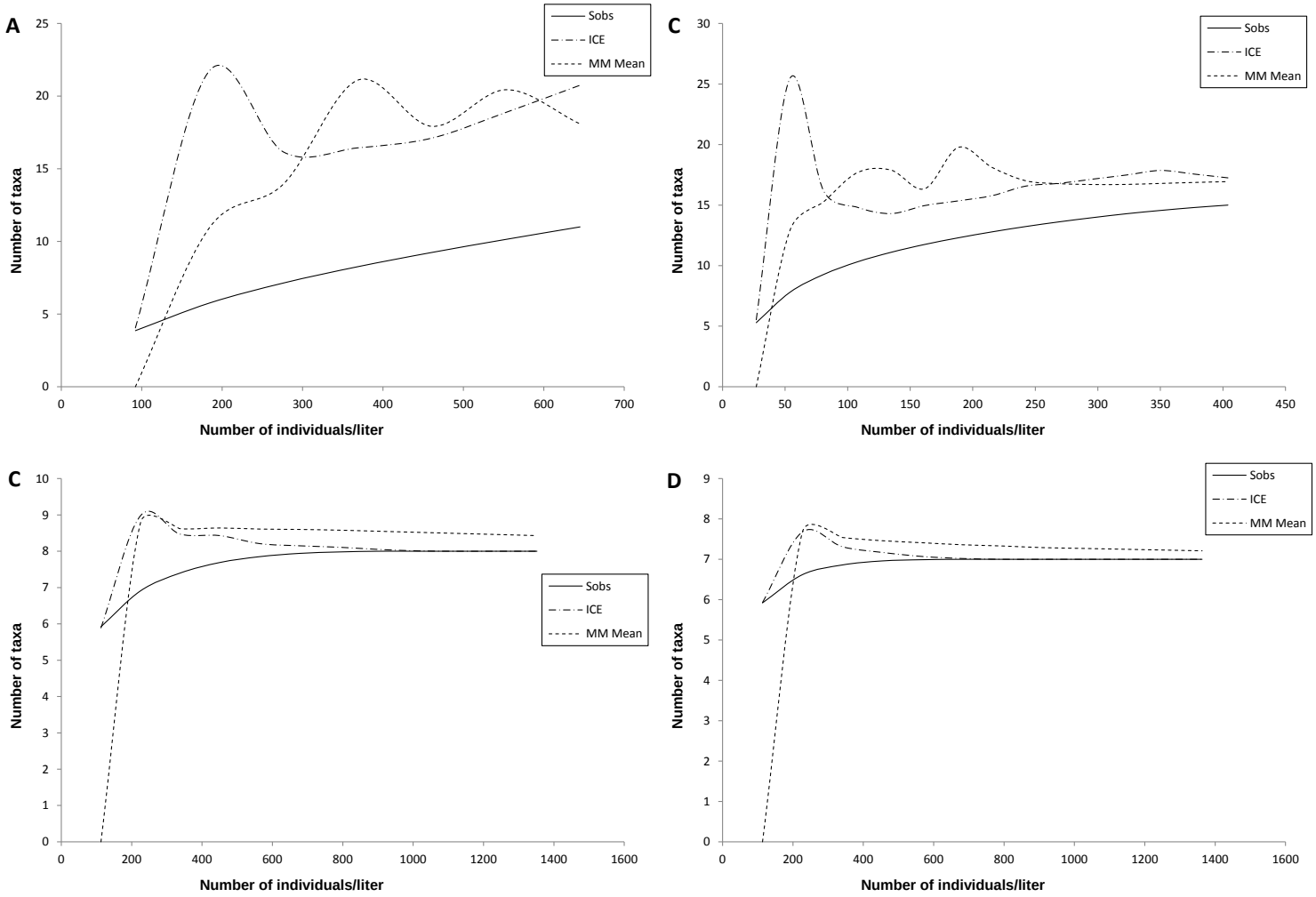

Figure 4.10 The species rarefaction curves for the free-swimming community recorded from the Vaalharts Weir, Vaal River for each sampling event (A: April 2012; B: November 2012; C: April 2013; D: November 2013), indicating the species observed (S_{obs}), Incidence-based coverage estimator (ICE) and the Michaelis-Menten Mean (MMMean) species richness estimators. Convergence of the estimators with the S_{obs} indicates adequate sampling effort.

4.3.5 Community analyses

The three species diversity measures were all significantly higher for the epiphytic community of Lake Sibaya than for the epiphytic community of the Vaalharts Weir for all sampling occasions (Table 4.5). The opposite was found for the diversity of the free-swimming invertebrate community, where the Vaalharts Weir was always significantly higher than that of Lake Sibaya (Table 4.5). This is reflected in the diversity measures for each sampling occasion for both systems. The species diversity measures were always significantly higher for the epiphytic community than the free-swimming community in Lake Sibaya (Table 4.5). The opposite was found for the Vaalharts Weir, where diversity of the free-swimming community was significantly higher than that of the epiphytic community (Table 4.5).

Table 4.5 The three diversity indices used to measure the diversity of both the epiphytic and free-swimming community. A GLM was employed to test for significant differences between the diversity indices between the sampling occasions and between Lake Sibaya and Vaalharts Weir at all the sampling occasions. The different letters indicate significant differences ($P < 0.05$) between the diversity indices.

System	Community	Date	Fishers α	GLM test statistic	Shannon-Wiener H'	GLM test statistic	Simpsons λ	GLM test statistic
Sibaya	Epiphitic	April 2012	2.38 (0.08)^{a}	$F_{(3,50)} = 11.67$; $P < 0.05$	3.97 (0.05)^{a}	$F_{(3,50)} = 230.20$; $P < 0.05$	2.58 (0.01)^{a}	$F_{(3,50)} = 422.27$; $P < 0.05$
		Nov. 2012	1.93 (0.02)^{b}		2.17 (0.01)^{b}		1.49(0.00)^{b}	
		April 2013	1.96 (0.07)^{b}		2.85 (0.07)^{c}		2.05(0.03)^{c}	
		Nov. 2013	2.01 (0.03)^{b}		3.61 (0.05)^{d}		2.29 (0.02)^{d}	
Sibaya	Free swimming	April 2012	0.47 (0.02)^{a}	$F_{(3,50)} = 33.85$; $P < 0.05$	1.09 (0.00)^{a}	$F_{(3,50)} = 19.65$; $P < 0.05$	1.03 (0.00)^{a}	$F_{(3,50)} = 17.76$; $P < 0.05$
		Nov. 2012	0.52 (0.01)^{b}		1.88 (0.01)^{b}		1.50 (0.00)^{b}	
		April 2013	0.56 (0.03)^{a}		1.56 (0.01)^{c}		1.27 (0.00)^{c}	
		Nov. 2013	0.57 (0.01)^{a}		1.43 (0.00)^{d}		1.20 (0.00)^{c}	
Vaalharts	Epiphitic	April 2012	1.36 (0.07)^{a}	$F_{(3,42)} = 5.94$; $P < 0.05$	2.99 (0.03)^{a}	$F_{(3,42)} = 2293.2$; $P < 0.05$	2.11 (0.06)^{a}	$F_{(3,42)} = 369.86$; $P < 0.05$
		Nov. 2012	1.56 (0.04)^{b}		1.92 (0.01)^{b}		1.39 (0.00)^{b}	
		April 2013	1.56 (0.04)^{b}		1.88 (0.01)^{b}		1.37 (0.00)^{b}	
		Nov. 2013	1.64 (0.02)^{b}		1.40 (0.00)^{c}		1.13 (0.00)^{c}	
Vaalharts	Free swimming	April 2012	1.53 (0.11)^{a}	$F_{(3,42)} = 497.24$; $P < 0.05$	2.25 (0.02)^{a}	$F_{(3,42)} = 165.22$; $P < 0.05$	1.67 (0.04)^{a}	$F_{(3,42)} = 627.49$; $P < 0.05$
		Nov. 2012	1.25 (0.02)^{b}		4.72 (0.09)^{b}		3.65 (0.04)^{b}	
		April 2013	2.97 (0.03)^{c}		5.23 (0.11)^{c}		3.45 (0.02)^{c}	
		Nov. 2013	1.11 (0.04)^{d}		4.79 (0.05)^{b}		4.16 (0.04)^{d}	
Sibaya	Epiphitic	Combined	2.08 (0.04)	$F_{(1, 98)} = 118.67$; $P < 0.05$	3.18 (0.10)	$F_{(1, 98)} = 102.61$; $P < 0.05$	2.13 (0.05)	$F_{(1, 98)} = 90.48$; $P < 0.05$
Vaalharts			1.55 (0.02)		1.92 (0.07)		1.43 (0.05)	
Sibaya	Free swimming	Combined	0.61 (0.02)	$F_{(1, 98)} = 106.67$; $P < 0.05$	1.47 (0.04)	$F_{(1, 98)} = 429.36$; $P < 0.05$	1.24 (0.02)	$F_{(1, 98)} = 375.54$; $P < 0.05$
Vaalharts			1.82 (0.12)		4.53 (0.15)		3.42 (0.12)	

An MDS plot grouped the total community (epiphytic and free-swimming combined) into four distinct sampling occasions for Lake Sibaya. This was confirmed by the ANOSIM results which showed a significant grouping between sampling occasions (Global R = 0.387, *P* = 0.001) (Figure 4.11).

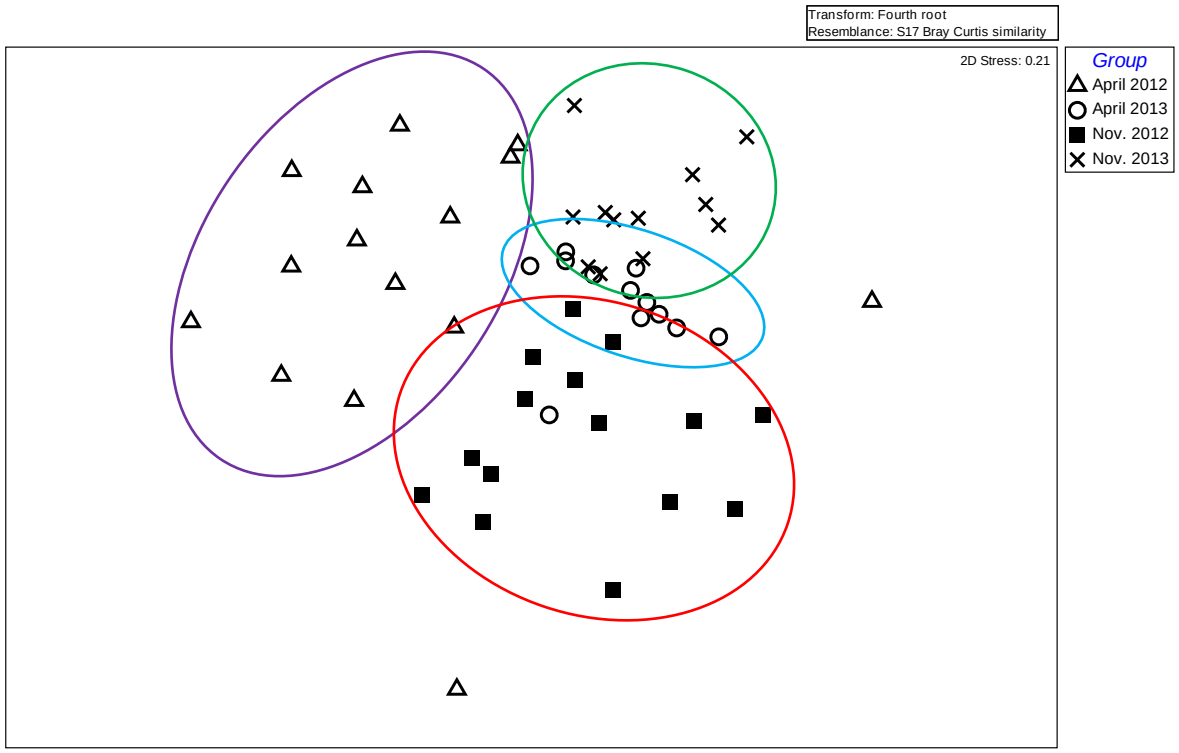

Figure 4.11 The MDS plot comparing the community structure of macroinvertebrates collected in association with submerged macrophytes in Lake Sibaya between the different sampling occasions (April 2012; November 2012; April 2013; November 2013).

The community comparison for each separate sampling occasion for the total community (epiphytic and free-swimming combined) grouped by location (i.e. basin) in Lake Sibaya, suggests that there was no community structure difference in the aquatic macroinvertebrates between the basins of Lake Sibaya dominated by different macrophyte species (Figure 4.12 A-D). The ANOSIM results confirm the lack of differences in community structure between the basins (April 2012 (Global R = 0.04, *P* = 0.57); November 2012 (Global R = 0.04, *P* = 0.61); April 2013 (Global R = 0.14, *P* = 0.76); November 2013 (Global R = 0.126, *P* = 0.74) (Figure 4.12 A-D)).

