AQUATIC INVASIONS OF THE NSELENI RIVER SYSTEM: CAUSES, CONSEQUENCES AND CONTROL

Thesis

A thesis submitted in fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

of

RHODES UNIVERSITY

by

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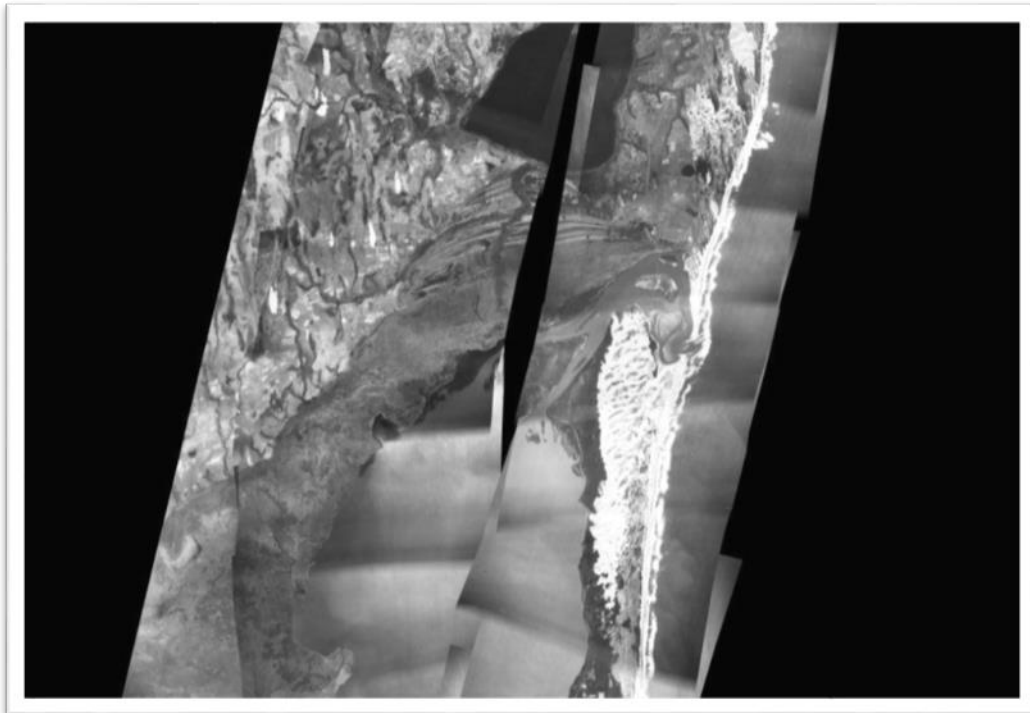Department of Zoology and Entomology
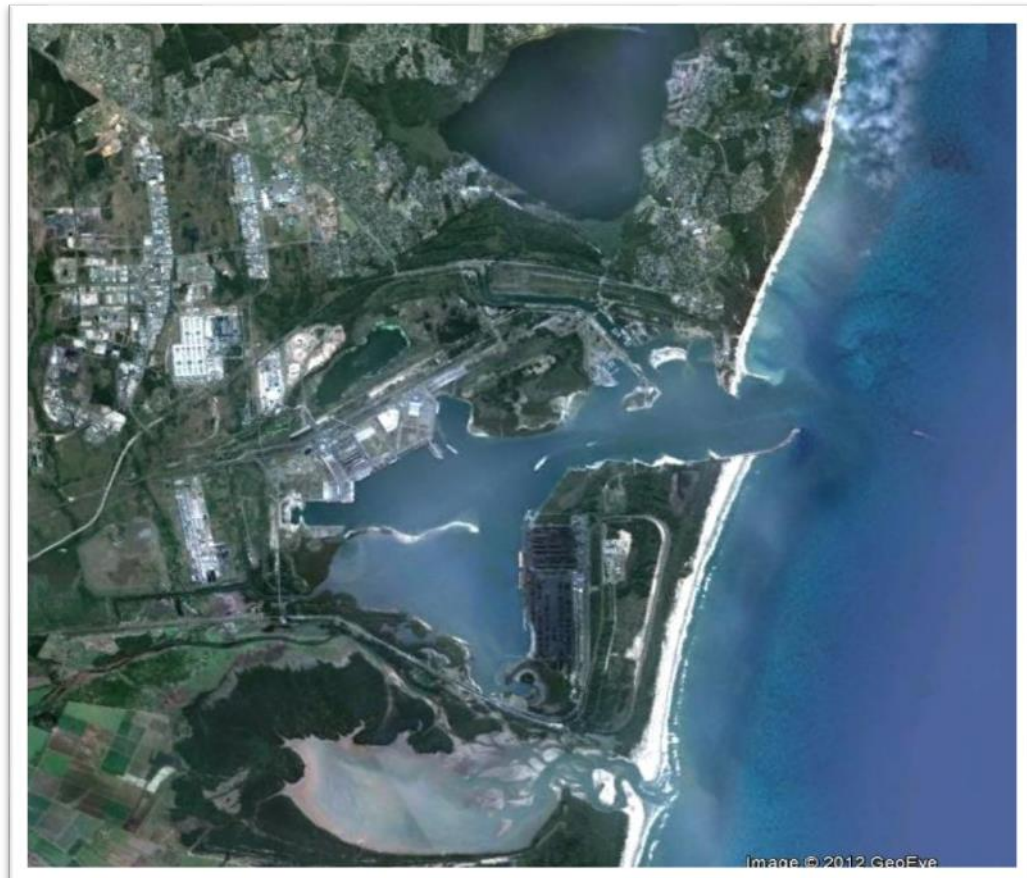
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Date: December 2014

Frontispiece

Richards Bay – 1937 (T.Ott – Richards Bay Minerals)

Richards Bay – 2012 (Google Earth)

QUOTATION
"We May Be Brothers After All".

In 1854, The Great White Chief in Washington made an offer for a large area of Indian land and promised a "Reservation" for the Indian people.

Chief Seattle's reply to the offer was:

"How can you buy or sell the sky, the warmth of the land? The idea is strange to us. If we do not own the freshness of the air and the sparkle of the water, how can you buy them?

Every part of this earth is sacred to my people. Every shining pine needle, every sand shore, every mist in the dark woods, every clearing and humming insect is holy in the memory and experience of my people. The sap which courses through the trees carries memories of the red man.

The white man's dead forget the country of their birth when they go to walk amongst the stars. Our dead never forget this beautiful earth, for it is the mother of the red man. We are of the earth and it is part of us. The perfumed flowers are our sisters; the deer, the horses, the great eagle, these are our brothers. The rocky crests, the juices in the meadows, the body heat of the pony, and man − all belong to the same family.

So when the Great White Chief in Washington sends word that he wishes to buy our land, he asks much of us. The Great White Chief sends word he will reserve us a place so that we can live comfortably to ourselves. He will be our father and we will be his children. So we consider your offer to buy our land, but it will not be easy. For this land is sacred to us.

This shining water that moves in the streams and rivers is not just water but the blood of our ancestors. If we sell you our land, you must remember that it is sacred, and you must teach your children that it is sacred and that each ghostly reflection in the

clear water of the lakes tells of events and memories in the life of my people. The water murmur is the voice of my father's father.

The rivers are our brothers, they quench our thirst. The rivers carry our canoes, and feed our children. If we sell our land, you must remember, and teach your children, that the rivers are our brothers, and yours, and you must henceforth give the rivers the kindness you would give your brother.

… But in you perishing you will shine brightly, fired by the strength of the God who brought you to this land and for some special purpose gave you dominion over this land and over the red man. That destiny is a mystery to us, for we do not understand when the buffalo are all slaughtered, the wild horses are tamed, the secret corners of the forest heavy with scent of many men, and the view of the ripe hills blotted by talking wires. Where is the thicket? Gone! Where is the eagle? Gone!

The end of living and the beginning of survival".

Chief Seattle (an Anglicization of Si'ahl)
1854

IN MEMORY OF THE LATE

Esther Joyce Amy Jones

(9 June 1927 - 1 September 2011)

You may no longer walk the world as we know it, but you will never be forgotten, as you look down on us from amongst the stars.

Thank you for all your encouragement in allowing me to pursue my interests, even though it took me far from home, at an early age.

Thank you for everything you did for our family, especially during the tough times.

Mbazwane Mhlongo

(15 October1956 – 22 June 2012)

Mbazwane joined the then Natal Parks Board, now KZN Wildlife, on the 1st of October 1976 and had always been stationed at the Enseleni Nature Reserve. Mbazwane started as a general assistant and worked his way up through the ranks to a Senior Field Ranger. All of his Officers-in-Charge saw the potential in him to progress within the conservation structure. At the time of his death, Mbazwane had 36-years of unbroken service. I was busy attempting to get him promoted to a Principal Field Ranger at the time of his untimely death. Mbazwane died in the line of duty when he and a colleague drowned on Lake Nhlabane, while recovering illegal gill nets. Mbazwane took every opportunity to join me on the river while carrying out various research, assisting students with research projects or boat patrols. Mbazwane showed a keen interest in controlling water hyacinth on the Nseleni River, not because it was his job, but because he knew the negative effect it was having on biodiversity. Mbazwane was fascinated by the biological control agents that had been introduced and always asked deep penetrating questions about them. Shortly before his death, Mbazwane received the highest award that a Field Ranger can receive, namely the **Magqubu Ntombela Award** for the Best Field Ranger in Ezemvelo KZN Wildlife. *Hamba Kahle, Mbazwane*.

Abstract

Globalization has seen an unprecedented dispersal of exotic and alien species worldwide resulting in worldwide homogenization and sometimes extinction of indigenous or endemic taxa. When an exotic species becomes established in a new habitat the invasive organisms are capable of having an impact on indigenous community dynamics and the overall structure and function of ecosystems. Furthermore, the impact of invasion is determined by the geographical range, abundance and the per-capita or per-biomass effect of the invader. However, the success of the introduced organisms is reliant on their ability to acclimate to the physiochemical conditions of the newly invaded environment.

Freshwater ecosystems are especially vulnerable to invasions because there are numerous potential routes of introduction including intentional pathways such as stocking, and unintentional pathways such as the release of ballast water and aquarium releases. Efforts to limit the introduction of invasive species or to manage established exotic populations are often hindered by insufficient understanding of the natural history of problematic species. Relatively little is known regarding the physiological tolerances of many taxa. Knowledge about specific species ecophysiological constraints allows for the prediction of future patterns of invasion more accurately, including where an introduced organism would probably survive, thrive and disperse. Furthermore, data on the physiological tolerances of an introduced exotic organism may provide data necessary for effective management and control.

This study investigated three invasive species in the Nseleni River system in a protected area in KwaZulu-Natal. The species studied were, *Tarebia granifera* (Quilted melania − Lamarck, 1822), *Pterygoplichthys disjunctivus* (Suckermouth armoured catfish - Weber, 1991) and *Eichhornia crassipes* (water hyacinth − (Martius) Solms-Laubach,). The Nseleni River flows into Lake Nsezi which is responsible for providing potable water to the surrounding towns and industry, as well as the surrounding rural communities. The Enseleni Nature reserve has become the centre for biodiversity dispersal in the immediate area, due to the change in landscape surrounding the protected area.

An important step in developing alien invasive species management strategies in protected areas is determining their extent and invasive traits. *Tarebia granifera* is a prosobranch gastropod originally from South-East Asia that has become invasive in several countries around the world including South Africa. Snail populations were sampled at nine sites throughout the Nseleni/Mposa river system every six weeks over a twelve month period. The snail was abundant throughout the system, especially in shallow waters of less than 1m in depth.

The first positive identification the loricariid catfish *Pterygoplichthys disjunctivus* for the Nseleni River was in 2006. The original introduction is believed to have been via the aquarium trade. The aim of the study was to assess the usefulness of the unified framework with regard to management of fish invasions by assessing the invasion stage of the loricariid population and identifying appropriate management actions using the Blackburn *et al*. (2011) framework. The fish were sampled at nine different sites and three different depths over a period of twelve months, as well as when two ichthyological surveys were carried out on the Nseleni River system. This invasive fish has been located throughout the system and both male and female fish were collected. The smallest fish sampled was a fingerling of a day or two old and the smallest pregnant female was a mere 270mm TL. This is a clear indication that this fish is breeding in the river system.

Although *T. granifera* and *P. disjunctivus* were abundant in the Nseleni/Mposa river system, it was not clear what their role in the system was, and in particular if they were competing with any of the indigenous species. Therefore, isotope samples were collected from numerous taxa over a two week period, with the exception of *Pterygoplichthys disjunctivus* samples, which were collected over 12 months. The $\delta^{13}C$ and $\delta^{15}N$ signatures of all samples were determined. The niche overlap between the invasive and indigenous snails was effectively zero ($1.02E^{-13}\%$), indicating no shared food resources. The medium ranges of dNR_b (7.14) and dCR_b (9.07) for the invasive fish indicate that it utilizes a wider range of food resources and trophic levels than the majority of indigenous fish. A medium CD_b value (2.34) for the invasive fish species, *P. disjunctivus*, describes medium trophic diversity, with three indigenous species possessing higher diversity and three possessing lower diversity. Further

results indicated that there was no direct dietary competition between *P. disjunctivus* and indigenous species.

Eichhornia crassipes was first recorded on the Nseleni River in 1978, and has been shown to have a significant negative impact on the biodiversity of the Nseleni/Mposa River system and therefore required a control intervention. Although biological control using the two weevil species *Neochetina eichhornia* (Warner) and *N. bruchi* (Hustache) has been credited with affecting a good level of control, the lack of a manipulated post-release evaluation experiments has undermined this statement. Five experimental plots of water hyacinth of $20m^2$ were sprayed with an insecticide to control weevils. After ten months the plants in the sprayed plots were significantly bigger and heavier than those in the control plots that had natural populations of the biological control agents. This study has shown unequivocally that biological control has contributed significantly to the control of water hyacinth on the Nseleni/Mposa River system.

The management plan for the Enseleni Nature Reserve identifies the need to control invasive and/or exotic organisms within the boundary of the protected area. In addition, set guidelines have been implemented on how to control these organisms, so that indigenous organisms are least affected. Lack of control of exotic organisms can have serious consequences for indigenous species. It is therefore of utmost importance that the population dynamics of the invading organism be understood, what the potential impact could be and how to control them. Furthermore, it has also acknowledged the threat of possible exotic species invasions from outside of the protected area that might result in threats to the protected area and that these must be investigated, researched and managed.

This thesis has identified *Tarebia granifera, Pterygoplichthys disjunctivus* and *Eichhornia crassipes* as being a threat to indigenous biodiversity within the protected area, as well as in adjacent areas to the protected area. The thesis will therefore investigate the hypothesis that both *Tarebia granifera* and *Pterygoplichthys disjunctivus* are having a direct negative effect on available food resources for indigenous species of organisms. In addition, this thesis will investigate if the

Neochetina species that have previously been introduced onto *E. crassipes* are having any negative effect on this invasive alien aquatic plant.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

The greater Richards Bay/Empangeni area (City of Mhlathuze) (Figure 1.1) has experienced a significant loss of biodiversity and an increase in invasive alien organisms since the area was identified by colonists as a suitable area for development mainly during the period of the Anglo – Zulu war (around 1900) and thereafter by industrialists. The need for a harbour during the late 1960's has led to the degradation of the coastal area through development.
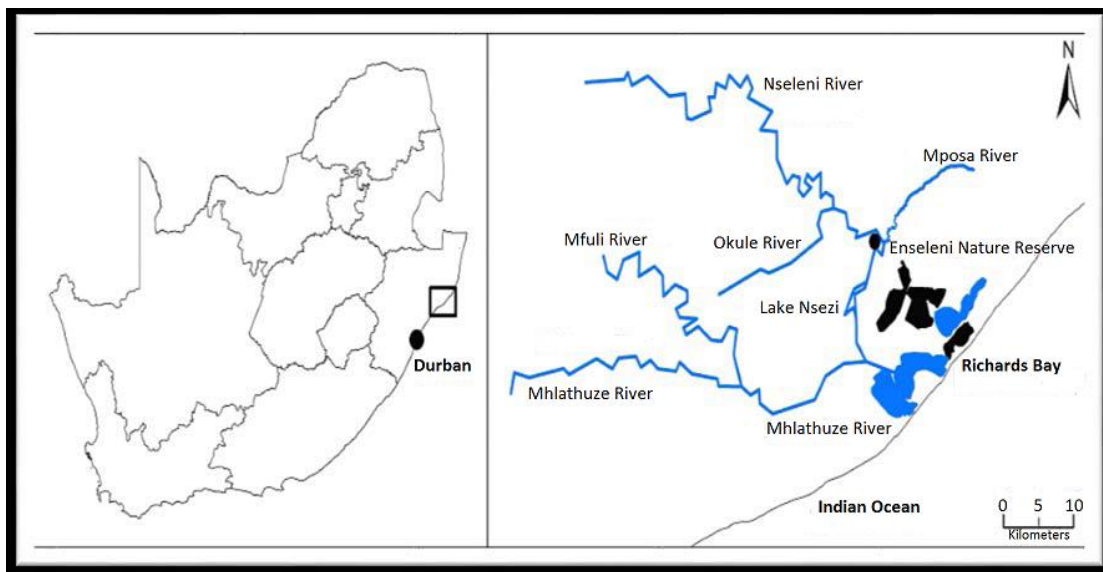
Fig. 1.1 Location of Richards Bay, KwaZulu-Natal, South Africa.

Richards Bay (originally called Mhlathuze Bay / Lagoon) was named after Admiral Sir F.W.Richards on 13 July 1879 (Figure 1.2). Due to the British defeat at Isandlwana, he set sail from the Cape and located a suitable harbour on the north coast of KwaZulu - Natal to be nearer the conflict area. He was present at the Battle of Gingindlovu and the relief of eShowe.
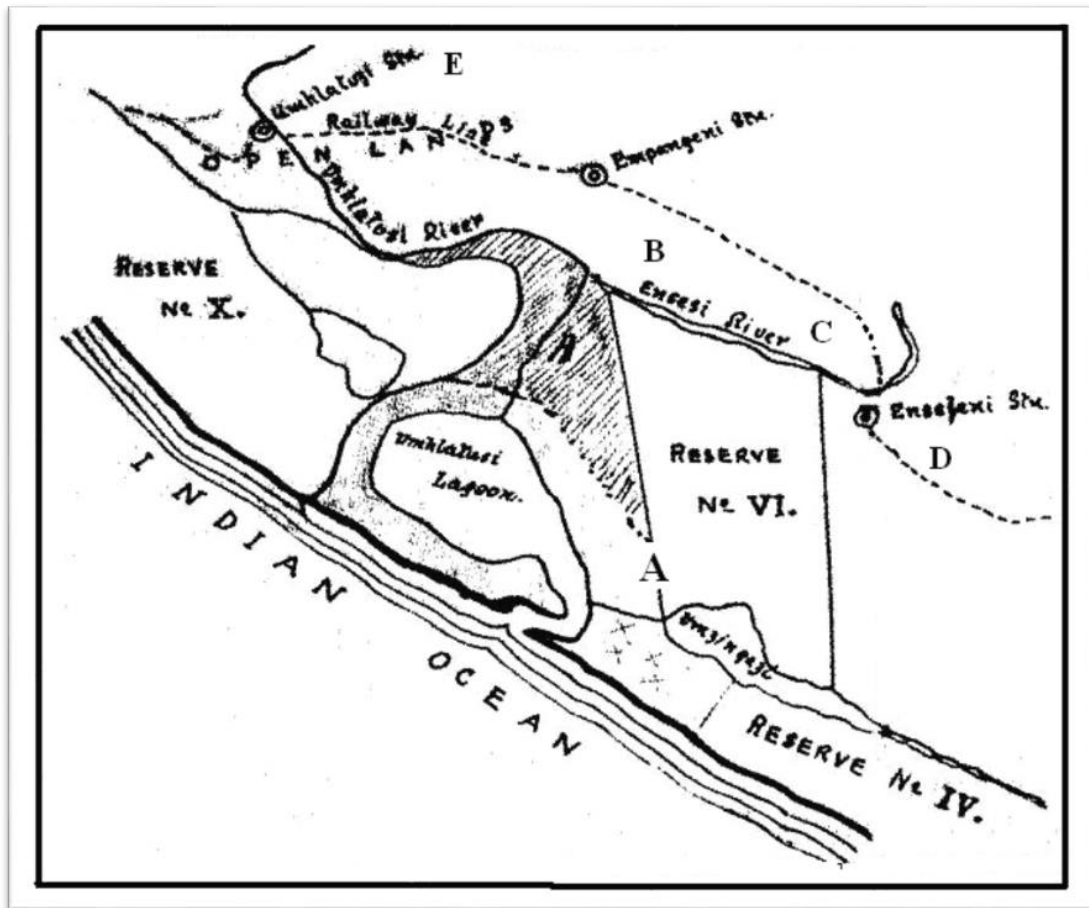
Fig. 1.2 Historical sketch map of Umhlatusi Lagoon (Richards Bay) – Cathcart Methven (1902). Label A on the map indicates the present locality of Richards Bay town. Label B indicates the position of the Nseleni River, where it flows into Lake Nsezi (not depicted on drawing). Label C is where the Enseleni Nature Reserve is situated. Label D is where the present day Nseleni railway station remains functioning, mainly for the purpose of loading timber and label E indicating the Mhlathuze station, is today referred to as the Felixton station which is mainly used for the unloading of sugarcane for the Felixton sugar mill.

1.2. Historical overview

In an attempt to understand the incursion of invasive exotic species of fauna and flora to the area, the historical background of the greater Richards Bay area is summarised.

1.2.1 The Nguni Tribe

The Nguni people originated from northern Central Africa. The Sahara Desert in the north of Africa was an impassable barrier for herdsman to cross with their livestock and so they had to move south. Furthermore, the Tropical Rain Forests and the Congo River proved to be a major barrier to migration from the North southwards. The subtropical savannah of Africa was home to great numbers of pastoralists. As their population numbers increased they had to move from their land known as "eMbo" and

find new land to occupy. They moved southwards through the Rift Valley and the Lake belt of Eastern Africa known as the "Interlacustrine Region or Belt", through what is now Mozambique under their leader, Dlamini. It was here that they came into contact with San hunters who produced the "click" sound, which is now characteristic of their language. As they moved southwards, following the Lebombo (Swazi) Ubombo (Zulu) Range, they finally found an ideal countryside in which to settle. Here the tribes dispersed and the North and South Nguni groups came about. The Zulu, the Swazi and the Ndbele are of the Northern Nguni group, while the Xhosa, Pondo and Thembu form the southern group. Ngwane, a leader, established himself in the Pongola River basin and founded what was to become the Swazi Nation (Havemann, 2011).

Of importance, is the trail of a chief of one of the small fragmentary tribes called Malandela (The Follower). Malandela with his family and followers settled in a valley named uMhlathuze (The Powerful One). Malandela had had two grandsons, Qwabe, the eldest and the youngest, Zulu (Heaven). Zulu or Nkosinkulu moved and settled in the eMakhosini Valley on the banks of the uMpembene stream. This is where the Zulu Nation was born (Havemann, 2011).

1.2.2 The Zulu Dynasty (Figure 1.3)

Some of the Northern Nguni people continued moving south to become the Zulu nation and their royal line starts with Luzumane. There are suggestions that the Zulu genealogy from, Mnguni to Nkosinkulu, to Mdlani to Luzumane. Luzumane had a son called Malandela, known as "Malandela ka Luzumane". In the Zulu language the prefix "ka" means "of" or "son of", hence Malandela son of Luzumane. (Havemann, 2011).

ZULU DYNASTY

LUZUMANE

MALANDELA

NTOMBELA

ZULU
(NKOINKULU)

PUNGU

MAGEBA

NDABA

JAMA

SENZANGAKHONA

SHAKA

(1781-1828)

Ruled:1816-1828
Killed by Dingane &
Mhlangana

DINGANE

(1795-1840)

Ruled:1828-1840

Killed by own people

MPANDA

(1810-1872)

Ruled:1840-1872

CETSHWAYO

(1832-1884)

Ruled:1873-1884

DINIZULU

(1868-19130

Ruled:1884-1913

SOLOMAN

(1890-1933)

Ruled:1913-1933

CYPRIAN
BHEKUZULU

(1924-1968)

Ruled:1948-1968

GOODWILL
ZWELITHINI

(1948 -   )

Ruled: 1971 to present

Fig. 1.3 The Zulu dynasty (van der Walt, 2007).

King Shaka was born during 1781 at Mbengi's Engugeni kraal on the north side of the Mhlathuze River, close to kwaNtoza hill. On 22 September 1828 his two half-brothers, Dingane and Mhlangana assassinated Shaka. It is said that Shaka's last word were "Your country, children of my father, will be ruled by white people who will come up from the sea" (van der Walt, 2007).

The Battle of Mhlathuze River was a battle fought between the Zulu and Ndwandwe tribes in 1820 following the Zulu civil war. The Ndwandwe hierarchy was demolished by the battle and largely scattered their population (van der Walt, 2007). King Dingane had a reputation as a man who ruled by fear, yet on 16 January 1836, he welcomed American missionaries to Zululand. On 16 September 1836 the first mission station was established. King Dingane recognized the need to educate himself and his subordinates (van der Walt, 2007). King Mpande welcomed Norwegian missionaries to Empangeni during 1850. However, prior to this in 1841, he gave permission to a French naturalist by the name of Adulphe Delegorgue to collect animal, bird, insect and plant specimens in Zululand (van der Walt, 2007). Cetshwayo became king on 1 September 1872. He expanded his army and readopted many methods used by Shaka. He also equipped his impis with muskets and banished European missionaries from his land. In 1878, Sir Henry Bartle Frere, British Commissioner for South Africa, began to demand reparations for border infractions. Although Cetshwayo was angered by these demands, he kept the peace until Frere demanded that he should effectively disband his army. Cetshwayo's refusal led to the Zulu War in 1879. After initial defeats (Battle of Isandlwana), the British eventually began to gain victories. On 4 July, Cetshwayo's capital Ulundi was captured and destroyed. Cetshwayo was deposed and exiled to London, returning only in 1883. By 1882 differences between two Zulu factions – pro-Cetshwayo *uSuthus* and three rival chiefs, *UZibhebhu* – had erupted into a blood feuds and civil war. The British tried to restore Cetshwayo to rule in 1883, but failed. He died in February 1884 as the last king of an independent Zulu nation (van der Walt, 2007).

King Dinizulu was born at a critical time in South African history. Europeans had firmly established themselves as the rulers of South Africa. When Bambata's rebellion broke out against the Europeans, it caused a danger to the security of the Europeans. Although the Zulu's had been vanquished, they continued to hope of one

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day becoming independent. Dinizulu was arrested for high treason and he was found guilty, due to the allegations of aiding and abetting and secretly assisting Bambata. He was banned for life from Natal and sent to Middleburg, Transvaal, in what is now the Mpumalanga Highveld, where he died. Before his exile and death, Dinizulu had offered rewards of land to Boer mercenaries who would fight on his side. This land was all in northern Zululand. By his death in 1913, Zululand was already being transformed from its natural appearance due to European influences (van der Walt, 2007).

1.2.3 European influences

Adulphe Delegorgue, a French naturalist, collected animal, bird, insect and plant specimens in Zululand from 1841, financed through hunting. Delegorgue's main fascination was with hippopotamus and he recorded the fact that his observations led him to believe that this species numbers were rapidly declining due to over hunting. He further wrote: "It is not difficult to predict that the species will disappear completely from the country, as it has done from every other place where white man has settled". Delegorgue constructed a solid warehouse on the north bank of the Umfolozi River for his vast collection of ivory, horns, skins, skeletons, butterflies, beetles, chemicals, illustrations and stuffed birds, prior to them being transported to Durban harbour for export (van der Walt, 2007).

On 14 December 1849, Robert Struthers arrived in KwaZulu-Natal. He became an ivory hunter in Zululand. By 1855, more than 25% of the total export value from Natal was ivory and hides, which was obtained from the Zulu kingdom. In 1854, Struthers sailed down the Mhlathuze River until he reached the vast Mhlathuze lagoon. Here he hunted hippopotamus. Struthers carried out numerous hunting trips around the Mhlathuze River, Lake Mzingazi and Lake Nsezi. Struthers wrote "The area (Richards Bay) presenting extensive tracts well fitted for European occupation and culture, and I think will yet be so occupied for it presents an unlimited field of enterprise" (van der Walt, 2007).

Rev. W.C.Holden describes the Colony of Natal as: "abounding in streams of pure water. Seldom does one travel an hour along the coast country, without crossing rivulets of transparent water. These vary in size from the merest brook, three miles in

length to 150 yards wide and 200 miles long.   The animals are most abundant, as are the serpents, insects, birds and birds of prey,   As to the botany of Natal, a person newly arrived from the higher latitudes of New England, the entire novelty of the general features of all vegetation makes him feel as if he has landed in a new planet" (Holden, 1963).

The Charter of Natal, promulgated by Letters Patent dated 15 July 1856, created Natal a separate Colony, excluding the Zululand area's which gave it a limited form of representative government (Brooks and Webb, 1965). This led to additional changes to the landscape within the Natal Colony.

The massacre of Lord Chelmsford troops at Isandlwana on 22 January 1879 started a chain of events that would lead to the total destruction of the Zulu Kingdom (van der Walt, 2007) and thereby opened the area to "invasion". It was in the same year, that Admiral Sir F.W.Richards renamed Mhlathuze Bay to Richards Bay. In addition, emaNgweni, the military inkhanda (stronghold) at Empangeni was destroyed. The British unleashed 9 200 troops to destroy the uMhlathuze area and thereafter in 1887, Zululand became a British Colony (van der Walt, 2007; Figure 1.4).
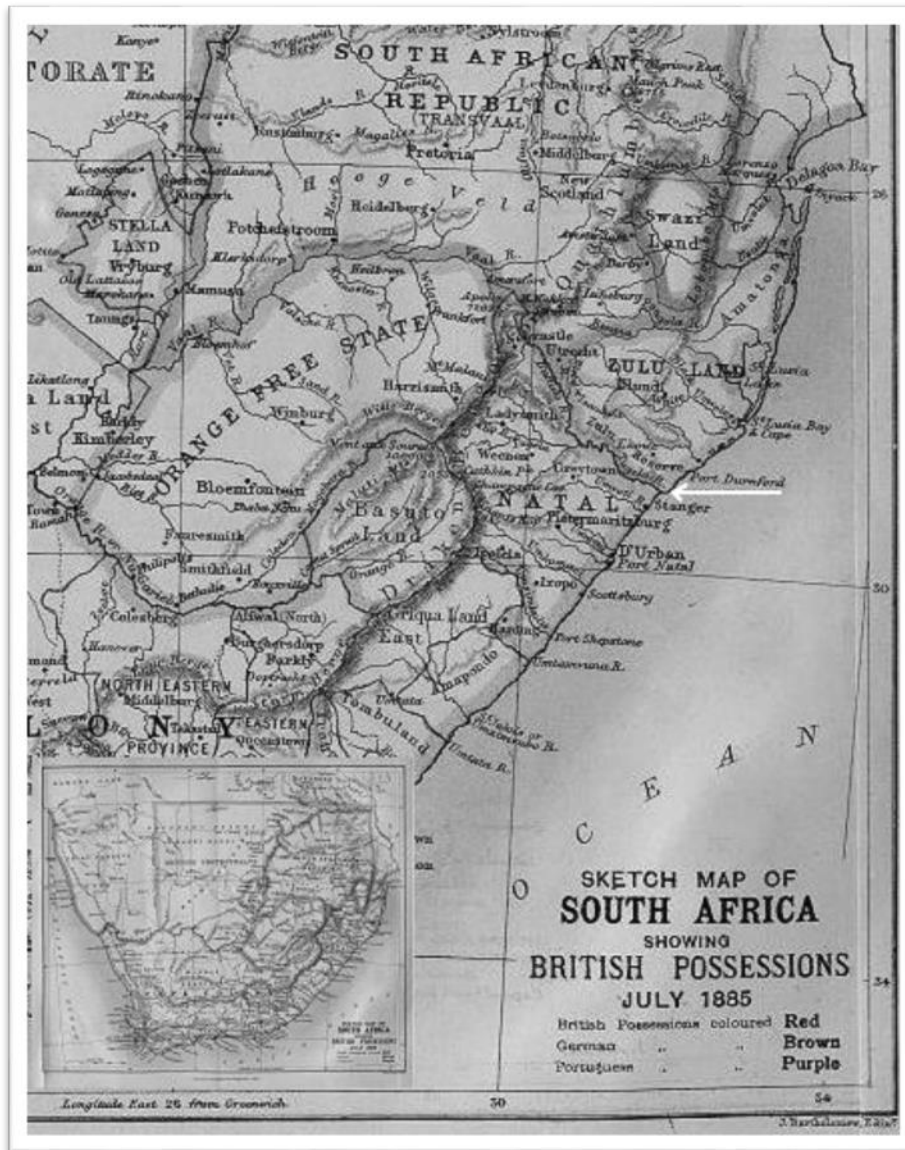
Fig. 1.4 Sketch map of South Africa showing British possessions – July 1885 (Scottish Geographical Magazine, 1885). The importance of this map is that a clear boundary is indicated, at the Thugela River (white arrow), between the Zululand region ruled by the Zulu Kingdom to the north and Natal, and governed by the British, to the south.

Coal was first discovered on the land of Chief Somkhele kaMalanda in 1892 and the Zululand Collieries Company started mining and conveying coal to Durban harbor. The mine, however, closed in 1909 due to it becoming non-profitable. Somkhele coal mine [operated by Tendele Coal Mining (PTY) Ltd.], situated 52 kilometers NNE of Richards Bay, was commissioned in 2007 and produces 40,000TPM valued at ZAR R63m/annum.

During the late 1890's, Sir James Liege Hulett was the most influential businessman in Natal. His main interest was in the development of sugar cane farming in

Zululand, as well as the development of a harbour at Richards Bay. At this stage the railway line had only reached Verulam, north of Durban. In 1884, Hulett and Sons were granted permission to extend the line to the border of Zululand, at the Thukela (Tugela) River. By 1902, the railway line had reached the Mhlathuze halt and Empangeni station by 1903, not far west of Richards Bay. The railway line connected Durban with Zululand which accelerated the settlement of white people to the area. The first sugar mill was opened in Amatikulu in 1908, followed by the Felixton sugar mill in 1911 (van der Walt, 2007).

Major Peter Millar Rattray (DSO) was the pioneer of the forestry industry in Zululand. During 1912, he purchased farms in the vicinity of the kwaMbonambi railway halt and felled his first crop of Saligna trees in 1928. The first commercial saw mill in Zululand was then opened (van der Walt, 2007). With the railway line now reaching kwaMbonambi, Zululand became more accessible to naturalists, hunters, hoteliers and gold mining. With the new railway line, coal exports, sugar cane crops, planting of *Eucalyptus* trees, houses and shops increased in the area. During the WWII era, Richards Bay was used by the South African air force to patrol for Japanese, Italian and German submarines (van der Walt, 2007). Thereafter it became an industrial port and soon became one of the quickest developing areas in South Africa (1990s). Construction for a harbour at Richards Bay commenced during 1972 and was officially opened on 1 April 1976.

The above synopsis is an indication of how the once 'pristine' landscape of rolling hills of natural vegetation for pastoralists, unpolluted rivers and vast amounts of game were transformed by the influx of 'colonists' who brought with them non-indigenous species of organisms accidentally, or on purpose. All of this development had a negative impact on the ecosystems in the area.

1.3.   Invasive alien species

All major southern African river systems are inhabited by alien species, both plants and animals (Moosa, 2000). Moosa (2000) estimates that freshwater systems in South Africa have been invaded by some 54 animal species of which thirty seven are considered harmful. The impacts of these invaders occur through competition for food

and space, predation, hybridisation, the introduction of parasites and diseases, disruption of breeding behaviour and habitat alteration. Of the thirty seven, eighteen are considered to have had a major detrimental impact on indigenous species and communities. Among the most harmful and widespread are fish parasites, gastropod snails and various fishes, including trout (*Oncorhynchus* spp.), carp (Cyprinidae), bass (*Micropterus* spp.), swordtails (*Xiphophorus* spp.**)** and guppies (*Poecilia reticulata*, Peters, 1859).

Many species of fish have been deliberately introduced around the world to provide food or sport (Gozlan, 2008). In addition to these deliberate introductions, a large number of fish species have been spread beyond their native range by releases from aquaria, bait buckets, and water gardens, as contaminants of fish intended for stocking, or in ballast water (Strayer, 2010). Some of these fish have caused large negative ecological impacts. Humans are also guilty of introducing fish into fresh waters that already contain fish, either accidentally or in a deliberate attempt to improve the fish community. The most obvious impacts have been losses of favoured prey species, especially in cases where the alien has no native trophic analogue in the system (taxonomic distinctiveness hypothesis). The global extinction of approximately 200 species of cichlids, belonging to the Family Cichlidae, from Lake Victoria following the invasion of the Nile perch (*Lates niloticus*, Linnaeus, 1758) is possibly the most dramatic example (Strayer, 2010).

Significant ecological concern has been raised due to the introduction of alien fish predators into freshwater ecosystems throughout the world, is due to their potential for causing deleterious, top-down effects on indigenous fish populations (Britton *et al.*, 2010). An example of this is *Micropterus salmoides* (Lacepède, 1802), a piscivorous largemouth bass, which has been introduced from North America for sport fishing and is established in South Africa. Once these fish have been introduced and become established, they have the potential to cause significant ecological impacts to the river system. Britton *et al.*, (2010), suggests that these ecological impacts may change the species composition and size structure of communities, shifts in habitat use, foraging behaviour and even somatic growth of prey populations. *Micropterus salmoides* is listed on the IUCN list of 100 of the world's worst invasive alien species (Lowe *et al.*, 2008).

1.3.1   Impact of Alien Invasive Species in a Protected Area

Prior to the establishment of the Enseleni Nature Reserve (the locality for this study), the area was Crown Land and was used by local farmers for grazing. In 1910, the land was identified as an industrial site suitable for the construction of a sugar mill, however, a site closer to Empangeni was eventually chosen for the mill. Known to local's as the "Beauty Spot", constant requests for permission to plant sugar cane on the land were received and refused by Mr. Jim Grantham, MEC. Instead, Mr. Grantham had other ideas and through his determined efforts the area was finally proclaimed a nature reserve on 22 January 1948 to be managed by the newly established Natal Parks, Game and Fish Preservation Board which had been formed in December 1947.

Although the area was now officially proclaimed a nature reserve in 1948, its development was slow until the arrival of the first Officer-in-Charge, Mr. Hennie van Schoor in 1963. The railway line was in use until the 1960's when, with the development of Richards Bay, a new line was constructed which bypassed Enseleni Nature Reserve. The old line had a siding adjacent to the reserve's western boundary, Hibberd Station. The eastern boundary of the Enseleni Nature Reserve consists of 6.2 kilometers of the Nseleni River. The Nseleni River forms an aquatic and terrestrial biodiversity corridor, from its source some 80 kilometers inland to the Indian Ocean.