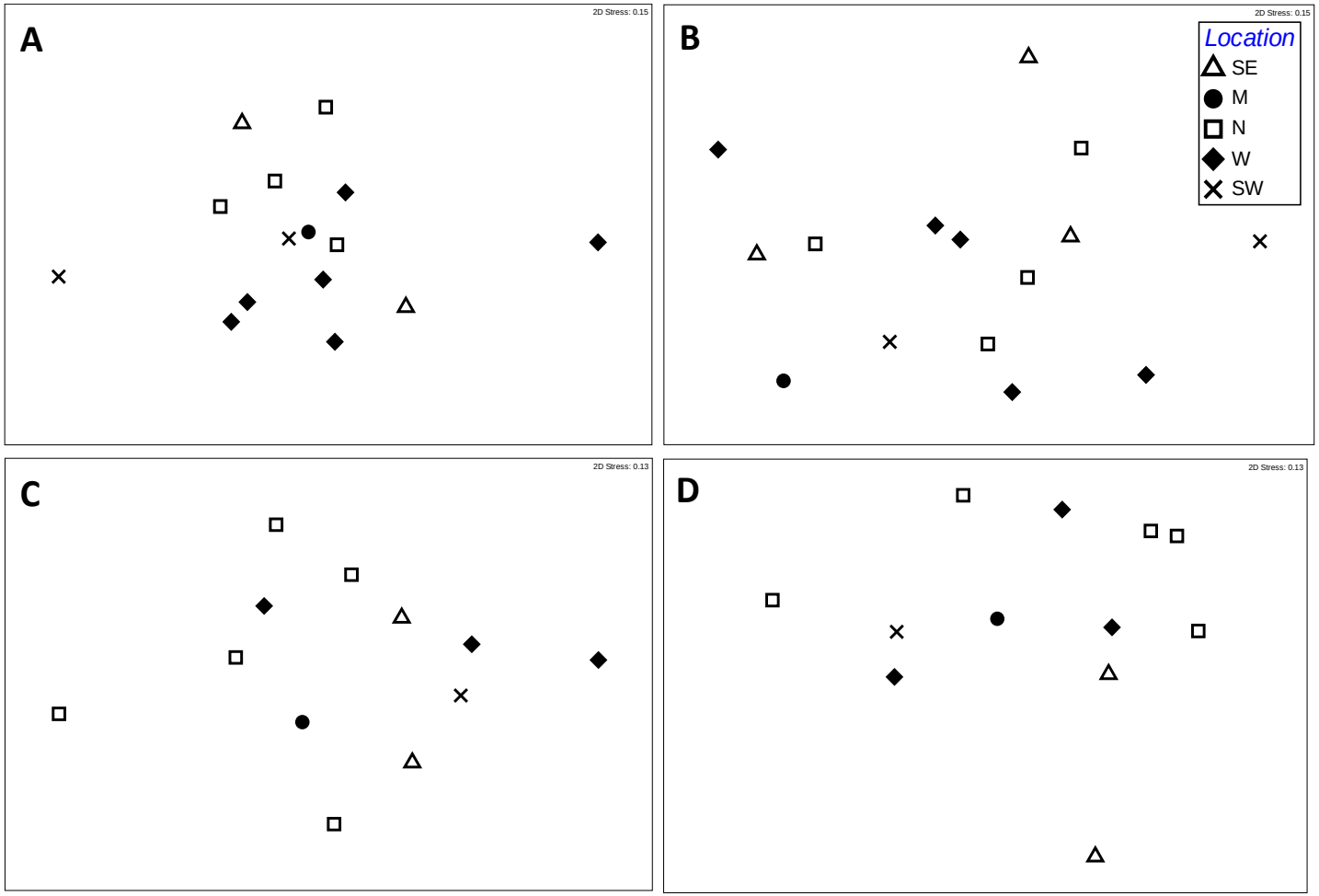

Figure 4.12 The MDS plot comparing the community structure of macroinvertebrates collected in association with submerged macrophytes between the basins of Lake Sibaya (SE = South East Basin, M = Main Basin, N= Northern Basin, W = Western Basin, SW = South Western Basin) at each sampling event (A: April 2012; B: November 2012; C: April 2013; D: November 2013). In all cases, the data were fourth root transformed and resemblance was based on the Bray Curtis similarity matrix.

An MDS plot grouped the total community (epiphytic and free-swimming combined) into four distinct sampling occasions for the Vaalharts Weir. This was confirmed by the ANOSIM results which showed a significant grouping between sampling occasions (Global R = 0.624, *P* = 0.001) (Figure 4.13).

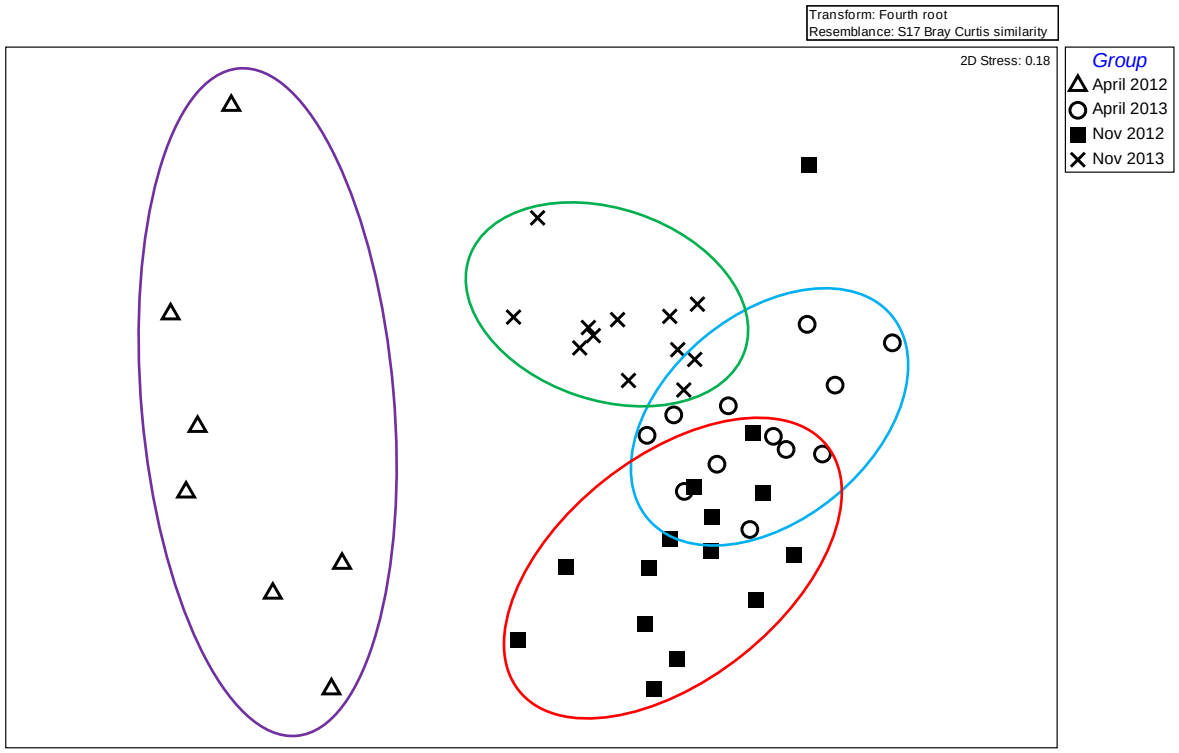

Figure 4.13 The MDS plot comparing the community structure of macroinvertebrates collected in association with *Myriophyllum spicatum* in the Vaalharts Weir, Vaal River between the different sampling occasions (April 2012; November 2012; April 2013; November 2013).

4.3.6 Macrophyte biomass

There was a significant positive relationship between the wet mass and dry mass for *M. spicatum* for both Lake Sibaya (R^2 = 0.9862; $P < 0.05$) (Figure 4.14) and the Vaalharts Weir (R^2 = 0.9794, $P < 0.05$) (Figure 4.15). *Ceratophyllum demersum* from Lake Sibaya also had a significant positive relationship between the wet mass and dry mass (R^2 = 0.9862, $P < 0.05$) (Figure 4.14).

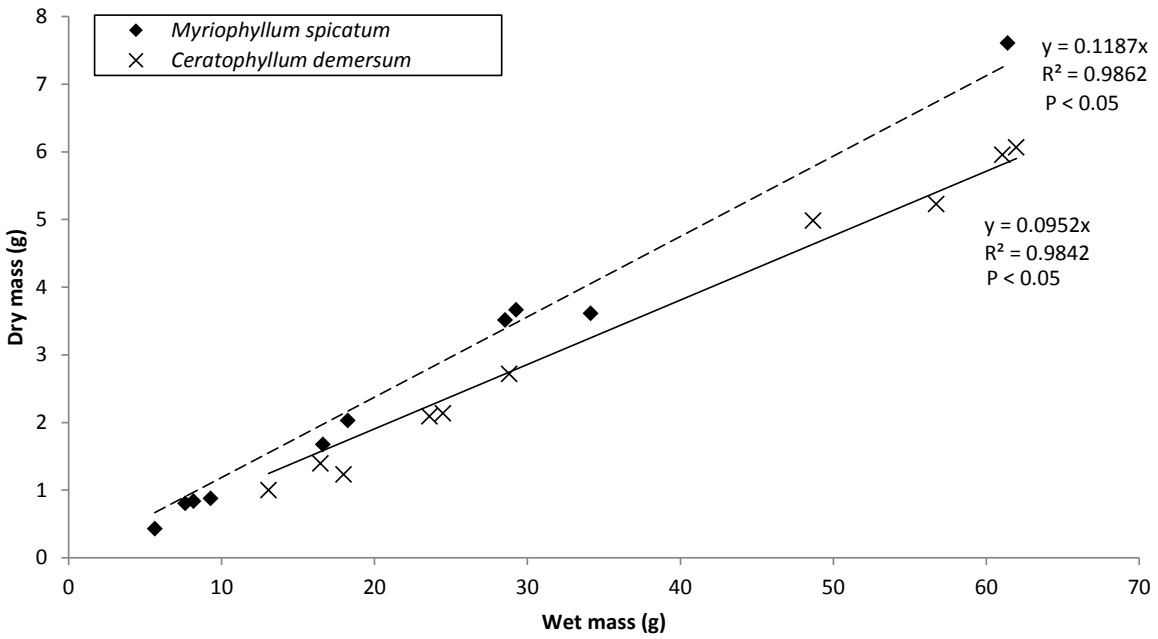

Figure 4.14 Wet mass to dry mass relationship of *Myriophyllum spicatum* (dashed line) and *Ceratophyllum demersum* (solid line) from Lake Sibaya.

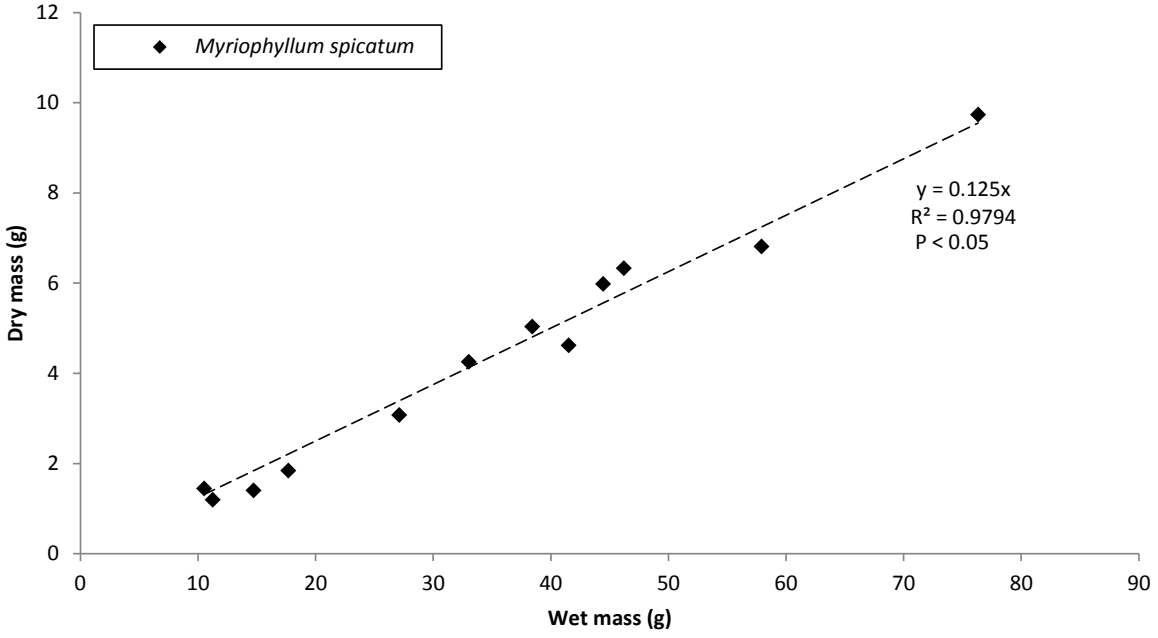

Figure 4.15 Wet mass to dry mass relationship of *Myriophyllum spicatum* (dashed line) from the Vaalharts Weir, Vaal River.

There were significant interactions between system*time ($F_{(3, 592)} = 25.59$, $P < 0.05$) for the *M. spicatum* biomass measures between the Vaalharts Weir and Lake Sibaya. The most noticeable significant difference in biomass was between the two systems in November 2013, where the biomass of *M. spicatum* from the Vaalharts Weir was significantly higher than that of Lake Sibaya, 953.1±127.1 g dry mass/m^2 and 163.4±16.8 g dry mass/m^2 respectively (Figure 4.16). The mean biomass of *M. spicatum* did not vary significantly on the first three sampling occasions between Lake Sibaya and the Vaalharts Weir, Vaal River and ranged between 200 – 400 g dry mass/m^2 (Figure 4.16). The biomass of *M. spicatum* did not vary significantly between sampling occasions for Lake Sibaya, where the biomass ranged between 163.4±16.8 g dry mass/m^2 in November 2013 and 376.9±57.1 g dry mass/m^2 in April 2012. There was a significant increase in the biomass recorded from the Vaalharts Weir in November 2013 (953.1±127.1 g dry mass/m^2) compared to the other sampling occasions which ranged between 188.2±40.2 g dry mass/m^2 and 434.4±67.4 g dry mass/m^2 (Figure 4.16).

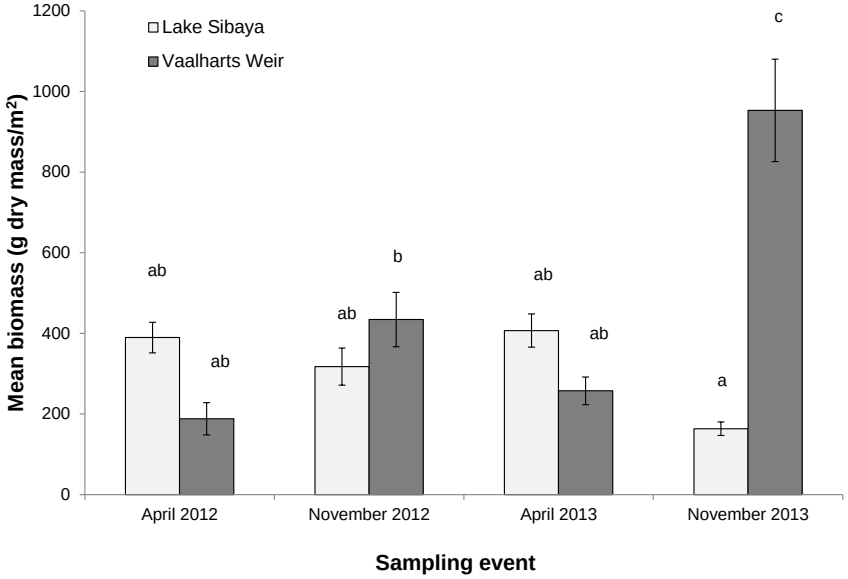

Figure 4.16 Mean *Myriophyllum spicatum* biomass for each sampling occasion, error bars indicate standard error around each mean. The letters indicate homogenous groups: system*time ($F_{(3, 592)} = 25.59$, $P < 0.05$).

At every sampling occasion, the biomass of *M. spicatum* was higher than that of *C. demersum* (Figure 4.17; Table 4.6). The biomass of these species were not evenly distributed between the different basins of Lake Sibaya, with the Northern and Western basins consistently having higher biomass and occurrence of both species. *Ceratophyllum demersum* biomass was significantly higher and was found consistently in the Northern and Western basins on all sampling occasions, while in the other basins, the biomass was low and occurrence was variable between sampling occasions ($H_{(4,80)} = 13.22$, $P = 0.01$) (Figure 4.17).

Table 4.6 Mean biomass ± standard error of *Myriophyllum spicatum* and *Ceratophyllum demersum* recorded for Lake Sibaya on each sampling occasion.

Date	Species	Biomass (g dry mass/m^2)	GLM test statistic
April 2012	*Myriophyllum spicatum*	376.9±57.1	$F_{(1, 214)} = 24.64$, $P < 0.05$
	Ceratophyllum demersum	207.8±92.7	
November 2012	*Myriophyllum spicatum*	317.4±86.8	$F_{(1, 178)} = 17.45$, $P < 0.05$
	Ceratophyllum demersum	82.4±63.6	
April 2013	*Myriophyllum spicatum*	407.1±74.4	$F_{(1, 142)} = 12.46$, $P < 0.05$
	Ceratophyllum demersum	173.1±102.0	
November 2013	*Myriophyllum spicatum*	163.0±24.38	$F_{(1, 142)} = 30.99$, $P < 0.05$
	Ceratophyllum demersum	34.76±27.54	

The biomass collected from the Vaalharts Weir was variable within as well as between sites, with no trend or location effect on the biomass recorded (Figure 4.18). During the first three sampling occasions the biomass was below 500 g dry mass/m^2, however during the November 2013 sampling occasion the biomass was just below 1000 g dry mass/m^2, more than double the previous sampling occasions (Figure 4.18).

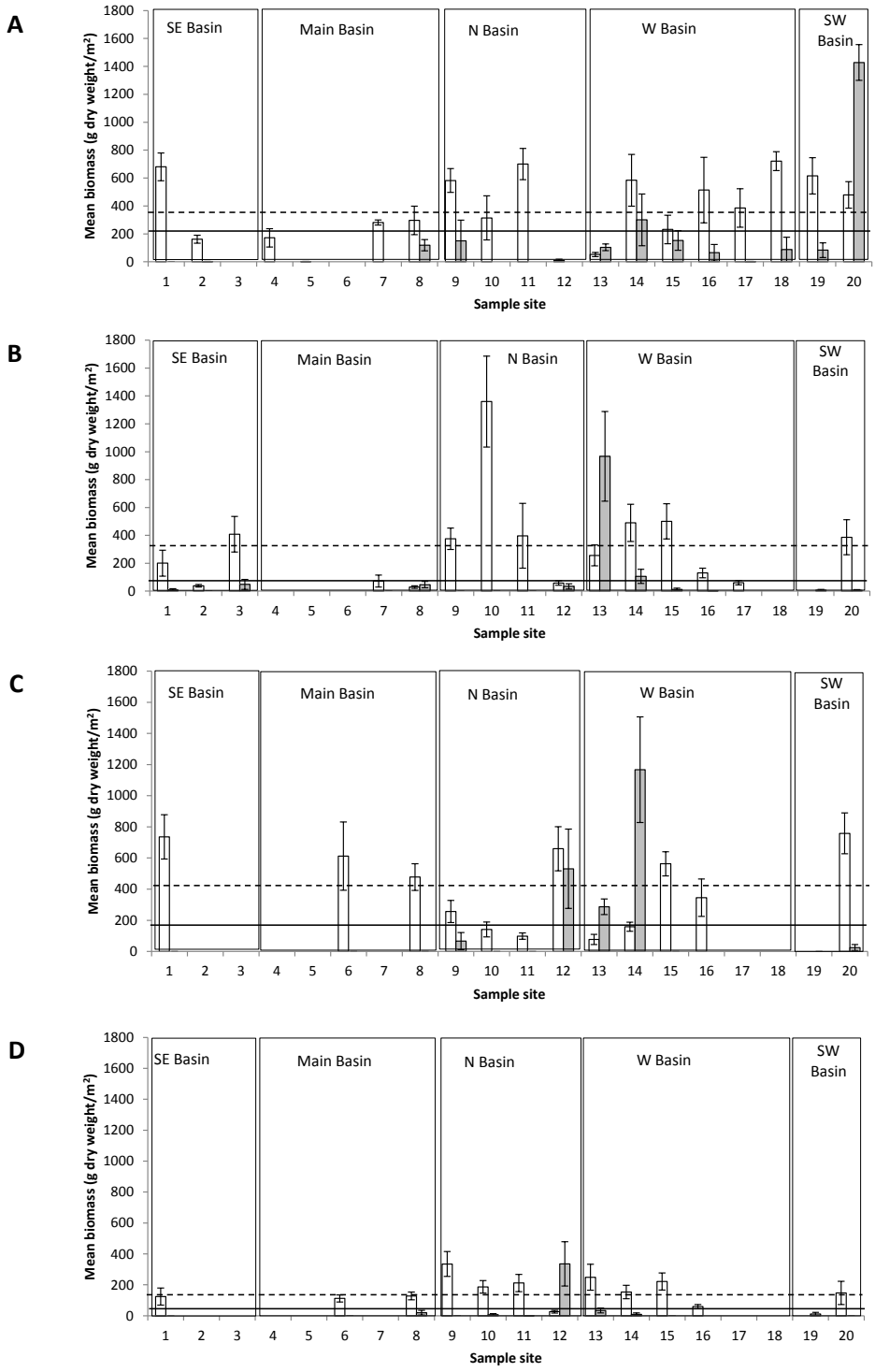

Figure 4.17 Biomass of both *Myriophyllum spicatum* and *Ceratophyllum demersum* during the four field surveys (A: April 2012; B: November 2012; C: April 2013; D: November 2013) in Lake Sibaya. The white bars show *M. spicatum* biomass at each site and the grey bars indicate *C. demersum* biomass at each site, the error bars indicate standard error of the mean. The hashed line indicates the mean biomass of *M. spicatum*, while the solid line indicates the mean biomass of *C. demersum* of each sampling event. Each graph is split between basins, where SE Basin = South East Basin; Main Basin; N Basin = Northern Basin; W Basin = Western Basin; SW Basin = South Western Basin.

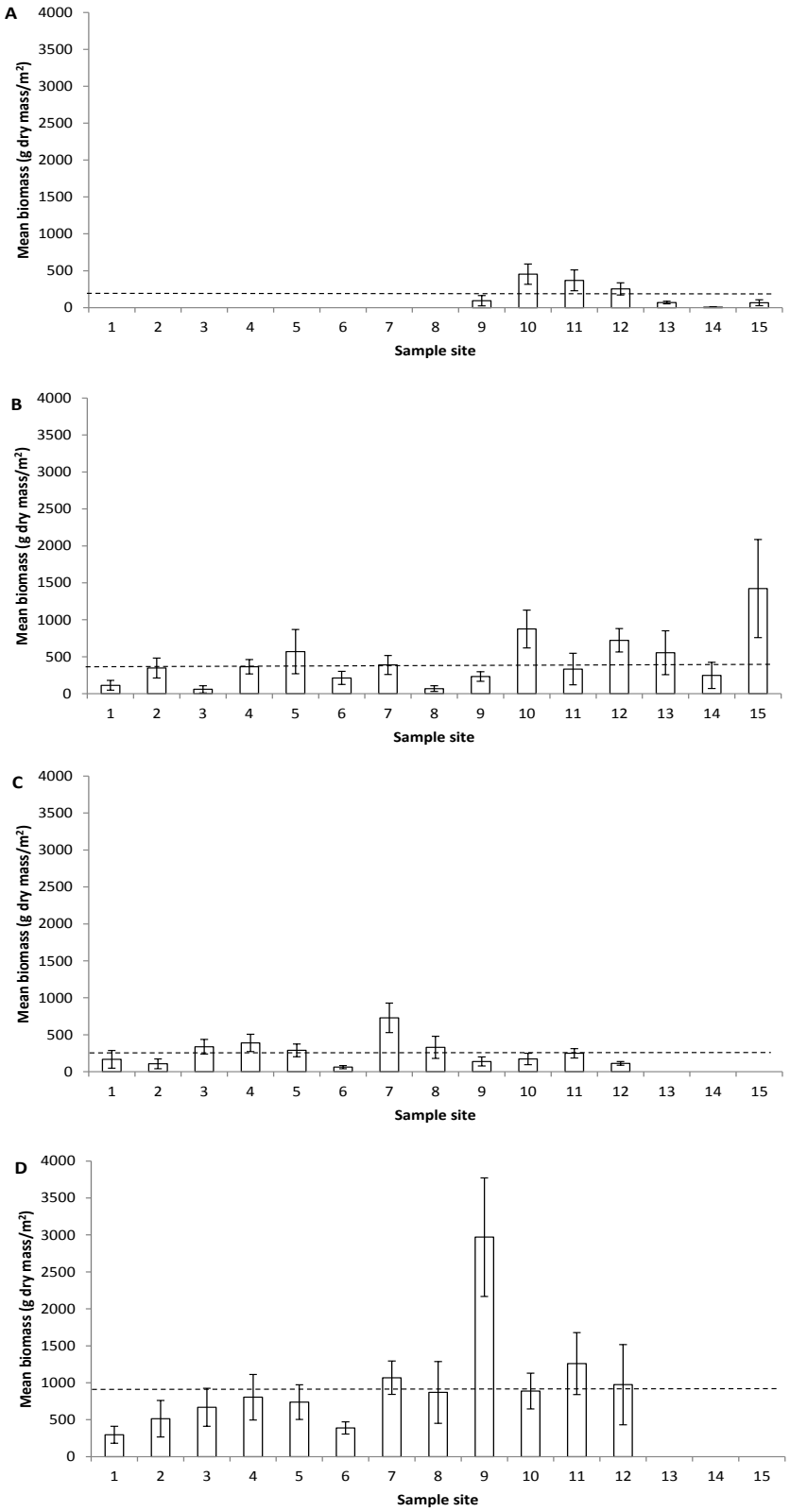

Figure 4.18 Biomass sampled of *Myriophyllum spicatum* during the four field surveys (A: April 2012; B: November 2012; C: April 2013; D: November 2013) in the Vaalharts Weir, Vaal River. Site 1 is closest to the dam wall and site 15 is farthest, on each sampling occasion. The error bars indicate standard error of the mean. The hashed line indicates the mean biomass of *M. spicatum* of each sampling event.

In summary, *M. spicatum* in the Vaalharts Weir and Lake Sibaya, has very few, if any, phytophagous invertebrates associated with it and there was a lack of consistent feeding damage recorded on the sprigs from each site and sampling occasion. There was a diverse community associated with *M. spicatum* at each sampling occasion for both systems, but again, this was not consistent between sampling occasions shown in the community analysis. The macrophyte biomass and abundance was on most occasions high, and for Lake Sibaya, *M. spicatum* had a consistently higher occurrence and was more abundant than *C. demersum.*

4.4 Discussion

4.4.1 Phytophagous invertebrates associated with *Myriophyllum spicatum* in southern Africa

The results from this study suggest that there are no phytophagous invertebrates using *M. spicatum* as a direct food source in these two systems in southern Africa, or they were in such low abundance that they were not detected. The species accumulation curves for both Lake Sibaya and the Vaalharts Weir reached an asymptote for each sampling occasion, so the probability of finding additional phytophagous invertebrates was low. Five phytophagous invertebrate taxa were collected in association with *M. spicatum* from both Lake Sibaya and Vaalharts Weir. However, the lack of feeding damage recorded on the sprigs suggests that these phytophagous taxa may have been incidental visitors that do not use *M. spicatum* as a direct food source. This could be considered a lack of evolutionary history in the southern African region as there is a rich diversity of phytophagous invertebrates that use *M. spicatum* as a food source in other regions. For example, in the native Eurasian distribution of *M. spicatum*, at least 44 species of phytophagous insect have been recorded to feed directly on it, including several specialist herbivores (Ghani *et al.* 1970; Spencer & Lekić 1974; Buckingham 1998; Cock *et al.* 2008). Of these 44 species, the majority comprised species in

the Curculionidae (15 species, of which six were in the genus *Bagous*) and Lepidoptera (12 species) (Cock *et al.* 2008). Despite the relatively high number of phytophagous insects, none of these was monophagous, however, there were surprisingly few oligophagous species comprising only 23% of the total herbivore species. This low number of oligophagous insects could be an underestimate as a result of the relatively narrow geographic range of the surveys in the native range, as well as short windows in the growing season that were sampled (Cock *et al.* 2008). Therefore it is possible that the number of phytophagous insects could increase with more comprehensive surveys in the Eurasian distribution over longer periods.

In North America, where *M. spicatum* is introduced, there are eight species of phytophagous insects (Sheldon & Creed 1995), of which at least two have been inadvertently introduced from Europe: the pyralid moth, *Acentria ephemerella* Denis & Schiffermüller (Crambidae), and the Chironomid, *Cricotopus myriophylli* Oliver (Chironomidae) (Buckingham *et al.* 1981; MacRae *et al.* 1990). Because of the high number of native *Myriophyllum* species in North America, a relatively large pool of potential phytophagous insects could use *M. spicatum* as a food source. The biological control programme against *M. spicatum* in North America has taken advantage of this by using the native North American weevil, *Euhrychiopsis lecontei* Dietz (Curculionidae) that usually feeds on *Myriophyllum sibiricum* Komarov. (Haloragaceae), but has shown a host switch to favour the introduced *M. spicatum* (Creed & Sheldon 1993; Sheldon & Creed 1995).

The lack of phytophagous insects associated with *M. spicatum* in southern Africa could also be linked to the possible lack of genetically similar plant species in the region (Strong *et al* 1984). The family Haloragaceae is represented by only two genera in southern Africa, *Myriophyllum*, which has two species – *M. aquaticum* introduced from South America in the early 1900s, and *M. spicatum*; and *Laurembergia* which has only two

subspecies, *Laurembergia repens* (L.) Bergius. and *Laurembergia repens* subsp. *brachypoda* (Welw. ex Hiern) Oberm. (Cook 2004). Because the only other species in the *Myriophyllum* genus, *M. aquaticum*, was introduced without any of its natural enemies, it is unlikely that it could have provided a source population of phytophagous insects for *M. spicatum.* The genus *Laurembergia* is considered amphibious, not truly aquatic, despite being able to withstand prolonged periods submerged (Moody & Les 2010). It is therefore unlikely, due to the major differences in the life histories of *Myriophyllum* and *Laurembergia,* that they would have the potential to share many phytophagous species. This is in contrast to North America, where despite it being the introduced range of *M. spicatum,* there are several species of native *Myriophyllum* with very similar life histories and a diverse associated herbivore fauna (Creed & Sheldon 1993; Sheldon & Creed 1995).

In the native Eurasian range there are 16 species in the genus *Myriophyllum* (Moody & Les 2010), and therefore the potential of a large pool of phytophagous insects is high (Agrawal & Kotanen 2003; Agrawal *et al.* 2005; Schoonhoven *et al.* 2005). In addition to this, several species of herbivores are recorded on *M. spicatum* in this region which also feed on several other species within the genus *Myriophyllum*, suggesting that the herbivores are using genetically similar species (Ghani *et al.* 1970; Spencer & Lekić 1974; Buckingham 1998; Cock *et al.* 2008).