During the period 1947 to 1963, local farmers continued to utilize the area for feed for their livestock, which had a detrimental effect on the vegetation, especially the grass species, as the "sweet veld" was over utilized and the veld type slowly changed to a "sour veld". Although small areas of sweet veld remain, the management of the reserve is attempting to reverse the negative impacts of the over grazing that occurred between 1947 and 1963.

During the late 1970's conservationists/ scientific communities became more aware of the threat of invasive alien plants, not only due to visual observations, but because of more awareness being created about the problems that could result if they were not controlled. During the latter 1980's and early 1990's, the then Natal Parks Board, recognized the threat of invasive alien plants in the Protected Areas under its jurisdiction and policies and management plans were formulated to control invasive

alien plants in all of the Protected Areas. This included the Enseleni Nature Reserve, where numerous alien plants were identified (Table 1.1) and due to the location (coastal and sub-tropical) the plants were thriving. The worst affected areas in the Enseleni Nature Reserve were located along the ecotone (forest / grassland) and within the riverine and coastal forests, where open areas were located. The invasion of alien invasive plants has had a detrimental effect on indigenous species of plants.

Table 1.1 Terrestrial Invasive alien plants identified within the Enseleni Nature Reserve, in terms of the Conservation of Agricultural Resources Act 43 of 1983.

Scientific name:	Common name:	Type:	*Category:
Psidium guajava	guava	Invader	2
Ageratum conyzoides	mexican ageratum	Weed	1
Chromolaena odorata	triffed weed	Weed	1
Nephrolepis exaltata	sword fern	Invader	3
Rubus cuneifolius	bramble	Weed	1
Ipomoea indica	morning Glory	Weed	1
Litsea glutinosa	indian laurel	Weed	1
Senna didymobotrya	cassia	Invader	3
Solanum mauritianum	bugweed	Weed	1
Canna indica	canna	Weed	1
Caesalpinia decapetala	mauritius thorn	Weed	1
Cardiospermum grandiflorum	balloon vine	Weed	1
Cestrum laevigatum	ink berry	Weed	1
Hedychium coronarium	wild ginger	Weed	1
Opuntia ficus-indica	sweet prickly pear	Weed	1
Ricinus communis	castor oil	Invader	1
Schinus terebinthifolius	brazilian pepper	Weed	1
Melia azedarach	syringa	Invader	3
Lantana camara	lantana	Weed	1
Senna didymobotrya	peanut butter cassia	Invader	3

Definitions of Categories: 1. Plants that may not occur on any land or inland water surface other than in biological control reserves. 2. Plants that may not occur on any land or inland water surface other than a demarcated area or a biological reserve. 3. Plants that may not occur on any land or inland water surface other than in biological reserve (Conservation of Agricultural Resources Act 43 of 1983).

Control of invasive alien plants was classified as: Initial, follow-up and maintenance levels. Initially, where high percentages of alien invasive plants occurred these areas were treated as containment areas ie. the plants were not allowed to spread any

further. The less dense stands of alien invasive plants were initially managed and once they were under control, then the higher density of alien invasive plants were brought under control (Conservation of Agricultural Resources Act 43 of 1983).

From the outset, a management decision was made to use minimum herbicides in the Protected Area as possible and to rely on manual removal, but this resulted in high labour costs. Herbicide application on species like *Psidium guajava* L. and *Litsea glutinosa* Lam. were unavoidable, due to the root structure.

The control programme for Invasive Alien Species on the Nseleni River has taken approximately ten years to achieve a maintenance level. The original annual cost has been reduced from approximately R268 000-00 to R42 842-00 in 2012. Unlike aquatic weeds, no terrestrial biological control of invasive alien species is carried out in the reserve.

1.3.2 Threats to the Nseleni River

The Nseleni River system has been altered to accommodate the fast growth of the area and the need for more water for both industry and domestic use. The Lake Nsezi outflow has been altered by means of an earthen weir constructed by the Mhlathuze Water Board (1979), to control the amount of water available. At a later stage, water was transferred from the Mhlathuze River to Lake Nsezi to ensure a sustainable quantity of water. This has led to the Nseleni River and Lake Nsezi becoming highly altered (Jones, 2009).

The banks of the Nseleni River have also been altered from coastal grasslands to either sugar cane or forestry plantations, with a high level of nutrient run-off. In addition, a sewerage works was constructed near to the Nseleni Township and the outflow from the sewerage works enters the Nseleni River, via the Mposa River increasing the nutrient levels (Jones, 2001). The river system has now become eutrophic. There are numerous threats to the biodiversity of the Nseleni River and these are listed below.

Urbanization

Human encroachment: Due to the development of the greater Richards Bay area, the Nseleni Township has grown exponentially over a short period of time. This has led to a decrease of important riverine forest which is important for bank stabilization, resulting in biodiversity loss and an increase in the use of freshwater fishes both by angling and illegal gill netting.

Forestry and Agricultural: Both private and large forestry companies have identified the immediate area adjacent to the Nseleni River as having suitable soil and climate conditions for the growing of *Eucalyptus* trees. Sugar cane has been planted in close proximity to the Nseleni River, with high nutrient run-off. In both cases, natural vegetation has been removed which has led to a direct and indirect loss of biodiversity.

Sewage: Water quality and quantity have been severely negatively impacted as a result of land change and development and the need for more water for industry and house hold use. The Mhlathuze Water Board implemented a water transfer scheme between the Mhlathuze River and Lake Nsezi in the 1990s to augment the natural flow of water into the lake. Nseleni Township has a sewerage works, near the township that discharges directly into the Mposa River has led to an increase in nutrient levels, and hence the eutrophication of the river system. This sewerage works was constructed in the 1970's, had a slight upgrade in the mid 1990's and has not had an upgrade since, even though the population for the township has more than doubled since the early seventies. Problems do occur at the sewerage works that directly influences the water quality of the Mposa River.

Alien invasions

Terrestrial weed invasions: Due to land use change, numerous species of alien vegetation have invaded the area immediately adjacent to the Nseleni River, which have a negative effect on the biodiversity of the habitat. Soil disturbance, and the clearing of coastal forest to make way for sugarcane and *Eucalyptus* plantations, has allowed for the invasion of species such as *Chromolaena odorata* L.(King and Robinson), *Caesalpinia decapetala* (Roth) Altson), *Lantana camara* L., *Ricinus communis* L., *Melia azedarach* L. and *Solanum mauritanium* (Scop). *Chromolaena*

odorata has been shown to negatively affect the success of breeding in Nile crocodile *Crocodylus niloticus* (Laurenti), due to shading (possibly causing a female-biased sex ratio), possible prevention of embryonic development and abandonment of nesting site due to encountering the fibrous root mats of *Chromolaena odorata* while digging egg chambers (Leslie and Spotila, 2001).

Aquatic weed invasions: Eichhornia crassipes, water hyacinth, originated in the New World tropics and is now widely distributed throughout the world (Hill *et al*., 1999). *Eichhornia crassipes* is presently one of the most important aquatic weeds in South Africa. The first records for this invader in South Africa are around 1900 (Hill *et al*., 1999). Since its introduction, it has been spread by gardeners, aquarium owners, and accidentally by boating enthusiasts. It has become establish throughout South Africa, due to not having any natural enemies, as well as the fact that many rivers in South Africa are highly enriched. This invader is capable of degrading aquatic ecosystems and limits their utilization (Hill *et al*., 1999).

Community composition of phytoplankton, zooplankton and fish in freshwater ecosystems is determined by the structure of a macrophyte community (Villamagna and Murphy, 2010). They further suggest that the interconnectivity among organisms within an aquatic system makes the plant's overall influence challenging to assess. Multiple trophic levels can be negatively directly and indirectly affected by a shift in the primary production base of a lake and thereby the ecosystem. Without the complete understanding of an existing system and the effects of invasions by exotic species, it becomes difficult to predict the impacts of the exotics on an ecosystem. This is due to the strong dependence amongst biological communities in an ecosystem (Villamagna and Murphy, 2010).

Eichhornia crassipes and *Azolla filiculoides* (Lam.) have been recorded on the Nseleni River. Successful control of *A. filiculoides* has resulted with the introduction of *Stenopelmus rufinasus* (Hustache). *Eichhornia crassipes* has been successfully brought under control using an integrated approach, which was initiated in 1995 (Jones and Cilliers, 1999; Jones 2009) (see Chapter 6).

Invertebrates: The snail *Tarebia granifera* (Lamarck) has been identified in the Nseleni River. This is a highly invasive invertebrate, but little is known about its distribution and affect on the biodiversity in the system (see Chapter 3 and 5). No control measures have as yet been identified (Appelton *et al*., 2009).

Tarebia granifera has invaded Puerto Rico and Venezuela, where populations reached high densities and has been associated with the disappearance of the indigenous species *Biomphalaria glabrata* (Say, 1818). In Cuba, it has been associated with the disappearance of the indigenous *Pachychilus violaceus* (Preston, 1911) (Pleuroceridae) (Appelton, 2003).

Fish: The locariid catfish, *Pterygoplichthys disjunctivus* (Weber) is endemic to neotropical South America. As with other invasive fish species, it too is common in the tropical fish trade. It has now been recorded in North American freshwaters and Asian inland waters such as the Philippines, Taiwan, Indonesia, and Singapore. Introduction is believed to have been introduced to these waters by aquarium release or by escaping from aquaculture farms (Yalçın-Özdilek, 2007).

Pterygoplichthys disjunctivus has recently invaded the Nseleni River (Jones *et al*., 2013). Little is known about the source, status or potential impact of this invasion (see Chapter 4 and 5). No control measures have as yet been identified. Habitat loss and degradation, flow modification, invasive alien species, over-exploitation and pollution are considered the most important impact factors acting on species extinction and threats to biodiversity (Allan and Flecker, 1993; Naiman and Turner, 2000; Revenga *et al*., 2005; Dudgeon *et al*., 2006; Agostinho *et al*., 2007a In: Júnior *et al.,* 2009). Introduction of alien species has impacted negatively on biodiversity, natural environments, economics, and even human health. Alien species become predators or competitors of native species (Hill and Lodge 1999), spread parasites or pathogens (Torchin *et al.,* 2003, Torchin and Mitchell 2004), cause unexpected hybridization (Gaskin and Schaal 2002; Mallet, 2007), and may ultimately cause a decrease in local biodiversity by eliminating local species (Chapin *et al*., 2000; Dick and Platvoet 2000).

1.4.   Study organisms

This study aims to investigate the Nseleni River, a highly invaded system, focusing on one invasive plant species, one invasive gastropod species and one invasive fish species, all of which are freshwater invaders thought to have negative impacts on ecosystem biodiversity.

1.4.1. *Eichhornia crassipes*

Water hyacinth is a free-floating aquatic plant, native to South America and was first recorded in South Africa (Cape and KwaZulu-Natal) in 1908 (Cilliers, 1991) (Fig 1.5). Water hyacinth became problematic on the Nseleni River in the late 1970's and by the early 1980's the entire system had reached 100% cover. Water hyacinth has infested approximately 21 kilometers of river and 267 hectares of lake. An integrated control plan was initiated in 1995, which has been successful in controlling the infestation (Jones and Cilliers, 1999; Jones, 2009).

Fig. 1.5 Water hyacinth, *Eichhornia crassipes (*Martius*)* Solms-Laubach (Drawn by Rita Weber, SANBI). Scale bar = 10cm.

The integrated control plan consisted of dividing the river system into manageable units and initiating control efforts at the source of the problem and working downstream towards Lake Nsezi. Each management unit was identified by placing a 28mm steel cable across the river, with plastic buoys placed at approximately 1 meter apart (Figure 1.6). Herbicide application was started at the source of the infestation and worked towards Lake Nsezi. This allowed for the introduced biological control agents to settle after introduction, in the management units that were not being treated chemically. During the course of herbicide application, entire mats of water hyacinth were not sprayed, to allow the biological agents to multiply and spread (Jones and Cilliers, 1999).

Fig. 1.6 Cable and buoys across Nseleni River to prevent water hyacinth spread upstream.

The first biological control agent, *Neochetina eichhorniae* (Warner) was introduced in 1995. In addition, the following agents have been released, *Niphograpta albiguttalis* (Warren), *Orthogalumna terrabrantis* (Wallwork), *Eccritotarsus catarinensis* (Carvalho), *Neochetina bruchi* (Hustcache) and the pathogen *Cercospora rodmanii (piaropi)* (Tharp). Two indigenous pathogens, *Acremonium zonatum* (Saw) and *Alternaria eichhornia* (Nag-Raj and Ponnappa) were found to be prevalent on the water hyacinth infestation, without any introductions being necessary.

1.4.2. *Tarebia granifera*

The Asian prosobranch snail *Tarebia granifera* (Figure 1.7) *w*as reported from South Africa (and Africa) for the first time in 1999 in northern KwaZulu-Natal though it was probably introduced sometime prior to 1996 (Appelton *et al*., 2009). *T. granifera* has spread from where it was first identified, to Mpumalanga Province, the Kruger National Park and Swaziland and has colonized different types of habitat, from rivers, lakes and irrigation canals to concrete lined reservoirs and ornamental ponds. It has been recorded at densities of up to 21 000 m^{-2} and would likely have a negative impact on the entire indigenous benthos of the natural waterbodies, at such high densities. Appelton *et al*. (2009) suggest that the indigenous thiarids *Thiara amarula* (Linnaeus)*, Melanoides tuberculata* (Müller), and *Cleopatra ferruginea* (Lea) are considered particularly vulnerable to such densities of invasion. Highest densities, apparently the highest yet measured anywhere, were recorded in the Nseleni River where the snails were on (and in) soft mud near the river's bank (Appelton *et al*., 2009).

The aim of this research is to determine the distribution and possible impact of the snail populations.

Fig. 1.7 Adult *Tarebia granifera* from Lake St Lucia, KwaZulu-Natal, South Africa - shell height 22.0 mm. (Appelton *et al*., 2009).

1.4.3 *Pterygoplichthys disjunctivus*

The 'armoured catfish' (Fig 1.8), which originates in South America, has recently been recorded in the Nseleni River (Jones *et al*., 2013). Loricariidae is the largest family of catfishes (Siluriformes) with about 80 genera and over 700 species. Loricariids are endemic to South America, Panama, and Costa Rica. Loricariids are characterized by having large bony plates and a ventral suctorial mouth with or without noticeable barbels (Armbruster and Page, 2006).

Fig. 1.8   Juvenile *Pterygoplichthys disjunctivus*, Nseleni River – May 2010. (R.Jones)

These fish have a distinctive appearance and a propensity for consuming algae from all submerged surfaces.   They are a popular fish in the aquarium trade, throughout the world.   Escape from aquaculture facilities or releases by aquarists when fish outgrow aquaria have resulted in numerous freshwater invasions where it is thought to compete with indigenous species for space and food (Mendoza Alfaro *et al*., 2009).

1.5.   Research aims

The overall aim of this study was to identify and quantify major invasion threats to the Nseleni River. More specifically, to identify their modes of introduction, the mechanisms driving the invasion, their impact and possible control interventions.

Chapter 2 provides an overview of the Nseleni River system and surrounds, highlighting the aquatic ecosystems in the area. Historically, Richards Bay area was a vast area of wetlands with high biodiversity value, both aquatic and terrestrial. With the expansion of Richards Bay, all of these habitats have come under severe threat. This chapter investigates the possible sources and modes of introduction and mechanisms driving the invasions of exotic invasive alien organisms. Furthermore it considers the ecological services provided by the Enseleni Nature Reserve and attempt to determine if they will be threatened by the invasions.

Chapters 3 assess the occurrence, distribution and potential impacts of the invasive freshwater snail *Tarebia granifera*, in the Nseleni River system. No research has been carried out to determine its abundance, habitat preference and if there is an impact on indigenous invertebrates in the system. Previous research on *Tarebia granifera* was confined to one locality on the Nseleni River, this research will determine its abundance and distribution in the system over time.

Chapter 4 aims to determine the state of the *Pterygoplichthys disjunctivus* invasion on aquatic biodiversity. In addition, this study will also determine if they are breeding successfully. Hereto, the hypothesis is that this invasive alien fish is competing directly with indigenous fish species and isotope studies will assist in testing this hypothesis.

Chapter 5 quantifies the impact of the two invaders using stable isotope analysis. This chapter tests the hypothesis that the invasive snail *Tarebia granifera* and the invasive fish *Pterygoplichthys disjunctivus* are directly competing with indigenous fauna in the Nseleni River system for dietary resources.

Chapter 6 considers the management strategies for *Eichhornia crassipes* over many years, from its initiation in 1995 to the current status. Previous research carried out on *Eichhornia crassipes* has not considered the effect that biological control agents are having (if any) on water hyacinth in an open system. The results of an insecticide exclusion experiment to quantify the impact of biological control as a tool will be presented, in an integrated weed management programme. All previous studies have been carried out in laboratory situations.

Chapter 7 provides an interpretation of the previous chapters' findings and makes recommendations on the management / control of the invasive organisms in protected areas throughout South Africa.

CHAPTER 2

SITE DESCRIPTION

2.1   Introduction: Enseleni Nature Reserve

The aim of this chapter is to provide a comprehensive description of the area in which the research was undertaken. This chapter thus provides a reference for subsequent chapters rather repeating the site descriptions for the following four chapters.

Ezemvelo KZN Wildlife is the provincial mandated conservation authority for the KwaZulu-Natal Province of South Africa, in terms of Nature Conservation Ordinance 15 of 1974 and the National Environmental Management: Protected Areas Act, No. 57 of 2003. The Enseleni Nature Reserve is situated at 28° 41'18.9"S and 32° 00'16.2"E (Figure 1.1; Chapter 1) and falls within the uMhlathuze local municipality and uThungulu district municipality. The reserve, situated inland from and north-west of Richards Bay, on the north coast of the province was established on 22 January1948 - Proclamation P4/48. This proclamation was amended on 16 October 1958– Proclamation P72/58. The reserve is approximately 300ha in size and is commonly known as the gateway to Zululand. It is a popular venue for outdoor enthusiasts. It is a well-known area for birding enthusiasts and boasts many rare or unusual species, many of which are aquatic species.

One of the reasons for the proclamation of this nature reserve in KwaZulu-Natal was the formal protection of the biodiversity of the immediate area. At the time of the proclamation of the nature reserve, considerable coastal grasslands / forests were being converted into sugar cane and timber farms, due to the national and international demand for these products.   Prior to proclamation, the area was classified as Crown Land and used by the surrounding farming community as grazing land, which caused significant damage to the vegetation due to over-grazing. The Enseleni Nature Reserve's eastern and northern boundary is the Nseleni River, which flows into Lake Nsezi. Since the proclamation of the nature reserve, there has been an increase in farming, industrial and urban development in the area, which has resulted in an increase of pressure on the biodiversity of the area.   There are numerous rivers, lakes and wetlands in this region of the coast and all experience the same pressure from development.

With the increase in industrial and urban development in the Richards Bay, Empangeni and Nseleni township area, the water in the area is being placed under severe pressure from extraction and nutrient input and this is placing severe pressure on the biodiversity of the area, especially the aquatic biodiversity.

2.1.1   Importance of Enseleni Nature Reserve

The Enseleni Nature Reserve comprises of five distinct habitats, namely: coastal grassland, coastal forest, riverine forest, swamp forest and an aquatic habitat. The most noteworthy terrestrial habitat is the riverine forest, which is possibly one of the rarest types of habitats in the province.   The riverine forest habitat is characterized by large canopies of *Barringtonia racemosa* (L.) Spreng and *Ficus* species. The fauna of the reserve is diverse in nature. Of importance, is the fact that the rare Cape clawless otter (*Aonyx capensis* Schinz, 1821) has been recorded and requires an open aquatic environment, free of invasive aliens, to survive. In addition, fish species that have been recorded in the Nseleni River are: oxeye tarpin (*Megalops cyprinoids* Broussonet,1782), estuarine round-herring (*Gilchristella aestuaria* Gilchrist,1913), bulldog (*Marcusenius macrolepidotus* Peters,1852), straightfin barb (*Barbus paludinosus* Peters,1852), Johnson's topminnow (*Aplocheilichthys johnstoni* Günther,1983) and the southern mouthbrooder (*Pseudocrenilabrus philander* Weber, 1897) (Skelton, 2001). All game occurring within the reserve depend on the Nseleni River, and access to the water, as there are no man-made drinking points.

In addition, there are numerous species of avi-fauna that are reliant on the protected area and Nseleni River system. This has come about due to habitat transformation in the greater Richards Bay area. The South African Red Data Book – Birds (SA National Scientific Programmes Report No.97) lists endangered (five species), vulnerable (seventeen species), rare (forty-two species) and intermediate (thirty-five species) birds of South Africa. These bird species include the blackrumped buttonquail (*Turnix hottentotta* Temminck, 1815) which is listed as endangered. Five species of birds on the vulnerable list namely: bittern (*Botaurus stellaris* Stephens, 1819), Natal/swamp nightjar (*Caprimulgus natalensis* Smith, 1845), ground hornbill (*Bucorvus*

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leadbeateri Lesson, 1830), African broadbill (*Smithornis capensis* Smith, 1840) and the spotted thrush (*Turdus fischeri* Nikolaus, 1982).

Forty-two bird species listed as rare, of which fourteen have been recorded in the region, namely: white pelican (*Pelecanus onocrotalus* Linnaeus, 1758), pinkbacked pelican (*Pelecanus rufescens* Gmelin, 1789), little bittern (*Ixobrychus minutes* Linnaeus, 1766), white stork (*Ardea ciconia* L.), woollynecked stork (*Ciconia episcopus* Boddaert, 1783), openbilled stork (*Anastomus lamelligerus* Bonnaterre, 1791), marabou stork (*Leptoptilos crumeniferus* Lesson, 1831),yellowbilled stork (*Mycteria ibis* Linnaeus, 1766), bat hawk (*Macheiramphus alcinus* Bonaparte, 1850), southern banded snake eagle (*Circaetus fasciolatus* Kaup, 1850), palmnut vulture (*Gypohierax angolensis* Gmelin, 1788), peregrine falcon (*Falco pelegrinoides* Ripley and Watson, 1963), lesser jacana (*Microparra capensis* Smith, 1839) and the pel's fishing owl (*Scotopelia peli* Bonaparte, 1850).

Thirty-five bird species were listed under the intermediate category of which five have been recorded in the region, namely: whitebacked night heron (*Gorsachius leuconotus* Wagler, 1827), greater flamingo (*Phoenicopterus roseus* Pallas, 1811), lesser flamingo (*Phoenicopterus minor* Geoffroy Saint-Hilaire, 1798), cuckoo hawk (*Aviceda cuculoides* Swainson, 1837), African finfoot (*Podica senegalensis* Vieillot, 1817) and mangrove kingfisher (*Halcyon senegaloides* Smith, 1834).

2.1.2   Ecological Services

During 2008, an analysis was made of six protected areas in KwaZulu-Natal, to identify the supply and demand for ecosystem services (Tables 2.1 to 2.3) from these areas (Mander *et al*. 2009). The analysis was based on Ezemvelo KZN Wildlife (EKZNW) expert workshops in each of the protected areas, which scored the levels of ecosystem services supplied and demanded for each protected area. Additional research was undertaken to highlight some of these services with economic values. The analysis showed that a wide range of ecosystem services were supplied by protected areas - influenced both by the size and habitat composition of the protected area. The work also showed that there are a wide range of users of protected areas who may never directly access the area, and are in most cases, ignorant of their dependence on the continued effective

management of the protected area. For example, the Hluhluwe Dam's operational life span is four times the national average, delaying an investment of between R50 and R250 million in Government expenditure for some 150 years, provided the Hluhluwe catchment in Hluhluwe-Imfolozi Park (HIP) continues to be managed effectively by Ezemvelo KwaZulu-Natal Wildlife. The analysis showed that in most cases, there were at the very least tens of thousands of households benefiting from the services supplied by a protected area, while Ukhahlamba-Drakensberg Park (UDP) has millions of benefiting households at the national level due to the water security provided by EKZNW.

The analysis consisted of identification of the ecosystem goods and services supplied by the following protected areas:

Hluhluwe-Imfolozi Park (HIP), Ukhahlamba-Drakensberg Park (UDP), Weenen Nature Reserve, Ithala Game Reserve, Enseleni Nature Reserve and Krantzkloof Nature Reserve. These nature and game reserves were selected by geographical distribution throughout KwaZulu-Natal. The key ecosystem services were identified and agreed upon by a management committee and external consultants prior to visiting the protected areas (Table 2.1).

Table 2.1 The presence or absence of services supplied per Protected Area.

Key Ecosystem Services Supplied	Protected Area					
	Enseleni	**HIP**	**Ithala**	**Krantz kloof**	**UDP**	**Weenen**
Climate management	√	√	√	√	√	√
Carbon sequestration	√	√	√	√	√	√
Seed dispersal	√	√	√	√	√	√
Soil stability	√	√	√	√	√	√
Sediment supply	√	√	√	√	√	√
Soil formation and fertility	√	√	√	√	√	√
Waste assimilation	√	√	√	√	√	√
Waste dilution	√	√	√	√	√	√
Salinity control	√	√	X	X	X	X
Flood attenuation	√	√	√	√	√	√
Disease control	√	√	√	√	√	√
Pest biocontrol	√	√	√	√	√	√
Water supply regulation	√	√	√	√	√	√
Water distribution	√	√	√	√	√	√
Groundwater recharge	√	√	√	√	√	√
Wind damage control	√	√	√	√	X	√
Fire damage control	√	√	√	√	√	√
Coastal storm damage control	X	X	X	X	X	X
Disaster damage control	√	√	√	√	√	√
Pollination provision	√	√	√	√	√	√
Settlement	√	√	√	√	√	√
Conservation	√	√	√	√	√	√
Recreation	√	√	√	√	√	√
Sacred (spiritual, religious)	√	√	√	√	√	√
Mental wellbeing (stress to inspired, wilderness, healing)	√	√	√	√	√	√
Natural heritage	√	√	√	√	√	√
Cultural heritage (archaeological, paleontological, historical, cultural)	√	√	√	√	√	√
Knowledge generation and learning sites	√	√	√	√	√	√
National icon	X	X	X	X	√	X
Water supply	√	√	√	√	√	√
Fibres	√	√	√	√	√	√
Energy	√	√	√	√	√	√
Biochemical and medicinal supply	√	√	√	√	√	√
Wildlife products	√	√	√	√	√	√
Genetic vigour	√	√	√	√	√	√
Live Game	√	√	√	√	√	√
Refugia or nursery for biodiversity	√	√	√	√	√	√
Ornamental plants resources	√	√	√	√	√	√
TOTAL NUMBER OF SERVICES	**36**	**36**	**35**	**35**	**35**	**35**

The dependence on the services supplied within the protected area are not a function of protected area size, but of the conditions in the surrounding or user population (Table 2.2).The dependence score presented in table 2.2 is the sum of two criteria, namely the difficulty of access to alternative services and the investment into accessing the services (relative to household income).

These two criteria combined serve to provide a clear indication of dependence. For example, people who do not have alternatives and those who have invested significantly at the household level to access the services will have a greater level of dependence. In reviewing the comparative list of dependence on services, Enseleni Nature Reserve again stands out as being the smallest supplier, but with a very high level of dependence on several of its services. The highly transformed landscape context of the protected area, makes its services scarce and of high value in the urban context.

Scoring criteria was discussed at length by the panel and it was decided not to use the "High", "Medium" or "Low" criteria scoring method, as it was felt that a broader type scoring criteria was required i.e. 1 to 10, due to the amount of services provided and more importantly because it was felt that the high, medium or low scoring criteria were too limited.   For example, Enseleni Nature Reserve scored a 9 for pollination provision, and the only reason that it did not score a 10 is because possibly Ithala and UDP had more species diversity.

Table 2.2 Comparison of the dependence levels of users on services supplied per Protected Area. Colour coding: green = highest score; yellow = moderately high score; orange = moderately low score and red = lowest score.

Key Ecosystem Services Supplied	Protected Area					
	Enseleni	HIP	Ithala	Krantzkloof	UDP	Weenen
Climate management	8	8	-	9	8	-
Carbon sequestration	7	7	7	7	7	7
Seed dispersal	9	8	9	5	5	6
Soil stability	10	10	8	9	10	9
Sediment supply	10	9	-	-	7	-
Soil formation and fertility	8	9	-	-	8	-
Waste assimilation	10	9	9	9	8	8
Waste dilution	9	9	10	8	-	-
Salinity control	5	7	-	-	-	-
Flood attenuation	8	9	9	7	8	9
Disease control	6	8	9	7	9	9
Pest biocontrol	5	9	-	8	6	9
Water supply regulation	10	10	9	9	10	7
Water distribution	6	10	9	10	10	10
Groundwater recharge	10	10	10	-	9	-
Wind damage control	8	7	7	8	-	-
Fire damage control	9	8	-	9	8	7
Coastal storm damage control	-	-	10	-	-	-
Disaster damage control	6	9	-	8	10	8
Pollination provision	9	7	10	7	10	6
Settlement	9	7	-	5	10	8
Conservation	10	10	-	8	10	7
Recreation	5	9	7	5	10	6
Sacred (spiritual, religious)	3	10	10	-	10	8
Mental wellbeing (stress to inspired, wilderness, healing)	5	7	10	5	8	6
Natural heritage	10	10	9	10	10	7
Cultural heritage (archaeological, paleontological, historical, cultural)	4	10	10	-	10	5
Knowledge generation and learning sites	6	10	10	7	10	3

National icon	-	-	9	-	6	-
Water supply	7	10	9	10	8	-
Fibres	8	7	10	8	5	8
Energy	-	-	-	-	-	-
Biochemical and medicinal supply	-	-	-	-	5	-
Wildlife products	4	7	9	-	6	8
Genetic vigour	7	10	10	7	10	7
Live Game	-	10	10	-	10	7
Refugia or nursery for biodiversity	7	8	10	7	7	9
Ornamental plants resources	-	3	-	2	8	10
TOTAL DEPENDENCE	**237**	**288**	**229**	**191**	**275**	**191**

The service worth highlighting for the Enseleni Nature Reserve is the flood attenuation service of the riverine forests, are important for the users or commuters who use the National Road N2 – as any damage to the Nseleni River bridge will have significant costs to N2 users. The riverine forests play a key role in protecting the bridge from floods. However, the dependence is only an 8 as there is an alternative road which can be used. There are also several services linked to water that are all relatively important. Soil stability, ground water recharge, water supply regulation (and storage), waste assimilation and waste dilution are all linked to both the Nseleni River and Lake Nsezi – as key water supply assets in the uMhlathuze Municipality. Sediment supply is also important for sustaining the river bank vegetation. Thus, the whole municipality (approximately 600,000 people) benefits significantly from the protected area's ecology in enhancing water security and by saving on the costs of alternative infrastructure. However, the waste dilution services and sediment supply services of the protected area is small, but still important, but, ironically, also driving much of the invasion in the river.

At the local level there are some 650 farming people who benefit from services such as fire damage control, seed dispersal, soil formation, local climate management and biodiversity refuge. These local farming community therefore benefit significantly from the protected area. The local neighbouring community also benefits from services such as access to fibre, pollination, wind damage control, but in small numbers.

In reviewing the comparative list of dependence on services, Enseleni again stands out as being the smallest supplier, but with a very high level of dependence on several of its services. The highly transformed landscape context of the protected area, makes its services scarce and of high value in the urban context.

2.1.3 Rivers and Lakes

The City of uMhlathuze relies on two rivers to supply water, the Mhlathuze River (indirectly) and the Nseleni River (Figures 2.1 and 2.2). Lake Nsezi is fed directly from the Nseleni River, while water is transferred via a weir on the Mhlathuze River to Lake Nsezi, to supplement water that is extracted from the lake for industrial and domestic use. Lake Mzingazi does not have any large rivers that feed it, but relies on a couple of small streams and precipitation.
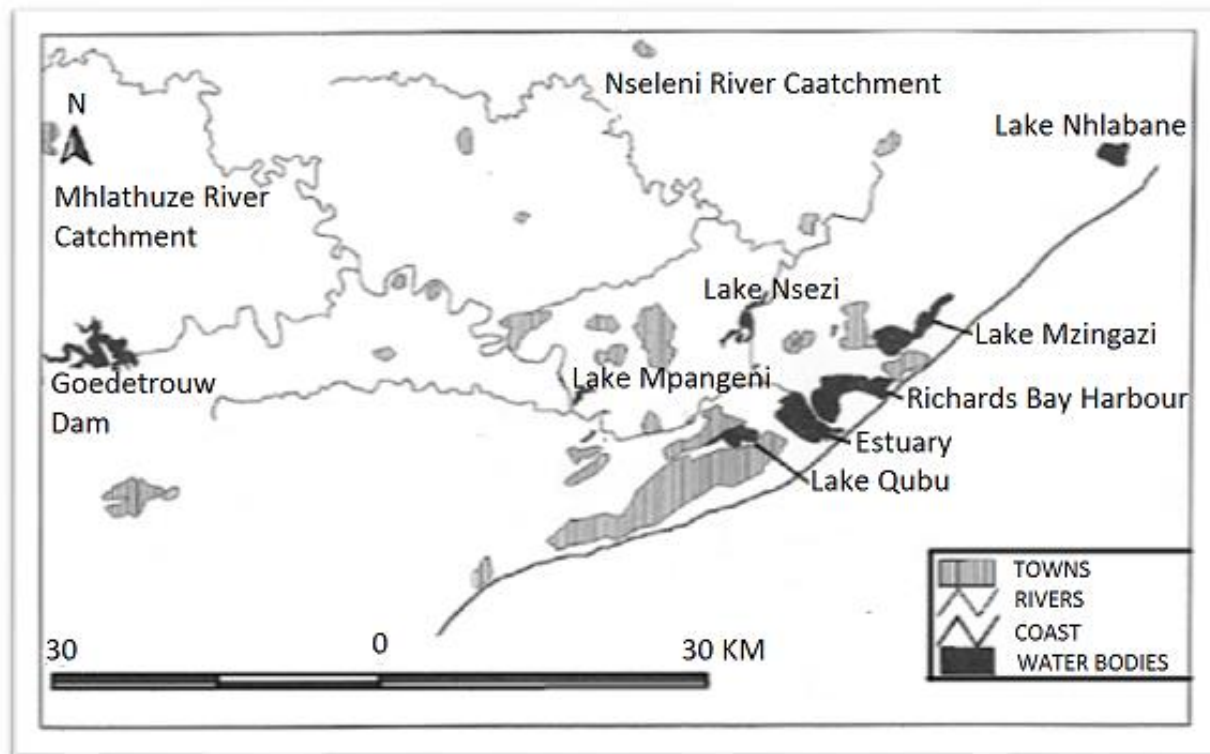
Fig. 2.1 Mhlathuze River catchment (Adapted from Water Research Commission Report No.720/1/01).
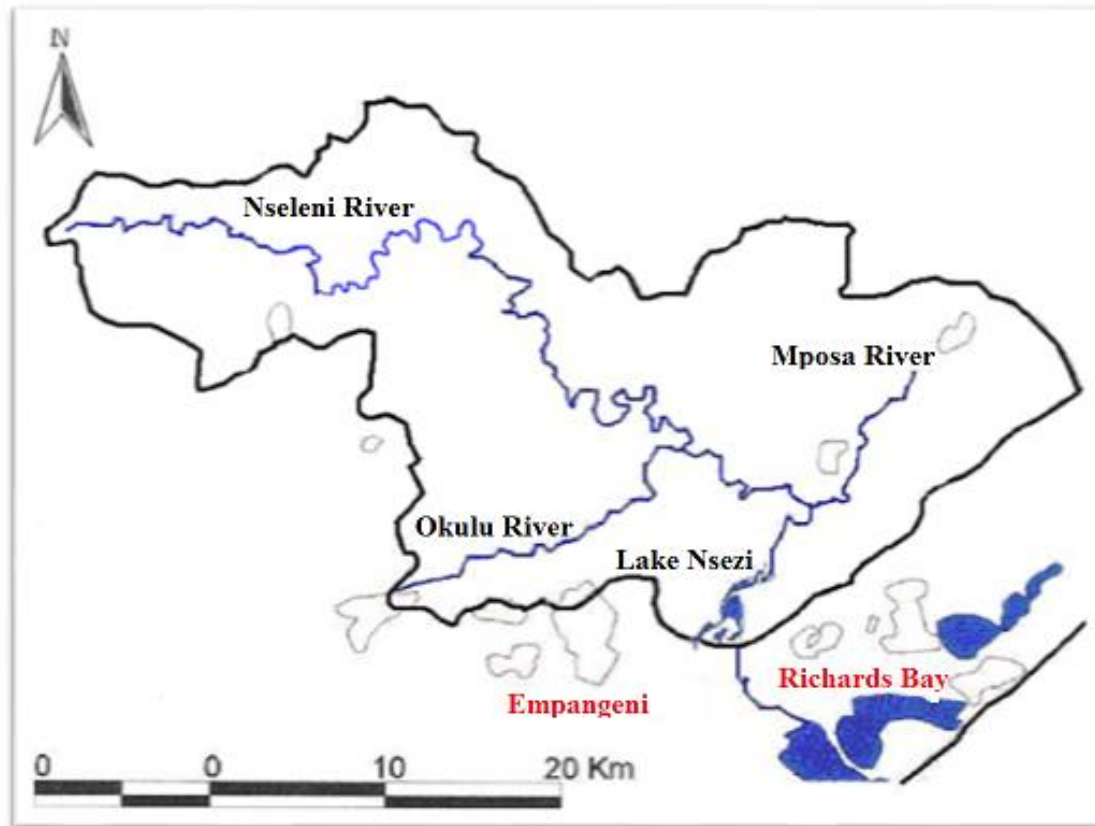
Fig. 2.2 Nseleni River catchment (Adapted from Water Research Commission Report No.720/1/01).

2.1.4 Nseleni River Catchment

The Nseleni River has a relatively small catchment, compared to that of the Mhlathuze River, which is fed from the Goedetrouw Dam. The Nseleni River has Two small rivers fall within the Nseleni River catchment, namely the Mposa River that reaches as far as Kwambonambi (this catchment covers a large area of rural land and private timber and sugar cane farms with a high nutrient run-off) and the Okulu River (this catchment covers the area to the west of the N2 Highway and Enseleni Nature Reserve and comprises mainly of sugar cane farms with a high nutrient run-off) (Figure 2.2).

The catchment of Lake Mzingazi is even smaller than that of Lake Nsezi and comprises of a couple of small streams that rely on precipitation to feed them. Commercial forestry and informal settlements surround the streams.

Lake Nsezi and Lake Mzingazi are presently unable to cope with the demand from industrial and domestic use in the area, due to the low natural flow of water into these two lakes. Additional water is transferred to Lake Nsezi, from a weir on the Mhlathuze River, which is controlled by the Mhlathuze Water Board situated in Richards Bay. Additional water requirement for the Mhlathuze Water Board (Lake Nsezi) is provided by the Goedetrouw Dam, which is situated on the Mhlathuze River in the vicinity of Eshowe. This, therefore, makes Lake Nsezi and the Nseleni River unique in the area in as much as the Nseleni River's natural flow is from west to east, but during times of water transfer from the Mhlathuze River into Lake Nsezi, water pushes back upstream i.e. east to west. The water transfer is carried out by the Mhlathuze Water Board, in an attempt to have a constant level of water at the Mhlathuze Water Board extraction point on Lake Nsezi. This two directional water flow has both negative and positive implications; negative influences include water hyacinth re-infestation in previously cleared areas, and the facilitation of the spread of the spiral snail *Tarebia granifera*, which uses water hyacinth as a mode of transport, by adhering to the root system (pers. obs. R.Jones). From a positive aspect, water hyacinth biocontrol agents are assisted in their dispersal by mats of water hyacinth moving up and down stream as the construction of the weir means that the river does not have a significant flow.

2.1.5 Water consumption in the area

Potable water in the area is under severe threat from development in the area. There are two sources of water for extraction for industrial and domestic use, for the City of uMhlathuze, namely Lake Nsezi and Lake Mzingazi. There are in addition, extraction points along the Nseleni River, that supplies Richards Bay Minerals, the rural township of Nseleni and private agricultural farms (Figure 2.3). Table 2.3 presents the of water requirements for the City of uMhlathuze, with special reference to the transfer of water from the Mhlathuze River weir into Lake Nsezi, as the lake is unable to cope with demand from natural inflow.
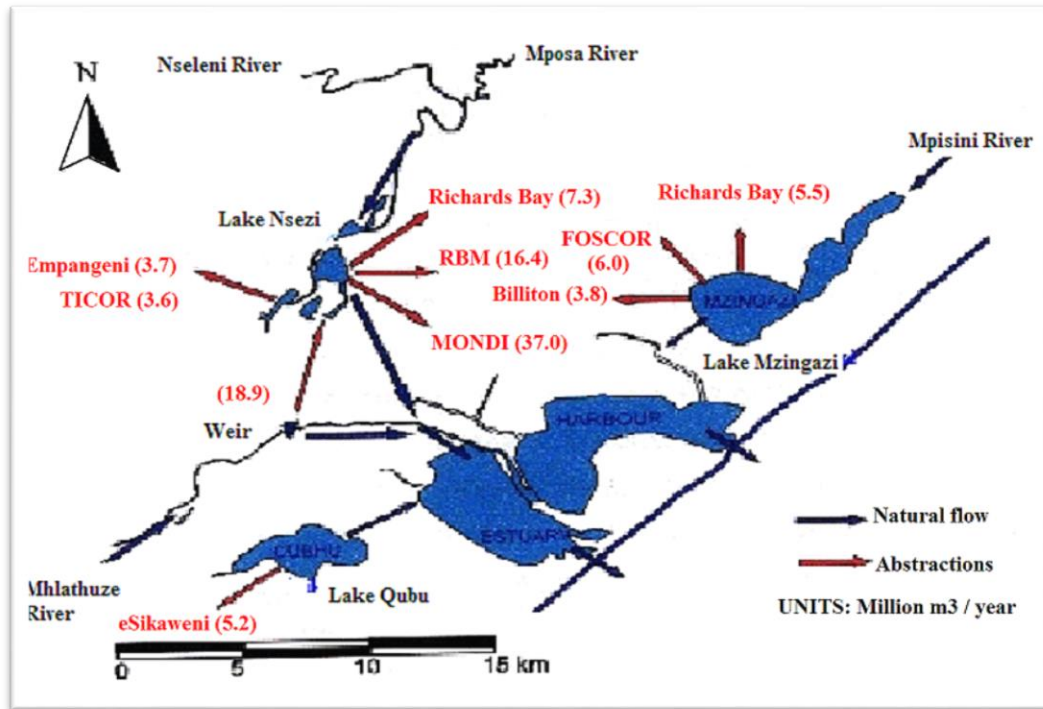
Fig. 2.3 Water extractions (million m³) from various sources (2001) – City of uMhlathuze (Adapted from Water Research Commission Report No.720/1/01). Figures reflected in black boxed brackets are 2010 data.

Table 2.3 Comparison of water extraction and transfer – City of uMhlathuze for 2001 and 2007 (Mander *et al*., 2009) and 2010 data were obtained verbally from Mhlathuze Water's Chief Operating Officer – S.G.Xulu.

Source	Authority	2001 Million m³/year	2007 Million m³/year	2010 Million m³/year
Lake Qubu	Richards Bay Municipality	5.2	9.4	11.9
Lake Nsezi	Mhlathuze Water Board	51.6	46.4	45.3
Nseleni River (*RBM)	Mhlathuze Water Board	16.4	1.2	8.4
Mhlathuze River Weir	Mhlathuze Water Board	18.9	24.0	62.5
Lake Mzingazi	Richards Bay Municipality	15.3	19.0	17.9
Lake Mzingazi	BHP Billiton (Bayside)	1.2	0.81	3.2

Although the rainfall for the Nseleni River catchment is not known (Richards Bay receives on average 1 200mm of rainfall / annum), the natural inflow to Lake Nsezi is insufficient for the demand, as can be observed by the fact that the total extraction from the Nseleni River at the Richards Bay Minerals and Mhlathuze Water Board extraction points for 2007 was 40.1 million m³, whereas only 24.0 million m³ was transferred from the Mhlathuze River weir to Lake Nsezi. This equates to 16.9 million m³ being extracted during 2007 or 1 408 million m³ / month, from the Nseleni river.

The Mhlathuze Water Board has recently reconfigured their water extraction and delivery piping in and around Richards Bay. The Empangeni water extraction plant has been closed down and now receives water directly from the Lake Nsezi pump-house, via the Mhlathuze weir transfer scheme. A higher volume of water is now extracted from the Mhlathuze weir pump for Richards Bay / Empangeni domestic use, as it has been found to be of a higher quality of water in Lake Nsezi, possibility due to the Nseleni sewerage works, rural pollution, agricultural and forestry practices along the Nseleni and Mposa Rivers.

The augmentation of water into Lake Nsezi from the Mhlathuze River weir (Figure 2.4) commenced in October 1992, as the Mhlathuze Water Board maintain Lake Nsezi level at 6.2m AMSL. This is carried out to ensure sufficient water for industrial and domestic use. The transfer of water, however, continues even when the lake is overflowing in order to dilute the very high dissolved solids concentration in the lake (V. Botes, CEO – Mhlathuze Water, pers comm.). It is estimated that the lake capacity is 834 000 m³.
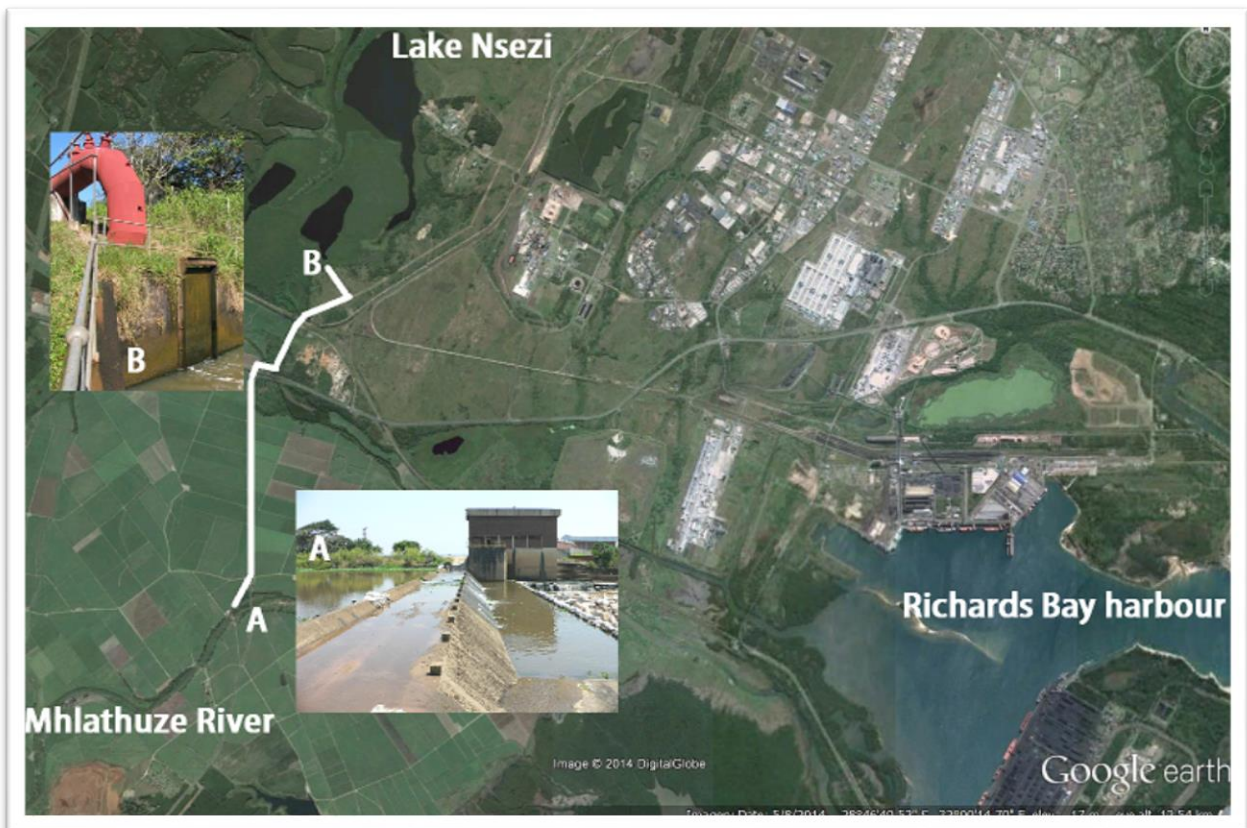
Fig. 2.4   Water transfer scheme between the Mhlathuze River and Lake Nsezi. (A – extraction point on the Mhlathuze River; B – inflow point on Lake Nsezi and C – Mhlathuze Water Board).

2.1.6 Climate

The climatological information is the normal values and, according to World Meteorological Organization (WMO) prescripts, based on monthly averages for the 30-year period 1961 – 1990 (Table 2.4).

Table 2.4 Climate for Richards Bay (www.richemp.org).

Month	Temperature (° C)				Precipitation		
	Highest Recorded	Average Daily Maximum	Average Daily Minimum	Lowest Recorded	Average Monthly (mm)	Average Number of days with >= 1mm	Highest 24 Hour Rainfall (mm)
January	41	29	21	11	172	12	317
February	39	29	21	13	167	12	145
March	39	29	20	14	107	10	253
April	37	27	18	8	109	8	130
May	35	25	15	7	109	7	88
June	35	23	12	6	57	6	82
July	31	23	12	4	60	6	135
August	37	24	14	5	65	7	62
September	40	25	16	6	77	9	65
October	42	25	17	10	105	12	99
November	43	27	19	11	114	13	135
December	42	29	20	13	86	11	78
Year	**43**	**26**	**17**	**4**	**1228**	**113**	**317**

2.1.7 Human settlement

The greater Richards Bay/Empangeni area (City of uMhlathuze) has experienced a significant loss of biodiversity and an increase in invasive alien organisms, since the area was identified by colonists as a suitable area for development mainly during the period of the Anglo – Zulu war (around 1900) and thereafter by industrialists (Chapter 1). The need for a harbour during the late 1960's has led to the degradation of the coastal area through development. Much of the area has been converted from pristine coastal vegetation to land that is now dysfunctional or irreversibly impaired due to sugarcane and forestry plantations, urban and rural development and a high demand for water (Figures 2.5 and 2.6).
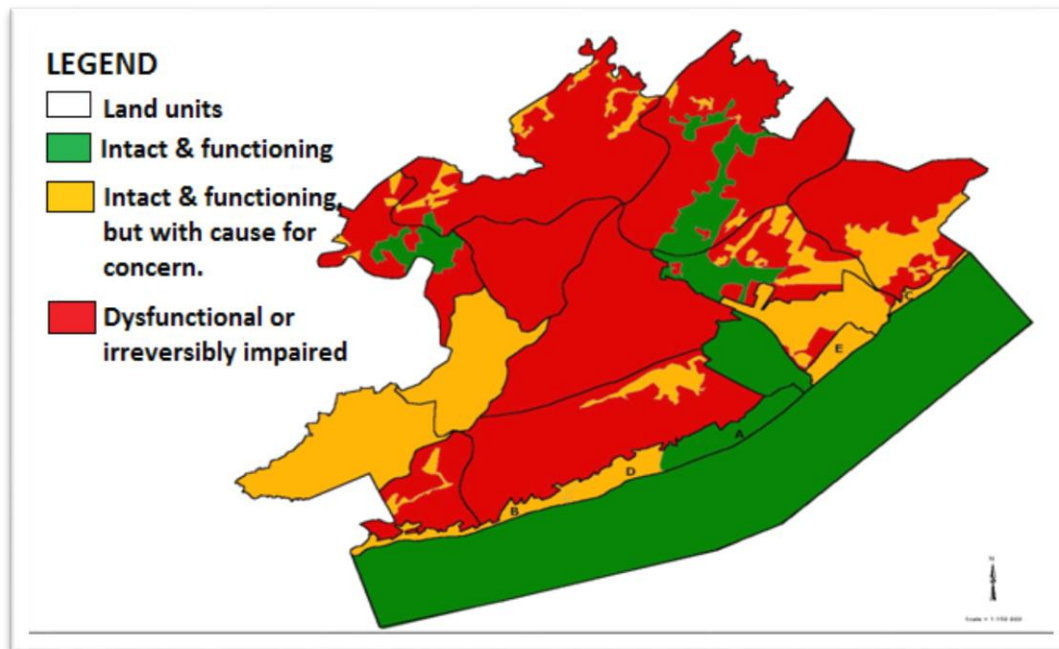
Fig. 2.5 The state of biodiversity within the uMhlathuze Municipal area (Mander *et al.*, 2003), of which a high percentage of the intact and functioning well category, is the Indian Ocean (green shading along coastline) and only a small percentage being terrestrial.
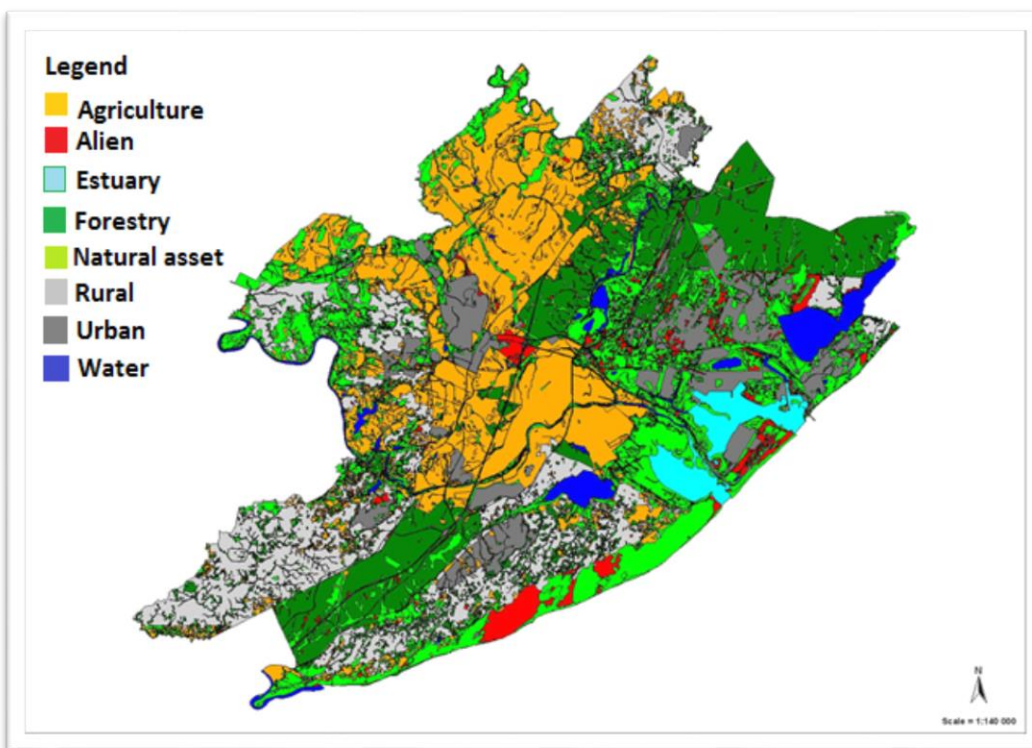
Fig. 2.6 Various landuse within uMhlathuze Municipality, indicating the high degree of land transformation.

The City of uMhlathuze covers an area of 7 959 km² and has an estimated 345 776 total population and about 81 008 households. It is characterized by highly developed urban areas surrounded by a poor and undeveloped hinterland, with a substantial number of the total population considered rural residents. The unemployment level in the area is 37.3% (uMhlathuze Municipality Integrated Development Plan, 2014/2015). This is a further indication on how biodiversity of the area has been negatively influenced historically.

2.2. Materials and methods

2.2.1 Study sites on the Nseleni River

All research for this project was carried out on the Nseleni and Mposa rivers (Figure 2.7). In total nine sites were chosen on the river system, three sites on the 'upper' Nseleni River (sites N1, N2 and N3) with another three on the 'upper' Mposa River (sites M1, M2 and M3). Site M1 is located north of the eNseleni township sewerage works, while the remaining two were south of it. The last three sites (NM1, NM2 and NM3) were situated south of the N2 road bridge downstream of the confluence of the Nseleni and Mposa rivers. The nine sites cover a distance of 11.5 kilometres. Each site was divided into three sub-sites, against the bank, the middle of the river and one sub-site in between (Figure 2.8).
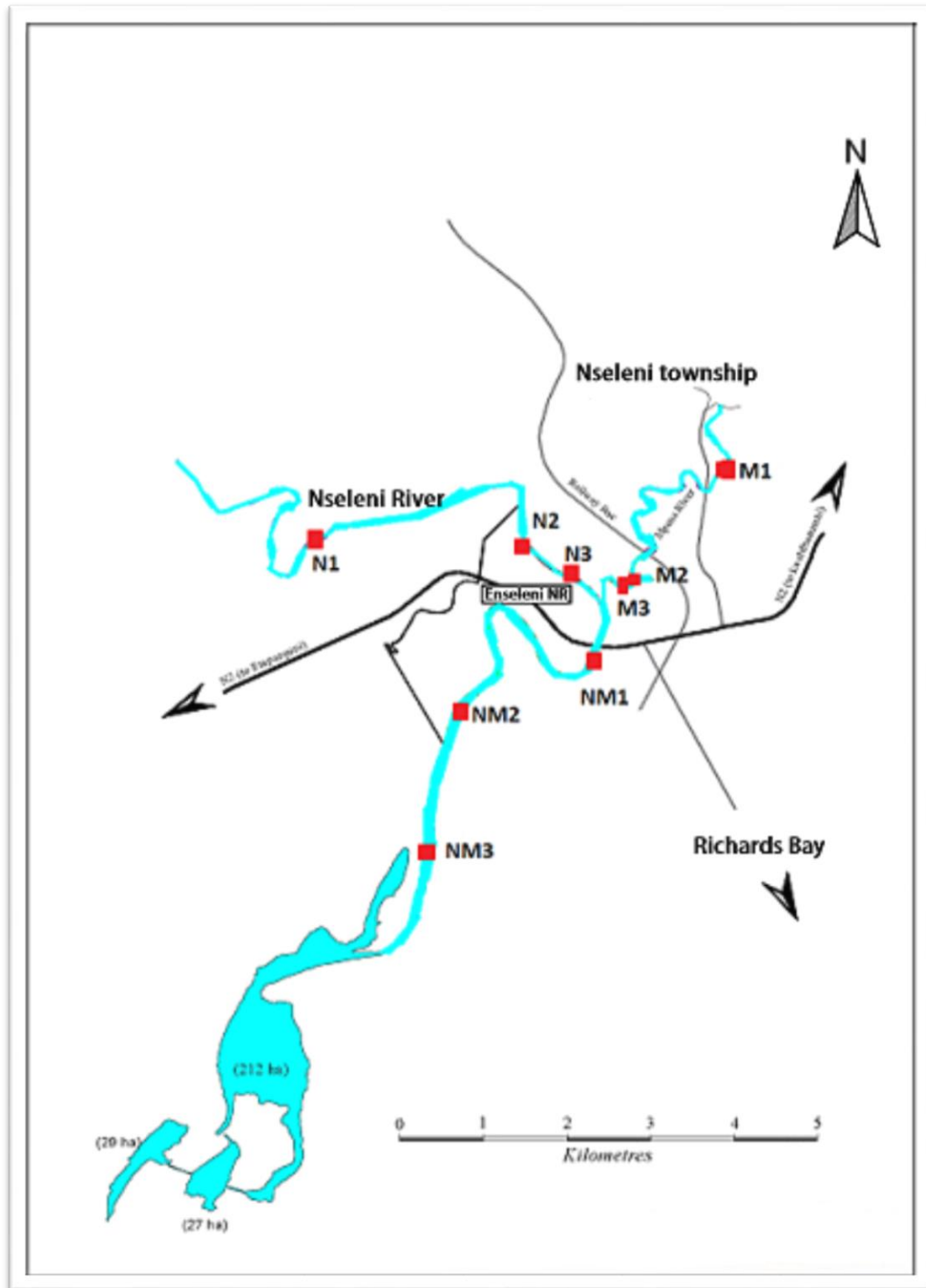
Fig. 2.7 Sampling sites on the Nseleni and Mposa Rivers. Refer Table 2.5 for co-ordinates. The natural flow of the river is from north to south, however, the reverse is applicable when the lake level is maintained via the water transfer scheme between the lake and the Mhlathuze River.

2.2.2 Site Descriptions

The study sites varied with respect to the riparian habitat (Table 2.5)

Table 2.5 Location and description of study sites.

Site	GPS location	Site description	Surrounding habitat
N1	28° 40' 51.43"S 31° 59' 33.88"E	Open water, with occasional water hyacinth mats.	Sparse riverine forest on both river banks.
N2	28° 41' 07.78"S 32° 00' 22.05"E	Open water, with occasional water hyacinth mats.	Sparse riverine forest on northern bank and dense riverine forest on southern bank.
N3	28° 41' 18.44"S 32° 00' 38.17"E	Open water, with occasional water hyacinth mats.	Sparse riverine forest on northern bank and dense riverine forest on southern bank.
M1	28° 40' 41.45"S 32° 01' 19.71"E	100% aquatic vegetation. Both alien and indigenous, floating and submerged.	Sparse riverine vegetation on either bank.
M2	28° 41' 22.05"S 32° 01' 00.52"E	Frequent water hyacinth mats and 90% submerged weeds.	*Typha capensis* beds with rural agriculture on northern bank and southern bank consists of *T. capensis* and reed beds.
M3	28° 41' 19.37"S 32° 00' 57.44"E	Frequent water hyacinth mats and 90% submerged weeds.	*Typha capensis* beds with rural agriculture on northern bank and southern bank consists of *T. capensis* and reed beds.
NM1	28° 41' 47.86"S 32° 00' 47.76"E	Open water, with occasional water hyacinth mats.	Modified due to the construction of the N2 road. Eastern bank has sparse riverine forest vegetation, whereas west bank has dense riverine forest.
NM2	28° 42' 02.47"S 32° 00' 04.72"E	Open water, with occasional water hyacinth mats.	Both banks have dense stand of riverine forest.
NM3	28° 42' 57.81"S 31° 59' 35.47"E	Open water, with occasional water hyacinth mats.	Both banks have dense stand of riverine forest.

2.2.3 Physicochemical data

2.2.3.1 Depth

Depth measurements were obtained for each site, at three distances from the river bank, at the bank (shallow), in the middle of the river (deep) and at a locality half way between the river bank and the middle of the river (mid). Depths per site were measured using a weighted nylon line and then measured with a tape measure.

2.2.3.2 Water chemistry

Physiochemical data were collected on a quarterly basis between 1 February 2011 and 30 January 2012 at each depth for all of the nine research sites. A Hanna H1 9828 multiparameter probe was used to collect total dissolved solids (TDS), conductivity, dissolved oxygen (DO), salinity and pH and temperature.

Water chemistry parameters were analysed within sites over time and between sites using a factoral analysis of variance using the software STATISTICA v10. All plots are the least squares means and standard errors. The error bars are all the same because this is similar to making predictions from the ANOVA model which assumes homogeneity of variance for the Nseleni and Mposa rivers.

2.2.4 Water nutrient analysis

At each site, 1 litre water samples were also collected in sterilized bottles at a depth of 1 meter below the surface and sent to Integral Laboratories, Empangeni, KwaZulu-Natal to obtain nitrate and orthophosphate concentrations for each site.

Results supplied by the laboratory (Integral Laboratories (Pty) Ltd.) are in mg/l. Nitrate values <0.14, are indicated as 0.14 and orthophosphate values <0.10, are indicated as 0.1.

2.3 Results
2.3.1 Depth

All research site depths were measured, with depths varying from 350mm to 2780mm (shallow); 550mm to 3330mm (middle) and 1600mm to 4310mm (deep). Of interest are the depths recorded at research sites at N2 and NM2, where the shallowest depth is greater than the deepest depth for sites M1, M2 and M3. The high shallow readings for N2 and NM2 are an indication of a sudden "drop-off" at the river's edge, which has possibly been caused by scouring in times of flood. M1, M2 and M3 overall shallow depths are indicative of a section of river that does not receive much scouring during periods of high rainfall (Figure 2.8).
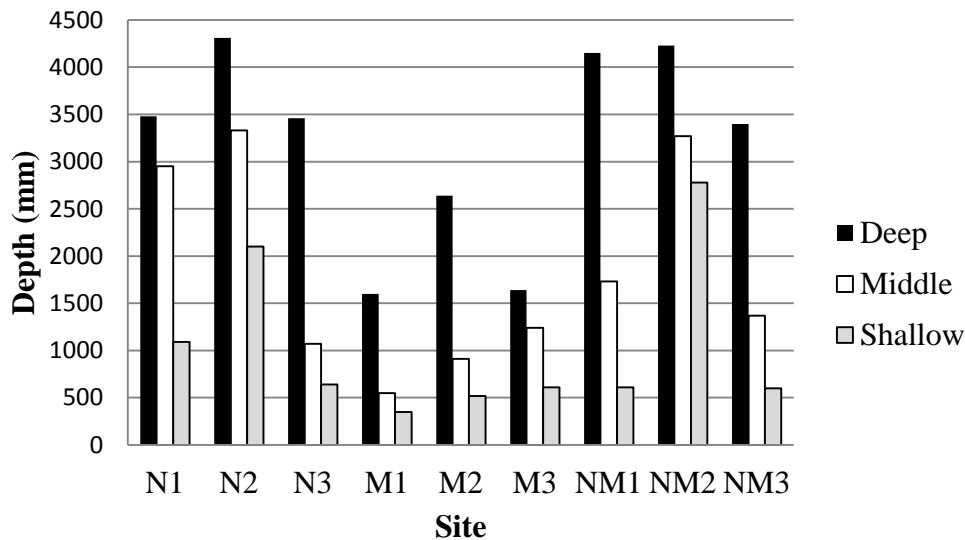
Fig. 2.8 Water depth at each of the research sites in the Nseleni (N), Mposa (M) and Nseleni/Mposa (NM) Rivers.

The Nsezi Lake (Figure 2.9) is situated at the southern extreme of the Nseleni River. It has become silted up over the years, due to poor farming practices inland, and presently only has a maximum depth of 2.5 meters. This has caused what used to be a single large lake to become fragmented. Lake Nsezi now consists of four "splintered" lakes due to aquatic vegetation (*Typha capensis* and *Eichinochloa pyramidalis*) that has taken advantage of the siltation and encroached into the former large lake area. The two extensive floods of the 1980's, (1984 and 1987 in particular), deposited a high volume of siltation along the entire river and lake system.
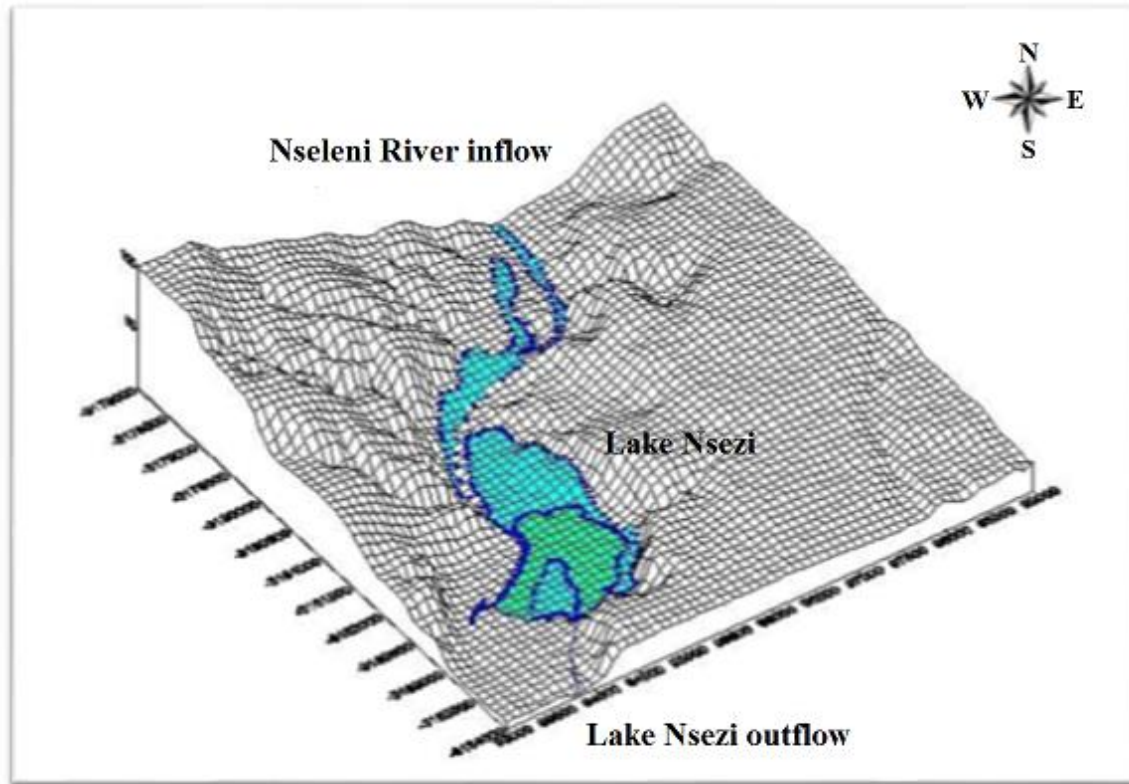
Fig. 2.9 3D Profile of Lake Nsezi. The green shaded area indicates a large papyrus swamp area. Note the small outlet, due to the man-made barrier in the south-east area of the lake (Adapted from Water Research Commission Report No.720/1/01).

2.3.2 Water Chemistry

2.3.2.1 Mposa River

Total dissolved solid results indicated no significant difference between sites over time, ($F_{(6, 24)}$=1.8135, p = 0.13885) (Figure 2.10a). Conductivity differed significantly between sites over time, ($F_{(6, 24)}$=5.6554, p=0.00089) (Figure 2.10b), where conductivity results were lower in the summer months, especially January 2012, compared to the winter months. Dissolved oxygen showed a significant difference between sites over time, ($F_{(6, 24)}$=60.645, p=0.000001) (Figure 2.10c). The cause of the high readings during the February 2011 data collection at site M2 and M3 are unknown, but could have been affected by the sewage outlet above these sites. Salinity results indicated a significant difference between sites over time, ($F_{(6, 24)}$=11.933, p=0.000001) (Figure 2.10d). The lowest readings were recorded in January 2012, compared to the previous results. There was no significant differences in pH between sites over time, ($F_{(6, 24)}$=2.3922, p=0.05918) (Figure 2.10e). Temperature results indicate a significant difference over time, with

significantly lower temperatures being recorded in the June sample ($F_{(6, 24)}$=8.1417, p=0.00007) (Figure 2.10f).
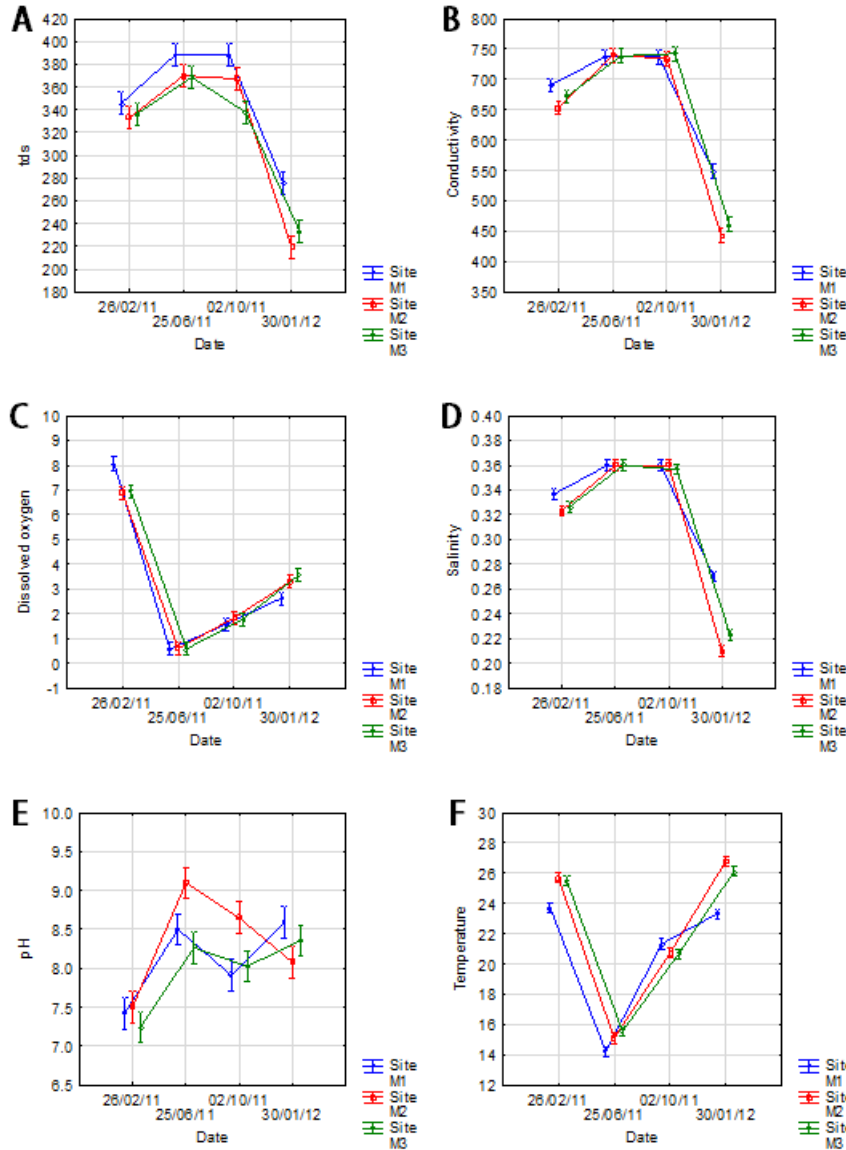
Fig. 2.10 Water chemistry results obtained between 26.02.2011 and 30.01.2012 (A) Total dissolved solids; (B) Conductivity; (C) Dissolved oxygen; (D) Salinity; (E) pH and (F) Temperature for the three sites on the Mposa River.

2.3.2.2 Nseleni River

The trends for the Nseleni River were similar to the Mposa River, where total dissolved solids differed significantly over time, ($F_{(6, 24)}$=0.3131, p=0.28944) (Figure 2.11a), conductivity showed no significant difference over time, ($F_{(6, 24)}$=.74530, p=0.61886) (Figure 2.11b) as did dissolved oxygen ($F_{(6, 24)}$=2.9079, p=0.02824) (Figure 2.11c), and salinity ($F_{(6, 24)}$=66963,

p=0.67499) (Figure 2.11d). pH results indicate a significant difference over time ($F_{(6, 24)}$=3.7609, p=0.00883), and higher pH readings were recorded in the winter months (Figure 2.11e). Temperature results indicate no significant difference over time ($F_{(6, 24)}$=.84394, p=0.54878) (Figure 2.11f).
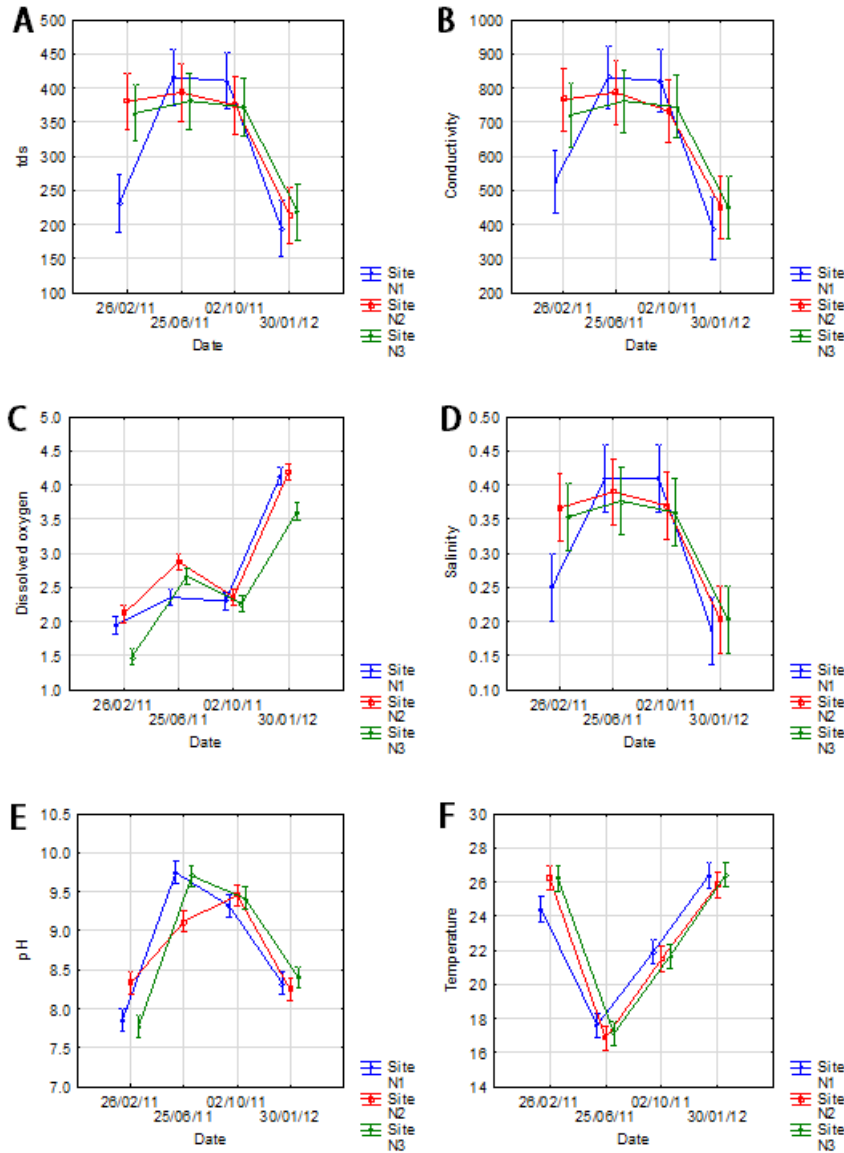
Fig. 2.11 Water chemistry results obtained between 26.02.2011 and 30.01.2012 (A) Total dissolved solids; (B) Conductivity; (C) Dissolved oxygen; (D) Salinity; (E) pH and (F) Temperature for the three sites on the Nseleni River.