Many of the aquatic phytophagous insects feeding on submerged macrophytes are known to be generalist feeders (Newman 1991). During surveys for phytophagous insects attacking *M. spicatum* in the Asian range, Buckingham (1998) identified 11 species. Of these, many are polyphagous and feed on a wide variety of macrophyte species including *M. spicatum* (Buckingham 1998). The lepidopteran *Parapoynx* (= *Nymphula*) *vittalis* (Park) Bremer (Crambidae) and the trichopteran *Leptocerus* sp. (Leptoceridae) are polyphagous and

were recorded feeding on *Hydrilla verticillata* (L.f.) Royle (Hydrocharitaceae) as well as *M. spicatum* (Buckingham 1998). In addition to these, there are host-specific insects which include many of the Coleopterans, with weevils in the genus *Bagous* and *Eubrychius* showing the highest specificity, and *Cricotopus myriophylli* Oliver (Oliver) (Chironomidae) being host specific as well (Buckingham 1998).

South Africa has been shown to have a complex phytophagous insect diversity associated with submerged macrophytes (Schutz 2007; Baars *et al.* 2010). This suggests that the potential pool of herbivores that could potentially use *M. spicatum* in southern Africa should not be limited. During surveys on *L. major*, several species of phytophagous insect have been recorded (Schutz 2007; Baars *et al.* 2010). Of those collected, several are polyphagous, including *Parapoynx* sp.and *Synclita* sp. (Crambidae). Schutz (2007) also surveyed two other species of macrophyte, *Lagarosiphon muscoides* Harvey (Hydrocharitaceae) and *S. pectinata*, and also found a relatively high herbivore diversity associated with these. With such a potential pool of herbivores, it would be expected to find some species using *M. spicatum*, if it was native from this region.

Myriophyllum spicatum is often considered less palatable than other species of macrophyte to many invertebrate herbivores (Lodge 1991; Newman 1991), which could potentially explain the lack of observed herbivores in southern Africa. In Europe, several species in the genera *Parapoynx*, *Acentria* (=*Acentropus*), *Limnephilus* (Limnephilidae) and *Phryganea* (Phryganeidae) have been observed feeding on both *Potamogeton* species and *M. spicatum*, but there was a preference for the *Potamogeton* species and feeding was sporadic and not consistent on *M. spicatum* (Soszka 1975). Lodge (1991) conducted large-scale feeding experiments to determine if the crayfish *Orconectes rusticus* (Girard) (Cambaridae) preferentially fed on particular species of macrophyte. The results suggested that this crayfish

was polyphagous and fed on at least 14 species of macrophyte, but it did reflect feeding preferences, and the genus *Myriophyllum* was considered of an intermediate preference (Lodge 1991). This suggests that although there may be a potentially large diversity of invertebrate herbivores available to feed on *M. spicatum* in southern Africa, it may be less preferred and not very consistently fed upon when other food sources are available. Based on the large number of detritivore taxa collected, this suggests that in Lake Sibaya and Vaalharts Weir, *M. spicatum* is more important as a detrital food source than as living material which is relatively common in many freshwater food webs (Fisher & Carpenter 1976; Carpenter & Lodge 1986; Harper 1986; Dawson 1988; Harrison 1989; Newman 1991; Lodge 1991).

4.4.2 Macroinvertebrate communities associated with *Myriophyllum spicatum*

The vast majority of macroinvertebrates that were associated with *M. spicatum* in Lake Sibaya and the Vaalharts Weir were predators, scrapers, and detritivores, which accounted for over 70% of the taxa recorded during this study. This supports the theory that macrophytes are used by many macroinvertebrates primarily as substrate (Cheruvelil *et al*. 2002; Ferreiro *et al.* 2011). These studies highlight the need for macrophytes in the aquatic ecosystem to provide complex habitat, which are used by several invertebrates as refuge, camouflage, feeding substrate and through senescence a direct food source. The community composition between the Vaalharts Weir and Lake Sibaya was very different, by taxa, but the functional diversity as described by Merrit & Cummings (1984) and Pennak (1978) was very similar in the two systems. This may suggest that the structural complexity of *M. spicatum* in these two systems provides a similar ecological role in these aquatic habitats.

There was a relatively diverse and comparable invertebrate fauna associated with *M. spicatum* between Lake Sibaya and the Vaalharts Weir. In both of these systems, the abundance was largely dominated by one or two taxa. In Lake Sibaya, the invasive snail, *T.*

granifera was extremely abundant and usually 2-3 orders of magnitude more abundant than any other invertebrate, except for the Chironomidae. In the Vaalharts Weir, the invertebrate community was largely dominated in abundance by Chironomidae, which reached densities in excess of 2000 individuals/kg of macrophyte wet mass. This is not completely uncommon for the distribution of abundances among aquatic macroinvertebrate taxa, where, in many cases, the diversity of taxa are dominated by one or a few taxa (Cheruvelil *et al*. 2000; 2002; van Donk & van de Bund 2002; Ferreiro *et al.* 2011).

It was not possible to determine the effects of different macrophyte species on the community composition of macroinvertebrates in the Vaalharts Weir because there were no other species of submerged macrophyte present during the study period. However, the community dynamics within Lake Sibaya in relation to macrophyte species was explored due to the frequent occurrence of *C. demersum* during the study period. The results from biomass measurements showed a significant contribution of *C. demersum* in the western and northern basins of Lake Sibaya and therefore the macroinvertebrate community was assessed between the basins. It was thought that if the different species of macrophyte supported a different macroinvertebrate community, there would be differences observed in the community analysis (ANOSIM) between the basins from each sampling occasion. However, this was not the case and there were no differences between the basins during any sampling event. This is not too surprising because both of these species provide complex habitat with a large surface area (Cheruvelil *et al*. 2002; Ferreiro *et al.* 2011). It does suggest that *M. spicatum* provides habitat, which is just as suitable as *C. demersum* in Lake Sibaya.

The subtle differences that may occur between macrophyte species, especially in this system, could be masked by the potentially large impacts that the invasion of *T. granifera* is having in the Lake. *Tarebia granifera* has been implicated in the disappearance of several gastropods and invertebrates throughout the world probably through competition for space

and resources (Prentice 1983; Samadi *et al.* 1997). To date, no studies have been conducted in South Africa on the potential impacts of this species, but due to the extremely high densities that it can achieve (over 1000 individuals per kg macrophyte wet mass), it must have had an effect on the community dynamics of other aquatic macroinvertebrates (Appleton *et al.* 2009). During the course of this study, it was noted that the surface of the plants was extremely clean or void of any periphyton growth. This is unlikely to be related to low nutrients as the water nutrients had moderate levels of ammonia in the system with a mean of 2.5mg/l which should be sufficient for periphyton growth (Hogan *et al.* 2014). The lack of periphyton could be related to the extremely high abundance of *T. granifera,* which feeds primarily on the periphyton (Appleton *et al.* 2009) and thus might be excluding or competing with other macroinvertebrates that would normally use this as a food source. This was not quantified during this study but should be considered for any future studies where the macroinvertebrate community is studied in the presence of *T. granifera.*

In the absence of other macrophyte species in the Vaalharts Weir, it is difficult to make any conclusions on the effect that *M. spicatum* has on the macroinvertebrate community. In addition to this, the Vaal River is considered to be an extremely impacted system (King *et al.* 2005) so it may be impossible to untangle the influence that pollution has on the macroinvertebrate community structure from that of *M. spicatum.* In a recent study on the macroinvertebrate abundance and community structure in the Vaal River, the macroinvertebrate abundance and diversity was very low and mainly dominated by Chironomidae (Fordham 2012). This was attributed to the pollution status of the Vaal River which was not expected to support a high diversity or abundance of macroinvertebrates. However, in the current study, the abundance was much higher and more in the region of that discussed by Wilson & Ricciardi (2009) in North America, where abundances were between 4000 – 5000 individuals /kg of macrophyte wet mass. The differences in diversity and

abundance could be due to different sampling methods, or could be related to sampling location. This study focused on getting a detailed understanding of the Vaalharts Weir, a comparatively small section of the river compared to the study by Fordham (2012), who collected samples for a much larger section of the Vaal River.

Interestingly, the species diversity indices for the free-swimming invertebrates were significantly higher than that of the epiphytic invertebrates on *M. spicatum* in the Vaalharts Weir. This is despite there being fewer species recorded in the free-swimming samples. They were collected more evenly and usually in a similar abundance, which could suggest that *M. spicatum* is not necessarily a suitable or preferred habitat for the epiphytic invertebrates found in the Vaalharts Weir. The epiphytic community was largely dominated by Chironomidae (in most cases by three orders of magnitude higher). It has been noted by several researchers that, despite having a complex architecture, *M. spicatum* is not always the most suitable habitat for macroinvertebrates and often other macrophyte species have higher species diversities and abundances (Cheruvelil *et al*. 2002; Wilson & Ricciardi 2009). This avoidance by macroinvertebrates of certain species of macrophyte has been linked to possible chemical exudates of macrophytes that affect the macroinvertebrates not only directly (Pennak 1973), but also indirectly (Marko *et al.* 2005). *Daphnia* sp., among others, has been shown to avoid certain macrophyte species, including *M. spicatum*, and toxicity tests suggest that this might be related to chemical deterrents (Pennak 1973; Dhillon *et al.* 1982). Macrophyte diversity in a stand has also been implicated in reducing the abundance and diversity of macroinvertebrates (Brown *et al.* 1988). Several studies have shown links between the diversity of invertebrates and macrophyte species richness, where mixed beds often have a higher diversity of invertebrates associated with them (Brown *et al.* 1988; Cheruvelil *et al*. 2002; Wilson & Ricciardi 2009; Stiers *et al.* 2011). However, in this study there were no other species of macrophyte in the Weir to compare diversity of

macroinvertebrates so it was not possible to determine whether there was a preference for another species of macrophyte. In Lake Sibaya, there were no differences observed in the invertebrate community when *M. spicatum* occurs alongside *C. demersum*, so it is possible that the differences observed in the Vaalharts Weir are related to other factors in the system that may affect the macroinvertebrate community, such as pollution.

4.4.3 Macrophytes associated with *Myriophyllum spicatum*

Myriophyllum spicatum was the dominant macrophyte in both Lake Sibaya and the Vaalharts Weir. In the Vaalharts Weir, it was the only macrophyte observed and sampled during the study period, despite other species (*C. demersum, S. pectinata* and *R. rionii*) being recorded in the Weir in the past (Personal Observations). In Lake Sibaya, *C. demersum* was commonly associated with *M. spicatum*, but in most cases *M. spicatum* had a higher biomass in the samples. A study by Howard-Williams (1979) in the late 1970s, on the community dynamics of macrophytes in Lake Sibaya, suggested that although *M. spicatum* was widely distributed in the lake, it was not the dominant species. Other species such as *C. demersum* and *P. schweinfurthii* were consistently higher in biomass and more widely distributed than *M. spicatum* (Howard-Williams 1979). The biomass distribution was also much lower than in the current study, 85-900 g/m^2 wet mass for 1979 and 1800-4000 g/m^2 wet mass for 2012/2013, where all submerged macrophytes sampled were grouped. This could be due to the recent water level rise just prior to the study of Howard-Williams (1979), while in 2012 and 2013, the water levels had been relatively stable for at least five years (Dlamini pers. comm.). These changes in water level would have resulted in depth changes for the submerged macrophytes and the community may have been in the pioneer stage and not fully developed (Howard-Williams 1979).

The possible changes in the community dynamics of Lake Sibaya may also be a result of increased anthropogenic activities around the lake, which include the grazing of cattle and increased populations of people living in the area. The nutrient analysis of both the water and the sediment suggest that there is, to a certain degree, some nutrient loading with relatively high levels of ammonia (80.6 mg/kg of sediment and 2.52 mg/l of water). This is much higher than many of the natural lakes in the past, for example in the 1970s, Lake Sibaya had a measured ammonia level of 0.03 mg/l in the 1970s (Hart & Hart 1977), which is much lower than what was measured during the current study. The ammonia and nitrate levels collected from the sample sites did not show any pattern and were evenly distributed in the Lake, despite close proximity to potential sources of pollution such as settlements or popular grazing areas. This could be explained by the relatively low runoff caused by the sandy catchment of Lake Sibaya and the importance of the large swamps of *Typha* and *Papyrus* at most of the inflows that may act as nutrient sinks for any incoming nutrients into the system (Howard-Williams 1979). There is also a considerable amount of mixing of water between the basins due to the strong currents that are primarily wind driven (Allanson 1979).

Fluxes in nutrient dynamics have been implicated in many other studies as a major determinant for the shift in dominance and abundance of particular species of macrophyte (Jeppesen *et al.* 2005; Scheffer & van Nes 2007; Sayer *et al.* 2010). The nutrient loading of the water column usually results in phytoplankton blooms, increased turbidity and a reduction in the biomass of submerged macrophytes (Scheffer & van Nes 2007; Sayer *et al.* 2010). Sayer *et al.* (2010) showed that this process can be a relatively slow one, with phytoplankton blooms dominating early in the growing season, then a slow development in the macrophyte community as the growing season progresses. However, as time progresses, it is possible that the macrophytes get gradually replaced (Sayer *et al.* 2010). The main difference with southern African systems is that macrophytes do not necessarily die back in winter due to the

freezing over of water bodies, so it is possible that the positive feedback loop that macrophytes have with water clarity is maintained year round. Nutrient loading in these systems would then affect the community dynamics of submerged macrophytes rather than a phytoplankton dominated system.

The dominance of *M. spicatum* in the Vaalharts Weir is less obvious as it is not found in every piece of suitable habitat in the Weir, but there are no other submerged aquatic macrophytes in the system. It is unlikely that *M. spicatum* has been able to outcompete these species to the point where they have been driven to local extinction in the Weir. A plausible argument is the recent invasion by grass carp, *C. idella* , into the Vaal River which has changed the dynamics of the system. Grass carp are aggressive feeders on macrophytes, especially favouring submerged species (Venter & Schoonbee 1991). For example, grass carp have been used for the purpose of submerged weed control and eradication in New Zealand (Hofstra & Clayton 2014).

In recent years, anglers on the Vaal River system have been reporting a large increase in the catch numbers of grass carp. First brought into the country in 1967 from Malaysia and again in 1975 from Germany, these fish were originally used for aquatic weed biological control (Venter & Schoonbee 1991). Grass carp are extremely selective in food preference and have been documented to completely remove a preferred food source before moving to less palatable items (Van Dyke *et al*. 1984; Fowler & Robson 1978), and large populations of this fish have potentially severe implications for ecosystem functioning. These include denudation, loss of biodiversity and habitat and increases in turbidity, nutrient loading and chlorophyll-a concentration (De Moor & Bruton 1988, Bonar *et al*. 2002). As grass carp need to migrate upstream to spawn on gravel riverbeds with very strong currents (Schoonbee *et al.* 1985, Stanley *et al.* 1978), it was believed that they would be effectively unable to reproduce under natural conditions in South Africa. As a failsafe to avoid establishment of the species in

South African waterways, National Environmental Management of Biodiversity Act (NEMBA) (2004) restricts the use of this species to sterile triploid hybrids. According to carp anglers, the population of grass carp is increasing in the Vaal River, which suggests a viable breeding population. *Myriophyllum spicatum* ranks extremely poorly on a scale of food preference for grass carp (Pine & Anderson 1991; McKnight & Hepp 1995; Fowler & Robson 1978). As it is highly likely that the relatively recent invasion of grass carp in the Vaal River is strongly linked to the current population explosion of *M. spicatum*, which has increased in abundance and density, taking advantage of decreased levels of competition, filling the niches left by other submerged macrophytes now completely removed by this fish. This however, has never been tested in southern Africa.