2.3.2.3 Nseleni/Mposa River

For most downstream sites, the trends were similar to the Nseleni and Mposa sites. Total dissolved solids differed significantly ($F_{(6, 24)}$=3.4367, p=0.01361) (Figure 2.12a), as did conductivity ($F_{(6, 24)}$=17.624, p=0.00001) (Figure 2.12b), dissolved oxygen ($F_{(6, 24)}$=5.9286, p=0.00066) (Figure 2.12c), salinity ($F_{(6, 24)}$=27.284, p=0.00005) (Figure 2.12d) pH ($F_{(6, 24)}$=2.6418, p=0.04124) (Figure 2.12e) and temperature ($F_{(6, 24)}$=3.5075, p=0.01237) (Figure 2.12f).
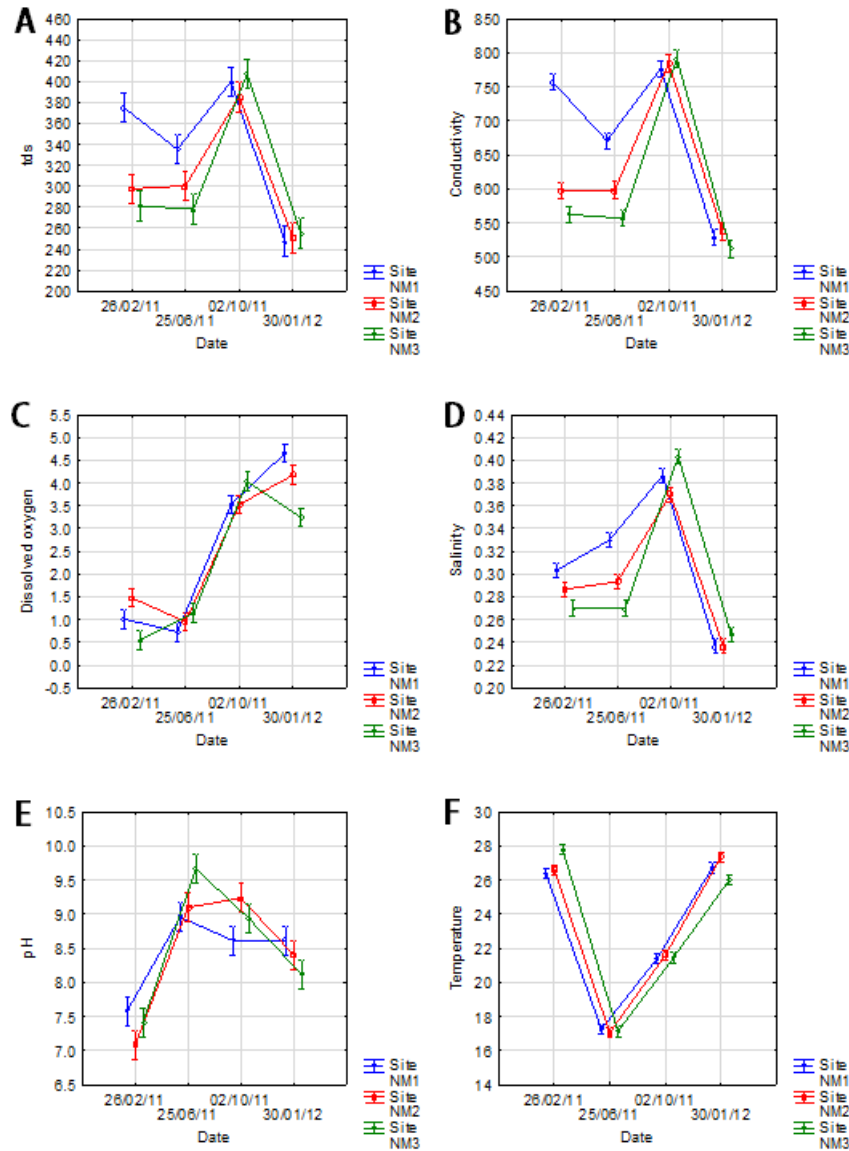
Fig. 2.12 Water chemistry results obtained between 26.02.2011 and 30.01.2012 (A) Total dissolved solids; (B) Conductivity; (C) Dissolved oxygen; (D) Salinity; (E) pH and (F) Temperature for the three sites on the Nseleni/Mposa River.

Water analysis was also carried out at the effluent outflow site on the Mposa River, in shallow water (Site 4 in Table 2.6) and compared to the other Mposa River shallow research sites for the same date. Of interest, is the fact that research site M1's bank vegetation differs from those of research sites M2 and M3. The river bank vegetation at research site M1 is very similar to that at all of the N and NM research sites, namely wooded riverine forest with either sugarcane and/or forestry plantations adjoining the riverine forest.   At sites M2 and M3 the immediate area comprises of swamp area, comprising mainly of *Typha capensis*. The results at the effluent outflow site (Site 4) are vastly different to the other shallow sites, excluding the temperature, within the Mposa River on the same date (Table 2.6).

Table 2.6 The differences in water chemistry between site M4 (sewerage outlet) and the other research sites above sewerage outlet (M1) and those sites below the sewerage outlet (M2 and M3), for the same date and carried out in the shallow section of the research site.

Research Site	TDS µS/cm	Conductivity µS/cm	DO mg/l	Salinity mg/l	pH	Temperature ^{o}C
M1	277	550	2.73	0.27	8.45	24.6
M2	220	442	3.28	0.21	8.05	27.6
M3	246	478	3.5	0.24	8.2	26.7
M4	**182**	**363**	**0.73**	**0.17**	**9.02**	**24.03**

2.3.3 Water Nutrient Analysis

2.3.3.1 Mposa River

Higher recordings for orthophosphate were recorded at sites below the sewerage outlet (M2 and M3) in July, October 2011 and June 2012 (M2), whereas higher recordings for nitrate were recorded above the sewerage outlet (M1) in July 2011 and June 2012 (Figure 2.13).
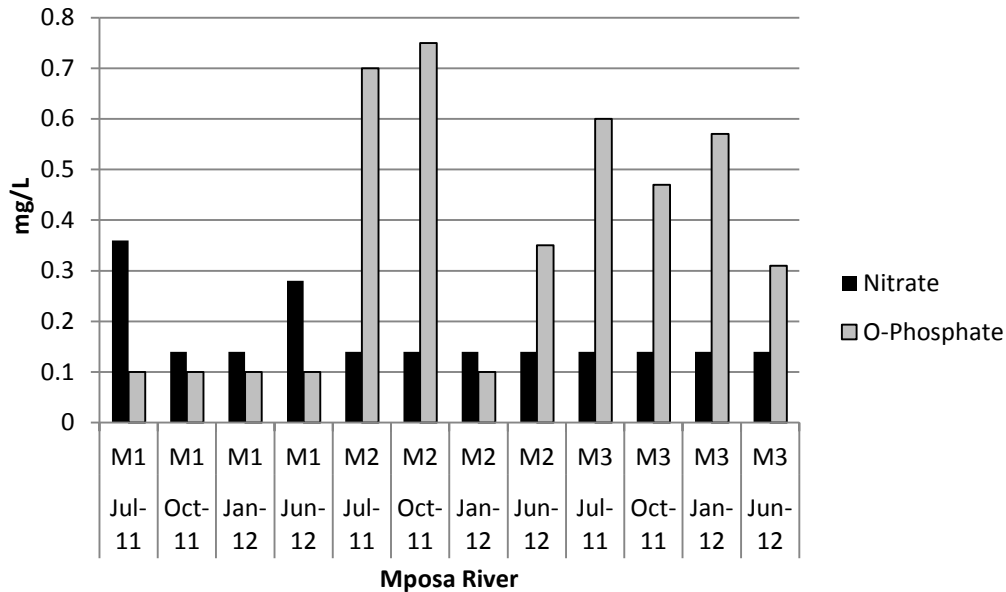
Fig.2.13 Nitrate and orthophosphate recordings for Mposa River.

2.3.2.2 Nseleni River

The Nseleni River had generally stable readings of both nitrate and orthophosphate, except for January 2012 (Site N1: nitrate = 0.53 mg/l), January 2012 (Site N2: nitrate = 0.50 mg/l) and January 2012 (Site N3: orthophosphate = 0.41). These high figures are related to high rainfall (226mm) and run-off from neighbouring sugarcane farms adjacent to the upper Nseleni River (Figure 2.14). Not surprising was the fact that orthophosphate recordings were higher in the Mposa River, below the sewerage works, compared to those in the Nseleni River (Figure 2.13). The only higher recording of orthophosphate in the Nseleni River was a site N3, being close to the confluence of the Mposa River. The reason for this single higher recording at Site N3 is that it could have coincided with Mhlathuze Water Board pumping water into Lake Nsezi i.e. water would have been pushed upstream from the lake. Nitrate recordings were generally stable between these two sites, except for the Nseleni River sites at N1 and N2 in January 2012 (Figure 2.14). These higher recordings coincided with rainfall and adjacent sugar cane farmers having placed fertilizers on their sugar cane fields.
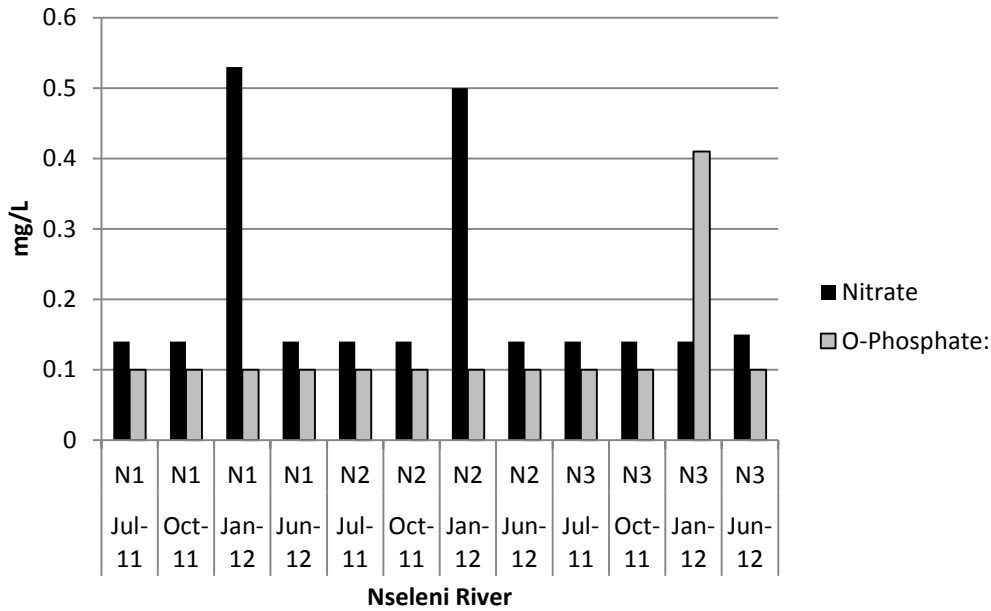
Fig. 2.14 Nitrate and orthophosphate recordings for Nseleni River.

2.3.3.3 Nseleni/Mposa River

The orthophosphate readings at all sites throughout the study period remained at <0.01 mg/l, however, the nitrate readings spiked in January 2012 (NM1, NM2 and NM3) and June 2012 (NM2). This too could be related to rainfall and runoff from the surround timber plantations and sugarcane farming activities (Figure 2.15). Orthophosphate recordings at these sites were stable compared to those of the Nseleni sites and in particular the Mposa sites. Although the nitrate recordings showed some spikes in levels recorded, they were far lower than the Nseleni and Mposa River sites.
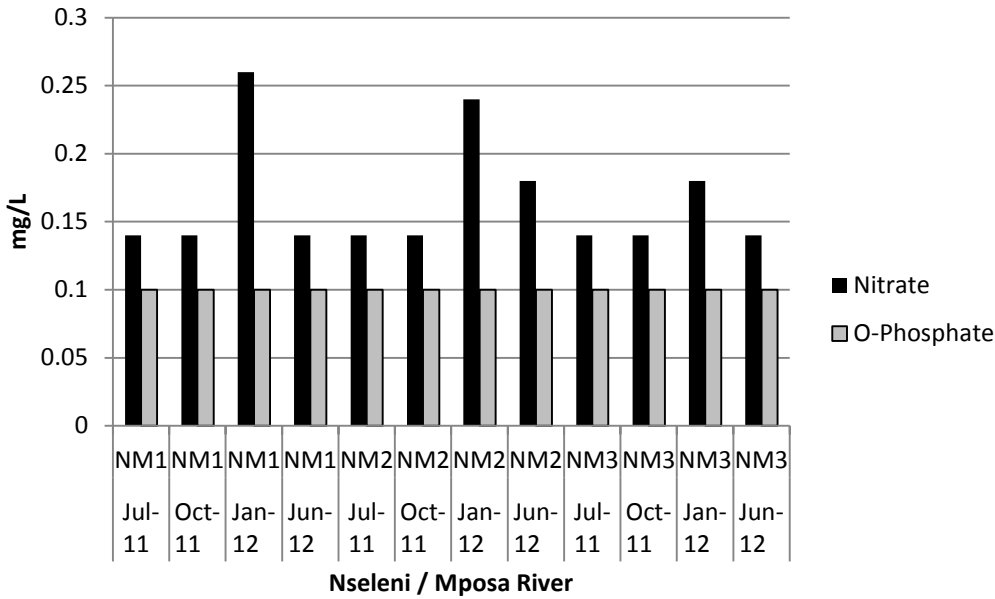
Fig.2.15 Nitrate and orthophosphate recordings for Nseleni/Mposa River.

2.4   Discussion

Three sites on each of the river sections were chosen to determine if there were any significant differences in depth profile, water chemistry and water nutrients, to assist in determining any possible significant differences in occurrence and abundance for the non-indigenous species being examined. Depth of river sections investigated mainly pertained to *Tarebia granifera* and *Pterygoplichthys disjunctivus* as *Eichhornia crassipes* is a free floating plant. The depths recorded in the Mposa River were found to be significantly different from the other two river sections, as has been described earlier in this chapter. The Nseleni/Mposa river section was the only river section to show significant differences, over time, for all of the water chemistry sampled. No significant differences over time in the Mposa River were observed in total dissolved solids and pH, whereas, the Nseleni River results in water chemistry indicated no significant difference over time in conductivity, dissolved oxygen, salinity and temperature. Overall water chemistry analysis indicated a difference between all of the river sections. Williams (1966) found a high correlation between specific conductance and concentrations of total dissolved solids. McNeill and Cox (2000) suggest that there is a correlation between colloidal and suspended matter and that it may contribute to measured values of water conductivity. Furthermore, Rhoades (1996) correlates salinity to electrical conductivity and total

dissolved solids. These findings are further reflected in the water chemistry findings of this study. This suggests that physiochemical data is inter-related. Both the Mposa River and the Nseleni River water chemistry are similar for total dissolved solids, conductivity and salinity. The Nseleni/Mposa River water chemistry were also very similar except salinity for site NM1 does not indicate the same "spike" as sites NM2 and NM3. This has probably occurred due to the fact that the river does not only flow in one direction (natural flow) but when the level of the lake is raised at Lake Nsezi, the water is pushed back up the system. Results also indicate that all research sites physiochemical features indicate stability between sites on the same sampling date, as well over the twelve month sampling period.

Water nutrient analysis (nitrate and orthophosphate) of the three river sections sampled was additionally carried out to determine if there were any significant differences between the river sections over time. The analysis of nitrates in the Nseleni/Mposa river section was comparatively higher than those recorded in the other two river sections (excluding the spikes that occurred within these two river sections) and was ascribed to forestry and agricultural practices adjacent to the Nseleni/Mposa river section. Orthophosphate recordings for the Mposa River were considerably higher compared to the other two river sections and were ascribed to the sewerage outlet situated between sites M1 and M2.

The South African Department of Water Affairs have provided guidelines for water quality in aquatic ecosystems in South Africa and this is used in water quality management as the primary source of reference information and decision support required for the management and protection of aquatic ecosystems (Tables 2.7 and 2.8).

Table 2.7 Inorganic Nitrogen guideline - South African Department of Water Affairs.

Average Summer Inorganic Nitrogen Concentration (mg/l)	Effects
< 0.5	Oligotrophic conditions; usually moderate levels of species diversity; usually low productivity systems with rapid nutrient cycling; no nuisance growth of aquatic plants or the presence of blue-green algal blooms.
0.5 - 2.5	Mesotrophic conditions; usually high levels of species diversity; usually productive systems; nuisance growth of aquatic plants and blooms of blue-green algae; algal blooms seldom toxic.
2.5 - 10	Eutrophic conditions; usually low levels of species diversity; usually highly productive systems, nuisance growth of aquatic plants and blooms of blue-green algae; algal blooms may include species which are toxic to man, livestock and wildlife.
> 10	Hypertrophic conditions; usually very low levels of species diversity; usually very highly productive systems; nuisance growth of aquatic plants and blooms of blue-green algae, often including species which are toxic to man, livestock and wildlife.

All three rivers (Mposa, Nseleni and Nseleni/Mposa) generally had readings of < 0.5 mg/l of inorganic nitrogen at all of the nine sites. The exception to this was the Nseleni River at site one, where in January 2012 it was > 0.5 mg/l and at site two in January 2012 where it = 0.5 mg/l. Theses readings coincide with heavy rainfall and the use of fertilizers by a neighbouring farmer adjacent to site one (Pers. obs. R.Jones).

Table 2.8 Inorganic Phosphorus guideline - South African Department of Water Affairs.

Average Summer Inorganic Phosphorus Concentration (mg/l)	Effects
< 5	Oligotrophic conditions; usually moderate levels of species diversity; usually low productivity systems with rapid nutrient cycling; no nuisance growth of aquatic plants or blue-green algae.
5 – 25	Mesotrophic conditions; usually high levels of species diversity; usually productive systems; nuisance growth of aquatic plants and blooms of blue-green algae; algal blooms seldom toxic.
25 - 250	Eutrophic conditions; usually low levels of species diversity; usually highly productive systems, with nuisance growth of aquatic plants and blooms of blue green algae; algal blooms may include species which are toxic to man, livestock and wildlife.
➢ 250	Hypertrophic conditions; usually very low levels of species diversity; usually very highly productive systems; nuisance growth of aquatic plants and blooms of blue-green algae, often including species which are toxic to man, livestock and wildlife.

The analysis of water quality carried out over a twelve month period, indicates a eutrophic system, especially the Mposa River.

Warmsly (2003) reported to the Department of Water Affairs that there did not appear to be any long-term eutrophication management programmes in place and that eutrophication water quality problems were being encountered throughout the country in varying degrees. de Villiers and Thiart (2007) carried out a comprehensive overview on the nutrient status on 20 of South Africa's largest river catchments. In all of the rivers surveyed, except one, were found to exceed nutrient levels as per the water quality guidelines set by the Department of Water Affairs. Furthermore, they concluded that upward trends in dissolved PO_4^{3-} levels in 60% of the rivers surveyed. They are of the opinion that dysfunctional and unsewered human settlements were the cause, as were the findings at sites M2 and M3, below the sewerage outlet. Byrne *et al*. (2010)

concluded that water hyacinth is the symptom of a larger problem of eutrophication of South African fresh water bodies. Of further concern is the fact that a consequence of a eutrophic river is blooms of cyanobacteria and therefore the possibility of cyanotoxin contamination and excessive growth of macrophytes (van Ginkel, 2011).

Mposa River sites below the sewerage outlet (M2 and M3) showed readings higher than site M1 (up-stream of the sewerage outlet). Recordings below the sewerage outlet rose as high as 0.75 mg/l, an indication that water samples had been taken after sewage had been released. All sites on the Nseleni River indicated readings of < 0.1 mg/l, except at site N3 in January 2012 where it increased to >0.4 mg/l. All readings for the Nseleni/Mposa River sites were <0.1 mg/l. Haller and Sutton (1973) in: Coetzee *et al.* (2012) found that phosphorous concentrations higher than 0.1 mg l^{-1} allowed for growth in water hyacinth, as well as the uptake of nutrients in excess of what the plant actually required, hence the prolific growth of this plant observed in the Mposa River. More importantly, however, Haller and Sutton ((1973) in: Coetzee *et al.* (2012)) found that concentrations below 0.1 mg l^{-1} halted the active growth of water hyacinth.

Wetland ecosystem structure and function is primary determined by the hydrological regime, whereas site-specific conditions of depth, duration and timing of inundation are formed within floodplain wetlands by the characteristics of the geomorphological form of the channel and riparian zones (Patrick and Ellery, 2006). Local-scale features such as levees, point bars, scroll bars, oxbow lakes and back water swamp habitats (hydrogeomorphical features) caused by the interactions of runoff and sedimentation, operate at the community level which influences the distribution of plant communities within the immediate landscape (Patrick and Ellery, 2006). The development of the town of Richards Bay, has without doubt, curtailed and negatively influenced the hydrological regime of the area. The Nseleni River is a source of fresh water for the City of uMhlathuze and is a pathway for biodiversity dispersal from a local, regional and national aspect.

Even though the South African Water Quality Guidelines set by the Department of Water Affairs (Department of Water Affairs and Forestry (DWAF) 1996) are higher than international standards, as laid out by the Organisation for Economic Co-operation and Development (Vollenweider and Kerekes, 1982), South Africa has numerous eutrophic systems, brought about by pollution, which are the result of increased economy and population growth (Coetzee and Hill, 2012). In terms of these guidelines, it appears that the Nseleni River system can be classified as a eutrophic system, especially the Mposa River (M) where orthophosphate recordings were much higher than the Nseleni (N) and Nseleni/Mposa sites (NM). Of concern, is the fact that non-indigenous aquatic weeds can cause the acceleration in the eutrophication process, as well as a range of additional impacts on water quality (Chamier *et al*., 2012). The Nseleni River system's water quality is therefore indicative of a river system with high nutrient loading (pollution) which has resulted in a river system benefitting the continual growth of the invasive non indigenous *Eichhornia crassipes* plant.

The increase in human population in the area has caused intentional and unintentional transformation and degradation of the area has led to serious consequences for loss of biodiversity. The population of South Africa is estimated to have reached the 50.59 million mark of which 21.39% of the population resides in KwaZulu-Natal. The present scenario in South Africa relating to economics and unemployment (25% of total population in South Africa), is not assisting the loss of biodiversity, as South Africa is on a drive to create more employment and development to those that are unemployed. This will lead to an increase in the pressure on protected areas while requiring more than ever the provision of ecosystem services from these areas.

Water quality has a direct effect on ecosystem services that is provided by the Enseleni Nature Reserve. It is therefore of utmost importance that factors affecting the provision of ecosystem services be managed and/or improved upon, and that all invasive non-indigenous species of organisms be eradicated where possible, or alternatively managed in such a way that there is no threat to indigenous biodiversity.

2.5   Conclusion

The Enseleni Nature Reserve is an important area in terms of biodiversity protection and ecosystem services, not only for the greater Richards Bay area, but also on a regional, provincial and possibly even on a national basis. This is one of the few remaining protected area's that still has intact coastal grassland, coastal forest, riverine forest and swamp habitats remaining, as much of the coastal area of the province has been transformed into urban and rural settlements, forestry and sugar cane plantations which have had a negative impact on biodiversity. In November 2013, the Enseleni Nature Reserve was awarded the best managed protected area for the province, at the Excellency Awards Gala evening, for 2012 / 2013. This study was therefore important to identify, and where possible to mitigate the impacts of exotic organism/s invasions.

Exotic invasions, in this protected area, were identified as early as the late 1970's when *Eichhornia crassipes* was first recorded. The hypothesis has always been that the Nseleni River system is highly polluted due to the sewerage outlet from the Nseleni township into the Mposa River and also the fact that much of the adjacent habitats have been removed to make place for agricultural plantations where large amounts of fertilizers are used. Runoff and seepage occurs during periods of high rainfall, which ends up in the Nseleni River system. This study has shown that the river system (excluding the Mposa River) is not as badly polluted in terms of the South African Water Quality guidelines, as was originally thought. This may be due to alien vegetation (*E. crassipes*) taking up the nutrients (see Chapter 6).

Although the Enseleni Nature Reserve is seen as the nucleus of biodiversity in the area, it is under constant external pressures, and it is therefore of utmost importance that the City of uMhlathuze fulfils its mandate by means of proclaiming the identified "future nature reserves" of the City. These nature reserves will linkup with the Enseleni Nature Reserve and will increase the area under formal conservation status. In terms of the Convention of Biodiversity, this will further assist the country to achieve its goal of having 18% of the countries viable representative biodiversity samples, under formal conservation.

CHAPTER   3

THE ABUNDANCE, DISTRIBUTION AND HABITAT PREFERENCE OF THE
INVASIVE FRESHWATER SNAIL *TAREBIA GRANIFERA* (LAMARCK, 1822)
(GASTROPODA: THIARIDAE)

3.1   Introduction

Tarebia granifera is a prosobranch gastropod originally from South-East Asia that has become invasive in several countries around the world including South Africa (Appleton *et al.,* 2009). Appleton (2003) undertook research on introduced freshwater snails in South Africa, in which he assessed the biology of invasive and potentially invasive species and reviewed the state of knowledge of the ecological and economic consequences of the spread of these species. A synopsis of the Appleton (2003) review follows as it sets the scene for this study.

Four of the ten freshwater gastropod species that have been introduced into South Africa are invasive, five are known only from artificial water bodies such as ornamental ponds, the aquarium trade and laboratories, and one, *Helisoma duryi* Wetherby, 1879, has become established in a number of natural habitats but has failed to spread and it thus not considered invasive at this stage (Table 3.1).

Table 3.1 Alien freshwater gastropod species found in South Africa, with their status and regions of origin (after Appleton, 2003).

Species	Status in South Africa	Region of origin	Main habitat types colonised
Pomacea bridgesii **Reeve, 1856**	Non-invasive	South America	Aquaria, ornamental ponds
Pomacea lineata **Spix, 1827**	Non-invasive	South America	Aquaria, ornamental ponds
Marisa cornuarietis **Linnaeus, 1758**	Non-invasive	South America	Laboratories
Tarebia granifera **Lamarck, 1822**	Invasive	Asia	Rivers
Lymnaea columella **Baker, 1911**	Invasive	North America	Rivers, streams, ornamental and natural ponds
Biomphalaria glabrata **Say, 1818**	Introduced	Caribbean, South America	Laboratories
Helisoma duryi **Wetherby, 1879**	Non-invasive	North America	Aquaria, ornamental ponds
Planorbella trivolvis **Baker, 1945**	Non-invasive	North America	Ornamental ponds
Physa acuta **Draparnaud, 1805**	Invasive	North America	Rivers, streams, ornamental and natural ponds
Aplexa marmorata **Guilding, 1828**	Invasive	Caribbean, South America	Ornamental and natural ponds

Globally, Madsen and Frandsen (1989) noted that the most commonly found invasive gastropod species belonged either to the family Thiaridae or to the Pulmonata group which presumably is a

result of their modes of reproduction whereby the Thiaridae are parthenogenetic and ovoviviparous, and the pulmonates are hermaphrodites. Madsen and Frandsen (1989) indicated that the aquarium trade was probably responsible for distributing several common gastropod species in association with ornamental fish and plants into Denmark (6 species); Brazil (7 species, Corrêa *et al.* 1980); Australia (5 species, Walker 1978) and South Africa (6 species, Madsen and Fransen, 1989). Locally, Madsen and Fransen (1989) recorded five introduced species of freshwater gastropod in aquarium shops in Durban, South Africa, including *Pomacea bridgesii*, *P. lineata*, *Helisoma duryi, Physa acuta*, and *Melanoides tuberculata.* They further suggest that the aquarium trade is likely also responsible for the introduction of *Lymnaea columella* and *Tarebia granifera* into South Africa. Two others, *Marisa cornuarietis* and *Biomphalaria glabrata*, were introduced for scientific research purposes and it is possible that *Aplexa marmorata* has been present in south-eastern Africa for at least 160 years, perhaps having been introduced via the Portuguese slave trade (Appleton *et al.* 1989). The source of *Planorbella trivolvis* is not known. *Melanoides tuberculata* is also distributed via the aquarium trade and is commonly found in aquaria in South Africa. Although indigenous to Africa, it has been introduced around the world including India, parts of Asia and northern Australia.

One of the recently introduced species, *T. granifera* has become invasive on at least three continents, North America, South America and Africa (Appleton *et al*., 2009). In South Africa, using criteria proposed in a unified framework by Blackburn *et al*. (2011), *T. granifera* can be classified as a fully invasive (category E), "with individuals dispersing, surviving and reproducing at multiple sites across a greater or lesser spectrum of habitats and extent of occurrence" as it has spread over approximately five degrees of latitude (25°S-30°S) since its 1999 discovery in South Africa (Appleton *et al.,* 2009). *Tarebia granifera* was first reported, in Africa, in 1999, where specimens were found in a concrete lined reservoir at Mandeni, northern KwaZulu-Natal, South Africa (Appleton, 2009). Appleton (2009) believed that the original introduction was as early as 1996 and surmises that the most likely pathway for its introduction was via the ornamental fish trade. Since its introduction, it has become widespread in the eastern half of South Africa, particularly the provinces of KwaZulu-Natal and Mpumalanga (Appleton, 2009). The snail was first recorded in an estuarine environment (St. Lucia Estuary, South Africa) in 2005. Thereafter, established populations have been found in increasing numbers of estuaries in northern KwaZulu-

Natal. *Tarebia granifera* has also been found in freshwater coastal lakes, such as the Kosi lakes and Lake Sibaya (Miranda *et al*., 2011).

Several mechanisms have been proposed for the rapid dispersal of *T. granifera* following its introduction (Appleton *et al*., 2009). Passive dispersal may occur via weed on boats and boat trailers and via water pumped from one water body to another for industrial and irrigation purposes (Appleton *et al*., 2009). Active dispersal could also occur through waterfowl as Appleton *et al*., (2009) recorded many small (5-7 mm) individuals in bird droppings on the bank of the Mhlali River, suggesting that waterfowl assisted with the dispersal from water bodies or river catchments. Freshwater gastropods have been observed in the diet of many waterfowl, including Whitefaced Duck, *Dendrocygna viduata* (Linnaeus, 1766) (Hockey *et al*., 2005). Van Leeuwen (2012) studied seventeen species of Anatidae (ducks, geese and swans) and Rallidae (crakes, coots and gallinules) and concluded that they assisted in the dispersal of thirty-nine species of macro-invertebrates. Van Leeuwen (2012) also concluded that a waterfowl flying at 75 km^{-1} would produce 6.1 droppings per hour, over a distance of seventy-five kilometers, and that this would equate to 3 viable organisms been excreted during the first hour after ingestion and a further 4.1 organisms over the next 3 hours. Food retention time in waterfowl is estimated to be between 2 and 6 hours and this therefore provides enough time for waterfowl to move between rivers and wetlands to disperse any possible ingested snails (Halse, 1984). Such dispersal has been demonstrated for several other snail species, for example, Carlquist (1981) recorded that small snails adhering to bird feathers; Green and Figuerola (2005) confirm that a variety of long-distance migrants carry invertebrates both internally and externally; *Balea perversa* (Linnaeus, 1758) was dispersed from Europe to the Azores and Tristan da Cunha islands by birds (Gittenberger *et al*., 2006) and Wada *et al*., (2012) showed that *Tornatellides boeningi* (Schmacher and Boettger, 1891) was able to survive passing through the digestive tract of the brown-eared bulbul (*Hypsipetes amaurotis* Temminck, 1830).

Miranda *et al*. (2010) suggest that both temperature and salinity play a role in the distribution and survival of *T. granifera*. They further suggest that *T. granifera* has a wide temperature tolerance, with an optimum temperature range around 30^{0}C. In addition, they described how *T. granifera* survived and reproduced at salinities of up to 20 psu, however, the snail was not able to survive

salinities between 30 psu and 40 psu for longer than a month and that this could be a barrier to the snail spreading and establishing in more saline waters.

In Florida, Tucker Abbott (1952) recorded a *T. granifera* density of 4444 m^{-2}, which is similar to ranges measured by Appleton and Nadasan (2002) at five sites in northern KwaZulu-Natal (Table 3.2) where mean (± standard deviation) densities ranged from 844 ±320 m^{-2} to 20764 ±13828 m^{-2}. Appleton and Nadasan (2002) determined that *T. granifera* densities were lowest on shallow (± 50 cm), sandy mud and sparsely vegetated shores that were exposed to wave action (Lake Mzingazi), while high densities were recorded in the non-flowing portion of the Nseleni River where the snails were located on/in soft mud substrate that was devoid of rooted vegetation and shaded by trees (*Barringtonia racemosa* (L.) Roxb.). This between site variability may be positively correlated to habitat heterogeneity and food availability (de la Vega *et al*., 2003; Snider and Gilliam, 2005).

Table 3.2 Densities (D, no.m^{-2}) and shell heights (H, in mm) ± 1SD of *Tarebia granifera* at five sites in northern KwaZulu-Natal (after Appleton *et al*., 2009).

Locality	D (mean ±sd) (m^{-2})	D (range) (m^{-2})	H (mean ±sd) (mm)	H (range)
Mandeni Reservoir (1999)	677 ± 445	120 - 1316	10.9 ± 2.5	0.8 - 22.0
Sokhulu Reservoir (2006)	3419 ± 1269	2664 - 4884	13.4 ± 2.8	7.6 - 20.5
Nseleni River (2006)	20764 ± 13828	5905 - 33256	10.7 ± 2.8	4.4 - 18.5
Mhlathuze River (2006)	15 ± 10	4 - 33	9.6 ± 2.5	3.4 - 16.4
Lake Msingazi (2006)	844 ± 320	577 - 1199	9.5 ± 1.1	6.3 - 11.5
Nhlabane estuary (2006)	4440 ± 2198	2264 - 6660	7.1 ± 2.6	2.7 - 18.8
Mhlali River (2008)	1200 ± 1810	0 - 4056	6.8 ± 2.3	1.8 - 15.5

Appleton *et al.* (2009) suggested that data obtained from several KwaZulu-Natal localities have provided evidence that severe population fluctuations occur from time to time, and that this scenario could occur every few years. This author (Jones), observed such a "crash" in the Nseleni River population in August to October 2007, whereby hundreds of thousands of dead *T.granifera*

shells washed up along several kilometres of both river banks. In such situations densities have been noted to drop by as much as 95%, i.e. from ± 1000 m^{-2} to ± 50 m^{-2}. Appleton *et al.*, (2009) suggested that this may indicate intermittent failure of the spring/summer breeding effort but that after a period of months, numbers build up again. Regrettably, no water analysis was carried out on the Nseleni River during the *T. granifera* "crash" between August and October 2007 that might have provided the possibility to assess for environmental influences on population abundance.

Freshwater snails are microphagous species. The diet comprises a variety of food sources and includes food such as microalgae on submerged surfaces, as well as detritus and plant material that is in the process of decaying (Appleton, 2003). As would be expected, these food sources are also utilized by indigenous grazers (snails and some insects) and once *T. granifera* reaches high densities there is the possibility that there will be competition for food sources and high numbers of the invasive snail may reduce food availability to indigenous invertebrates (Appleton, 2003; see Chapter 5). Appleton (2003) suggested that once the invasive snail reaches high densities, there are bound to be physical interactions between invasive snails and indigenous invertebrates, as has been demonstrated in laboratory experiments by Madsen (1979; 1992 in: Appelton, 2003) whereby fewer egg masses, reduced hatching and slower growth rates were recorded amongst occurred in other species. Pointier (1999) showed in the field that when *T. granifera* reached high population densities, indigenous species *Biomphalaria glabrata* (Say, 1818) in invaded habitats in Puerto Rico and Venezuela and *Pachychilus violaceus* (Preston, 1911) in Cuba disappeared. Locally the indigenous thiarid *M. tuberculata* has become less common, possibly due to pressure from the spread and increased abundance of *T. granifera* (Appleton *et al.*, 2009).

Tarebia granifera has spread into the saline St Lucia estuary system (Appleton *et al.*, 2009; Miranda *et al.*, 2011) and other estuaries and lagoons along the KwaZulu-Natal coast. It is therefore highly likely that *T. granifera* will impact another indigenous thiarid, *Thiara amarula* (Linnaeus, 1758), which to date, has not received much attention. Appleton *et al.*, (2009) recorded that both *T. amarula* and *T. granifera* occurred in the Nhlabane estuary, north of Richards Bay and in 2006 a survey showed that *T. granifera* was present at between 2000 and 6000 m^{-2} and only two specimens of *T. amarula* were recorded while previous sampling at the same site prior to the *T. granifera* invasion found *T. amarula* to be abundant.

Of further concern, is the fact that Appleton (1977) reported that three mollusc species, *viz. Cleopata ferruginea* (Lea and Lea, 1850), *Lanistes ovum* (Peters, 1845) and *Chambardia wahlbergi* (Krauss, 1848), reach the southern limits of their African distributions in north-eastern KwaZulu-Natal, where *T. granifera* has now been recorded. Being at the limits of their ranges, these species may be vulnerable to interference from dense populations of *T. granifera*, and Appleton (1977) therefore suggested that studies on the ecological impact of *T. granifera* are urgently needed.

In order to fully understand the impacts of *T. granifera* in the Nseleni River system, the extent of the invasion by this exotic snail must be determined. While several assessments have shown that *T. granifera* is present in the Nseleni River system, and elsewhere in KwaZulu-Natal (e.g. Appleton *et al*., 2009; Miranda *et al*., 2011), no research has been carried out to determine its abundance, distribution and habitat preference. The aims of this study were therefore to determine the seasonal abundance of *T. granifera* throughout the system, including the upper Nseleni River (N), Mposa River (M) and the lower Nseleni River (NM).

3.2   Materials and Methods

This study was carried out at nine sites in the Nseleni/Mposa river system with three sites on the Nseleni River, three on the Mposa River and a further three downstream of the confluence of the two rivers (see Chapter 2 for details). At each site, three sub-sites were chosen to assess snail populations, one up against the bank (shallow site), one in the middle of the river (deep site) and one half-way in between (middle site). Assessments of relative abundance of *T. granifera* were undertaken using colonization rates of artificial substrates as is described by Midgley *et al.* (2006) and Coetzee *et al*. (2014) and were constructed using mesh bags (20cm x 50cm). The mesh used was a coarse shade cloth, which allowed recruitment of macroinvertebrates. Each bag was filled with 2.0kg of small pebbles. This method of sampling invertebrates and in particular gastropods differs to that used in the study by Appleton *et al.* (2009) who used a Van Veen grab, and thus the densities recorded here are not directly comparable with their study. The bags were closed using a cable tie and attached to each other, from the river bank (shallow) to the centre of the river (deep),

using 2.4mm nylon string. A marker was placed on the river bank to identify the research site. The bags were dropped at a site, after having measured the depth from water surface to substrate.

The sample bags were then left for a period of six weeks, to allow for complete colonization by invertebrates (Thirion, 2000; Midgley *et al.* 2006; Coetzee *et al.* 2014) and then collected with the use of a boat. This was repeated on ten occasions, every six weeks, with the first set of bags being placed in the river in March 2011 and the last in March 2012. On each sampling event, the artificial substrate bags were carefully removed and placed into large plastic bags. The plastic bags were placed below the water surface with the openings slightly above the water level until the artificial substrate bag reached the surface, at which point the plastic bag was dropped below the water level to ensure no loss of specimens. Each artificial substrate bag was placed into a separate plastic bag and labeled with site number and depth: s (shallow), m (middle) or d (deep). Each artificial substrate bag was then replaced with a new one.

All bags were then returned to a working area and individually opened. The contents (pebbles) were emptied into a sorting tray, including the sample bag, and were washed with clean water to separate any invertebrates from the bag and pebbles. Each pebble was individually cleaned with fresh water and a small paint brush. Both the sample bag and individual pebbles were examined before being removed from the sorting tray. The contents of the plastic bag were mixed with fresh water and then emptied into the sorting tray. The contents of the sorting tray (water and substrate) were then poured through a sheet of mosquito gauze (1mm mesh size) and invertebrates and invertebrate body parts were removed with forceps and placed into a glass vial, containing 20% formalin. Each vial was emptied into a dish and examined for invertebrates with a light sourced magnifying lamp. All gastropods collected, were placed into a separate vial containing 20% formalin.

A General Linear Model (GLM) was used to analyse in Statistica v.12 (Statsoft 2006) analysed patterns of *T. granifera* abundance between the three rivers, over the sample period. Abundance data were log + 1 transformed because of the number of zero abundances recorded. Thereafter, a General Regression Model (GRM) investigated the relationship between *T. granifera* abundance and river, sample sites, depth and the physico-chemico properties of the sites (dissolved oxygen (DO), conductivity, pH, temperature, TDS and salinity) (see Chapter 2). After examination of the

residuals, the abundance data were square-root transformed, and a multiple regression modelled the dependent transformed abundance data against the categorical predictors; river, sample site, and depth; and the continuous physico-chemico predictors. GLMs were followed by Fisher's least significant difference (SD) post hoc tests and then analysed for the effects of the significant variables on the abundance data. A Principal Components Analysis in PAST v2.17 was used to analyse whether there was any relationship between the shallow sample sites (see below) and the physico-chemico variables to further explain the patterns of snail abundance.

3.4. Results

Tarebia granifera, was abundant in the Nseleni and Mposa River system with a total of 9287 individuals collected from all of the artificial substrates placed at the different sample sites over ten sampling events, between March 2011 and March 2012. Densities of up to 300 individuals/2kg bag of pebbles were recorded. Generally, more snails were collected from the Nseleni-Mposa system over time, with significant increases in the snail populations during the summer months, while significantly fewer snails were collected from the Mposa River (Figure 3.1).
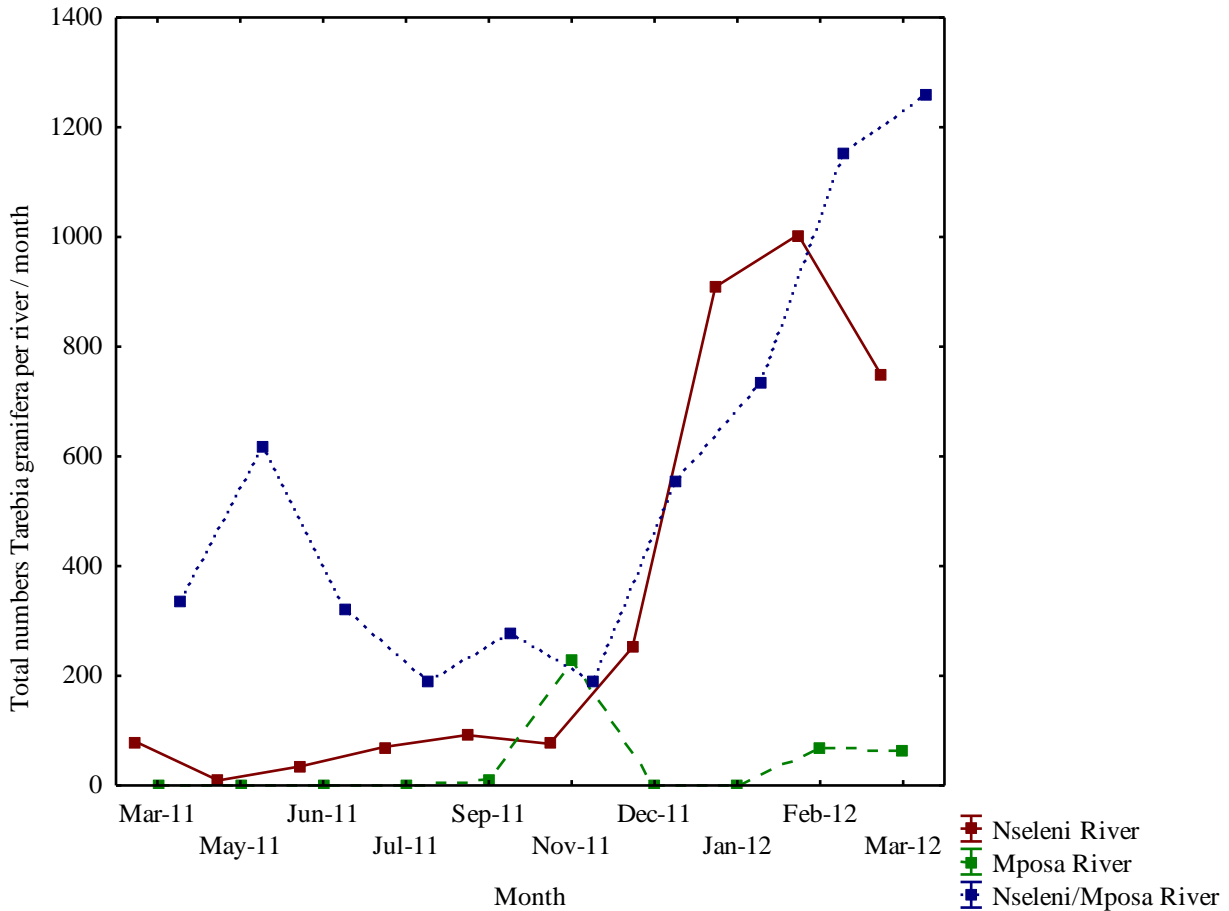
Fig. 3.1 The total abundance of *Tarebia granifera* in the Mposa, Nseleni and Nseleni/Mposa rivers over a twelve month sampling period (31/03/2011 to 30/03/2012). Depths and sites for each river are combined.

Multiple regression analysis showed that all three categorical predictors - river, sample site and depth - significantly affected *T. granifera* abundance, while the only significant physico-chemico variable was pH (R^2=0.456, $F_{19, 88}$=3.885, *P*<0.0001) (Table 3.1).

Table 3.1 Multiple regression analysis of the effects of river system and physico-chemico properties on *Tarebia granifera* abundance in the Nseleni-Mposa River system (R^2=0.456, $F_{19, 88}$=3.885, *P*<0.0001). Significant variables are highlighted in bold.

	SS	DF	MS	F	P
Intercept	33.663	1	33.6626	2.11668	0.149260
River	**150.049**	**3**	**50.0165**	**3.14500**	**0.029119**
Sample site	**335.611**	**8**	**41.9514**	**2.63787**	**0.012208**
Depth	**347.804**	**2**	**173.9021**	**10.93485**	**0.000057**
Dissolved oxygen	1.058	1	1.0577	0.06651	0.797096
Conductivity	0.654	1	0.6536	0.04110	0.839816
pH	**75.614**	**1**	**75.6136**	**4.75453**	**0.031890**
Temperature	0.327	1	0.3267	0.02054	0.886356
TDS	0.047	1	0.0467	0.00294	0.956908
Salinity	0.397	1	0.3974	0.02499	0.874761
Error	1399.506	88	15.9035		

Further analysis showed that over the entire sampling period, *T. granifera* abundance was significantly higher in the Nseleni-Mposa River sites than the other river sites, and significantly more snails were recorded from shallow sites than from middle and deep sites from all systems (Figure 3.2). Site M3 was the only locality with more *T. granifera* recorded from the deep site, most likely due to high cover of submerged weeds at the shallow and middle depths at this site. The highest abundance of snails was recorded from site NM2 at the shallowest depth.
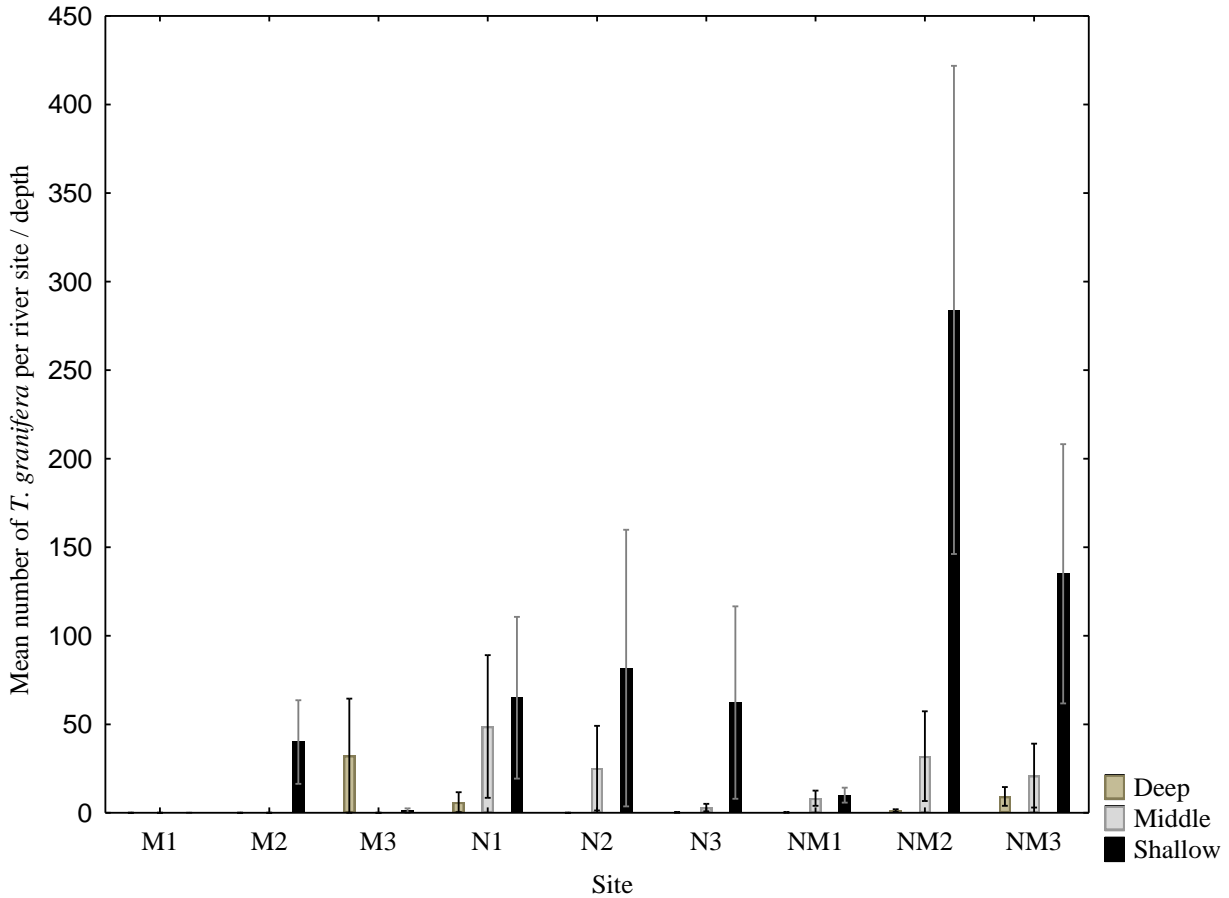
Fig. 3.2 Mean abundance of *Tarebia granifera* per river sample site and depth, for the entire sample period. Mposa River (M1 to M3); Nseleni River (n1 to N3) and Nseleni/Mposa River (NM1 to NM3).

As pH was the only significant physico-chemico variable affecting *T. granifera* abundance (Table 3.1), differences in mean pH of the three river systems was examined. There was no significant difference in pH measurements within rivers, however, there was a clear difference in means between rivers, with the Mposa sites significantly less alkaline than the Nseleni and Nseleni/Mposa sites ($F_{(8, 99)}$ =2.0309, p=0.0503) (Figure 3.3). While these differences appear small, pH is measured on a log scale and thus even a small difference might be biologically significant.
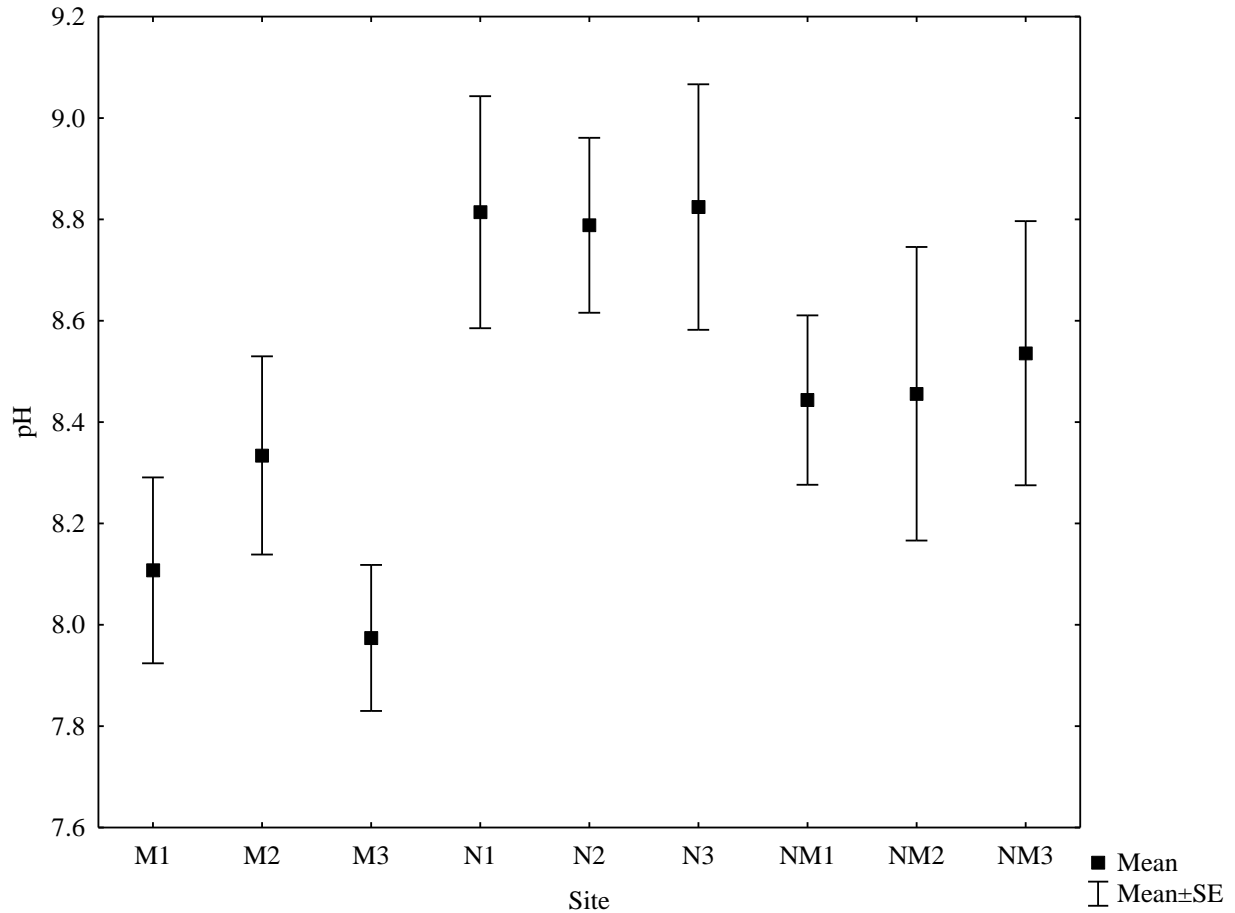
Fig. 3.3 Mean and mean ±SE (error bars) pH for all sites sampled at each site (Mposa River (M1 to M3); Nseleni River (n1 to N3) and Nseleni/Mposa River (NM1 to NM3) between 26.02.2011 and 30.01.2012.

As significantly more *T. granifera* were recorded from shallow sites than middle and deep sites, a PCA of the physico-chemico properties of the shallow depths alone was conducted. Results of the PCA do not show any grouping of shallow sites by river as a result of their physico-chemico properties (Figure 3.4). More than 80% of the variance is explained by the first two principal components, suggesting that the environmental differences between sites do not account for the patterns in abundance of the snails in the shallow sites.
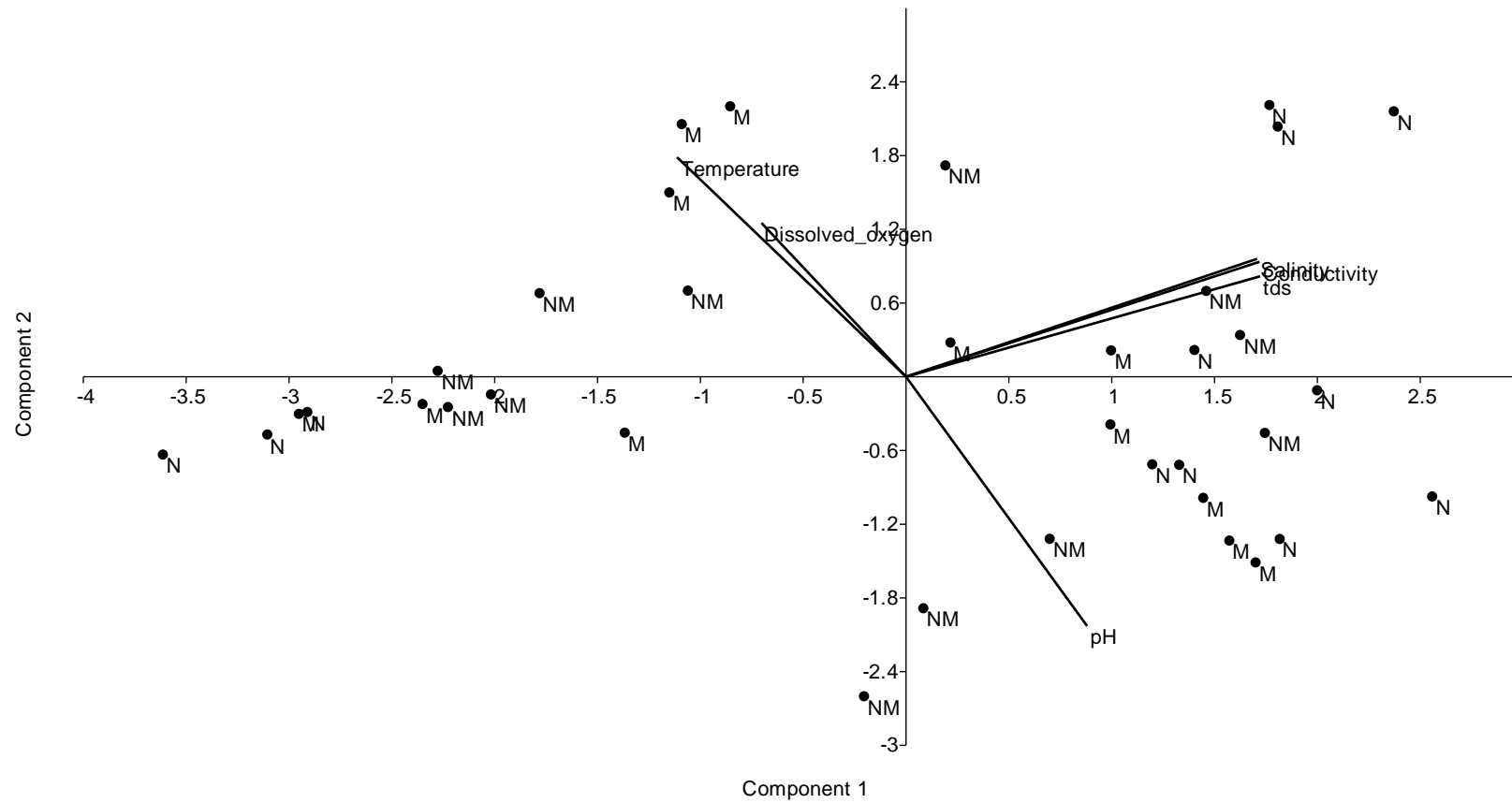
Fig. 3.4 Principal Component Analysis of shallow sample sites of the Mposa, Nseleni and Nseleni-Mposa Rivers, showing the lack of spatial relationship with water quality.

3.4   Discussion

The origin of the first identified *T. granifera* population at the Mandeni site in September 1999 was believed to have been introduced via the aquarium trade and could well have originated from Hong Kong or Singapore (Appelton and Nadasam, 2002) (voucher specimens have been deposited at the Natal Museum, Pietermaritzburg – NMSA V9231). The assumption of their origin is due to the colour pattern, which is very light compared to other samples collected from around the World (Appelton and Nadasam, 2002).

Results from this study have shown that *T. granifera* has become invasive throughout the Nseleni river system, with the highest abundance occurring at shallow river depths in the Nseleni/Mposa river section during the months of austral summer. This could be have serious repercussion on indigenous gastropods (see Chapter 5). This supports the work carried out by Appelton *et al*. (2009), where high densities along the Nseleni River banks were also recorded. However, the high densities found in the Nseleni River are not unique to this river system, as similarly high densities of *T. granifera* (and other invasive Mollusca) and low densities of indigenous snails have been recorded worldwide e.g. *Dreissena polymorpha* (Pallas, 1771) commonly known as the zebra mussel, has become extremely invasive in North American waters since its introduction in the mid-1980's. In addition, *D. polymorpha* has been implicated in the demise in densities of indigenous freshwater bivalves (Allen and Ramcharan, 2001). Wolmarans and de Kock (2006), found that in comparing the status of freshwater molluscs in the Kruger National Park, South Africa, between 2001 and 2006 that *T. granifera* had invaded a further twelve habitats in sufficient numbers to record their concerns of further habitat invasions.

This study concurs with work carried out by Miranda *et al*. (2011; 2014) which showed that *T. granifera* was found to invade and become dominant in shallow waters (<2.6m) within the estuaries and lakes of the iSimangaliso Wetland Park, an UNESCO World Heritage Site in north-eastern South Africa. Miranda *et al*. (2014) additionally investigated a combination of environmental factors to assist in determining the best correlation with benthic assemblages, namely: salinity, dissolved oxygen content, pH and sediment particle size, as did this study. Miranda *et al*., (2014) noted that although *T. granifera* was capable of maintaining densities of over 1000 ind.m^{-2}, variable environmental conditions did in fact play a role in their population

fluctuations. They also found a relationship between low pH and a lack of invasion by *T. granifera* at one of the numerous sites investigated in their study and suggested that lower pH could have a direct impact on shell deposition. The differences in pH between the sites on the Nseleni river system could also have an indirect effect on snail populations through influencing the abundance of food availability (i.e. microalgae). However, the main difference between the Mposa River and the other two rivers sampled may be the availability of allochthonous inputs from surrounding vegetation. The Nseleni River upstream and below the confluence with the Mposa is lined with large *Barringtonia racemosa* trees that produce a constant supply of leaf material into the water column, while the Mposa River has none of these trees and is large lined by *Phragmites australis* (see Chapter 2). Appleton *et al*., (2009) suggested that the variability in *T. granifera* population sizes could be linked to habitat heterogeneity and food availability with allochthonous input (e.g. leaf litter) playing a significant role.

Tarebia granifera is well-established in high numbers in the Nseleni River, however can it be controlled and by what method? Mechanical and chemical control at this stage is not feasible, due the financial and environmental implications involved. Cowie (2001) has reviewed the implications of using snails as a biological control against other snails. One of the most investigated possibilities is that of Ampullariid and thiarid snails being used against snail vectors of human schistosomes (parasites that cause bilharzia). Although successful control of the bilharzia vectoring snails has been recorded using molluscicides, Cowie (2001) suggests that little has been done to determine the impact on indigenous biota. Another example of a snail biological control agent occurred when *Euglandina rosea* (Férussac, 1821) was introduced on Pacific and Indian Ocean islands to combat *Achatina fulica* (Férussac, 1821), the giant African land snail (Cowie, 2001). Unfortunately in this case, Cowie (2001) records that not only did it fail to control *A. fulica*, but that it had negative impacts on endemic island species due to its lack of specificity. Cowie (2001) also produced a table outlining snail species that were used as putative biocontrol agents around the World. A total of twenty snail control agents were released on forty-four target species of which only nine were completely successful. These are alarming statistics and one is therefore cautioned that pre-release testing must include all possible negative effects on indigenous biodiversity.

Blackburn *et al*., (2011) proposed a unified framework which divides the invasion process into a series of four stages (transport, introduction, establishment and spread), each of which is confounded by barriers (geography, captivity, survival, reproduction, dispersal and environmental) which need to be overcome before passing on to the next invasion stage. Each stage has associated management actions (e.g. prevention of introduction, containment once introduced, mitigation of spread once established in the wild and eradication when feasible). The *T. granifera* invasion has been categorized as a stage "D2" invasion (Jones, *et al*., 2013): "*self-sustaining population in the wild, with individuals surviving and reproducing a significant distance from their original point of introduction*" (Blackburn *et al.,* 2011). The only barrier to it becoming a fully invasive species (stage "E") is its inability to cross catchments without human assistance. Regrettably, as numerous research papers have indicated, *T. granifera* has spread widely, since its first African identification in 1999 in Mandeni, KwaZulu-Natal, South Africa. It is therefore suggested that future research should be aimed at reducing the densities of *T. granifera* to an acceptable manageable level, whereby associated indigenous biodiversity is not negatively affected, but, probably more importantly curbing the spread through the aquarium trade, inter-basin transfers and other movement between catchments.

CHAPTER   4

A FIRST RECORD OF AN INVASION BY A LORICARIID CATFISH IN THE NSELENI RIVER SYSTEM, KWAZULU-NATAL – SOUTH AFRICA AND IN AFRICA

4.1 Introduction

The number of alien fish species introduced worldwide has more than doubled compared to nearly three decades ago with growth in global trade and human mobility principally responsible. Alien fish species have primarily been introduced into new ecosystems through human activity, either deliberately or unintentionally. Non-native species are reared locally in farms or imported from abroad for commercial reasons based on the most cost-efficient species in terms of production costs to output revenues, resistance to environmental stressors and pre-existing knowledge of rearing methodologies and technologies. This includes the genetic improvement of farmed species, promotion of species pathogen-resistant stocks and stock enhancement practices and ease of reproduction (Gozlan *et al.,* 2010). In more recent times, non-native fish species (6%) have been intentionally introduced into open waters to manipulate the ecosystems through physical or biological actions to control unwanted organisms, especially mosquito fishes *Gambusia affinis* (Baird and Girard) and *Gambusia holbrooki* Girard, for mosquito control, grass carp *Ctenopharyngodon idella* (Valenciennes) for macrophyte control and silver carp *Hypophthalmichthys molitrix* (Valenciennes) for controlling phytoplankton. While these introductions appear to have been highly successful, particularly relative to more costly and environmentally unacceptable alternatives such as insecticides and herbicides, the effects on the recipient habitats have yet to be fully evaluated (Gozlan *et al.,* 2010). Several species have also been imported and stocked live for use in research. This is mainly for new, alien species imported for experimental aquaculture trials, or for bio-control assays or other research issues. Generally, these types of introductions have a lower risk of dispersal because the research institute applies quarantine measures to experimental trials, which are conducted in closed systems (Gozlan *et al.,* 2010).

Among non-native fish introductions, those that may have the most severe ecological consequences are ecosystem engineers that directly modify their ecosystems (Gozlan *et al.,* 2010).   These effects are often achieved through a change in physical habitat and

consequences are typically proportional to long-term stability and complexity of the ecosystem. A change in habitat and particularly microphytes or phytoplankton community or biomass, would typically result in a substantial modification of ecosystem function with long-term implications for many species and the overall integrity of the ecosystem (Gozlan *et al.,* 2010).

Koehn and O'Connor (1990) record that in Australia, fragmentation of the distributions of the mountain and climbing galaxies, due to predation by trout, has been documented. In many areas these species are absent where tout are present. The introduction of exotic fish species may be responsible for changes to freshwater fish communities in New Zealand as they have been elsewhere. The presence and abundance of the common river galaxias (*Galaxias vulgaris* Stokell, 1949), found only in the Canterbury region of New Zealand, were best predicted by the absence of trout. In most cases they were only found above waterfalls large enough to inhibit trout migration (Cambray, 2003). In some New Zealand streams, the entire annual production of the benthic invertebrate fauna is eaten by alien brown trout, impacting on the feeding behaviour of the indigenous galaxiids and that the introduction of these trout has resulted in trophic cascades, leading to periphyton build-up as well as to changes in the behaviour of both indigenous fish and invertebrates.

When alien predators are introduced there are often negative impacts on indigenous fish and invertebrate communities. Brown trout *Salmo trutta* Linnaeus, 1758 were released into New Zealand streams in the 1860s and resulted in a severe decline in native fish populations and a change in invertebrate community structure (Flecker and Townsend (1994) in: Weyl *et al.*, 2010). A field experiment in Northern Japan demonstrated that predation by invasive rainbow trout *Oncorhynchus mykiss* Walbaum, 1779 had a cascading effect that extended across the aquatic–terrestrial boundary via a reduction in emerging insects (Baxter *et al.*, 2004, in: Weyl *et al.,* 2010).

Lowe *et al.,* (2008) studied the impacts of smallmouth bass *Micropterus dolomieu* Lacépède, 1802 in the Rondegat River, South Africa, and demonstrated ecosystem level effects that included changes in invertebrate community structure, alterations in grazing patterns and pressure and concomitant differences in algal biomass between invaded and non-invaded sections of the river. Largemouth bass *Micropterus salmoides* Lacépède, 1802, introduced into South Africa for recreational angling in 1928, can be a highly invasive predator. While

predation by *M. salmoides* on indigenous freshwater fishes in southern Africa is fairly well documented (Weyl *et al.,* 2010), there is little information on the effects of this species on invertebrate communities.

Many species of fish have been deliberately introduced globally to provide food or sport. In addition to these deliberate introductions, a large number of fish species have been spread beyond their native range by releases from aquaria, bait buckets, and water gardens, as contaminants of fish intended for stocking, or in ballast water. Some of these fish have had large ecological effects. Humans also often introduce fish into fresh waters that already contain fish, either accidentally or in a deliberate attempt to improve the fish community. Again, the most obvious impacts have been losses of favoured prey species, especially in cases where the alien has no native trophic analogue in the system. Perhaps the most dramatic example is the global extinction of ±200 species of cichlids from Lake Victoria following the invasion of the Nile perch *(Lates niloticus* Linneaus) (Strayer, 2010). Alien fish that are not piscivores may also have large effects on their food. For example, introduced alien planktivores such as *Alosa* spp. and kokanee salmon (*Oncorhynchus nerka* [Walbaum]) can greatly alter zooplankton, introduced salmonids have had large direct and indirect effects on stream invertebrates, even when introduced to sites where fish already live; and even tiny mosquitofish (*Gambusia* spp.) have reduced densities of native invertebrates and outcompeted native fish (Pyke, 2008).

Herbivorous alien species such as grass carp (*C. idella*) likewise can have strong effects on the amount and composition of aquatic vegetation. As is now well appreciated, indirect effects of alien fish can be propagated through the food web and affect many parts of the ecosystem. Thus, both alien piscivores and alien invertivores can have large effects on primary producers and exchanges with neighbouring ecosystems, and bioturbation and nutrient excretion by alien fishes may alter light and nutrient availability. Alien fish have had large, far-reaching effects on almost all parts of freshwater ecosystems, both lentic and lotic. It is possible that these effects have been so dramatic because most fish are able to disperse so poorly (if at all) on their own between continents and drainage basins that many sites support naturally depauperate fish faunas. Introductions of new species into such sites are therefore likely to bring in functionally distinctive species, which often have large ecological effects in insular ecosystems (Strayer, 2010).

In South Africa, alien invasive species (AIS) management is a high priority in national legislation (National Environmental Management: *Biodiversity Act* (*Act 10 of 2004*) and the National Environmental Management: *Protected Areas Act* (*Act 57 of 2003*). On 13 July 2013, the National Department of Environmental Affairs published regulations pertaining to Alien and Invasive Species (Regulation Gazette No. 9993, Vol. 577, No. 36683 – Government Notice R506). In addition, they further published a national list of: Invasive species (GN – R507), prohibited alien species (GN – R508) and exempted alien species (GN – R509). *Pterygoplichthys disjunctivus* is listed as a Category 1b invasive species that requires control by means of an invasive species management plan. The 2007 discovery of a loricariid catfish in a protected area, the Enseleni Nature Reserve, was therefore cause for concern. An important step in developing alien invasive species management strategies in protected areas is determining their extent and traits (Spear *et al.,* 2011). A unified framework such as that proposed by Blackburn *et al.,* (2011), which combines previous stage-based and barrier models, and provides a terminology and categorisation for populations at different points in the invasion process is therefore a potentially valuable tool. According to the framework the invasion process can be divided into a series of four stages (transport, introduction, establishment and spread), each of which is confounded by barriers (geography, captivity, survival, reproduction, dispersal and environmental) which need to be overcome before passing on to the next invasion stage; and each stage has associated management actions (e.g. prevention of introduction, containment once introduced, mitigation of spread once established in the wild and eradication when feasible).

4.1.1 Loricariid catfish invasion in the Nseleni River.

The first record of a loricariid catfish record for the Nseleni River (Jones *et al.,* 2013) was in 2006 (SAIAB83178). The specimen was found by a local farmer, farming adjacent to the Nseleni River, who was walking along the river bank and came across the specimen. It is believed to have been removed from an illegal gill net and left on the river bank.

With about 80 genera and over 700 species, Loricariidae is the largest family of catfish (Siluriformes). Loricariids are endemic to South America (absent in Chile), Panama, and Costa Rica and are characterized by having large bony plates and a ventral suctorial mouth with or without noticeable barbels. A group of loricariid catfishes with 10 or more dorsal-fin

rays are commonly referred to as sailfin catfishes. Weber (1991; 1992) reviewed variation among sailfin catfishes and assigned them to three genera. Species with an elevated supraoccipital process were assigned to *Glyptoperichthys* Weber 1991, those lacking the elevated supraoccipital process and with the supraoccipital bone bordered posteriorly by one large plate were assigned to *Pterygoplichthys* Gill, 1858, and those lacking the elevated supraoccipital process and with the supraoccipital bone bordered posteriorly by three plates were assigned to *Liposarcus* Günther, 1864 (Armbruster and Page, 2006).

The sailfin catfish, comprising several morphologically similar species from the suckermouth armored catfish family Loricariidae, are among one of the alien fish groups most seriously threatening tropical and subtropical freshwater regions. Widely believed to be an excellent algae grazer, these fish have been introduced all around the world from South America by the aquarium trade (Figure 4.1).

Fig. 4.1 Sale of *Pterygoplichthys disjunctivus* in a pet shop in Richards Bay November 2011 (R. Jones).

Nevertheless, occasional escapes due to carelessness of owners provided a chance for these fish to establish populations in tropical and subtropical freshwater bodies. Ecological impacts of this fish include competition with native algae-grazers, direct predation on bottom-attached eggs of other fish, bank destabilization, increased turbidity, and economic loss to fisheries (Wu *et al.,* 2011). Fishermen blame them for decreasing marketable fish in the waters of Laguna de Bay – Philippines (Chavez *et al.,*, 2006) and destroying fishing

equipment from 12 localities in 10 Taiwanese drainage basins (Wu *et al.,*, 2011). In Florida, *P. disjunctivus* are known to disturb resting endangered Florida Manatees (Gibbs *et al.,,* 2013) The invasion of sailfin catfish in Taiwan can be traced to the late 1970s, when this fish first appeared in the local ornamental fish trade (Liang *et al.,* 2005). Originally, some of the fish were directly imported from South America, presumably Brazil or Peru. Some others may have been introduced from farm-raised individuals, possibly from Florida, USA. In the early 1980s, captive breeding was successfully established in Taiwan, and the offspring of such populations have become available to meet the great demand of local pet shops (Wu *et al.,* 2011).

The aim of the study was to assess the usefulness of the unified framework with regard to management of fish invasions by assessing the invasion stage of the loricariid (Figure 4.2) population in the Nseleni River and identifying appropriate management actions using the Blackburn *et al.,* (2011) framework.

Fig. 4.2 First live *Pterygoplichthys disjunctivus* (juvenile) captured in the Nseleni River in 2010 whilst carrying out separate research (R. Jones).