In conclusion, there are neither specialist nor generalist phytophagous invertebrates that inflict consistent or substantial damage in either the Vaalharts Weir or Lake Sibaya. There is, however, a relatively diverse macroinvertebrate community associated with *M. spicatum* suggesting it is an important component of the ecosystem of both the Vaalharts Weir and Lake Sibaya. However, because it was the dominant macrophyte in Lake Sibaya, it appears to be capable of outcompeting other macrophytes in the conditions present. These findings suggest that there is a lack of evolutionary history of this species in both the Vaal River and Lake Sibaya. Chapter 5 therefore investigates the genetic relationships that the populations of *M. spicatum* in southern Africa have with populations in the known native range, Eurasia, and given the potential lack of evolutionary history described in this chapter, attempts to determine a region of origin.

Chapter 5: Do the genetic relationships between *Myriophyllum spicatum* L. (Haloragaceae) in southern Africa and the Eurasian native range suggest a recent introduction?

5.1 Introduction

The phylogenetic placement of a species into geographic regions is an important aspect of invasion biology, and genetic assessments have provided a unique tool to investigate phylogeographic relationships (Les *et al.* 2003). These techniques have been applied to several introduced plant species to determine their geographic origins (e.g. Madeira *et al.* 1997; Novack & Mack 2001; Gaskin *et al.* 2005; Goolsby *et al.* 2006; Taylor & Keller 2007; Paterson *et al.* 2009; Gaskin *et al.* 2011; Thum *et al.* 2011; Paterson & Zachariades 2013). Species introduced to a region will show a genetic origin based in the native range (Petit *et al.* 2003), making it possible to gain information about an introduced species history using multiple, highly variable molecular markers.

The different morphological varieties of *Myriophyllum spicatum* L. (Haloragaceae) in southern Africa suggest that there is a high level of genetic diversity within the southern African populations (Chapter 3). These local adaptations observed in southern Africa could have arisen in two ways, either: 1) the plants had a long evolutionary history in the region and these local adaptations have evolved, or 2) the plants were recently introduced from multiple populations that were phenotypically and genetically distinct (Chapter 3). Evidence obtained from DNA markers of *M. spicatum* from both the native range and southern Africa may resolve the mechanisms of these local adaptations and could possibly determine a region of origin.

Morphological variations based on local adaptations are common in aquatic plants, especially populations that have had a long evolutionary history within a geographic region and are considered native (Santamaria *et al.* 2003). Introduced macrophyte populations that exhibit morphological variation are usually characterised by high levels of phenotypic plasticity coupled with low genetic variation (Geng *et al.* 2007; Lambertini *et al.* 2010; Riis *et al.* 2010). A low level of genetic variation is a common trait of introduced macrophyte populations, usually linked to the introduction of few propagules, clonality and/or small founder populations (Gornall *et al.* 1998; Parker *et al.* 2003; Thum *et al.* 2011).

Recently there has been a growing body of literature to suggest that genetic diversity in the introduced range can be diverse (Taylor & Keller 2007; Crawford & Whitney 2010). This is linked to either a large number of propagules, the number of source populations and/or the number of introduction events from the native range, or a combination of both (Kolar & Lodge 2001). Multiple introductions from different source populations are common in aquatic plants, which could result in high levels of genetic diversity (Thum *et al.* 2011; Zuellig & Thum 2012). For example, there have been two distinct genotypes of *M. spicatum* identified in North America, suggesting multiple independent introduction events from different regions of the native range (Zuellig & Thum 2012). This supports the hypothesis by Couch & Nelson (1985) who suggested multiple introductions post 1940s, based on the disjunct distribution as well as arbitrary spread of *M. spicatum* through North America.

Introduced species carry a genetic footprint into the introduced range that can provide useful information on the invasion history that can be traced back to the native range (Taylor & Keller 2007; Thum *et al.* 2011; Paterson *et al.* 2009; Paterson & Zachariades 2013). The internal transcribed spacers (ITS) regions of the nuclear ribosomal DNA, and more recently chloroplast DNA (cpDNA) (Shaw *et al.* 2007; 2014), have been used successfully to assess interspecific and intraspecific phylogeographic relationships between species of

Myriophyllum (Moody & Les 2002; 2010; Thum *et al.* 2011; 2012; Zuellig & Thum 2012; Chen *et al.* 2014). These markers are variable at the species level and the combination of ITS and cpDNA has been successful at identifying multiple genotypes in introduced populations (Thum *et al.* 2011; Zuellig & Thum 2012), as well as identifying the region of origin (Thum *et al.* 2011). For example, *Myriophyllum heterophyllum* Michx. (Haloragaceae), native to south eastern North America, was first recorded in Connecticut in 1932, outside the native range, after which it spread rapidly into new water bodies (Les & Mehrhoff 1999). The use of genetic markers in the phylogeographic study by Thum *et al.* (2011) demonstrated that the native population is separated into two primary biogeographic regions – the Atlantic Coastal Plain and Continental, with each of these regions characterised by their own distinct genotype of *M. heterophyllum* (Thum *et al.* 2011). This species has two introduced ranges, one in the north-eastern U.S.A. and one in the western U.S.A. which appear to have had multiple independent introduction events from both biogeographic regions in the native range (Thum *et al.* 2011).

The aim of this study was to investigate the relationships between populations of *M. spicatum* in southern Africa with the native Eurasian range, using ITS and cpDNA sequence data to determine a region of origin. Comparing the genetic data collected in southern Africa to potential source regions will determine whether multiple introductions or a long evolutionary history are responsible for the variety of ecotypes present in the region.

5.2 Materials and methods

5.2.1 Sample collection and DNA extraction

The collection of *M. spicatum* samples from across the native range, both Europe and Asia, was a collaborative effort with colleagues from all over the globe. A total of 40

independent populations of *M. spicatum* were sampled through the native range as well as southern Africa (Table 5.1; Figure 5.1). At least 10 apical sections ~ 5-10 cm long were collected from field populations, loosely wrapped in paper towel and placed in a Ziplock bag with a generous amount of silica gel. In some cases, when a freeze drier was available, the apical sections were freeze-dried initially then stored in silica gel. The plants were cleaned before drying them, to be sure to remove any periphyton or other organisms that may contaminate the sample. When silica drying, the paper towelling was changed regularly for seven days and the silica gel was replaced when necessary. In most cases a photograph of the plants was taken for identification purposes. A handheld GPS was used to obtain coordinates of the field population where the *M. spicatum* sample was collected. The samples were then shipped to Grand Valley State University, Michigan, U.S.A., where the DNA extraction, PCR, cloning and sequencing was done from the apical growth tip as soon as possible.

Table 5.1 The locations of the samples of *Myriophyllum spicatum* that were collected for the ITS and cpDNA analysis. The "Y" indicates whether the DNA sequencing was successful for each of the markers, ITS and cpDNA.

Code	Location	Latitude	Longitude	ITS	cpDNA
AF003	Vaalharts Weir, Vaal River, South Africa	-28.0916	24.9682	**Y**	
AF004	Barkley West, Vaal River, South Africa	-28.5503	24.5317	**Y**	
AF005	Lake Sibaya, Zululand, South Africa	-27.36	32.7132	**Y**	**Y**
AF007	D. Impson pond ex. Nuwejaars River, South Africa	-34.676	19.914	**Y**	**Y**
AF008	Vaalharts Weir, Vaal River, South Africa	-28.09164	24.96824	**Y**	**Y**
AF009	Klipplaat River, Hogsback, South Africa	-32.49387	26.94959	**Y**	
AF011	Prieska, Orange River, South Africa	-29.65969	22.74911		**Y**
BELG001	Dessel/SchötenChannel, Retie, Belgium	51.27733	5.15074	**Y**	
BELG002	Dessel/SchötenChannel, Turnhout, Belgium	51.32692	4.92699	**Y**	
BELG004	Dessel/SchötenChannel, Sint-Job-in-t'-Goor, Belgium	51.29949	4.56313	**Y**	
DEN001	Gudenå, Denmark	56.383561	9.727819	**Y**	**Y**
DEN004	Stilling Lk, Denmark	56.06448	10.02274	**Y**	

DEN005	Knudsø, Denmark	56.104162	9.783341	**Y**	
DEN007	Margrethe Kos, Denmark	54.937196	8.703486		**Y**
FR001	Yvonand, France	46.802845	6.732063	**Y**	**Y**
FR002	Camargue, France	43.68	4.63	**Y**	**Y**
FR003	Lac du Couze, France	45.098948	1.462235	**Y**	**Y**
FR004	Bandeville, France	48.57626	2.026683	**Y**	**Y**
FR005	Herepian, France	43.589261	3.102808	**Y**	
FR007	Lacoste, France	43.560184	3.302629	**Y**	
FR008	Gignac, France	43.652202	3.53083	**Y**	
GRM001	Were River, Lohne, Germany	52.18489	8.66707	**Y**	
GRM004	River Oker, Braunschweig-WatenbYttel in Lower Saxony, Germany	52.31056	10.46528	**Y**	**Y**
SCO001	Loch Leven, Scotland	56.206069	-3.37728	**Y**	**Y**
SWE001	Hudiksvall, Sweden	61.720232	17.13665	**Y**	**Y**
SWL001	River Treene, Ipland (near Treia) in Schleswig-Holstein	54.53034	9.28958	**Y**	
IND001	Kasmir University	34.133122	74.84200	**Y**	
CH1825	Behai, TengChong, Yunnan province, China	25.1205	98.5586	**Y**	**Y**
CH1775	Laogao yu tang, Mjan ning, Sichuan province, China	28.538727	102.1569	**Y**	
CH3592	Daga Town, Qushui county, Tibet	29.336746	90.68417	**Y**	**Y**
CHN002	Wuhan Botanical Gardens, Wuhan City, Hubei Province, China	30.545264	114.4180		**Y**
CHN003	Kunming Lake of Summer Palace, Beijing, China	39.993662	116.2610		**Y**
CHN004	Nahu Park, Changchun City, Jilin Province, China	43.851125	125.2991		**Y**
CHN005	YanQi Lake, Yanquing District, Bejing City, China	40.396699	116.6768		**Y**
WU 40	Bosten Lake, Xinjiang, China	41.9067	86.7314	**Y**	
WU 49	Bosten Lake, Xinjiang, China	41.9067	86.7314		**Y**
WU 120	Fuyang, Zhejiang, China	29.9944	119.6944	**Y**	**Y**
WU 127	Tai Lake, Jiangsu, China	31.2228	120.4461		**Y**
WU 128	Tai Lake, Jiangsu, China	31.2228	120.4461		**Y**
KOR001	Shihwa Lake, Seonah Jeong, Jihee Kim, South Korea	37.2792	126.6242		**Y**

The DNA extraction and analysis followed the protocols outlined by Moody & Les (2002), Thum *et al.* (2011) and Wu *et al*. (2013). For each population, DNA was extracted

from the apical meristem using DNEasy Plant Mini Kits (Qiagen) following the manufacturers' protocols.

Figure 5.1 The distribution of *Myriophyllum spicatum* populations sampled through Eurasia and southern Africa. The purple squares indicate successful sequencing for both the ITS and cpDNA regions, the yellow circles indicate only ITS region and the red stars indicate only cpDNA region.

5.2.2 PCR amplification and sequencing

The PCR reactions for the nuclear ribosomal DNA Internal Transcribed Spacers 1 and 2 and the intervening 5.8S ribosomal subunit (hereafter ITS) region followed the standard protocol outlined in Thum *et al.* (2011), using the universal primers ITS1 and ITS4 (Soltis & Kuzoff 1995). The reaction contained the following: 1 µl of PCR buffer (Invitrogen), 2 mM

$MgCl_2$, 2 µmol each primer, 0.2 mM each dNTP, 1 unit of *Taq* DNA polymerase (Invitrogen), 1 µl template DNA and brought to a total volume of 10 µl with sterile, distilled and deionized water. Thermal cycling protocol was as follows: one cycle at 94ºC for 2 min followed by 25 cycles of 94ºC for 1 min, 53ºC for 30 s, 72ºC for 1 min and a final extension at 72ºC for 8 min. Amplification of the cpDNA generally followed the protocol of Shaw *et al.* (2007) outlined as follows. The chloroplast DNA trnQ-rps16 intron region (hereafter cpDNA) showed the highest variation among the cpDNA markers recommended by Shaw *et al.* (2007), tested on DNAs from *M. spicatum* in its native range and southern Africa. The primers $trnQ^{(UUG)}$ and rps16x1 (Shaw *et al.* 2007) were used for the amplification of the cpDNA. The PCR reactions for cpDNA region contained the following: 1 µl of PCR buffer (Invitrogen), 2 mM $MgCl_2$, 2 µmol each primer, 0.2 mM each dNTP, 1 unit of *Taq* DNA polymerase (Invitrogen), 1 µl template DNA and brought to a total volume of 10 µl with sterile, distilled and deionized water. The PCR cycling conditions were template denaturation at 80ºC for 5 min followed by 30 cycles of denaturation at 95ºC for 1 min, primer annealing at 50ºC for 1 min, followed by a ramp of 0.3ºC/s to 65ºC, and primer extension at 65ºC for 4 min; followed by a final extension step of 5 min at 65ºC.

The PCR products were visualized on 1.5% agarose gel in TAE buffer stained with Ethidium Bromide (ITS) or SYBR Safe (Life Technologies) (cpDNA) to check for size and purity. The remaining DNA was treated with the enzymes Exonuclease I (New England Biolabs) and Antarctic Phosphatase (New England Biolabs) or ExoSAP-IT (Affymetrix) to eliminate unincorporated primers and dNTPs before sequencing. The PCR products were sequenced using BigDye terminator chemistry (Applied Biosystems) on ABI 3130xl and 3730xl DNA sequencers.

In the cases where the PCR products did not produce clean and unambiguous sequences for ITS, the PCR products were cloned using the TOPO TA cloning kit

(Invitrogen) and sequenced with 5–10 positive inserts. Sequencing of the cpDNA region was performed using the rps16x1 primer only as sequencing with the trnQ$^{(UUG)}$ encountered a long PolyA region.