4.2 Material and Methods

4.2.1 Sample collection

To determine the extent and state of invasion, both community engagement and direct sampling were carried out. Community engagement included the encouragement of the local community to report and donate specimens of loricariid catfishes. This was done through a newspaper article in May 2010 and through personal communication with rural community members (RJ). Direct sampling were undertaken during two ichthyological surveys during which the fish fauna in the Nseleni River were sampled using valve traps (Figure 4.3a), double ended fyke nets with anchors at each end to maintain it in position (Figure 4.3b), gill nets (25mm, 38mm, 50mm, 60mm, 75mm and 100mm stretched mesh size) as well as by electro fishing at multiple locations. Habitat traps (Figure 4.3c) comprising of PVC pipes, each pipe with six 5mm holes drilled into it, to allow it to sink, were set in the Nseleni and Mposa rivers at nine different sites and three different depths at each site and monitored from February 2011 to February 2012. Traps were inspected every six weeks. In addition, whilst carrying out the above research, data were collected on distribution, impact on other fish/invertebrates via collection of isotope samples (Refer Chapter 5), sex ratios, weights and sizes, as well as to determine where they fit into the 'food web' assess for niche competition with other aquatic fauna.
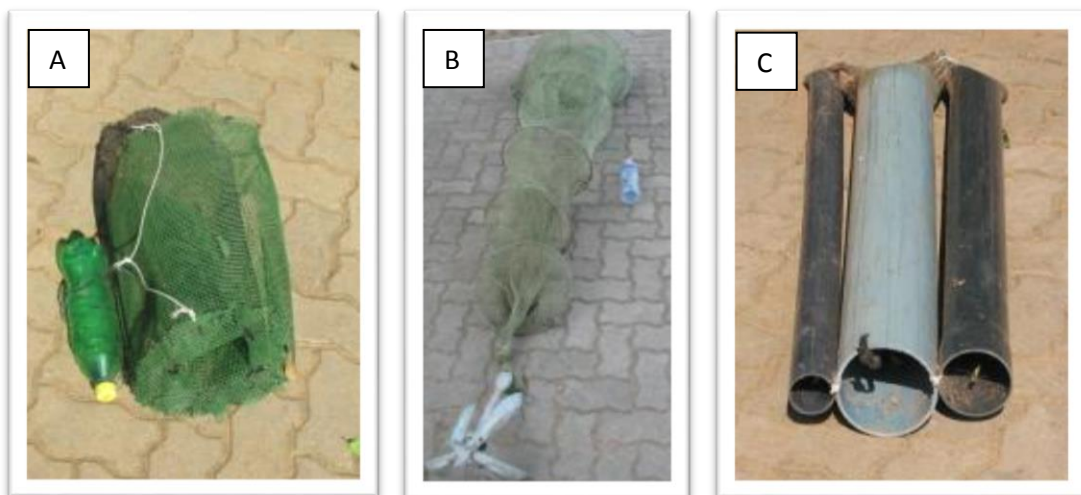
Fig. 4.3(a) Homemade valve trap made from cylindrical plastic mesh with an internal cylinder also made of plastic mesh. Each trap had an external opening of approximately 10cm and narrowing to an end diameter of approximately 5cm, inside the main trap, (b) double ended fyke net with an 8-m guiding net and a first-ring diameter of 55 cm and a cod end mesh size of 12mm and (c) homemade

habitat traps made from PVC pipe measuring 55mm, 70mm and 110mm diameter and each pipe 1 meter in length. All traps had flotation devises attached to enable detection for recovery.

4.2.2 Identification

Due to the complexity of the Loricariidae, final species identification often depends on sampling locality and colour patters (Armbuster and Page 2006) (Figure 4.4a and b). Selection for desirable traits in the pet trade, has made invasive populations of this already taxonomically complex group of fishes difficult to identify (Wu *et al.,* 2011). As a result of this complexity, identified specimens using the taxonomic keys to genera provided by Armbuster (2003) were used and the key to the species for this genus provided by Armbuster and Page (2006) and then confirmed these identifications by barcoding two specimens.

Fig.4.4 Colour variation of two adult *Pterygoplichthys disjunctivus*, (a) being a darker shade with less white stripes and (b) a lighter shade with more white stripes (R. Jones).

4.3 Results

4.3.1 Assessing invasion stage

Stage 1: Transport

The first stage in the invasion process is the breaching of a geographical barrier. The pet trade is a major pathway for introducing alien fish into South Africa and more than 1200 alien fish are permitted for importation and trade (Anonymous 1994). According to two ornamental fish wholesalers, loricariid catfish have been imported and traded into South Africa at least since the 1970's (N. Stallard, owner of Fish Designs and ornamental fish wholesale business). In this case the reliable identification of the invading species was complicated by the complex taxonomic diversity of the Loricariidae, where final species identification often depends on sampling locality and colour pattern (Armbuster and Page 2006). Selection for desirable traits in the pet trade has further complicated identification of invasive populations of this already taxonomically complex group of fish (Wu *et al.,* 2011). For this reason the taxonomic keys (Armbuster and Page 2006) and DNA barcoding (as part of the FISH-BOL project, described by Swartz *et al.*, 2008) to attempt to identify specimens.

Specimens were identified as *P.disjunctivus* on the basis of morphology and ventral surface coloration (Armbuster and Page 2006). For DNA-Barcoding, six loricariid individuals from the introduced population were sequenced for the part of the mitochondrial cytochrome oxidase subunit I (COI) gene used in DNA barcoding, following the standard protocols implemented for fish barcoding by the Canadian Centre for DNA Barcoding at the University of Guelph (methods summarised by Ward *et al.,* 2005). BLAST searches done in Genbank and subsequent phylogenetic analysis revealed that sequences from the six Nseleni specimens (612 base pairs; Genbank numbers KC170030 - KC170035) matched 100% (same haplotype) with individuals referred to as *Hypostomus plecostomus* (JN026851 from April *et al.,*, 2011), *P.disjunctivus* (JF498722 - JF498724 and JF769355 - JF769356 from Jumawan *et al.,* 2011 and AP012021 and NC_015747 from Nakatani *et al.,* 2011) and *P. pardalis* (JF498752, JF769358 and JF769360 from Jumawan *et al.,* 2011). The genetic results could therefore neither reject nor confirm the morphological identification.

The morphological and genetic identifications were consistent with the findings of Wu *et al.,* (2011), suggesting that there could be confusion with the identification of introduced loricariid populations and/or that hybridisation (Figure 4.5a, b and c) has occurred between closely related species (especially considering potential mixing in the ornamental trade). Positively identified specimens from the type localities of *H. plecostomus*, *P. pardalis* and *P. disjunctivus* are required to resolve the taxonomic confusion surrounding introduced loricariid populations. Given that the genetic analysis could not reject or refute the morphological identification, the most likely identity of the South African invasion is *P.*

disjunctivus, but the possibility remains that the population has a hybrid history (Wu *et al.,* 2011).
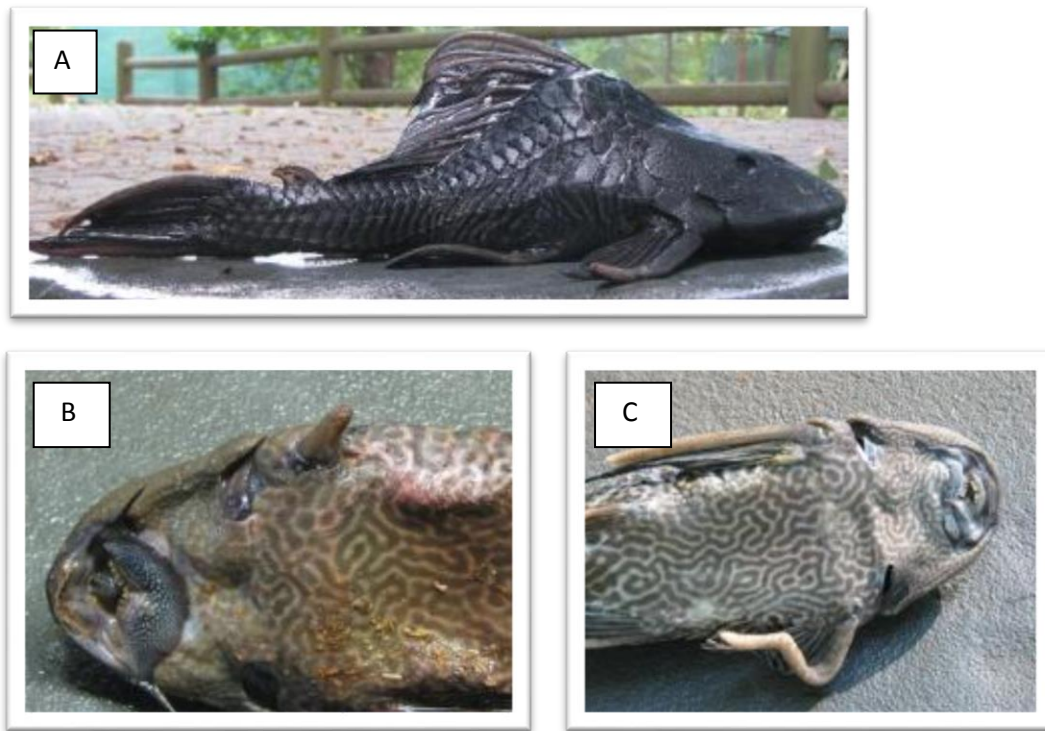
Fig. 4.5a, b and c Deformed *Pterygoplichthys disjunctivus* that were observed during data capture, possibly due to hybridization (R. Jones).

Pterygoplichthys disjunctivus is native to the Madeira River drainage of the Amazon basin in Brazil and Bolivia and is the main nuisance species in Mexico (Capps *et al.,* 2011), the USA (Nico *et al.,* 2009), the Philippines (Vallejo and Soriano, 2011) and Taiwan (Wu *et al.,* 2011). Interestingly, *P. disjunctivus* is not on South Africa's permitted fish list which includes three species of the closely related genus *Hypostomus*. It is therefore likely that *P. disjunctivus* was erroneously imported as one of these species. Such misidentifications are common in the pet trade and often result in the inadvertent importation of invasive organisms (Keller and Lodge, 2007).

Stage 2: Introduction

The history of loricariid site locality records is illustrated in Figure 4.6. The first report was from Lake Mpangeni in 2000 (Skelton and Weyl, 2011) but the first specimen-linked evidence of introduction into the wild was from Mhlathuze River in 2004 (South African Institute for Aquatic Biodiversity, National Fish Collection voucher specimen: SAIAB83643). Then in 2006 from the Nseleni River (SAIAB83178), where a total of 368

specimens have now been collected from numerous localities including its Mposa River tributary (Figure 4.6). Although the exact introduction pathway from captivity into the Mhlathuze and Nseleni Rivers is not known, the most likely source of introduction was through release by aquarists or escape from an ornamental pond into Lake Mpangeni; from the lake into the Mhlathuze River and then via an inter basin water transfer pipe line into Lake Nsezi and upstream into the Nseleni and Mposa Rivers (Figure 4.6)**.**
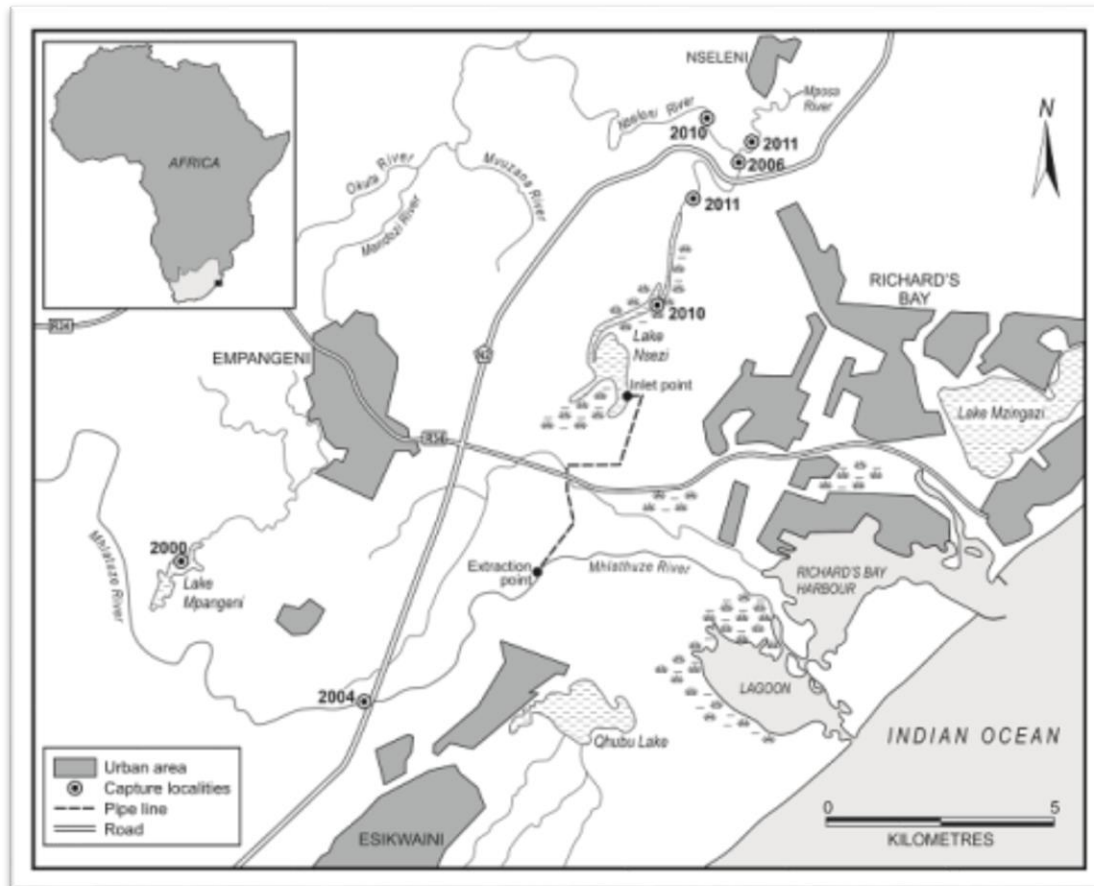
Fig. 4.6 The history of Loricariid site locality records for the greater Richards Bay area.

Stage 3: Establishment

A strong indicator of establishment was the sampling of specimens throughout the 15-month sampling period and the presence of multiple length cohorts in the population including small juveniles (12-130 mm TL) and large >300 mm TL adults (Figure 4.7). It was also determined that spawning capable fish were present in the population using established criteria to determine maturity (Gibbs *et al.,* 2008). Three hundred and forty six sampled fish were dissected and their gonads were examined to determine whether fish were immature (no ova visible and testes small and tubular in shape) or spawning capable (yolky ova visible and

testes distended and lobular). Of the fish examined, 64 were juveniles (Figure 4.8), 91 were mature males and 191 spawning capable females. The smallest mature female fish was 270 mm TL (Figure 4.9) and the largest was a 490 mm TL. The smallest mature male fish measured 305 mm TL and the largest was 480 mm TL. Female spawning capability was also inferred from a gonadosomatic index $I_G > 1$ ($I_G = 3.9 \pm 2.4$) calculated

$(I_G = {gonad\ mass(g)}\Big/{whole\ body\ mass(g)} \times 100$ from a sample of 54 female fish collected during austral summer (December 2011-January 2012). Estimates of maturity and GSI were consistent with those determined for the invasive population in Florida (Gibbs *et al.,* 2008). In the Nseleni River, the large number of mature specimens and the concurrent occurrence of both adult and juvenile fish therefore suggest that *P.disjunctivus* has successfully established.
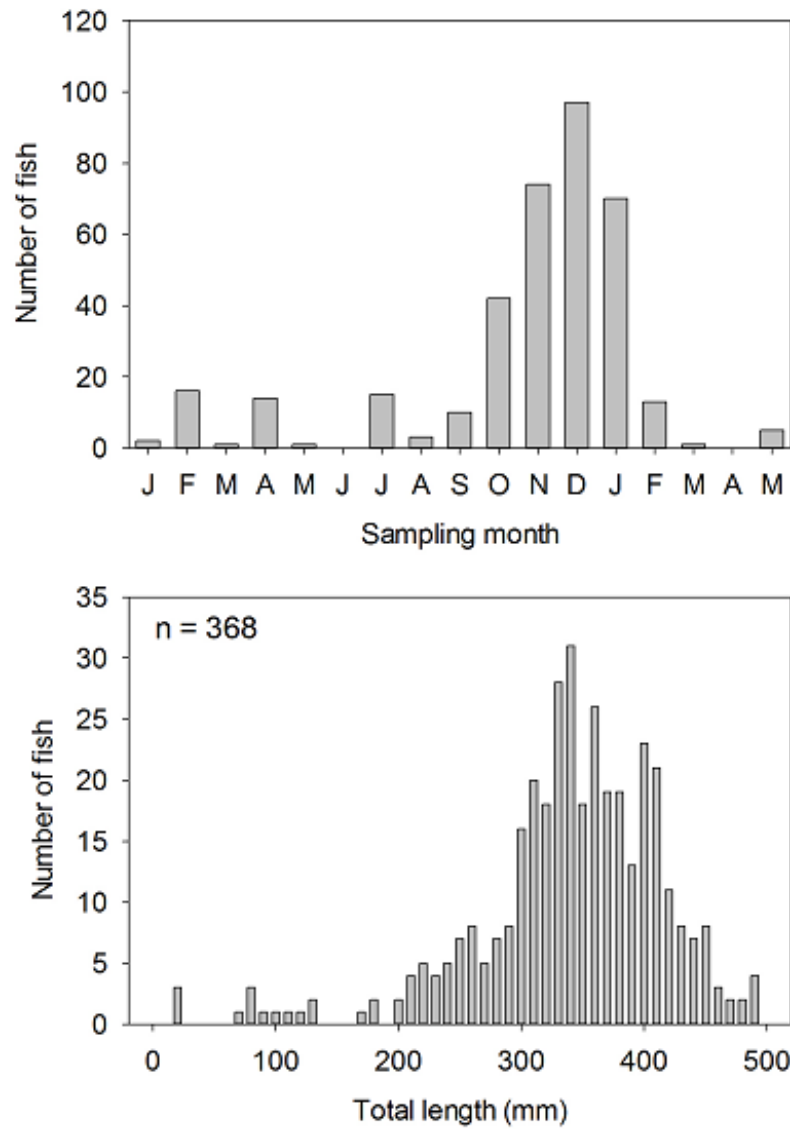
Fig. 4.7 Length frequency of *Pterygioplichthys disjunctivus* collected (January 2011 to May 2012) from the Nseleni River system.

Fig. 4.8 Seven juvenile *Pterygoplichthys disjunctivus* recovered from a homemade habitat trap, measuring from 78mm – 126mm in length (R. Jones).

Fig. 4.9 Smallest mature female *Pterygoplichthys disjunctivus* was 270 mm TL (14/12/2011- R. Jones).

Stage 4: Spread

There is strong evidence that *Pterygoplichthys disjunctivus* is in a stage of spread having dispersed from the Mhlathuze River into the Nseleni River system. This spread was most likely facilitated by the inter basin water transfer pipe which transports water from the Mhlathuze weir into Lake Nsezi (Figure 4.6 and Figure 4.10). Figure 4.8 is an indication that *P. disjunctivus* are breeding successfully in the Nseleni River system.
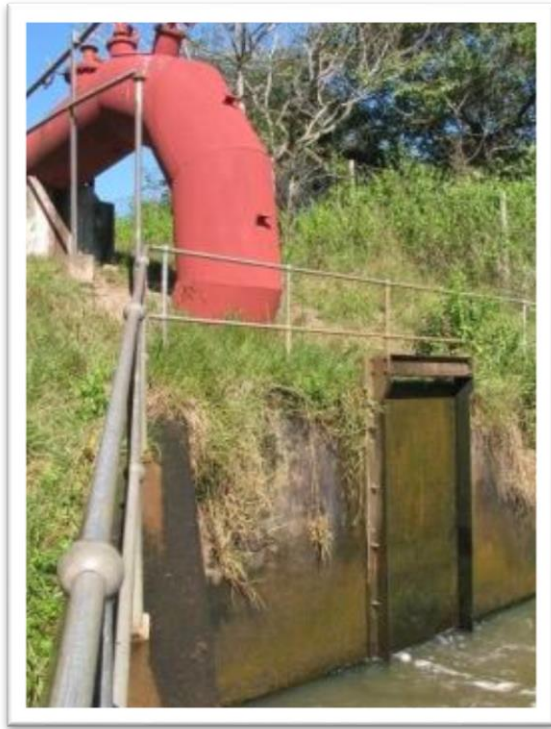
Fig. 4.10 Water transfer scheme. Exit point into Lake Nsezi from the Mhlathuze River.

4.3.2 Morphometric analyses

Morphometric data (Figure 4.11a, b, c and d) collected during the study to assist in any future research to be carried out on this system, or elsewhere. In addition, data was also collected on capture of certain length classes (Figure 4.12a, b, c and d) at different times of the year.

a

b

$y = 1E-05x^{2.9938}$
$R^2 = 0.9027$

FL - mm

$y = 0.8919x - 4.5939$
$R^2 = 0.9845$

TL - mm

c

d

$y = 0.9263x - 18.327$
$R^2 = 0.973$

SL – mm

$y = 0.8428x - 28.188$
$R^2 = 0.9626$

SL - mm
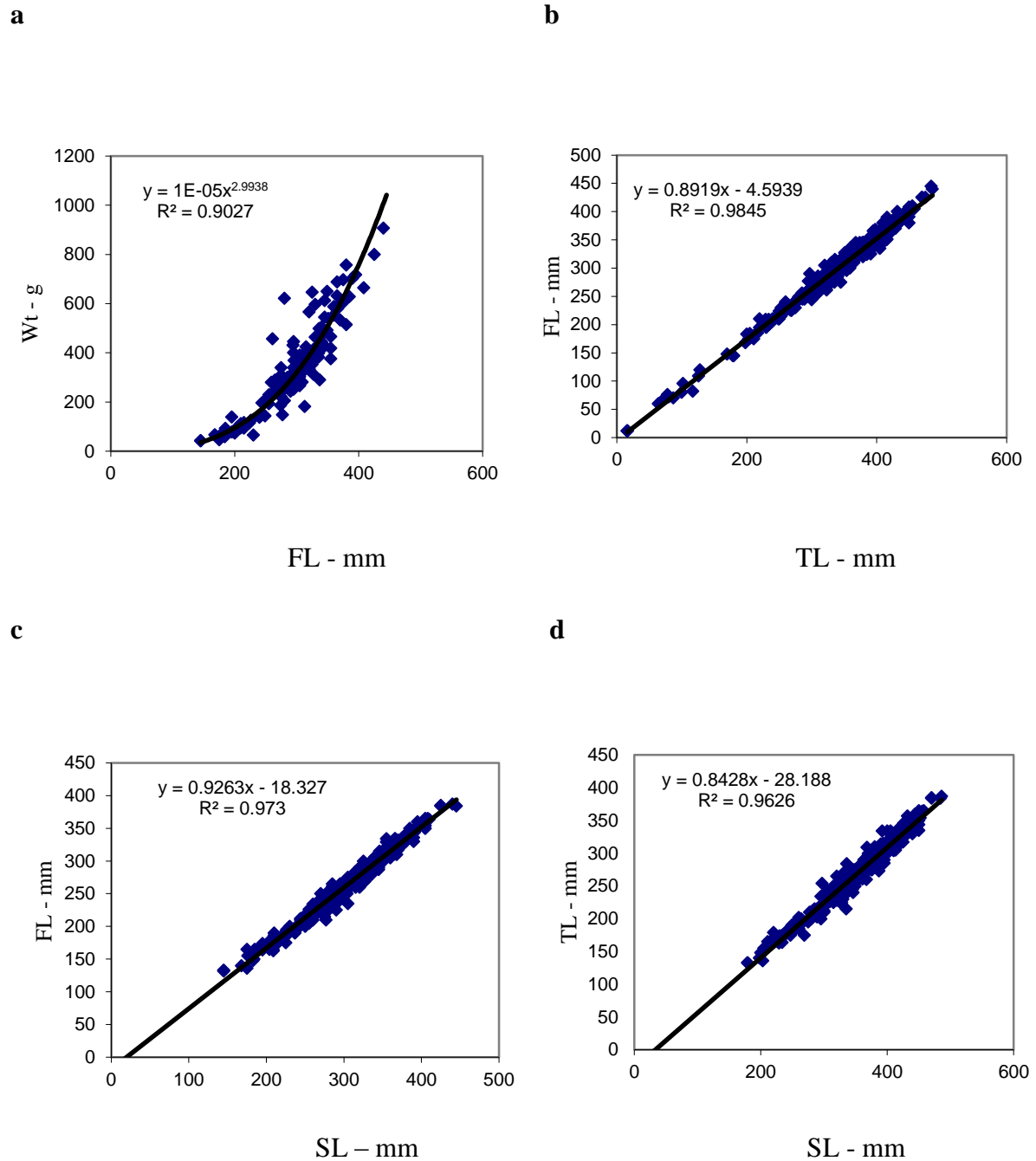
Fig.4.11a, b, c and d   Morphometric data collected in length and weight indicating the relations between (a) Fork Length: Weight (g); (b) Fork Length: Total Length; (c) Fork Length: Standard Length and (d) Total Length: Standard Length.
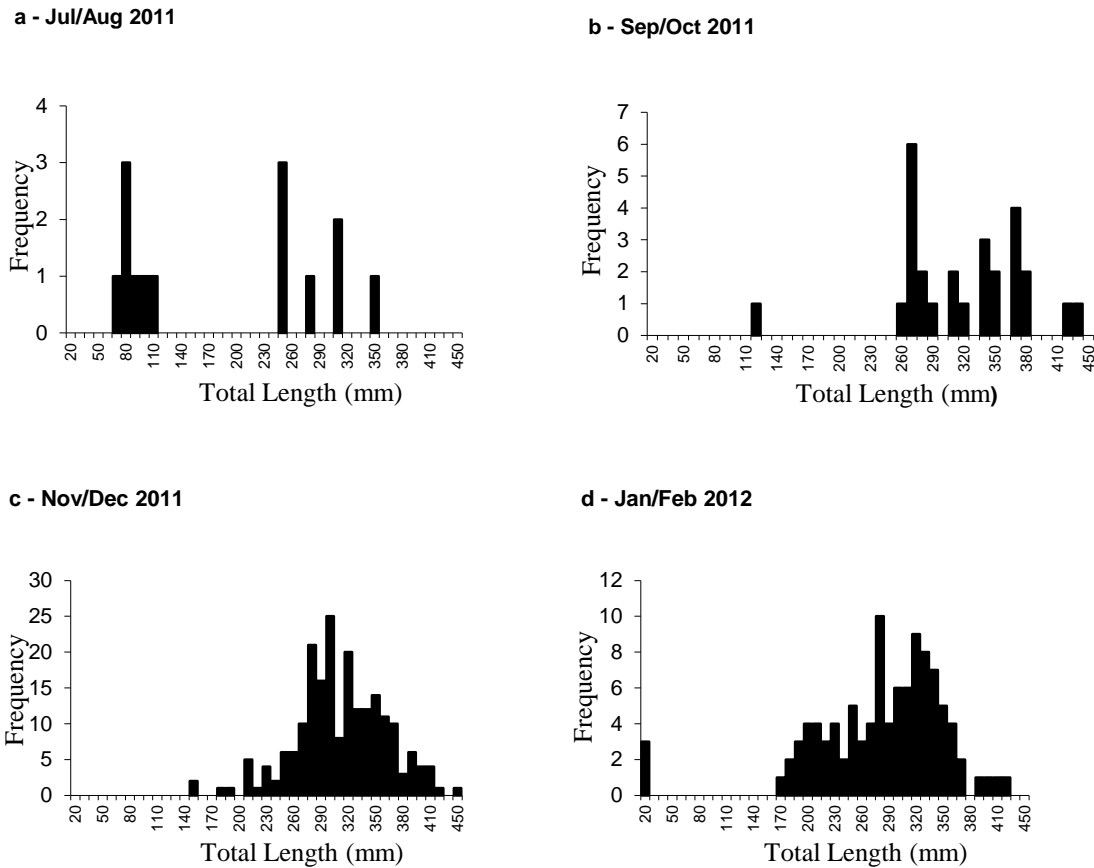
Fig. 4.12a, b, c and d Graphs indicating the frequency of capture of total length (mm) classes every two months, between July 2011 and February 2012.

4.4. Discussion

Using the unified invasion framework proposed by Blackburn *et al.,* (2011) Africa's first loricariid invasion could be categorized as a stage "D2" invasion: "*self-sustaining population in the wild* (Figure 4.13) *, with individuals surviving and reproducing a significant distance from their original point of introduction"* (Blackburn *et al.,* 2011). The only barrier to it becoming a fully invasive species (stage "E") is its inability to cross catchments without human assistance.

It is obvious that legislative prevention mechanisms (Anonymous, 1994) failed to stop the importation of this species and its eventual escape from captivity was inevitable given its proven establishment ability on several continents. The consequences of this invasion in the recipient ecosystem are not yet known but due to negative impacts reported elsewhere (Mendoza *et al.,* 2009), management action is necessary. The Nseleni and Mhlathuze rivers are relatively large rivers in a South African context, which makes the eradication of *P.*

disjunctivus neither logistically nor economically feasible and the management measures containment and mitigation suggested by Blackburn *et al.,* (2011) for stage D invasions is the most appropriate response strategy.

The effective management of non-native fishes begins with preventing introduction, as this optimizes the potential to minimize subsequent adverse effects and their associated costs (Myers *et al.,*, 1998, 2000; Smirnoff, 2002 In: Gozlan *et al.,*, 2010) and is complementary to the precautionary principle (Sandin, 1999; Foster *et al.,* 2000 In: Gozlan *et al.,*, 2010). Finnoff *et al.,* (2007) suggests that although managers might intuitively be expected to choose the prevention of introductions as their preferred form of management, Finnoff *et al.,* (2007), argues that instead managers frequently wait until non-native species have been introduced and only then act to limit their effect. This relates to a perception that post-introduction management is a safer choice than prevention because its productivity is relatively less risky (Gozlan, 2008).

To contain the *P. disjunctivus* population in the Mhlathuze and Nseleni rivers and mitigate against further spread it is suggested an education campaign to inform the general public on the dangers associated with fish introductions. Given the taxonomic complexity of this group of fishes it is suggested that the trade of *P. disjunctivus* be prohibited and that the trade in, at least, closely related Loricariid species with proven invasion histories elsewhere (e.g. *H. plecostomus* and *P. pardalis*) should also be discouraged.

CHAPTER 5

ISOTOPIC NICHE WIDTH COMPARISONS IN THE NSELENI RIVER SYSTEM: RESOURCE COMPETITION BETWEEN INDIGENOUS AND INVASIVE SPECIES

5.1 Introduction

Freshwater ecosystems are particularly vulnerable to invasion, as they represent biogeographic islands whose ecological space is unsaturated with native species and therefore more likely to favour the establishment of invaders (Oberdorff *et al*., 1997; Rahel, 2002; Leprieur *et al*., 2006; Strayer, 2010; Kadye and Booth, 2012). Ecological impacts may include behavioural shifts in native species, alteration of habitat, alteration of ecosystem food webs and trophic dependencies and in some cases extirpation of native biota (Cambray, 2003a, 2003b; Simon and Townsend, 2003; Grabowski *et al*., 2005). Chapter 3 confirmed that the alien invasive snail *Tarebia granifera* has been found throughout the Nseleni River system in varying numbers, utilising different depths of the river system, with significantly higher abundances within shallower regions of the river. This suggests that shallower regions are preferred habitat for *T.granifera* and its high rates of abundance indicate it may be competing with indigenous invertebrates for food. The invasion of this alien snail may thus pose potential threats to aquatic biodiversity in the Nseleni River system; however its effects on indigenous species have not been quantified.

Appelton *et al*., (2009) suggest from anecdotal reports and observations, that in KwaZulu-Natal, the indigenous thiarid *Melanoides tuberculata* is becoming less common and pressure, from the spread of *T. granifera,* particularly at high densities, is a possible explanation. Like *T. granifera*, *M.tuberculata* is parthenogenetic and ovoviviparous and grows to a similar size. Data from several habitats where the species occur sympatrically, shows that *T. granifera* always becomes numerically dominant (Appelton *et al*., 2009). In view of its spread into the St Lucia estuary system and other estuaries and lagoons along the KwaZulu-Natal coast, *T. granifera* is likely to impact on another indigenous thiarid, the poorly known *Thiara amarula* (Appelton *et al*., 2009). Both *T. amarula* and *T. granifera* occur in the Nhlabane Estuary (a mere 26km's from the Nseleni River system) and in July 2006, the latter was present at between 2000 and 6000 m^{-2} while only two specimens of the former were found. Previous collections at the same site showed *T.amarula* to be fairly common, and its growing presence

in South Africa suggests that studies on the ecological impact of *T. granifera* are urgently needed (Appelton *et al*., 2009).

Top-down effects of grazers (such as *T. granifera*) are known to be significant in marine and freshwater ecosystems (Pillay *et al*., 2009), and preliminary measurements undertaken *in situ* at the St Lucia Estuary, by Miranda *et al.,* (2011) indicated that *T. granifera* had relatively high gut pigment contents, indicating that although it was reported as being a detritivore by Larned *et al*. (2001), it may also significantly impact on microphytobenthos (MPB) biomass, and thus potentially compete with indigenous species for food resources (Miranda *et al.,* 2011). Indeed, *T. granifera*'s feeding impact can be deleterious to indigenous fauna due to its ability to utilize large amounts of MPB (March and Pringle, 2003) as well as detritus and coupled with very high feeding rates (Miranda *et al*., 2011) can potentially cause significant top-down and cascading effects within an ecosystem. The invasion by this alien snail may thus pose potential threats to aquatic biodiversity in the Nseleni River system and its potential impacts on indigenous species need to be quantified.

In comparison, very little dietary work has been done on the invasive armoured sailfin catfish *Pterygoplichthys disjunctivus.* Chapter 4 confirmed their establishment in the Nseleni River system and identified this population as a D2 invasion according to the Blackburn *et al*. (2011) unified framework for describing biological invasions (see Jones *et al*., 2013 and Chapter 4). Economically, the introduction of sailfin catfish has caused numerous problems, particularly within subsistence fisheries, where established populations have resulted in damage to aquaculture cages and fishermen's nets in the Philippines and Mexico (Chavez *et al*., 2006; Wakida-Kusunoki *et al*., 2007; Levin *et al*., 2008), and in Taiwan, where fishermen blame them for decreasing marketable fish in the river systems, and for the destruction of fishing equipment (Wu *et al*., 2011). Very little is known however, about the actual ecosystem impacts of loricariid invasions. Numerous studies suggest potential detrimental effects including increased turbidity (Chavez *et al*. 2006; Nico, 2010), bank destabilization due to tunnelling (Devick, 1989; Hoover *et al*., 2004; Page and Robins, 2006; Nico *et al*., 2009), predation of native fish eggs (Cook-Hildreth, 2009) and alteration of food web dynamics (Nico and Martin, 2001). Anecdotal data from Puerto Rico in the Caribbean Sea, mentions that piscivorous birds attempting to consume sailfin catfish are occasionally strangled (Bunkley-Williams *et al*., 1994). Page and Robins (2006) suggested that large populations of *P. disjunctivus* can significantly alter the ecology of a water body by reducing

the amount of energy available to other herbivores such as aquatic insects and other arthropods, and in Sri Lanka, the scraping habits of these invasive fish may be changing habitat quality, leading to detrimental effects on co-occurring species (Amarasinghe, *et al.,* 2006). Similarly, Levin *et al*., (2008) reported that the introduction of sailfin catfish in the USA has resulted in the disruption of aquatic food chains and declines in the abundance of native species. The U.S. Fish and Wildlife Service's Ecological Risk Screening Summary (2012) indicated that *Pterygoplichthys* spp. may compete for resources with native fish, which is supported by work done by Nico (2006) showing that high abundances of *P.multiradiatus* in Hawaii may have resulted in competition with indigenous stream species for food and space. *Pterygoplichthys* spp. have also been recorded in India, and are blamed for creating serious negative impacts on periphyton feeding and bottom spawning fishes (Krishnakumar *et al*., 2009). Furthermore *Pterygoplichthys* spp. are believed to displace several species of minnow in Texas, including the Federally threatened and 'Vulnerable (VU)' Devils River minnow (Cohen, 2008). These data suggest direct food competition between *P. disjunctivus* and indigenous fauna, however, this has been difficult to quantify.

Quantifying the impacts of invasive species can be challenging due to the complexity of ecological interactions (Miranda and Perissinotto, 2012), particularly in aquatic ecosystems. Recently, stable isotope analysis have been shown to be a powerful tool for tracking changes in trophic structure and energy flows within a system that can contribute to the further understanding of how an ecosystem can be affected by indigenous and non-indigenous species (Post, 2002; Caut *et al*., 2006). Stable isotope analysis also provides a time-integrated view of trophic connectivity, whereas traditional gut content analysis can only reveal snapshots of feeding activity (Hill *et al*., 2006; German and Miles, 2010; Miranda and Perissinotto, 2012). As such, stable carbon (δ^{13}C) and nitrogen (δ^{15}N) isotope ratios are increasingly used to provide time-integrated information about feeding relationships and energy flow through foodwebs (Peterson and Fry, 1987; Kling *et al*., 1992; Cabana and Rasmussen 1994; Vander Zanden and Rasmussen, 1999). The basic principle of stable isotope analysis uses the abundance of stable isotopes in nature to follow pathways of organic matter through successive trophic levels (DeNiro and Epstein 1978; DeNiro and Epstein 1981; Fry and Sherr 1984) and these isotope ratios are conserved up through the trophic food web, with predictable isotopic shifts (or fractionation) for every trophic step. δ^{13}C values of consumers for example, become 1‰ enriched relative to their food with every step up the trophic food web (Rau, 1980), whereas consumer δ^{15}N values enrich relative to their food by

3–4‰ (DeNiro and Epstein, 1981; Minagawa and Wada, 1984; Peterson and Fry, 1987; Cabana and Rasmussen, 1994; Vander Zanden and Rasmussen, 1999). Because $\delta^{13}C$ values are conserved up the food chain but vary at the base, the $\delta^{13}C$ of aquatic consumers can provide information about the energy (food) sources of higher consumers (Vander Zanden and Rasmussen, 1999) while $\delta^{15}N$ values provide time-integrated indicators of trophic position, based on the pathways of energy flow (Fry, 1988; Kling *et al.*, 1992; Cabana and Rasmussen 1994; Vander Zanden and Rasmussen, 1999). Because $\delta^{13}C$ and $\delta^{15}N$ values isotopes can vary both temporally and spatially, they can be used to conceptualize trophic niches among communities and habitats (Bearhop *et al.*, 2004; Layman *et al.*, 2007; Newsome *et al.*, 2007; Kadye and Booth, 2012). Stable isotopes can therefore provide a vigorous and synthetic approach to predicting the invasion impacts of non-indigenous species and the degree of dietary competition pressure felt by endemic species.

Using stable isotope analysis this chapter will focus on investigating the hypothesis that the invasive snail *Tarebia granifera* and the invasive fish *Pterygoplichthys disjunctivus* are directly competing with indigenous fauna in the Nseleni River system for dietary resources.

5.2 Material and Methods

Although previous chapters have divided the Nseleni River system into three different sections, this chapter will consider the Nseleni and Mposa rivers as one ecosystem for the following reasons: (1) fish are highly mobile and can migrate between biomes (see Chapter 4), (2) physiochemical parameters were very similar throughout the system (see Chapter 2) and (3) *Tarebia granifera* are also mobile throughout the system by "hitching a ride" via *Eichhornia crassipes* which can often be wind dispersed (see Chapter 3; R. Jones, pers. obs.).

5.2.1   Sample Collection

Isotope samples were collected from numerous taxa (Table 5.1) over a two week period in February 2012, with the exception of *Pterygoplichthys disjunctivus* samples, which were collected over 12 months from March 2011 – March 2012. In KwaZulu-Natal, freshwater gill netting is illegal and no permits are issued for the use of gill nets, unless it's for research purposes. To enable the author to capture freshwater fish from the Nseleni River system, with the use of a gill net, permit number: OP 4868/2011 was obtained. Fish muscle tissue

samples were taken from white muscle just before the caudal fin, above the lateral line. All flesh samples were scraped to remove skin and scales. All samples were then rinsed in distilled water, placed in labeled Eppendorf vials and oven dried for 48 hours at 50°C.

Each sample was then ground into a homogenous fine powder and weighed out (0.8-1.5 mg) into tin capsules. Prior to isotope analysis, a subsample of tissues were acidified using methods described by Cloern *et al*. (2002) and Jacob *et al*. (2005). No effervescence was apparent upon the addition of 1N HCl under a dissection microscope (Olympus SZ51) at 15x magnification and accordingly no acid washing was performed on any sample. The δ^{13}C and δ^{15}N signatures of all samples were determined using a Europa Scientific 20-20 IRMS interfaced to an ANCA SLElemental Analyser at the IsoEnvironmental Laboratory, Dept.Botany at Rhodes University, Grahamstown, South Africa. All δ^{13}C and δ^{15}N values were reported as *‰* vs Vienna PeeDeeBelemnite (VPDB) and air respectively and normalized to internal standards calibrated to the International Atomic Energy reference materials (IAEA-CH6 for δ^{13}C and IAEA-N2for δ^{15}N). Results are expressed in standard delta notation, δX ($[R_{sample}/R_{standard}]$-1) x1000, where X is the element in question and R is the ratio of the heavy over the light isotope. Precision of replicate determinations for both δ^{13}C and δ^{15}N were ± 0.06 and ± 0.09*‰* respectively.

5.2.2 Data analysis

5.2.2.1 Stable isotope metrics and niche widths

New developments in isotope ecology have provided statistical Bayesian frameworks for investigating variation in isotopically defined groups (Turner *et al*., 2010; Hammerschlag-Peyer, 2011). Layman *et al*. (2007) proposed a series of quantitative stable isotope metrics to define the trophic ecology and structure of a system using δ^{13}C and δ^{15}N population values. Jackson *et al*., (2011) refined these techniques and bolstered their ability to cope with sample size disparities (see also Jackson *et al*., 2012). Using δ^{13}C and δ^{15}N values of individual species (n > 6) within the Nseleni River population, these quantitative stable isotope metrics were calculated to compare the trophic ecology, structure and niche widths between indigenous and invasive fishes and snails. All metrics were bootstrapped (n = 500; indicated with subscript '$_b$') based on the minimum sample size in the data set (n = 6). These metrics included;

(1)   Nitrogen (dNr$_b$) and carbon (dCr$_b$) ranges – the distance between the most depleted and enriched values of δ^{15}N and δ^{13}C, indicating the total nitrogen and carbon range exploited by a species.

(2)   Mean distance to the centroid (CD$_b$) – the mean Euclidean distance of each individual to the δ^{15}N- δ^{13}C centroid for that species, providing a description of trophic diversity.

(3)   Standard deviation of mean nearest neighbour distance (SDNND$_b$) – the mean of Euclidean distances to each point's nearest neighbour in bi-plot space, providing a measure of trophic evenness.

(4)   Standard ellipse area (SEAc) – sample size corrected standard ellipse areas which are bivariate equivalents to standard deviations in univariate analysis, providing a measure of mean core isotopic niche. This approach identifies differences in niche widths of 'typical' members of a population and is robust to sample size variation (but may not encompass outlier individuals in isotopic space; see Jackson *et al*., 2011, Layman and Allgeier, 2012).

The calculation of SEAc subsequently allows for a measure of the degree of niche overlap which can then be used as a quantitative measure of dietary similarity between populations (Jackson *et al*., 2012; Jackson and Britton, 2013). Isotopic dietary overlap (%; absolute limit of 100% indicating complete overlap) between species of fish and species of snail were quantified according to Jackson *et al*., (2012). See Jackson *et al.,* (2011; 2012) for detailed methodology and Layman *et al.*, (2007) for original descriptions of stable isotope metrics. All metrics were completed using the Stable Isotope Bayesian Ellipses in R (SIBER; Parnell *et al*., 2010) model and the Bayesian Stable Isotope Analysis in R program (SIAR; Jackson *et al*., 2011) in the R environment (R Development Core Team, 2007; available at http://cran.r-project.org/web/packages/siar/index.html).

5.2.2.2 Isotopic mixing models

SIAR (Parnell *et al*., 2010) provided an estimate of the relative contribution of various dietary resources assimilated by the invasive *Pterygoplichthys disjunctivus* and *Tarebia granifera*. This model integrates variability in resource and consumer isotope values and separate single group models were run for the invasive fish and snail. Probability estimates of the relative

contribution of dietary resources assimilated by the invasive *Pterygoplichthys disjunctivus* and *Tarebia granifera* were obtained using bivariate, separate single group mixing models in the Stable Isotope Analysis in R package (SIAR; Parnell *et al.*, 2010), incorporating elemental source concentrations as per Inger *et al*. (2010). All food resources used in the model had sufficiently distinct isotope values. Fractionation factors between resources and consumers for *P. disjunctivus* were assumed to be -0.1 ± 0.15 for δ^{13}C and 5.01 ± 0.15 (German and Miles, 2010) and 0.4 ± 0.17 for δ^{13}C and 2.3 ± 0.28 for *T. granifera* (McCutchan *et al.* 2003).

5.3 Results

Primary producers sampled from the Nseleni River system had highly variable isotopic ranges; with *Azolla filiculoides* (-29.82 ‰) and *Eichinochloa pyramidalis* (-12.32 ‰) showing the minimum and maximum range for δ^{13}C and the *Nymphaea* sp. (7.85 ‰) and *Ceratophyllum demersum* (19.12 ‰) showing the minimum and maximum range for δ^{15}N (Figure.5.1). The isotopic ranges of fish sampled were much smaller; with *Gilchristella aestuaria* (-30.96 ‰) and *Clarias gariepinus* (-24.13 ‰), and *Pterygoplichthys disjunctivus* (12.66 ‰) and *Gilchristella aestuaria* (17.76 ‰) showing the minimum and maximum ranges for δ^{13}C and δ15N respectively (Figure 5.1).
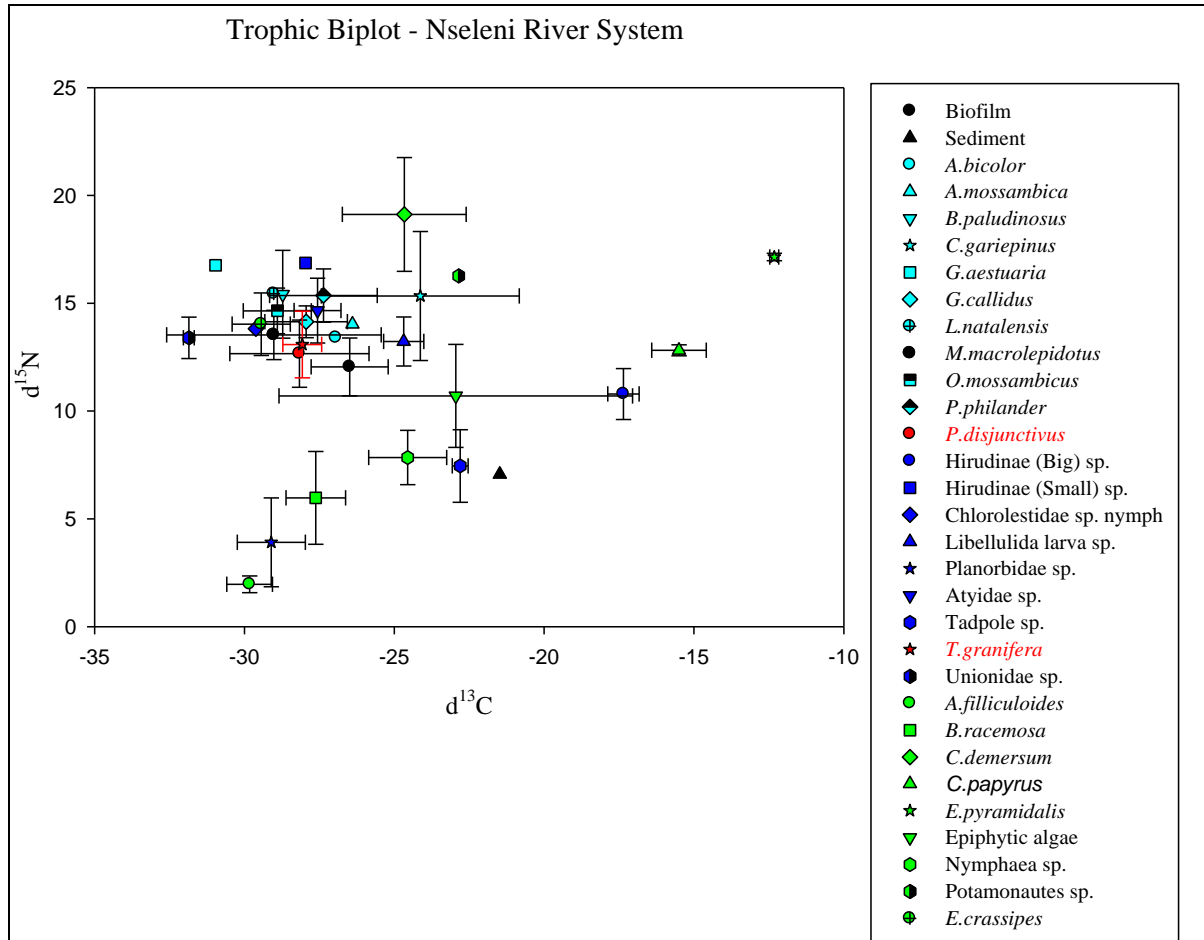
Fig. 5.1 δ^{13}C and δ^{15}N biplot of organisms collected in the Nseleni River system.

5.3.1 Stable isotope metrics

Stable isotope metrics described the invasive fish *P. disjunctivus* as occupying a medium size niche with a more diverse diet than four (*Barbus paludinosus*, *Glossogobius callidus*, *Marcusenius macrolepidotus* and *Pseudocrenilabrus philander*), but less than two (*Clarias gariepinus* and *Oreochromis mossambicus*) indigenous fish species (Table 5.1; Figure 5.1). The medium ranges of dNR$_b$ (7.14) and dCR$_b$ (9.07) for the invasive fish indicate that it utilizes a wider range of food resources and trophic levels than the majority of indigenous fish, with the exception of *Clarias gariepinus* and *Oreochromis mossambicus* (see Table 5.1; Layman *et al*., 2007). This is consistent with the SEAc value for *P. disjunctivus* describing a large isotopic niche space (10.8; Table 5.1). Concurrently, a medium CD$_b$ value (2.34) for the invasive fish species describes medium trophic diversity, with three indigenous species possessing higher diversity and three possessing lower diversity (Table 5.1). SDNND$_b$ values for the invasive fish were much lower than any of the indigenous fish (Table 5.1) representing a lower degree of trophic evenness. Niche overlaps (ie % overlap of standard

ellipse areas (SEAc)) between the invasive fish and its indigenous counterparts were very low, with the majority of shared food resources being <26.00 %, with the exception of *Marcusenius macrolepidotus*, which had a moderate niche overlap with *P. disjunctivus* of 48.02%. Interestingly, the niche overlap between the invasive fish and the invasive snail (*T. granifera*) was high at 85.59% (Table 5.1; Figure 5.2).

Comparison of stable isotope metrics between the invasive and indigenous snail showed similar dNR_b and dCR_b ranges. The isotopic niche area occupied by *T. granifera* (SEAc = 2.97) was larger than the indigenous *Planorbella* species (1.73) and *T. granifera* possessed a lower degree of trophic diversity (CD_b = 1.40) and evenness ($SDNND_b$ = 0.25) (Table 5.1). The niche overlap between the invasive and indigenous snail was effectively zero ($1.02E^{-13}$%), indicating no shared food resources (Table 5.1; Figure 5.2).

For a more comprehensive description of the ecological traits associated with the stable isotope metrics described in Table 5.1, see Layman *et al*., (2007); Jackson *et al.*,(2011) and Jackson and Britton (2013).

Table 5.1 Species, tissue type, sample size, and their mean stable isotope metrics where dNR_b = $\delta^{15}N$ range; dCR_b = $\delta^{13}C$ range; CD_b = mean distance to centroid; $SDNND_b$ = SD mean nearest neighbour distance; SEA_c = standard ellipse area; % overlap = the percentage of *Pterygoplichthys disjunctivus* niche area (SEA_c) which overlaps with indigenous fish and mollusc species * or % overlap = the percentage of *Tarebia granifera* niche area (SEA_c) which overlaps with the indigenous mollusc species **. Numbers in parentheses represent the 2.5 to 97.5 percent quantile range (see Jackson and Britton (2013)).

Species	Tissue type	n	dNR_b	dCR_b	CD_b	$SDNND_b$	SEA_c	% Overlap
Fish								
Barbus paludinosus	Muscle tissue	7	4.52 (2.93-5.10)	2.56 (1.36-2.90)	1.87 (1.21-2.35)	0.93 (0.32-1.46)	6.3	17.15*
Clarias gariepinus	Muscle tissue	9	10.24 (5.81-11.91)	11.06 (4.94-12.95)	3.21 (1.66-5.06)	1.07 (0.31-2.30)	9.14	9.09E^{-17}*
Glossogobius callidus	Muscle tissue	7	1.57 (0.64-2.13)	3.03 (1.07-3.98)	1.13 (0.55-1.72)	0.66 (0.00-1.25)	2.43	18.89*
Marcusenius macrolepidotus	Muscle tissue	24	4.54 (2.52-5.68)	12.57 (6.42-15.92)	2.76 (1.70-4.10)	0.97 (0.24-2.03)	8.83	48.02*
Oreochromis mossambicus	Muscle tissue	18	10.21 (5.81-11.91)	11.00 (4.94-12.95)	3.19 (1.63-5.03)	1.07 (0.31-2.30)	9.11	25.05*
Pseudocrenilabrus philander	Muscle tissue	21	4.16 (2.84-4.72)	6.27 (3.23-7.72)	1.77 (1.19-2.36)	0.6 (0.26-0.98)	5.86	8.95E^{-16}*
Pterygoplichthys disjunctivus	**Muscle tissue**	**42**	**7.14 (4.70-8.36)**	**9.07 (6.75-9.72)**	**2.34 (1.83-2.88)**	**0.51 (0.31-0.80)**	**10.8**	**-**
Anguilla bicolor	Muscle tissue	1	-	-	-	-	-	-
Anguilla mossambica	Muscle tissue	1	-	-	-	-	-	-
Gilchristella aestuaria	Muscle tissue	1	-	-	-	-	-	-
Labeobarbus natalensis	Muscle tissue	1	-	-	-	-	-	-
Invertebrates								
Tarebia granifera	**Whole body, pooled (n=3)**	**17**	**4.54 (3.80-4.73)**	**2.13 (1.55-2.29)**	**1.40 (0.91-1.79)**	**0.25 (0.14-0.35)**	**2.97**	**85.59***
Planorbella sp.	Whole body, pooled **(n=3)**	7	3.9 (0.64-5.45)	2.19 (0.44-2.78)	1.58 (0.44-2.63)	1.08 (0.59-2.04)	1.73	1.78E^{-11}* 1.02E^{-13}**
Dragonfly larva sp.	Whole body	3	-	-	-	-	-	-
Dragonfly sp.	Whole body	2	-	-	-	-	-	-
Potamonautes **sp.**	Muscle tissue	1	-	-	-	-	-	-

Freshwater shrimp sp.	Whole body, pooled (n = 5)	6	-	-	-	-	-	-
Plants								
Azolla filiculoides	Degrading plant tissue	3	-	-	-	-	-	-
Barringtonia racemosa	Degrading plant tissue	6	-	-	-	-	-	-
Ceratophyllum demersum	Degrading plant tissue	6	-	-	-	-	-	-
Echinochloa pyramidalis	Degrading plant tissue	3	-	-	-	-	-	-
Eichhornia crassipes	Plant tissue	3	-	-	-	-	-	-
Epiphytic algae	Plant tissue	6	-	-	-	-	-	-
Nymphaea **sp.**	Degrading plant tissue	6	-	-	-	-	-	-
Cyperus papyrus	Degrading plant tissue	3	-	-	-	-	-	-
Other								
Biofilm	-	4	-	-	-	-	-	-
Sediment	-	1	-	-	-	-	-	-
Amphibian								
Tadpole sp.	Whole body	2	-	-	-	-	-	-
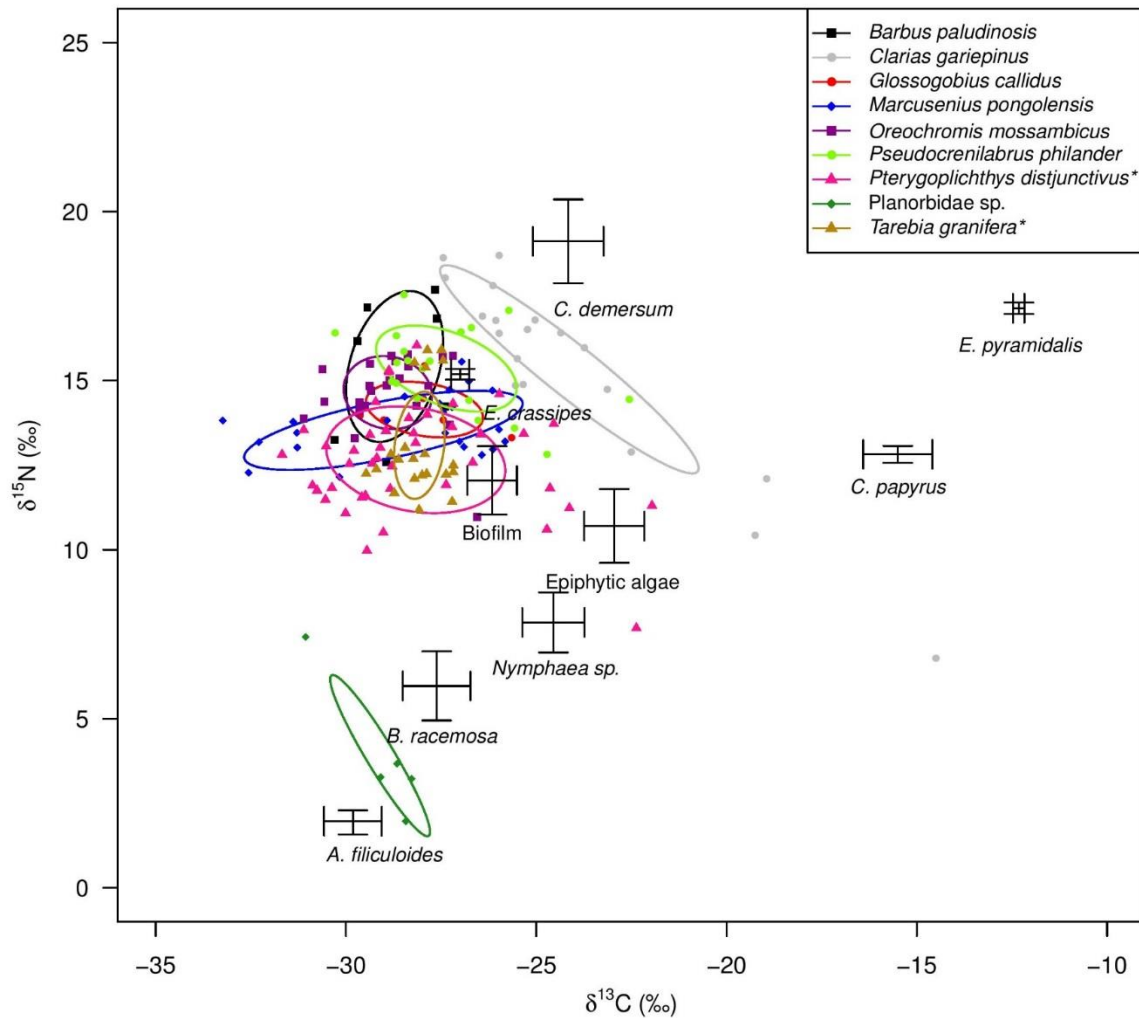
Fig.5.2 Niche widths (SEAc) as determined via SIBER metrics (Jackson *et al*., 2011; 2012) for the invasive and indigenous fish and snails. Convex hulls of total niche widths are depicted with dashed black lines, whereas corrected standard ellipse areas (SEAc) representing core isotopic niche space of each sampled organism, are depicted with solid coloured lines (Layman and Allgeier, 2012). Boxes indicate the invasive fish and snail.

5.3.2 Isotope mixing models

SIAR mixing models indicated that detritus from the freshwater mangrove *Barringtonia racemosa* likely contributes the largest proportion (on a scale of 0.00 – 1.00) to *Pterygoplichthys disjunctivus* diet (0.12 – 0.58), with proportional contributions of remaining sources ranging between 0.00 - 0.21 (Figure 5.3A). Conversely, SIAR models showed the diet composition of *Tarebia granifera* was more mixed, with detritus from the invasive plant *Eichhornia crassipes* contributing the largest dietary proportion (0.24 – 0.60), followed by

detrital *Azolla filiculoides* (0.09 – 0.33) and detrital *B. racemosa* (0.00 – 0.30). Remaining sources contributed proportionally less, with all inputs falling below 0.20 (Figure 5.3B).
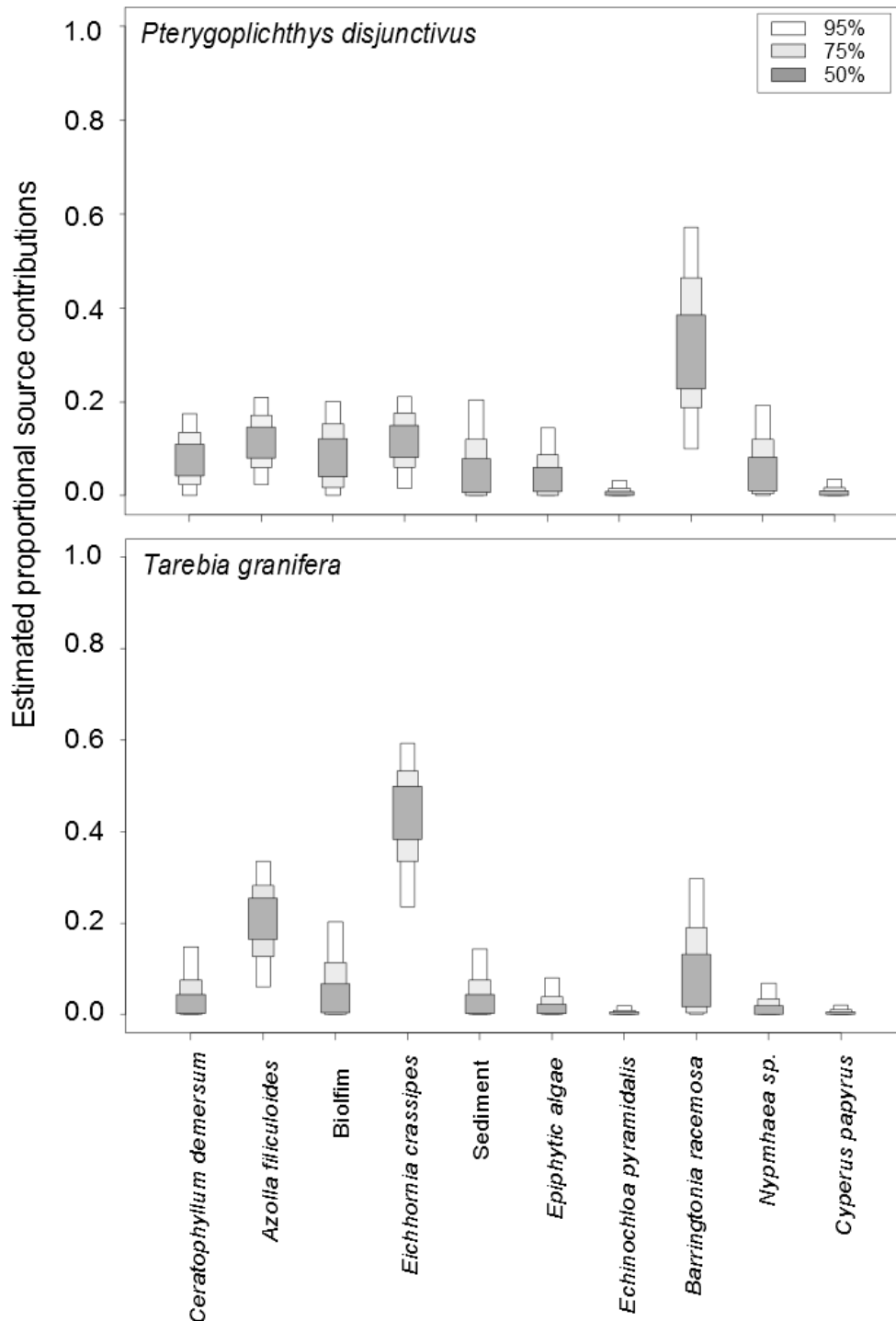
Fig. 5.3 Estimated proportional source contributions (with 50; 75; and 95% credibility intervals) as determined by SIAR (Parnell *et al.,* 2010) to *Pterygoplichthys disjunctivus* and *Tarebia granifera* diet.

5.4 Discussion

The hypothesis that both *Pterygoplichthys disjunctivus* and *Tarebia granifera* are directly competing with indigenous fauna for dietary resources is not supported by the results from this study.

Community-wide metrics reflect specific aspects of trophic structure within a food web, with the trophic niches of individual species determining metric values (Layman *et al.,* 2007). This allows for the extent of resource sharing between sympatric species to be quantified more easily (Jackson and Britton, 2013). Using these metrics, in terms of competition between the exotic and indigenous fish species in the Nseleni river system, there does not appear to be any direct competition for dietary resources.

The stable isotope metric values for the invasive *P. disjunctivus* were consistently midrange when compared to those of the indigenous species, with one exception (SDNND$_b$). The dNR$_b$, dCR$_b$ and CD$_b$ metrics for *P. disjunctivus* were higher than some indigenous species (e.g. *G. callidus* and *B. paludinosus*) but less than others (e.g. *C. gariepinus* and *O. mossambicus*), describing the utilization of a medium range of resources and trophic levels and an intermediate level of trophic diversity when compared to its indigenous counterparts. The SDNND$_b$ metric for every indigenous fish however, was higher than for *P. disjunctivus,* suggesting that the invasive fish is less evenly spread in isotopic space than any of the indigenous fishes. It is possible that this high level of packing in isotopic space may be explained, in part, by the fluctuating resources theory of invasibility (Davis *et al*., 2000; Davis and Pelsor, 2001). Davis *et al*., (2000) suggests that successful invasions rely on the simple assumption that an invading species will have a higher degree of invasion success if it does not encounter intense competition from resident species for necessary resources. This unevenness of isotopic spacing may describe an entire fish species exploiting a series of resources otherwise unavailable to indigenous fish fauna. Despite the intermediate level of resource use, trophic level use and trophic diversity and the lowest measure of trophic evenness, the invasive *P. disjunctivus* had the largest isotopic niche width (SEA$_c$) of all the species sampled.

Comparatively, the invasive *T. granifera* also had a bigger isotopic niche width (SEA$_c$) than the indigenous snail and the other metrics described a species feeding on more trophic levels than the indigenous *Planorbella* sp., but with fewer food resources and a marginally lower

degree of trophic diversity. The markedly smaller measure of evenness, however, may also be attributable to the exploitation of resources not available to indigenous fauna.

Overall, despite the large niche widths seen for both invasive species, the other intermediate stable isotope metric values suggest that neither *P. disjunctivus* nor *T. granifera* have direct dietary competition with indigenous fauna. This is possibly due to the fact, that although they may have more food sources and eat on more trophic levels than some indigenous fauna, and are more diverse and less evenly spread, this is not true for all, suggesting that the effects of these invasive species maybe mediated by its accessibility to resources. This would therefore suggest that they are not likely to have any immediate detrimental effects on indigenous fauna, but longer term indirect effects, which will be covered in more detail below, are potentially problematic.

Pterygoplichthys disjunctivus showed minimal levels of direct dietary competition with indigenous fish species in the Nseleni River system. With the exception of *Marcusenius macrolepidotus* (% SEA$_c$ overlap = 48.02%, a moderate value of competition (as described by Knickle and Rose, 2013)), the invasive fish displayed less than a 26% niche overlap with all other fish species, suggesting that *P. disjunctivus* likely occupies a different isotopic niche space than indigenous river inhabitants. Although information on proportional abundance of dietary items was not collected in this study, consumer isotopic ratios are directly linked to an organisms' diet and thus may be substantiated using Schoener's index of diet similarity (Schoener, 1968); where 0 = no overlap and 1 = complete overlap between two species. Typically, values > 0.6 (> 60%) infer ecologically significant dietary overlap (Schoener, 1968; Layman and Allgeier, 2012) and the dietary overlap (SEAc) between the invasive *P. disjunctivus* and any of the indigenous fish from the Nseleni River system was less than 60.00% (<0.60) in all comparisons. As such, *P. disjunctivus* is clearly not competing directly for food with indigenous species, exploiting instead, a food resource currently under-utilized by or unavailable to the indigenous fish community and may help to explain the recent explosion of *P. disjunctivus* in the Nseleni River system. This exploitation of under-utilized resources has been recently identified as one of the driving factors behind successful invasions (Davis *et al*., 2000; Davis and Pelsor 2001).

Similar results were found in the niche width (SEA$_c$) comparisons between the invasive snail *T. granifera* and the indigenous *Planorbella* species, with a complete lack of dietary overlap

between the two gastropods. SIBER analyses show that these two species occupy entirely different isotopic niche spaces and in particular, they feed at different trophic levels, and thus utilize entirely separate food resources. This is perhaps not surprising as the indigenous *Planorbella* species is a pulmonate gastropod and likely occupies a separate microhabitat (aquatic vegetation) to the invasive *T. granifera* (substrate grazer) (Seuffert and Martin 2010), however there is some evidence to suggest that thiarids and sympatic pulmonates can have similar diets (Madsen, 1992) and in some cases can outcompete native pulmonates, despite their different microhabitats (Perez *et al*., 1991). With the clear lack of isotopic niche overlap between the invasive *T. granifera* and the indigenous *Planorbella* sp. found in this study, any negative effects on *Planorbella* specie populations are more likely the result of competition for space, rather than competition for dietary resources. Some recent work completed by Miranda and Perrissinotto (2012) reported that although the diet of *T. granifera* is highly variable, (microphytobenthos, filamentous algae, detritus, decaying and/or sedimentary organic matter), it differs significantly from that of sympatric indigenous species. Furthermore, there is some evidence to suggest that chemical cues, possibly water soluble pheromones (Gomez *et al*., 1990) or biogenic volatile organic compounds (VOC's; Raw *et al*., 2013), are secreted by *T. granifera*, which induce avoidance behaviour and negative taxis in heterospecifics (Perez *et al*., 1991; Raw *et al*. 2013), resulting in increases in thiarid competitive fitness and the displacement of native species (Raw *et al*., 2013). *Tarebia granifera*'s separate microhabitat, generalist diet, (although they can have a variable diet in the Nseleni River, they appear to be specializing on a previously unexploited food source) and its ability to repel indigenous snails through chemical cues may therefore explain the separate isotopic niche space (and even trophic level) occupied by this invasive gastropod relative to the indigenous *Planorbella* species in the Nseleni River.

The lack of direct competition for food resources between both invasive and indigenous fauna in this study is surprising based on the potential for ecological damage speculated or reported by numerous studies on both *P. disjunctivus* and *T. granifera* (Cambray, 2003a, 2003b; Simon and Townsend, 2003; Grabowski *et al*., 2005). These results are in contrast for example, with work on the Asian cyprinid, topmouth gudgeon *Pseudorasbora parva* (Temmnick and Schlegel, 1846), which is highly invasive in Europe. Previous dietary studies based on gut contents analysis suggested that the invasive *P. parva* exploits food resources which are shared with local indigenous fishes (Britton *et al*., 2010). A more recent stable isotope–based expanded further on the trophic interactions of this fish, showing that they

shared food resources with both the common roach *Rutilus rutilus* (Linnaeus, 1758) and common carp *Cyprinus carpio* (Linnaeus, 1758) (Jackson and Britton 2013), with negative growth consequences for *R. rutilus* (Britton *et al.*, 2010). Jackson and Britton (2013) concluded that the stable isotope metrics of *P. parva* were consistently higher than any indigenous sympatric cyprinids, indicating that they occupied a larger niche area and had a more diverse diet than the native species of carp. Their study further suggests that there was the potential for *P. parva* to increase interspecific competition, and that it might occur in benthic-feeding species such as *Abramis brama* (Linnaeus, 1758) and *C. carpio*. This is currently not the case in the Nseleni River, where this study has shown that *Pterygoplichthys disjunctivus* is not directly competing with indigenous fish for food resources.

Interestingly, SEA$_c$ comparisons between the two invasive species show an ecologically significant dietary overlap of > 85%, indicating both the invasive *P. disjunctivus* and the invasive *T. granifera* are directly competing with each other for dietary resources, (but not with their respective indigenous counterparts). It is likely however, that there is strong indirect dietary resource competition as both *P. disjunctivus* and *T. granifera* and the majority of indigenous fish and snails occupy a similar range in $\delta^{13}C$ values (a descriptor of food source), and only really separate out based on $\delta^{15}N$ values. This suggests that the majority of the animals sampled in the Nseleni River system are ultimately dependent on the same food resources, but utilise the derived energy at different trophic levels. Although *P. disjunctivus* and *T. granifera* are both not in direct competition with their indigenous counterparts for the same food resources, their sheer numbers (see Chapters 3 and 4) are likely to be reducing the energy available to individuals further up the food chain, thus significantly altering the ecological food web. The results from this study therefore supports the suggestion by Page and Robbins (2006) that large populations of *P. disjunctivus* reduce the amount of energy available to other trophic levels and this limiting of energy to other food web members could have drastic, albeit indirect effects on fluvial population composition and structure.

As the isotopic niche space of *P. disjunctivus* and *T. granifera* overlap > 85%, are we then able to determine what food resources comprise the majority of their diet? Bayesian mixing models (SIAR) indicated that both diets are heavily dependent on riparian vegetation, including substantial contributions from water hyacinth, mangrove leaves and in some cases benthic invertebrates; however the largest single source contribution for *P. disjunctivus* was the freshwater mangrove *Barringtonia racemosa* and for *T. granifera* was *Eichhornia*

crassipes. Populations of both species are fortunate from a food source point of view in as much as *B. racemosa* is an indigenous species and are prolific amongst the riverine vegetation, both inside and outside of the protected area. Although *E. crassipes* is an invasive exotic and there is a management plan to control it, the management plan allows for a maximum of 10% coverage within the river system, as it has been recognised that while high nutrient levels prevail this plant will not be totally eradicated. There will therefore be for the foreseeable future always be a food source for *T. granifera*. *Pterygoplichthys disjunctivus* has recently been documented as grazing large amounts of algae from rocky and woody substrates (Hoover *et al*., 2004; Gibbs *et al*., 2013) and has been found to be very efficient at digesting the soluble components of detritus (German and Bittong, 2009; German and Miles, 2010). Investigations into *T. granifera*'s feeding dynamics also show that *T. granifera* can utilize large amounts of microphytobenthos in addition to detritus (Miranda *et al*., 2011) and can potentially cause significant top-down and cascading effects on the ecosystems food web.

Both the long and short term effects of ecosystem invasion by both *P. disjunctivus* and *T. granifera* are not yet well understood. However it is clear that unlike the situation reported by Nico (2006) in Hawaii and Cohen (2008) in Texas, in the Nseleni River system, there is clear lack of direct dietary competition between the invasive and indigenous species. There are however numerous other factors to consider when investigating invasive species impacts, particularly in terms of biodiversity. Firstly, abundance is a major concern; as previously mentioned, high numbers of invaders might restrict the energy available to higher consumers in the trophic food web, however increased numbers may also result in fewer young, reduced hatching and/or survivorship and slower growth rates in indigenous species (Appelton, 2003; Wu *et al*., 2011). Secondly, invasive species can play important functional roles in the nutrient dynamics of freshwater systems (Capps and Fletcher, 2013), with work done by McIntyre *et al*., (2008) showing that the aggregate excretion of *P. disjunctivus* populations can be sufficient to turn over the entire ambient pool of N in the water column. Both McIntyre *et al*., (2008) and Capps and Fletcher (2013) have recorded and reported on 'hotspots' or biogeochemical activity, pertaining to this fish. It is reported that this occurs as fishes attain high population densities, both indigenous and alien species, whereby they can generate areas of enhanced nutrient recycling rates and that this could influence primary productivity in both terrestrial and aquatic ecosystems. It is further suggested that for organisms to generate biogeochemical 'hotspots' within an ecosystem, their population densities must vary through space / and or time and that the contribution of the species to

nutrient remineralization rates must be significant relative to ecosystem demand. McIntyre *et al*., (2008) found that the aggregate excretion of fishes was sufficient to turn over the entire ambient pool of N in a water column. Capps *et al*., (2013) recorded that water samples collected within Loricariid aggregations had almost double the concentrations of N and P, compared to water samples collected away from the aggregation. Furthermore, they suggest that elevated ambient nutrient availability may locally alleviate some nutrient limitation and enhance algal growth and primary productivity immediately downstream of Loricariid aggregations. The question that follows is if *T. granifera* in high densities are also capable of causing 'hotspots', and together with high aggregations of *P. disjunctivus*, what are on the combined effects on the ecosystem? The above question is partially answered by Moslemi *et al*., (2012) concerning *T. granifera*. Although their study mainly concentrated on assemblages of *T. granifera* between open and closed river bank canopy, they reported that primary production in a Trinidadian lotic system can be co-limited by the availability of N and P. They further suggest that changes in relative and absolute availability of nutrients mediated by snail excretion may influence the identity of the nutrient that limits primary productivity, as well as the growth and community composition of primary producers, and that the impacts on basal resources may alter food web dynamics.