5.2.3 Sequence analysis

Chromatograms were assembled in Sequencher 4.2 and sequence alignments were proof read manually and aligned using Mega 3.1 (Kumar *et al.* 2004) using ClustalW set to default parameters for ITS. The cpDNA was manually aligned using MacClade 4.08 (Maddison & Maddison 2005) and indels were determined following Moody & Les (2010). Where singletons were present in the ITS dataset for cloned individuals (one base change in a single sequence), these were replaced with a "?" to take a conservative approach and reduce the possibility of cloning error. When the ITS sequence was identical for different individuals from the same location, only one sequence was used for further analysis. A haplotype network was constructed using statistical parsimony (Tempelton *et al.* 1992), as implemented in the program TCS version 1.21 (Clement *et al.* 2000), with a 95% connection limit.

Similar to the analysis of the ITS sequence, the cpDNA intron data were represented as a haplotype network constructed in TCS version 1.21 (Clement *et al.* 2000), with a 95% connection limit. Before analysis, the indels present in the sequence were expressed as binary characters (T/A) at the end of the sequence and the option in TCS to treat gaps as missing data was selected. There were a total of 18 indels identified in the cpDNA region for the *M. spicatum* samples that were used.

5.3 Results

The final ITS alignment of all the ITS sequences resulted in a 671 bp sequence. There were eight unique ITS alleles identified among the samples of *M. spicatum* which was

represented as a network (Figure 5.2). One loop was identified in the ITS haplotype network between the H_6, H_7 and H_8 haplotypes, all of which occur in South Africa.

The proposed ancestral allele, H_1, contains several populations of *M. spicatum* primarily originating from western Europe. These include populations from Belgium, France, Germany, Denmark, Scotland and Sweden (Figure 5.1; Figure 5.2). The southern African populations have unique ITS alleles, H_6, H_7 and H_8, that are not shared with any other populations sampled in the geographical regions from this study. There is a wide diversity within the South African populations and many of the ITS alleles are shared between geographically distant populations (Figure 5.2; Table 5.2). Lake Sibaya, Western Cape and Vaal River share the same ITS allele, H_7, and the Hogsback and the Vaal River populations also share an ITS allele, H_6, and where they do not, they are always less than two mutational steps apart (Figure 5.2).

There was little diversity at the ITS region for populations in Europe or Asia. The France population, FR03, shares the H_5 allele with the Scotland population, SC01, with both of these populations being one mutational step away from the hypothesized ancestral allele (Figure 5.2; Table 5.2). The populations collected in Asia are between two and three mutational steps from the hypothesized ancestral allele, H_1, separated by the H_2 allele, which is European dominated, between the Asian H_3 and H_4 alleles and the ancestral allele, H_1. Overall there was very little ITS sequence diversity amongst plants from the Eurasian region (Figure 5.2).

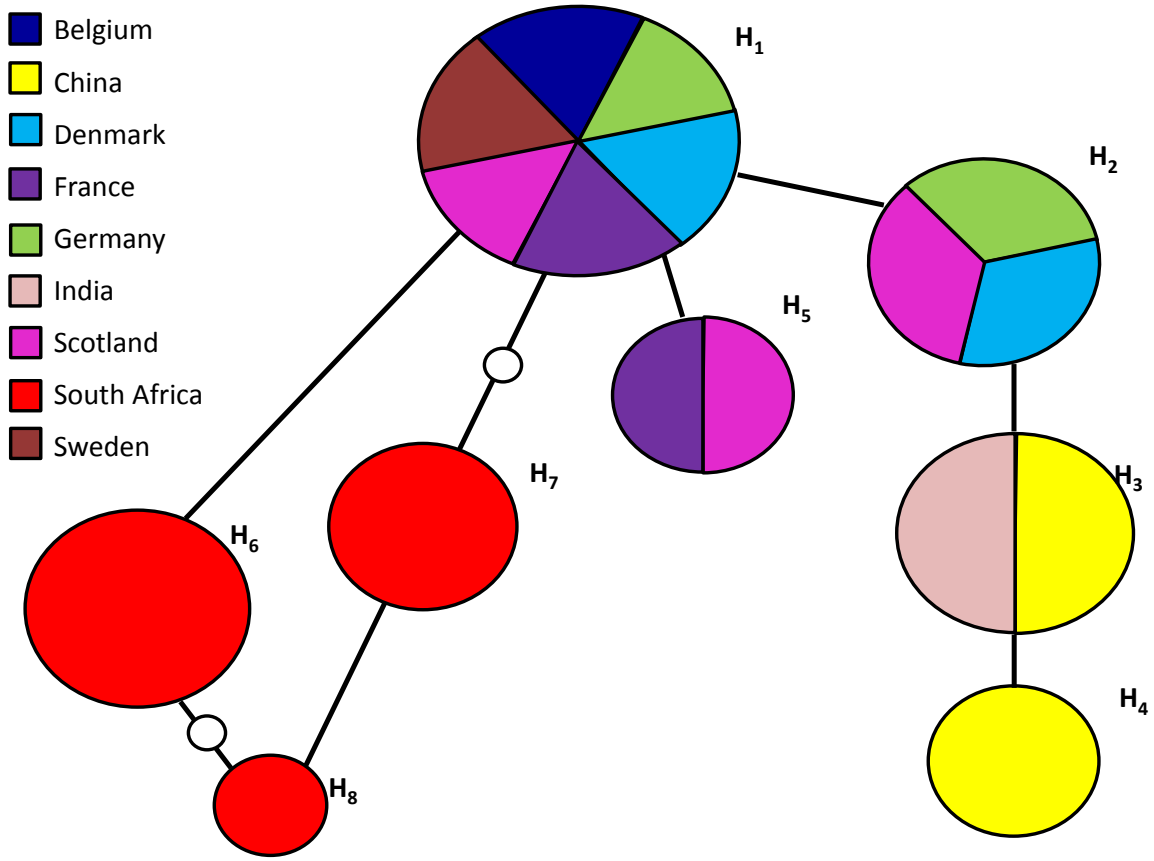

Figure 5.2 Statistical parsimony haplotype network constructed in TCS version 1.21 (Clement *et al.* 2000) using the ITS sequence of *Myriophyllum spicatum.* The haplotypes are colour coded by country, and each population that contains a haplotype is presented in Table 2. The small uncoloured circles indicate an additional mutational step between haplotypes detected. The circle sizes are proportional to the number of populations where that haplotype was found.

Table 5.2 The *Myriophyllum spicatum* populations which contain each ITS haplotype identified in the statistical parsimony haplotype network (Figure 5.2).

Code	H_1	H_2	H_3	H_4	H_5	H_6	H_7	H_8
AF003	-	-	-	-	-	✓	-	-
AF004	-	-	-	-	-	✓	-	-
AF005	-	-	-	-	-	-	✓	-
AF007	-	-	-	-	-	-	✓	-
AF008	-	-	-	-	-	✓	✓	-
AF009	-	-	-	-	-	✓	-	✓
BELG001	✓	-	-	-	-	-	-	-
BELG002	✓	-	-	-	-	-	-	-
DEN001	✓	-	-	-	-	-	-	-
DEN004	✓	-	-	-	-	-	-	-
DEN005	-	✓	-	-	-	-	-	-
FR001	✓	-	-	-	-	-	-	-
FR002	✓	-	-	-	-	-	-	-
FR003	-	-	-	-	✓	-	-	-
FR004	✓	-	-	-	-	-	-	-
FR005	-	-	-	-	-	-	-	-
FR007	✓	-	-	-	-	-	-	-
FR008	✓	-	-	-	-	-	-	-
GRM001	✓	✓	-	-	-	-	-	-
GRM004	✓	-	-	-	-	-	-	-
SCO001	✓	✓	-	-	✓	-	-	-
SWE001	✓	✓	-	-	-	-	-	-
SWL001	-	✓	-	-	-	-	-	-
IND001	-	-	✓	-	-	-	-	-
CH1825	-	-	✓	-	-	-	-	-
CH1775	-	-	✓	-	-	-	-	-
CH3592	-	-	✓	-	-	-	-	-
WU 40	-	-	-	✓	-	-	-	-
WU 120	-	-	-	✓	-	-	-	-

The final alignment of the cpDNA resulted in a 727 bp sequence with 18 indels treated as binary characters. There were 13 unique haplotypes represented in the network (Figure 5.3; Table 5.3). One loop was identified in the haplotype network involving the European and Asian lineages (Figure 5.3).

The hypothesized ancestral haplotype from the cpDNA network, H_A, included populations from France, Denmark and Scotland (Figure 5.3; Table 5.3). Two main lineages arose from the hypothesized ancestral haplotype, an Asian group containing samples from a wide geographic area of China (H_B, H_C, H_D, H_E, H_F and H_G) and a European group containing samples also from a wide geographic area (H_H, H_I, H_J and H_K) (Figure 5.3; Table 5.3). These two lineages are linked by a loop in the network. This suggests it is equally parsimonious as the Asian ancestral haplotype was H_A or H_J. The southern African samples do not share a haplotype with any of the populations sampled from Europe or Asia, but form a unique lineage that stems from the European haplotype H_J. The three populations in southern Africa are geographically isolated (Vaal River, AF008, flows directly into the Orange River, AF011), but consist of one haplotype, H_L, that is distinct from the European populations by at least 4 mutational steps (Figure 5.3). The South Korean haplotype, H_M, also stems from the European haplotype H_J differing by five mutational steps. There is a relatively large amount of variability in the cpDNA region for both the Asian and European populations, while there is no variability between any of the southern African populations sampled (Figure 5.3).

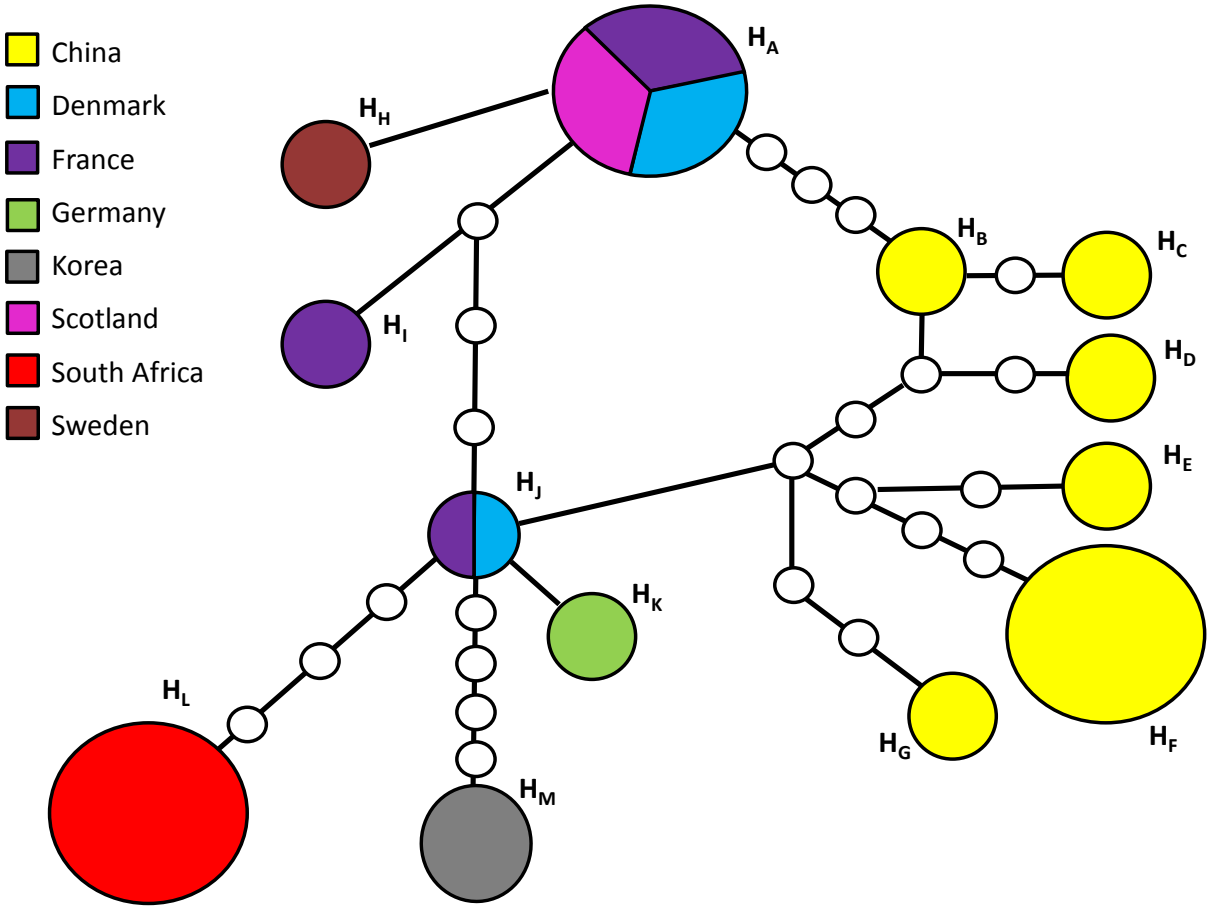

Figure 5.3 Statistical parsimony haplotype network constructed in TCS version 1.21 (Clement *et al.* 2000) using the cpDNA sequence of *Myriophyllum spicatum.* The haplotypes are colour coded by country, and each population that contains a haplotype is presented in Table 5.3. The small uncoloured circles indicate an additional mutational step between haplotypes detected. The circle sizes are proportional to the number of populations where that haplotype was found.

The southern African populations were unique at both the ITS and cpDNA regions compared to the Eurasian populations, but they showed a closer association with the European populations than with the Asian populations. The ITS region had a high diversity within the southern African populations compared to the populations from Eurasia, while the cpDNA region showed no diversity, but in both cases formed a distinct lineage stemming from a European ancestor.

Table 5.3 The *Myriophyllum spicatum* populations which contained each cpDNA haplotype identified in the statistical parsimony haplotype network (Figure 5.3).

Code	H_A	H_B	H_C	H_D	H_E	H_F	H_G	H_H	H_I	H_J	H_K	H_L	H_M
AF005	-	-	-	-	-	-	-	-	-	-	-	✓	-
AF007	-	-	-	-	-	-	-	-	-	-	-	✓	-
AF008	-	-	-	-	-	-	-	-	-	-	-	✓	-
AF011	-	-	-	-	-	-	-	-	-	-	-	✓	-
DEN001	✓	-	-	-	-	-	-	-	-	-	-	-	-
DEN004	✓	-	-	-	-	-	-	-	-	-	-	-	-
DEN005	-	-	-	-	-	-	-	-	-	✓	-	-	-
FR001	-	-	-	-	-	-	-	-	-	✓	-	-	-
FR002	✓	-	-	-	-	-	-	-	-	-	-	-	-
FR003	-	-	-	-	-	-	-	-	✓	-	-	-	-
FR004	-	-	-	-	-	-	-	-	-	✓	-	-	-
GRM004	-	-	-	-	-	-	-	-	-	-	✓	-	-
SCO001	✓	-	-	-	-	-	-	-	-	-	-	-	-
SWE001	-	-	-	-	-	-	-	✓	-	-	-	-	-
CH1825	-	-	-	✓	-	-	-	-	-	-	-	-	-
CH3592	-	-	-	-	-	-	✓	-	-	-	-	-	-
CHN002	-	-	-	-	-	✓	-	-	-	-	-	-	-
CHN003	-	-	✓	-	-	-	-	-	-	-	-	-	-
CHN004	-	✓	-	-	-	-	-	-	-	-	-	-	-
WU 49	-	-	-	-	✓	-	-	-	-	-	-	-	-
WU 120	-	-	-	-	-	✓	-	-	-	-	-	-	-
WU 127	-	-	-	-	-	✓	-	-	-	-	-	-	-
WU 128	-	-	-	-	-	✓	-	-	-	-	-	-	-
KOR001	-	-	-	-	-	-	-	-	-	-	-	-	✓

5.4 Discussion

The results from both the ITS and cpDNA analyses demonstrate that the southern African populations of *M. spicatum* sampled in this study are unique compared to populations

sampled throughout the European and Asian range. The southern African populations do not share any haplotypes with either the European or Asian populations, despite the wide geographic distribution of European and Asian alleles at both the ITS and cpDNA regions. This could suggest either that the populations in southern Africa have had a long evolutionary history in the region, allowing for the development of these unique alleles in isolation from European and Asian populations, or that the populations were introduced from a region that was not sampled in this study or does not currently exist.

The populations of *M. spicatum* sampled from southern African and Europe share a common ancestor. It was not possible to obtain cpDNA for all the populations, however sequences from each of the major ITS alleles, H_6 and H_7, Lake Sibaya/Western Cape/Vaal River and Vaal River/Hogsback, were obtained. The two distinct genetic lineages that occurred in the native range were characterised by low variability at the ITS region with relatively high variability at the cpDNA. The highly divergent ITS and cpDNA alleles from the European lineage are more consistent with long-term geographic isolation from this European lineage. What is unclear is whether this isolation has taken place within southern Africa or another region of the native range that was not sampled during this study. The morphological varieties in the southern African *M. spicatum* populations identified in Chapter 3 suggested that it is possible that these populations are genetically distinct. The results from this chapter did not identify patterns in the southern African populations with a mixed diversity within and between populations at the ITS, and no variability at the cpDNA region. The use of different molecular markers that are focused on within population variation, such as AFLPs (Freeland 2005), may shed light on this.

Most Asian populations formed a distinct lineage in the analysis of both data sets, and shared the European ancestral allele in all analyses. The South Korean population, KOR01, at the cpDNA region, groups with the European lineage. The Asian samples share a common

allele H_J, with Europe and have a similar level of divergence, so it is possible that the Korean haplotype was achieved without originating from the European lineage. However, this could be linked to the invasive tendency of *M. spicatum,* which means that the population sampled in Korea was possibly an introduced population from Europe. Displacement of native genotypes by invasive genotypes of the same species has been shown for several aquatic species (Saltonstall 2002; Les *et al.* 2013), where for example an invasive genotype of *Phragmites australis* (Cav.) Trin. Ex Steud. is progressively displacing native genotypes (Saltonstall 2002). Obtaining ITS data from this South Korean population should shed some light on this, however it was not possible for this study.

The analysis of the ITS and cpDNA regions from this study have been unable to identify a region or population of origin for *M. spicatum* in southern Africa. However, the divergence stemming from the European lineage for both the ITS and cpDNA regions suggests that the European and southern African plants share a common ancestor. The question that is raised from this analysis is the timing and mechanisms of dispersal for this species into southern Africa. If *M. spicatum* was anthropogenically introduced relatively recently, it would be expected that it would share ITS and cpDNA alleles with a potential region of origin, which is the case for the North American invasion (Thum, Moody, Coetzee & Weyl unpubl.). However, if this species range originally extended to southern Africa or if there was a more ancient natural dispersal event, a higher level of divergence would be expected than that found in the present study.

It is not uncommon for aquatic plants to have wide geographical ranges, and in many cases have intercontinental disjunct distributions (Santamaria 2002; Les *et al.* 2003). Many species found in both Eurasia and southern Africa have long evolutionary histories, with divergence times dating back to between the Cretaceous and the Eocene, with many of these distributions linked to continental drift (Les *et al.* 2003; Mao *et al.* 2012; Baker & Couvreur

2013). However, the divergence of *M. spicatum* and its sister species, *Myriophyllum sibiricum* Komarov. (Haloragaceae) was in the Quaternary approximately 5 million years ago (Chen *et al.* 2014) which is considered recent in geological time. This is relatively common in aquatic taxa, where many species with recent divergence times, 2.5 million years or less, have disjunct distributions between continents or major geographical barriers (Les *et al.* 2003). There has been a lot controversy over the mechanisms of long distance dispersal between the continents (Sculthorp 1967; Clausen *et al.* 2002), however, dispersal from Europe to southern Africa via historical water bodies connecting north of the Sahara to the rest of Africa (Drake *et al.* 2011) would be possible (although rare) through bird dispersal (Clausen *et al.* 2002) in the last 5 million years.

South Africa has a long history of aquatic plant invasion (Coetzee *et al.* 2011a; Coetzee *et al.* 2011b) and many of the more recent introductions have been linked to the aquarium plant trade (Martin & Coetzee 2011). *Hydrilla verticillata* (L.f.) Royle (Hydrocharitaceae), for example, is invading KwaZulu-Natal in the tropical east of South Africa. The South African population (currently restricted to one reservoir) was compared to several populations in both the native and invaded ranges of the world (Madeira *et al.* 2007). The South African population shared an allele that matched populations from Indonesia and Southeast Asia, demonstrating that this is likely to be the source of the introduction into South Africa. The potential pathway of introduction of *H. verticillata* into South Africa was the aquarium trade, as most of the aquatic plant trade in South Africa originates from Malaysia (Madeira *et al.* 2007). The introduction of *M. spicatum* before the first record in 1829 (Chapter 2) is unlikely to be linked to the aquarium trade, however, South Africa does have a long history of exchange with Europe (Les & Mehrhoff 1999), and the first botanist to record *M. spicatum* was of European origin (Ecklon 1830).

The data presented thus far do not provide evidence for the introduction of *M. spicatum* from any of the native regions sampled during this study, despite the extensive sampling across the native range. This provides evidence to suggest that this has not been a recent anthropogenic introduction. However, this study did provide some important insights into the phylogenetic relationships of *M. spicatum* with the native range. Firstly, the southern African populations share a common ancestor with plants from Europe rather than Asia, based on the sampled populations presented in this study. Secondly, the highly divergent ITS and cpDNA regions from the European lineage, coupled with the large amount of shared genetic information in the southern African populations, is consistent with geographically isolated populations that shared a common ancestor. What is unclear from this study is whether the common ancestor is a result of a recent introduction from a common source population, or a native ancestral population. Thirdly, the high diversity around the ITS, much of which is shared between populations, may suggest multiple introductions from genetically distinct populations, but this could also be a result of the development of local adaptations to the conditions experienced in the southern African region.

The following general discussion chapter will synthesise the evidence collected from Chapter 2 through to this chapter in an attempt to resolve the status of *M. spicatum* in southern African as a native species or not. Depending on the outcome, biological control as an appropriate management strategy will be discussed for the southern African region, as this species is considered a nuisance despite its controversial status.

Chapter 6: General discussion

Resolving the status of a species as native or exotic is no easy task, especially when the mode of introduction is unknown or unclear. There is no checklist or set of characters that can be ticked off, but rather a holistic approach of understanding the interactions of a species in the environment is required to make an informed decision. This thesis took a multifaceted approach in trying to resolve the status of *Myriophyllum spicatum* L. (Haloragaceae) in southern Africa, where several aspects of its history, biology and ecology were studied.

6.1 The status of *Myriophyllum spicatum* in southern Africa

This thesis did not provide evidence for the anthropogenic introduction of *M. spicatum* into southern Africa, indicating that it is most probably native to the region. An investigation into the history and distribution of this species in southern Africa (Chapter 2) failed to identify a mode of introduction into the region but did suggest an anthropogenic means of spread. The basis of this conclusion was the disjunct nature of the distribution throughout southern Africa between major catchments, as well as several populations possibly failing to establish, with local extinctions being fairly common (Chapter 2; Weyl & Coetzee 2014). However, it is possible that the local extinctions or poor performance of *M. spicatum* in many southern African aquatic systems are linked to it being at the edge of its native range, where the remaining populations are relics, rather that thriving populations. This is not uncommon with submerged macrophytes, for example the status of *Najas flexilis* (Willd.) Rostk. & Schmidt (Hydrocharitaceae) in Ireland was questioned, due to the disjunct populations that were coupled with local extinctions at sites (Wingfield *et al.* 2004). However, fossil evidence proved that this species had a long history in Ireland and the

populations were probably remnants from a once much wider distribution in Europe (Godwin 1975). In the case of *M. spicatum*, the fossil pollen evidence for southern Africa was never properly identified to species level, but rather to family level, and inferences were made to species level (Scott 1987; Neumann *et al.* 2008). The other genus in the family, *Laurembergia* Obermeyer (Haloragaceae), has an overlapping distribution (Cook 2004) with the sites where pollen was collected by Scott (1987) and Neumann *et al.* (2008), which suggests that the conclusion that *M. spicatum* has been present for up 12000 years could be confounded.

Indeed, evidence from the morphological study of *M. spicatum* (Chapter 3) supports the disjunct relic population hypothesis, where each ecotype occurs in distinct biogeographic regions under similar climatic conditions. In some cases these ecotypes are separated by large distances and major catchments. A classic example of this is the high altitude 'bottlebrush' ecotype recorded in the Mooi River, Drakensberg Mountains, KwaZulu-Natal and the Klipplaat River, Amathola Mountains, Eastern Cape, approximately 500 km apart as the crow flies (see distribution map in Chapter 2). Despite the large distance across major catchments and the lack of populations between the Mooi River and the Klipplaat River, the populations are morphologically similar and are found in a similar mountain stream, high altitude, cool climate habitat (Mucina *et al.* 2006). This suggests that the morphological ecotypes could have evolved under similar conditions, and that the bottlebrush type appearance (described in Chapter 3) is an adaptation to high altitude streams.

On the other hand, the lack of specialist herbivores provides convincing evidence for the introduction of *M. spicatum* into southern Africa, especially in comparison to the high numbers of specialist herbivores associated with *M. spicatum* in the native Eurasian range (Ghani *et al.* 1970; Spencer & Lekić 1974; Buckingham 1998; Cock *et al.* 2008). But the complete lack of herbivores, including generalists, may actually weaken that argument.

Strong *et al.* (1984) suggest that the number of herbivore species should reach equilibrium on the novel host relatively quickly, usually between 100-300 years. *Myriophyllum spicatum* has been recorded in southern Africa for at least 184 years and has been in the Vaal River for at least 136 years (Chapter 2), therefore it is expected that some herbivores would feed on it. The lack of phytophagous invertebrates may be a consequence of something inherent about *M. spicatum*, such as phylogenetic isolation or physical and chemical defences, which the southern African herbivores have not been able to overcome. Phylogenetic isolation has been shown to play an important role in the determination of the number of herbivores associated with the novel host for many species, where the greater the phylogenetic distance, the smaller the number of herbivores (Soldaat & Auge 1998; Agrawal & Kotanen 2003; Agrawal *et al.* 2005; Schoonhoven *et al.* 2005; Brändle *et al.* 2008). Only two genera in the Haloragaceae occur in southern Africa, *Myriophyllum* and *Laurembergia,* and *M. spicatum* has no native congeners, while *Laurembergia* has completely different life history traits (Cook 2004). In addition to this, *M. spicatum* is known to rank very poorly on a preference scale for many phytophagous invertebrates (Soszka 1975; Sheldon 1987; Sand-Jensen & Madsen 1989; Lodge 1991) and even herbivorous fish (Fowler & Robson 1978; Pine & Anderson 1991; McKnight & Hepp 1995). This may be attributed to chemical exudates that *M. spicatum* has been implicated in releasing (Pennak 1973; Marko *et al.* 2005). It is possible that the lack of phytophagous invertebrates is irrespective of time spent in the southern African region and may confound the conclusion of a lack of evolutionary history suggested in Chapter 4. Thus, the lack of phytophagous invertebrates can not be considered clear evidence that *M. spicatum* was introduced in southern Africa.

The dominance of *M. spicatum* in southern African water bodies in which it occurs is likely to be a response to changes in the system, such as nutrient loading, pollution, lack of competition and biological invasions of plants and animals. Some macrophytes respond

positively to disturbance in the aquatic environment (Tracy *et al.* 2003; Wersal & Madsen 2011), resulting in high biomass and a problematic status (Vitousek *et al.*1996; Chapin *et al.* 2000). This suggests that the high biomass of *M. spicatum* could be a symptom of an underlying cause rather than an exotic status.

Both the Vaal River and Lake Sibaya are impacted systems, albeit in different ways. The Vaal River is highly eutrophic, with nutrient inputs from both point and non-point sources, as well as various pollutants from mine dump wastes, including heavy metals (King *et al.* 2005). In addition, the Vaal River has been heavily invaded by both plants and animals, with the most notable species including water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laub. (Pontederiacae) (Coetzee *et al.* 2011b) and grass carp, *Ctenopharyngodon idella* Cuvier and Valenciennes (Cyprinidae) (Skelton 2001).

The introduction of grass carp into South Africa, between 1967 and 1975 (Skelton 2001) and its subsequent invasion of the Vaal River, may have diminished other native macrophytes to the point where *M. spicatum* has been released of its competitors. During previous surveys by the Biological Control Research Group (Rhodes University), it was noted that, although *M. spicatum* was dominant in the Vaal River, other species of macrophyte including *Ceratophyllum demersum* L. (Ceratophyllaceae) and *Stuckenia pectinata* L. syn. *Potamogeton pectinatus* L. (Potamogetonaceae) were present. However, during the study in Chapter 4, no other macrophytes were recorded in the samples and not one was seen in the Vaalharts Weir during the surveys from this thesis. This release from competition, coupled with high nutrients in the Vaal River (King *et al.* 2005) could explain the high biomass and dominance of *M. spicatum* in this system.

Lake Sibaya, on the other hand, is not eutrophic, but the levels of nitrogen in the system have increased since the 1970s. The Lake is also heavily invaded by the aquatic snail,

Tarebia granifera Lam. (Thiaridae) (Chapter 4). This could have changed the dynamics in the system and driven species of both macroinvertebrate and macrophyte to below detectable numbers (Prentice 1983; Samadi *et al.* 1997). The increase in biomass of submerged macrophytes in Lake Sibaya, from the 1970s (Howard-Williams 1979) and the current study (Chapter 4), suggests a shift in the dominance of species of macrophyte. Lake Sibaya is currently dominated by submerged macrophytes, including *M. spicatum* and *C. demersum*, where in the 1970s, submerged macrophytes were sparse and low in abundance (Howard-Williams 1979). This is probably linked to the increase in nutrient pollution of the Lake, where in the 1970s, ammonia levels were approximately 0.03 mg/l (Hart & Hart 1977), while in 2012 ammonia levels were approximately 2.5mg/l (Chapter 4). Lake Sibaya is a relatively deep system with limited habitat suitability for the emergent species which dominated the Lake in the 1970s after the recent lake level rise (Howard-Williams 1979). During this study period between April 2012 and November 2013, the lake level was considered relatively low and had been stable for some years. This, coupled with nutrient loading, has shifted the dominance to the submerged aquatic species.

The development of local adaptations between populations of *M. spicatum* in southern Africa (Chapter 3), and the distinct, but diverse genetic lineage identified (Chapter 5), are characteristic of a population which has been isolated for a significant period of time. What is unclear from this study is whether this occurred in southern Africa or was an artefact of multiple introductions from an unknown/unsampled source population. Multiple introductions have been responsible for genetically distinct genotypes in the introduced range for several species, including *M. spicatum* in North America (Zuellig & Thum 2012). But if the high genetic diversity observed in southern Africa was a result of multiple introductions, the chances of identifying a source population or region should increase, especially considering the lack of genetic diversity in the native range and extensive sampling effort

(Chapter 5). The Asian and European populations formed lineages distinct from one another, while the southern African populations always stemmed from the European lineage, suggesting a common ancestor. Understanding that Europeans have moved species around the globe for hundreds of years (Crosby 1986), the genetic evidence does not suggest a recent introduction from Europe, but rather an ancient association.

Despite the conclusion from this thesis that *M. spicatum* is probably not introduced to southern Africa, this species is still considered problematic and is invasive in certain southern African systems (Weyl & Coetzee 2014). The most important impacts in southern Africa are considered socio-economic, where *M. spicatum* forms dense mats (Figure 6.1) that are impenetrable by boats, block irrigation canals and pipes and preclude recreational activities, such as fishing (Coetzee *et al.* 2011a). In light of these impacts and the problematic status of this species, management strategies need to be initiated.

Figure 6.1 A dense mono-specific mat of *Myriophyllum spicatum* on the Vaalharts Weir, April 2012.

6.2 Native weedy species

Native species are known to respond to changes in land use patterns, disturbance, and even climate change to a point where they are considered problematic (Symoens & Triest 1983; Newman *et al.* 1998; Glen *et al.* 1999; Buitenwerf *et al.* 2012; Lukomska *et al.* 2014; Puttick *et al.* 2014), suggesting that although a species is native, it can still be considered invasive and may warrant management and control. The invasion of native *Typha* spp. into the Everglades, Florida, has been clearly linked to anthropogenic changes in fire regime, hydrology and nutrient loading (Newman *et al.* 1998). Management strategies in this case need to take a holistic approach in an attempt to return the system to as close to pre-invasion conditions as possible, where the control of these native *Typha* spp. would occur naturally (Newman *et al.* 1998). The major implication of resolving the status of *M. spicatum* in southern Africa, as a native species, largely dictates how or what management strategies can be implemented against it.

Southern Africa has a long successful history in the management and control of exotic floating macrophytes (Hill 2003), especially through the integration of several control options, including manual removal, herbicide application and biological control (Coetzee *et al.* 2011b). The control of submerged macrophytes in southern Africa has tailed behind that of the floating macrophytes, and in many cases is much more difficult and less predictable. This is in contrast to the rest of the world, where the control of submerged macrophytes has received much attention, especially in Ireland, New Zealand, Australia and the U.S.A. (Hershner & Havens 2008; Hofstra & Clayton 2012; Xu *et al.* 2014). The control options have included physical control through active mechanical removal or the use of jute matting (Caffrey *et al.* 2010, 2011; Hofstra & Clayton 2012), chemical control through the use of herbicides (Parsons *et al.* 2001; Shearer & Nelson 2002; Jones *et al.* 2012) and biological

control (Balciunas *et al.* 2002; Shearer & Nelson 2002; Newman 2004). South Africa has initiated mechanical control programmes against submerged macrophytes in a few systems, including against *H. verticillata* in Pongolapoort Dam, KwaZulu Natal (Kruger 2006) and against *E. densa* within the city of Cape Town, Western Cape (Stafford 2014). However, in most cases, the disadvatages of manual removal usually outweigh the benefits in the southern African situation and alternative control options are necessary (Stafford 2014). The use of insect biological control agents for the management of submerged macrophyte species has been shown in many cases to be an effective method of control (Wheeler & Center 2001; Clayton *et al.* 1995; Sheldon & Creed 1995; Newman 2004; Hofstra & Clayton 2014), but has never been implemented in southern Africa (Coetzee *et al.* 2011a). The use of an insect biological control agent for the management and control of a native species also has ethical implications that need to be considered before a decision is made to release the biological control agent.

6.2.1 Ethics of biological control

The ethical boundaries of the biological control of weeds will not allow the release of an exotic insect to control a native plant. Weed biological control has its grounding in classical biological control (McFadyen 1998), which is, by definition, the intentional introduction of an exotic natural enemy, which has coevolved with the target species, for the establishment of permanent, long-term control (Harris 1991; Eilenberg *et al.* 2001). Weed biological control has historically taken this conservative approach (Delfosse 2005), which is quite the opposite of the biological control of agricultural pests, where new associations are often sought after (Hokkanen & Pimentel 1984). A new association is the use of natural enemies that have not coevolved with the target species and may not originate from the native range of the pest (Hokkanen & Pimentel 1984). New associations are generally avoided in

weed biological control (Goeden & Kok 1986), and biological control practitioners most often prioritise regions in the native range to select appropriate biological control agents that have coevolved with the target species (McEvoy & Coombs 1999; Goolsby *et al.* 2006; Paterson *et al.* 2009; Paterson *et al.* 2014).

The use of new associations is not completely absent in weed biological control (Sheldon & Creed 1995), despite the arguments around the use of natural enemies that have not coevolved with their new host (Goeden & Kok 1986). However, there is only a single record where weed biological control practitioners have intentionally used an exotic natural enemy for the biological control of a native weedy species. This was the release of *Cactoblastis cactorum* Berg (Pyralidae), for the control of the native cactus, *Opuntia triacantha* (Willdenow) (Cactaceae), which was invading pasture lands on the island of Nevis, West Indies (Simmonds & Bennett 1966). This programme was a great success, with massive reductions in the target species just a few years later (Simmonds & Bennett 1966). However, the non-target effects were far reaching, where *C. cactorum* spread to other Islands and into North America where several native non-target *Opuntia* species were attacked and populations have been driven to threatening levels (Stiling 2002).

6.3 *Myriophyllum spicatum* in a southern African context

The control and management of *M. spicatum* in southern Africa poses an interesting scenario. A lot of time has been spent on developing a biological control programme for *M. spicatum* in southern Africa, using the North American weevil, *Euhrychiopsis lecontei* Dietz. (Curculionidae). The use of *E. lecontei* as a biological control agent in North America is a new association, where a native insect has been used to control an exotic weed (Newman 2004). The original host of *E. lecontei* is *Myriophyllum sibiricum* Komarov (Haloragaceae) however, there is a certain degree of preference for *M. spicatum* and the hybrids between *M.*

spicatum and *M. sibiricum* (Solarz & Newman 2001). The augmentative releases of this weevil into water bodies throughout the continent have been associated with declines in the abundance of *M. spicatum* (Newman & Biesboer 2000; Alwin & Cheruvelil 2009), and experimental evidence suggests that it is extremely damaging (Jester *et al.* 2000).

The host specificity testing of this weevil in South Africa was relatively simple, based on the lack of congeners and the large phylogenetic distance between *M. spicatum* and other native species. The results from the host specificity testing concluded that *E. lecontei* was highly damaging and safe for release with no predicted non-target effects on any other native species in southern Africa (Coetzee & Newman unpubl.). The decision to release this biological control agent in southern Africa has, however, been withheld until the controversial status of *M. spicatum* in southern Africa has been resolved (Coetzee pers. comm.).

6.4 Biological control of *Myriophyllum spicatum* in southern Africa

Ignoring the ethical boundaries of weed biological control, the risks of releasing an insect biological control agent for the control of *M. spicatum* in a southern African context may be be limited and justified.

6.4.1 Non-target effects on other native species

The proposed biological control agent for *M. spicatum* in southern Africa, *E. lecontei*, is completely host specific in a southern African context. This weevil is a *Myriophyllum* specialist (Sheldon & Creed 1995; Sheldon & O'Bryan 1996) and cannot complete its development on any other species native to southern Africa (Coetzee & Newman unpubl.). But, because this thesis suggests that *M. spicatum* is native to southern Africa, targeting it for control raises some concerns. These concerns may be alleviated by targeting populations of

M. spicatum in particular water bodies where it is problematic. Because the dispersal of *E. lecontei* between systems may be limited, the risk of natural dispersal from a system where it was intentionally released to a system where *M. spicatum* may need to be conserved would be mitigated.

The distribution and history of *M. spicatum* in southern Africa suggests that dispersal events of propagules between catchments are rare based on the disjunct distribution and no connectivity between populations (Chapter 2). The development of distinct locally adapted ecotypes in southern Africa (Chapter 3) supports the theory of limited mixing between populations and demonstrates that populations of *M. spicatum* are, to a certain degree, isolated. This suggests that if *E. lecontei* were released into a system, dispersal to a new locality where *M. spicatum* exists would have to be active dispersal of the insect. While the dispersal of *E. lecontei* within lakes and water bodies is relatively efficient, (Newman *et al.* 2001), dispersal between water bodies is inefficient and restricted to a radius of approximately 50km based on its limited flight ability (Tamayo *et al.* 2000). Thus, if *E. lecontei* were to be released in a particular water body where *M. spicatum* is problematic, for example the Vaal River, it is unlikely to disperse between catchments, where the closest living population is the Klipplaat River 500km away. This will limit the risk of *E. lecontei* spreading to populations of *M. spicatum* that are not problematic.

6.4.2 Revenge effects

Revenge effects are the direct or indirect negative effects of releasing a biological control organism to control a weed in a novel environment (Delfosse 2005). The much debated *Rhinocyllus conicus* Froehlich (Coleoptera: Curculionidae) in North America is a classic example of an indirect revenge effect, where the feeding of this weevil on native thistles is displacing the native insects that would normally feed on these species (Louda *et*

al. 1997; Louda 2000). The potential revenge effects of releasing a biological control agent for the control of *M. spicatum* are relatively small, but need to be considered. The biodiversity associated with *M. spicatum* in southern Africa is limited at best and *M. spicatum* is important in the aquatic environment as complex habitat, for epiphytes to grow and refuge for aquatic macroinvertebrates (Chapter 4). *Myriophyllum spicatum* has no phytophagous invertebrates that actively and consistently feed on it in the two systems tested in southern Africa (Chapter 4). This is relatively common in aquatic macrophytes and there are several examples of species that are only important as complex habitat and detritus (Fisher & Carpenter 1976; Carpenter & Lodge 1986; Harper 1986; Dawson 1988; Harrison 1989; Newman 1991; Lodge 1991), which suggests that any species or suite of species of aquatic macrophyte could fulfil this niche in southern Africa. In addition to this, *M. spicatum* dominates in certain aquatic systems in southern Africa (e.g. Lake Sibaya) and could be competitively excluding other macrophyte species (Chapter 4). The release of a biological control agent against *M. spicatum* in this case may reduce its competitive ability and allow other macrophyte species to contribute to the biodiversity of these systems.

Arguably, the proper functioning of aquatic ecosystems and the conservation of biodiversity hinges on a balance where macrophytes play an extremely important role (Jones *et al*. 1994; Dibble *et al*. 1996; Jones *et al.* 1997; Duarte 2000; Cheruvelil *et al.* 2002; Kufel & Kufel 2002; Schultz *et al*. 2003; Ferreiro *et al*. 2011). Thus, the biological control of *M. spicatum* needs to be considered with caution and each system needs to be understood before control options go forward. For example, it is suspected (albeit on anecdotal evidence) that the recent invasion of grass carp, *C. idella*, in the Vaal River could be completely removing all other macrophytes in the system. Thus, the biological control of *M. spicatum* in this system could leave the sediment of the Vaal River completely barren and exacerbate

problems associated with a complete lack of macrophytes, including sediment destabilisation and reduction in water quality and clarity.

6.4.3 Conflicts of interest

The conflict of interest around *M. spicatum* in southern Africa is limited, because this species is not considered endangered or currently targeted for conservation and there is no economic gain from this species in southern Africa. The lack of economic gain is based on the classification of *M. spicatum* as a Category 1 weed under the Conservation of Agricultural Resources Act, 1983, and the National Environment Management: Biodiversity Act (NEMBA), which has precluded any legal trade of this species in South Africa.

The risks of releasing *E. lecontei* for the control of *M. spicatum* in southern Africa are limited but there are still the ethical boundaries of weed biological control that need to be considered before a decision is made. The control of native *Opuntia* species in the West Indies is an example that cannot be repeated, but the non-target effects were predicted as *C. cactorum* will feed on most species of *Opuntia* (Simmonds & Bennett 1966). There are no direct non-target effects from the biological control of *M. spicatum* expected in southern Africa because the weevil *E. lecontei* is a *Myriophyllum* specialist (Sheldon & Creed 1995; Sheldon & O'Bryan 1996) and there are no other native *Myriophyllum* species on mainland Africa (Cook 2004). Benefits from the reductions in biomass of *M. spicatum* are likely to be high, where it will mitigate socio-economic as well as the ecological impacts in southern Africa.

6.5 Conclusions

The management and control of noxious weeds using biological control agents has in most cases taken the conservative approach where the risks are always carefully calculated,

and if there is doubt about the agent's safety and/or effectiveness, it is not released (Delfosse 2005). In the case of *M. spicatum* in southern Africa, the agent has proved host specific and highly damaging with little associated risks, but there were concerns about the status of the weed in southern Africa, as native or exotic. This thesis aimed to identify whether this plant was recently introduced to southern Africa and therefore could be considered exotic. Despite taking a holistic approach, investigating various aspects of its history, biology and ecology, there was no evidence to suggest a recent anthropogenic introduction. Until new evidence is brought forward, southern African weed biological control practitioners should take the "innocent until proven guilty" point of view with this plant, as it is probably an indigenous component of the southern African aquatic flora.

The only reason that this risk of biological control needs to be considered is the perceived negative impacts of the high biomass of *M. spicatum*. But, this increase in biomass is probably a symptom of an underlying cause, related to water resource management and an increase in nutrients in aquatic ecosystems, as shown with the *Typha* spp. in the Everglades, Florida (Newman *et al.* 1998). In recent years, southern African water resources are becoming more and more disturbed through changes in hydrology and nutrient enrichment as a result of poor water resource management (Van Ginkel 2011). Ultimately the control of native macrophyte species that become problematic, especially in impacted systems, should be a water resource management issue and not a biological control issue. It would be expected that if the control of *M. spicatum* were to take place through biological control, there would be another species (native or exotic) ready to take advantage of the vacant niche, in a resource-rich environment. However, if the water resource management issues surrounding southern African water bodies were dealt with, natural control of this native species, among others, should be inherent in the system.

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