Pterygoplichthys disjunctivus in particular, has become a successful invader, due in part to its nest guarding behaviour (Gibbs *et al*., 2008), tolerance to hypoxia (Gibbs *et al*., 2008**)** and its ability to store large coelomic fat and can survive several days of desiccation in burrows dug into stream or riverbanks, displacing native fishes that would otherwise use the aquatic vegetation for spawning and refuge (Gibbs *et al*., 2008). Although Wu *et al*., (2011) further suggests that *P. disjunctivus* may predate on bottom-attached eggs of other fish, cause bank destabilization due to tunnelling and increased turbidity due to feeding behaviour. Therefore, direct competition for dietary resources is not the only concern when it comes to the impacts of invasive species on trophic food webs. Simberloff and Von Holle (1999) have come up with a term 'ínvasional meltdown'. They suggest that this term be used for the process by which a group of non-indigenous species (*T. granifera* and *P. disjunctivus*) facilitate each other's invasion in various ways, which will ultimately increase the likelihood of survival and/or of ecological impact.

CHAPTER 6

**CONTROL EFFORTS AGAINST THE DAMAGING INVASIVE ALIEN WEED
WATER HYACINTH ON THE NSELENI RIVER**

6.1   Introduction

Recent work suggests that invasive alien species are the second biggest threat to biodiversity after habitat destruction (e.g. Mooney and Hobbs 2000; Richardson and Van Wilgen 2004). Chapters three and four of this dissertation have shown that both the invasive *Tarebia granifera* and *Pterygoplichthys disjunctivus* occur in high densities and are well distributed throughout the Nseleni River system, however chapter five suggested that, through the use of stable isotope metrics and mixing models, there appears to be very little direct competition for dietary resources between *T. granifera* and *P. disjunctivus* and indigenous gastropods and fish. Despite a lack of direct food competition it is likely that both invasive species are impacting on indigenous communities in other ways, such as indirect competition or resource limitation through high invader abundances. Coetzee *et al*. (2014) demonstrated that the third major invader in the river system, water hyacinth (*Eichhornia crassipes*) has, since its introduction to the Nseleni River system in the 1970s, had a significant and measurable negative impact on the river's aquatic biodiversity and thus requires controlled intervention.

Eichhornia crassipes is a declared weed in South Africa and falls under the Conservation of Agricultural Resources Act (Act 43 of 1983), administered by the Directorate of Resource Conservation of the National Department of Agriculture. The Act states clearly that this weed must be controlled, with the South African Department of Water Affairs mandated to co-ordinate the control of water hyacinth and to execute measures in situations where the weed threatens state water works. In other cases it becomes the responsibility of the provincial and local water authorities (Jones, 2001).

Ad hoc control efforts to manage water hyacinth infestations were practiced on the Nseleni River between the late 1970s and 1994 by various interested and affected parties. By 1982, stretches of the Nseleni and Mposa rivers had 100% cover of water hyacinth and the KwaZulu-Natal Nature Conservation Service initiated regulated control of the weed. In 1984, a heavy flood alleviated the problem, as most of the water hyacinth was washed away before an aerial spraying operation could be implemented. Thereafter, little was done to the

remaining island populations of water hyacinth, because the decreased level of infestation was no longer considered a threat (Jones, 2001).

To co-ordinate the control efforts against water hyacinth on the Nseleni River, an Integrated Water Hyacinth Control Committee was formed in 1995. The first objective of this committee was to collate all the work that had previously been carried out on the water hyacinth infestation, the second objective was to formulate an integrated approach using various control available options; i.e. chemical, biological and mechanical, and lastly to formulate a management plan. The river was divided into management units to facilitate the evaluation of the clearing programme (Table 6.1).

Table 6.1 Water hyacinth infestation on the Nseleni River, determined via a 1994 aerial photo (1:5000) and ground-truthing by boat, with both initial and follow-up control options (after Jones, 2009).

Management Unit	Hectares	Length (km)	Initial Infestation %	Initial Control Option	Follow up interventions
1	20.89	6.7	40	Biological/Chemical/ Mechanical	Biological/Chemical
2	12.14	1.7	40	Biological/Chemical/ Mechanical	Biological/Chemical
3	5.59	1.4	100	Biological/Chemical/ Mechanical	Chemical/Biological
4	11.44	2.3	100	Biological	Chemical/Biological
5	8.9	1.2	100	Biological	Chemical/Biological
6	34.91	4.6	60	Biological	Biological/Chemical
7	20.58	2.9	60	Biological	Biological/Chemical
8 (Lake Nsezi)	268	-	80	Biological	Biological

The first attempt to control the infestation of water hyacinth on the Nseleni River was the application of herbicide. At the time, a "quick-fix" solution was needed due to the threat of damage to the N2 highway road and important extraction points. At the same time as herbicide was being sprayed on the water hyacinth infestation, seven steel cables with a twenty-eight mm diameter were erected across the river at strategic places, to prevent any mats of water hyacinth being blown upstream on the Nseleni River. The intention was to allow mats of water hyacinth to accumulate at the cables, which in turn made herbicide applications by boat and mechanical removal easier and more cost effective.

Numerous biological control agents have been released in the past at various sites on the Nseleni River system, with the first release of the weevil *Neochetina eichhorniae* in December 1985 (Table 6.2). To-date, all released biological control agents available for control of water hyacinth in South Africa have established and spread throughout the system.

Table 6.2 Record of biological control agents released by ARC-PPRI (Pretoria). * Identified on *Eichhornia crassipes* without being intentionally introduced (after Jones, 2009).

Date:	Agent:	Number Released:	Management Unit:
12/1985	*Neochetina eichhorniae*	1400	MU 5
01/1994	*Niphograpta albiguttalis*	150	MU 7 & 8
	Orthogalumna terrabrantis	800	MU 7 & 8
03/1996	*Eccritotarsus catarinensis*	550	MU 7 & 8
06/1996	*Eccritotarsus catarinensis*	500	MU 7 & 8
	Neochetina bruchi	800	MU 5
10/1996	*Eccritotarsus catarinensis*	10 infested plants	MU 7 & 8
1997	*Eccritotarsus catarinensis*	300	MU 8
09/1995	*Cercospora rodmanii (piaropi)* *Acremonium zonatum* *Alternaria eichhornia*	5 x 20L plastic bags with infested plants.	MU 4

With the advent of integrated control on the Nseleni River, much of the reduction in *Eichhornia crassipes* populations on the river (the lower reaches in particular) and the lake was ascribed to biological control, most notably to the two weevils (*Neochetina eichhorniae* and *N. bruchi*). There was however no empirical evidence to prove this. Historically, weed biological control has suffered from a lack of empirical data to prove causation, relying on before and after scenarios. This approach has been regularly criticized and most recently so regarding the biological control programme on water hyacinth on Lake Victoria. Williams *et al.* (2005) suggest that weevils alone were not responsible for the rapid reduction in weed biomass on Lake Victoria, as the rapid decrease in water hyacinth abundance in only two years was extraordinarily fast. They suggest the decline was more likely due to decreased light availability caused by the continual cloudy weather during the El Nin˜o event of 1997/1998.

In rebuttal, Wilson *et al.* (2007) suggested that while much of the evidence points to classical biological control as the major factor, the El Nin˜o associated weather pattern of the last quarter of 1997 and the first half of 1998 confused the issue. Wilson *et al.* (2007) argue that the reductions in water hyacinth on Lake Victoria were ultimately caused by the widespread and significant damage to plants by *Neochetina* spp., although the increased wind and wave action would have increased the mortality of insect stressed plants. This example illustrates how the lack of sound empirical data in the field undermines the scientific acceptance and validity of biological control. Quantifiable follow-up field evaluations and empirical tests are thus imperative to improve the science supporting biological control. One method of evaluating the efficacy of weed biological control agents is through insecticide exclusion experiments. While there has been some research carried out on insecticide exclusion trials (Tipping and Center, 2002; Tipping *et al.*, 2012), this has mainly been on terrestrial weeds.

Therefore, the aim of this study was to conduct a series of insecticide exclusion trials to provide empirical evidence quantifying the contribution of biological control to the integrated control of water hyacinth on the Nseleni River system. This study was carried out in two phases. The first phase of the study was an outdoor common garden experiment to a) confirm that the chosen insecticide excluded biological control agents, b) determine how long it was effective (how often would it have to be applied) and c) determine whether it had any phytotoxic effects on water hyacinth plants. Although there are six biological control agent species established on water hyacinth on the Nseleni River, *Neochetina eichhorniae* was chosen as the agent of interest because it is the most ubiquitous and damaging agent on the river, and Hill *et al.* (2012) showed this insect to be far more tolerant of herbicide applications than some of the other established agents. The second phase of the study was a large-scale field experiment aiming to quantify the damage effected by biological control agents and their impacts on water hyacinth growth.

6.2   Materials and Methods

6.2.1   Insecticide and Wetter

There are numerous insecticides and wetters / stickers on the market that are utilized by the Department of Agriculture and farmers in the Zululand region of KwaZulu-Natal, for various reasons on a multitude of different crops, to prevent insect damage. Prior to making a

decision on which insecticide and wetter to use for the treatment trials, a literature search was conducted on the available insecticides. In addition, Mr. M. Butler, (who has over 30 years' experience in this field) from Ngoye Farmers cc was consulted on numerous occasions as were fruit farmers in the area. Numerous products were suggested in terms of pricing and effectiveness.

Actara SC was chosen as a suitable insecticide, after further discussions with Mr. Butler and Mr. J. Ross from Syngenta©, who produce the insecticide. The details on the insecticide are listed below and in Table 6.3:

- Insecticide group: D

- Active ingredient: Thiamethoxam (neonicotinoid)

- Registered No.: L7207 Act No.36 of 1947 N-AR0815 (Namibia)

- Safety data sheets available with purchase of the product, which includes: Precautions, Resistance management, Use restrictions, Directions for use and Application rates.

- Bioaccumulation: Thiamethoxam has low potential for bioaccumulation.

- Stability in water: Thiamethoxam is <u>not persistent in water</u>.

- Stability in soil: Thiamethoxam is not persistent in soil.

- Mobility: Thiamethoxam has medium mobility in soil.

Table 6.3 Safety and Environmental Profile of Thiamethoxam (Ecotoxicity) as provided by Syngenta[©].

Acute Toxicity	Test Species	Results	EPA Toxicity Category
Avian oral LD$_{50}$	Bobtail quail Mallard duck	1552 mg/kg 575 mg/kg	Slightly toxic Slightly toxic
Avian dietary LC$_{50}$	Bobtail quail Mallard duck	>5200 ppm >5200 ppm	Practically non-toxic Practically non-toxic
Freshwater fish LC$_{50}$ (96 h)	Rainbow trout Bluegill	>100 mg/l >114 mg/l	Practically non-toxic Practically non-toxic
Marine fish LC$_{50}$ (96 h)	Sheepshead minnow	>111 mg/l	Practically non-toxic
Freshwater invertebrate EC$_{50}$ (48 h)	*Daphnia magna*	100 mg/l	Practically non-toxic
Aquatic insect NOEC (30 d)	*Chironomus riparius*	0.1 mg/l	(no classification scheme for this test)
Marine invertebrate EC$_{50}$ (96 h)	Mysid shrimp Eastern oyster	6.9 mg/l >119 mg/l	Moderately toxic Practically non-toxic
Algae EC50 (96 h)	Green algae	>100 mg/l	None
Earthworm EC$_{50}$ (14 d)	*Eisenia foetida*	>1000 mg/kg soil	None
Honeybee LD50 (48 h) (LD50)	Honey bee	0.024	µg/bee Highly toxic

Discussion also revolved around a suitable wetter / sticker to use with the insecticide. Nu-Film 17[©] is a non-ionic extender sticker-spreader and was identified as an appropriate candidate. Nu-Film 17[©] is manufactured by Miller Chemical & Fertilizer Co., USA and is registered and distributed by Hygrotech Properties (PTY) Ltd. South Africa. The details on Nu-Film 17[©] are listed below:

- Active ingredient: Di-1-p-Menthene (905g/l)
- Compatibility: Nu-Film 17[©] is compatible with most insecticides, fungicides, herbicides and foliar fertilizers.
- Material data sheet: Provided.

In discussions with the representatives of Syngenta[©] and Ngoye Farmers cc, it was suggested that the spray/mixture rate should be 200/300 ml per hectare for Actara SC (insecticide) and 300 ml per hectare for Nu-Film 17 (wetter / sticker), used with a nozzle that projects a fine spray when being applied. Windless conditions would be best to minimise any possible spray

drift. The recommended spray rate for this research was 60ml / Actara SC, 60ml / Nu-Film and 3 litre of water / 20m^2 water hyacinth plot.

6.2.2 Phase 1: Common Garden Experiment

The experimental set-up for phase one comprised 6 x 20L plastic buckets filled with river water, to which 5g of Multicoat (6) fertilizer, with an N:P:K ratio of 5:1:4, was added to each bucket. A single water hyacinth plant was subsequently added to each bucket, and allowed two weeks to acclimate. During the two week acclimation period, the plants were inspected at 19:00 and again at 21:00 every night for any signs of *Neochetina eichhorniae,* or other agents present as the plants were sourced from the river. All observed agents were removed. In addition, leaf scarring and weevil damage was recorded and each feeding scar marked with Eco-blue water soluble dye (Ecoguard) to assist in determining weevil activity. Each of six treatments was replicated on ten occasions.

Treatment 1: a single adult plant; no insecticide; no weevils (Control).

Treatment 2: a single adult plant; no insecticide; a pair of *Neochetina eichhorniae* weevils introduced at the end of the two week acclimation period.

Treatment 3: a single adult plant; insecticide applied at the end of the two week acclimation period; no weevils. This was to determine the effect of insecticide on plant growth.

Treatment 4: a single adult plant; insecticide applied at the end of the two week acclimation period; a pair of *Neochetina eichhorniae* weevils introduced one week thereafter.

Treatment 5: a single adult plant; insecticide applied at the end of the two week acclimation period; a pair of *Neochetina eichhorniae* weevils introduced two weeks thereafter.

Treatment 6: a single adult plant; insecticide applied at the end of the two week acclimation period; a pair of *Neochetina eichhorniae* weevils introduced three weeks thereafter. All plants were then left for a further week, after the weevils were introduced in treatment 6, before examination for weevil presence and feeding activity.

Treatments 4, 5 and 6 tested the residual effect insecticide applications, aiming to determine how often the insecticide should be sprayed in the field. Longest petiole length (cm) was measured and the number of feeding scars on leaf 2 were counted at the beginning of the trial and again after five weeks. Longest petiole length was measured in order to investigate

possible phytotoxic effects of insecticide application and feeding scars on leaf 2 are an indication of the efficacy of the insecticide. These parameters are standard measurements used in the water hyacinth biological control programme throughout South Africa (see Byrne, *et al.* 2010).

Longest petiole length data met assumptions of normality and homogenous variances, while count data (feeding scars on leaf 2) was square root transformed. Data were analyzed using a general linear model repeated measures analysis of variance (GLM RM-ANOVA) in STATISTICA v11.0 (Statsoft 1984-2011). Treatment type (1-6) was considered the categorical predictor, time was the repeated measure (before and after) and longest petiole length and the number of feeding scars on leaf 2 were considered dependent variables. The RM-ANOVA was followed by Tukey's HSD post hoc tests where appropriate.

6.2.3 Phase 2: Field Trial

The experimental set-up for phase 2 consisted of a series of field manipulations whereby experimental and control plots were examined to quantify the impact of *N. eichhorniae* through insecticide exclusion plots in a field environment. A total of ten x 20m² plots were demarcated in the Nseleni River using 4.8 meter poles, which were sunk into the river bed and nylon string was placed around the plot at different heights to contain the water hyacinth plants (Figure 6.1). The plots were filled with water hyacinth plants from surrounding mats. The plots were constructed in August 2012 and the water hyacinth was left for an acclimation period of two weeks before the first insecticide spraying event took place. The first data set was collected after five weeks from the time that the water hyacinth was first placed within the demarcated plots, and thereafter every three weeks for a total of ten sampling events.

Fig. 6.1 Example of one set of field enclosures, in the Nseleni River at experimental start. The insecticide exclusion plot is on the left, and the biological control plot is on the right, with plants of equal size in each 20m² enclosure (August 2012).

The insecticide was applied every three weeks using a 16L back sprayer from a boat. Three litres of insecticide (60ml of ACTARA SC and 60ml of NU-FILM / 20m²) and water were pre-mixed (15 litres total) and put into a plastic container. A measuring jug was used to pour out 3 litres of mixture into the back sprayer at each of the required plots. A very fine nozzle was attached to the spray equipment to assist in reducing any run-off into the river. The insecticide was applied in the early mornings when there was little wind, after first having collected the required water hyacinth and insect growth parameters (see below).

Five of the plots were sprayed every three weeks at a dosage prescribed by the manufacturer for the control of weevils in crops, which was supported by phase 1 results of the common garden experiment. The remaining five plots were not sprayed, but left for biological control agents to carry out normal activities. The populations of agents were not manipulated in this experiment and thus population levels represented the ambient levels found throughout the river. Prior to each of the spraying events, ten mature water hyacinth plants were removed from each of the experimental and control plots. Morphological parameters measured on these plants included; longest petiole length (cm), number of weevil feeding scars on leaf two, total wet biomass (kg) per m², above surface biomass (kg), below surface biomass (kg) and dead material biomass (kg). Although all the above mentioned parameters can be

influenced by variation in nutrient regime and hydrology, physio-chemistry of the water at all plots was similar (see chapter 2).

Collected data met assumptions of normality and homogenous variances, with the exception of the count data (feeding scars on leaf 2) which was subsequently square root transformed. A general linear model repeated measures analysis of variance (GLM RM-ANOVA) was completed, with treatment (biological control and insecticide exclusion) as the categorical predictor, time (before and after) as the repeated measure and longest petiole length and the number of feeding scars on leaf 2 as the dependent variables. The GLM RM-ANOVA was followed by Tukey's HSD post hoc tests where appropriate. The remaining morphological measurements (total biomass, above surface biomass, below surface biomass and dead biomass) before experimental start were analyzed using a general linear model one-way analysis of variance (GLM ANOVA); with treatment type (biological control and insecticide exclusion) as a categorical predictor and all biomass parameters (see above) as the dependent variables to confirm there were no significant differences between measurements of plants in all plots prior to experimental start. As the data set of interest is how biological control plots compare with insecticide exclusion plots after experimental finish, the final (T10) data set was then analyzed using a general linear model one-way analysis of variance (GLM ANOVA). Treatment type (biological control and insecticide exclusion) was considered the categorical predictor and the biomass measurements (see above) were considered dependent variables. The GLM ANOVA was followed by Tukey's HSD post hoc tests where appropriate. All statistics were completed in STATISTICA v11.0 (Statsoft 1984-2011).

6.3 Results

6.3.1 Common Garden Experiment

Significant differences were seen between before and after measurements of longest petiole length (cm) in all treatments ($F_{5,54}$ = 938.43, p < 0.05; Figure 6.1), including controls (treatments 1 and 2). Tukey's HSD post hoc tests indicated that despite some variability, all measurements taken before experimental start belonged to the same homogenous grouping. Measurements taken after experimental finish demonstrated less variability and shared one homogenous group.
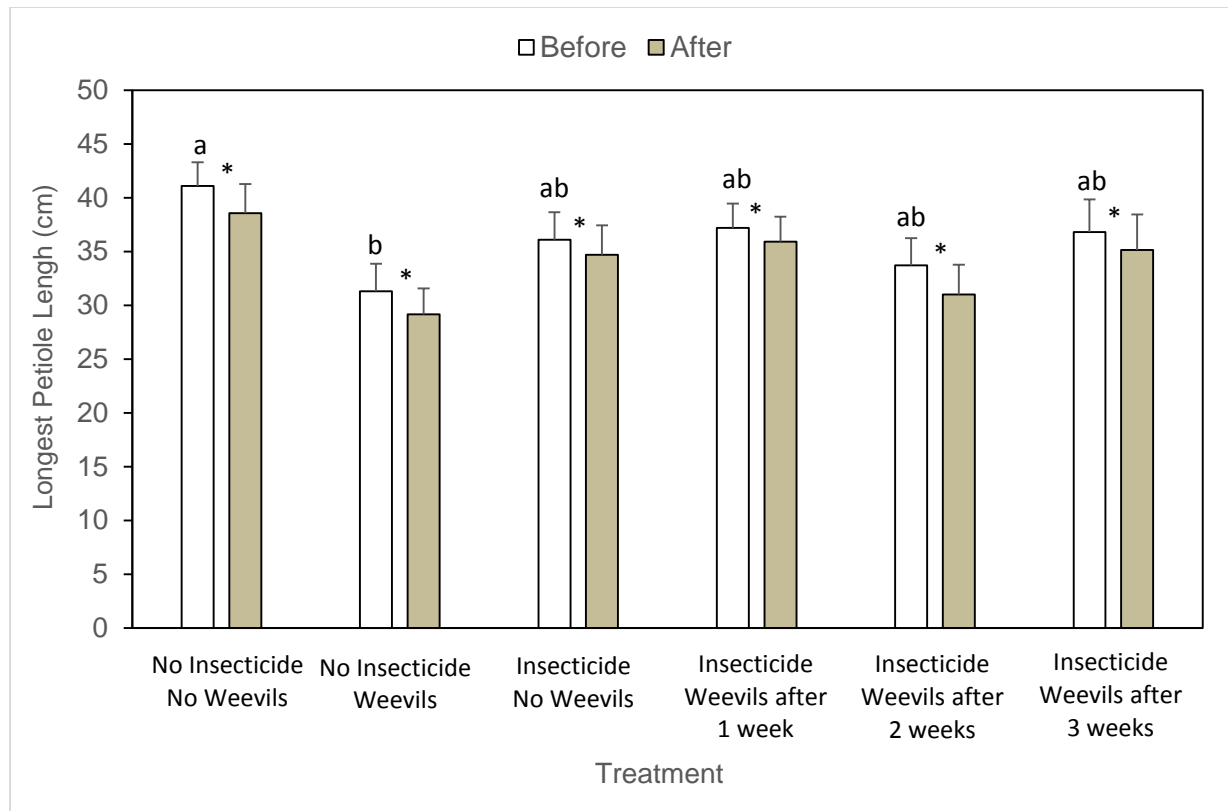
Fig. 6.2 Mean length of the longest petiole (cm) of *Eichhornia crassipes* plants; measuring phytotoxicity for the six experimental treatments. Lowercase letters denote homogenous groups (p < 0.05) between treatments for measurements before experimental start; * denotes significant differences (p < 0.05) between before and after measurements among treatments. Error bars indicate +1SE.

Significant differences were also seen between before and after measurements of the number of feeding scars on leaf 2 in all treatments ($F_{5,54}$ = 464.12, p < 0.05; Figure 6.2), including controls (treatments 1 and 2). Tukey's HSD post hoc tests indicated that despite some variability, all measurements taken before experimental start belonged to the same homogenous grouping. However clear differences were apparent between treatments for

measurements taken after experimental finish, with plants in treatment 2 (no insecticide, with weevils) showing much higher numbers of weevils scars relative to any other treatment.
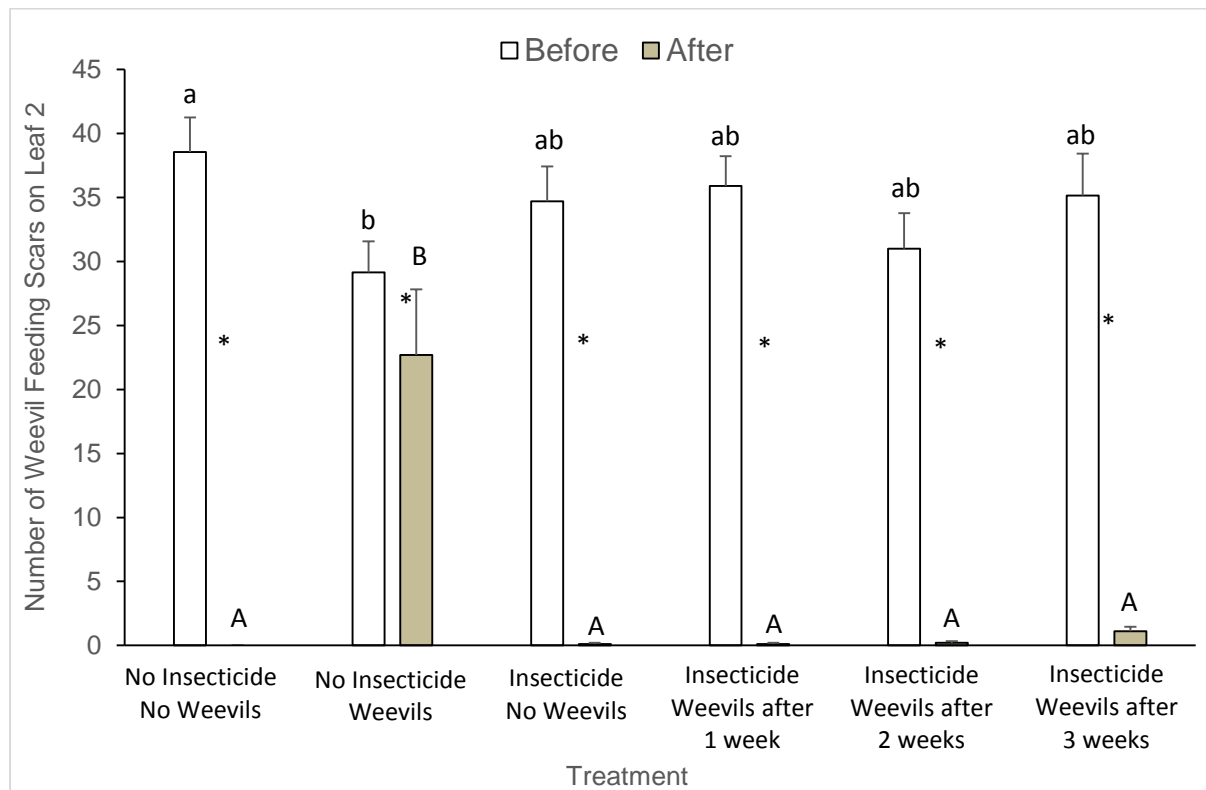
Fig. 6.3 Mean number of feeding scars on leaf 2 of *Eichhornia crassipes* plants; measuring insecticide efficacy for the six experimental treatments. Lowercase letters denote homogenous groups ($p < 0.05$) between treatments for measurements before experimental start; uppercase letters denote homogenous groups ($p < 0.05$) for measurements after experimental start; * denotes significant differences ($p < 0.05$) between before and after measurements among treatments. Error bars indicate +1SE.

6.3.2 Field Trial

Prior to the experimental start, the length of the longest petiole was significantly higher for plants in the insecticide exclusion plots (~34.0 cm) compared with those in the biological control plots (~22.0 cm) ($F_{1,98} = 192.97$, $p < 0.01$). After 33 weeks of growth, plants in biological control plots showed small increases in petiole length (~4.0 cm increase). Plants in insecticide exclusion plots however showed more dramatic increases in petiole length between experimental start and finish, gaining ~55.0 cm. Comparisons of longest petiole length between plants from biological control and insecticide exclusion plots after experimental finish clearly indicate significant effects ($F_{1,98} = 192.97$, $p < 0.01$) of weevil herbivory (Figure 6.4A & Figure 6.5).

The number of feeding scars on leaf 2 was also significantly different between biological control (~13) and insecticide exclusion (0) plots at both experimental start and finish. More importantly the number of feeding scars increased dramatically in the biological control plot over the course of the experiment, while no significant increase was seen in feeding scars on plants in the insecticide exclusion plots ($F_{1,98} = 580.30$, $p < 0.01$) (Figure 6.4B & Figure 6.5). Figure 6.6 is a visual indication of feeding scares found in a biological control plot, to that of the insecticide exclusion plot.
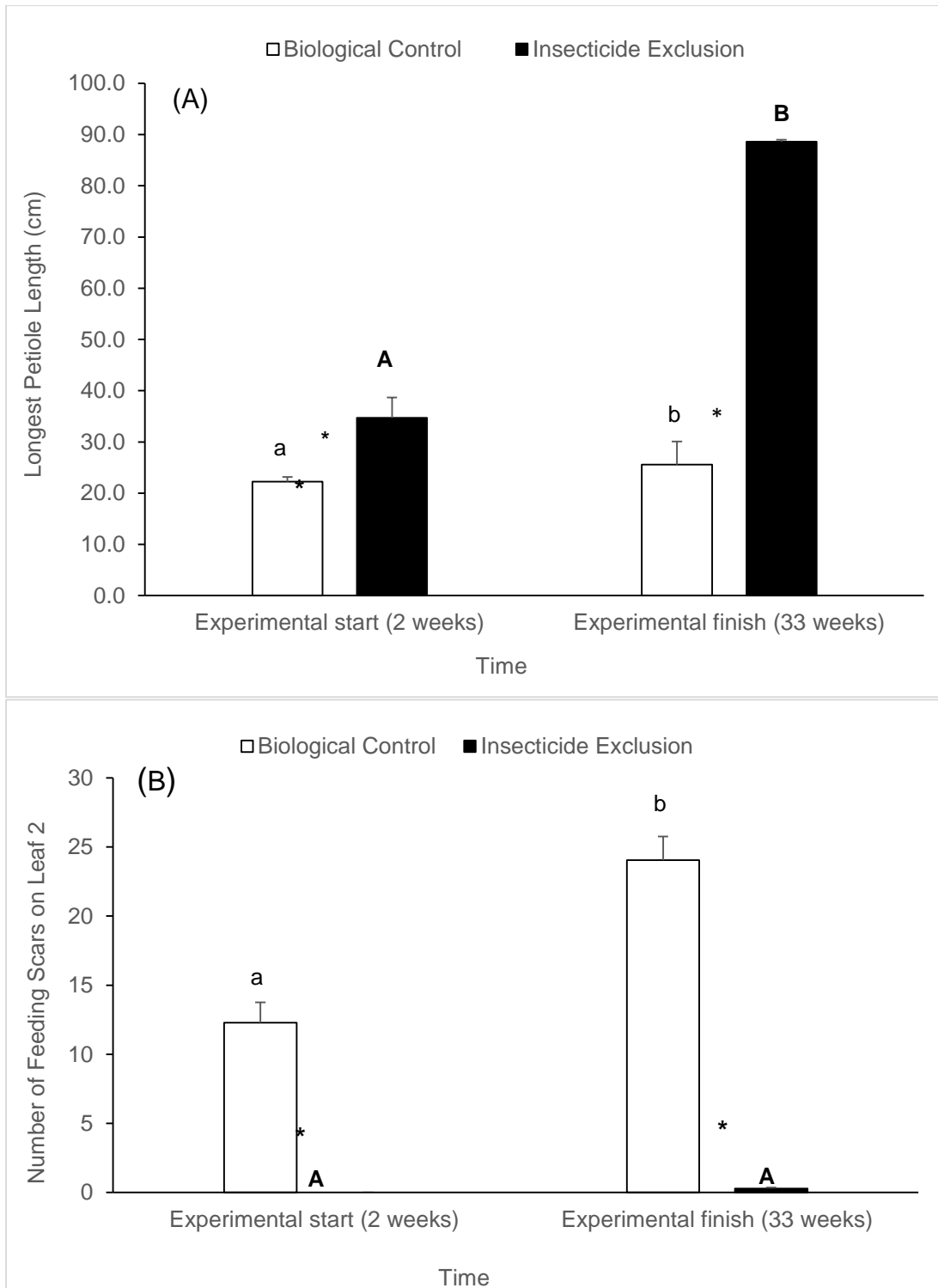
Fig. 6.4 Mean length of the longest petiole (cm) of *Eichhornia crassipes* plants (A) and the number of feeding scars on leaf 2 (B) before experimental start (after a 2 week acclimation period) and after experimental finish. Lowercase letters denote homogenous groups ($p < 0.01$) among biological control treatments over time; upper case letters denote homogenous groups among insecticide exclusion treatments over time.* denotes significant differences ($p < 0.01$) between treatments. Error bars indicate +1SE.

Fig. 6.5 Visual representation of the differences in longest petiole length between plants in the insecticide exclusion plot (left) and plants in the biological control plot (right) Plants within the insecticide exclusion plot (poles covered by water hyacinth) are clearly bigger, with longer petioles than those within the biological control plot on the right (December 2012).

Fig. 6.6 *Eichhornia crassipes* plants within an insecticide exclusion plot (left), with no weevil feeding scars and within a biological control plot (right), showing substantial weevil feeding scars in October 2012.

Total biomass/m^2 ($F_{1,8} = 227.95$, p < 0.01), above surface biomass (per 10 plants; $F_{1,8} =$ 1951.48, p < 0.01) and below surface biomass (per 10 plants; $F_{1,8} = 5.66$, p < 0.05) of plants grown in insecticide exclusion plots were significantly higher than those from plants grown in biological control plots after 33 weeks of growth. Biomass of dead material (per 10 plants) however was high in all biological control plots ($F_{1,8} = 53.44$, p < 0.01) (Figure 6.7 & 6.8).
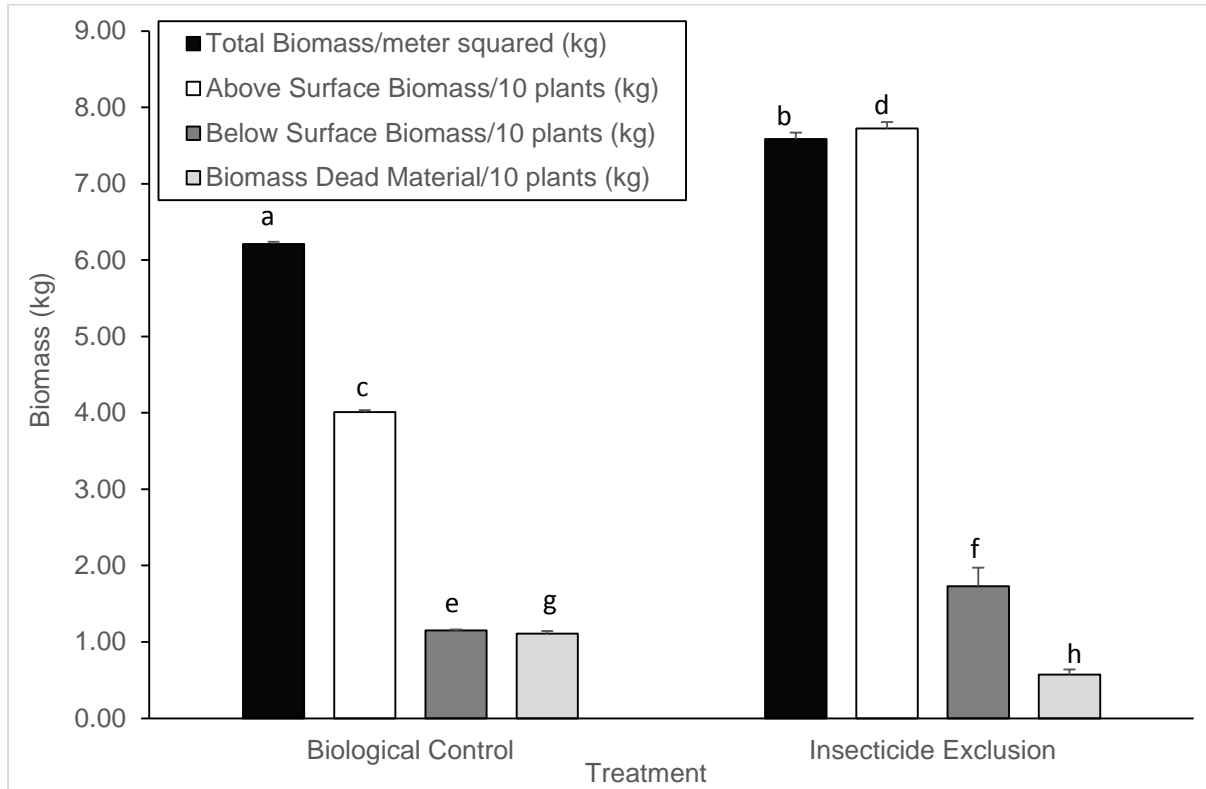
Fig. 6.7 Mean values of *Eichhornia crassipes* biomass after experimental finish (33 weeks). Lowercase letters denote different homogenous groups (p < 0.05) for total biomass (a,b), above surface biomass (c,d), below surface biomass (e,f) and dead material biomass (g,h) between biological control and insecticide exclusion treatments. Error bars indicate +1SE.

Fig. 6.8 Photographs of Ezemvelo KZN Wildlife staff members holding *Eichhornia crassipes* plants collected in January 2013. Photograph A is of a single water hyacinth plant removed from an insecticide exclusion plot (note the height of plants outside the boat on left) showing: long petioles, short roots, no weevil damage and very little dead material, compared to a single *Eichhornia crassipes* plant removed from a biological control plot in photograph B (note the height of plants outside the boat on left) showing: short, stressed leaves and petioles, root length similar to the plant in (A), high percentage of weevil feeding damage and dead material.

6.4. Discussion

Water hyacinth is highly invasive and damaging to biodiversity in the Nseleni River system (Coetzee *et al*. 2014). Biological control is one of the tools that have been used in an integrated approach to management on this system, and biological control has been reported to have been highly effective against aquatic weeds, including water hyacinth (Hill 2003). However, biological control has suffered from a lack of empirical evidence linking cause and effect. This study clearly showed that in the absence of the water hyacinth biological control agents on the Nseleni river system the plants were more vigorous, as measured through plant height and biomass. These data support the results obtained by Coetzee *et al*. (2005) and Tipping *et al.* (2008; 2010) where they confirmed that different biological control agents introduced onto invasive alien plant species, also had negative effects on this plant species.

One of the measures of success used in water hyacinth biological control is the reduction in surface area covered by the weed (Albright *et al.* 2004). In the present study, percentage cover did not differ and remained at 100% in the treatment and control plots. This was due to the fact that the water hyacinth was constrained within the plots and the short duration over which the experiment was conducted. Biological control agents, and in particular *N. eichhorniae*, are the cause of smaller and lighter plants that sit lower in the water, due to herbivory and they often require wind and wave action to break up the mats and cause them to sink (Hill and Olckers 2001; Wilson *et al.* 2007). Water hyacinth biological control in South Africa has not been as successful as it has on the large tropical lakes and rivers elsewhere in Africa and this has been ascribed to enriched waters and a temperate climate that impacts the life histories of the agents (Byrne *et al.* 2010). However, Hill and Olckers (2001) also suggested that the relatively small size of the water bodies in South Africa, and the fact that most of them are impounded thereby reducing natural flow regimes, prevents the necessary wind and wave action to cause large mats to sink. So, while biological control might reduce plant vigour and prevent it from spreading, it may still require another intervention such as manual or mechanical control to remove mats. However, it is unlikely, given water hyacinth growth rate, that manual and/or mechanical control would be successful in the absence of biological control.

In many systems around the world the integrated management of water hyacinth has been suggested (Jones 2009; Hill and Coetzee 2008). However, Hill and Olckers (2001) have warned against the large-scale and injudicious use of herbicide application as this disrupts the plant/insect interaction on which biological control depends. Further, Hill *et al.* (2012) showed that some of the herbicides used in water hyacinth control are toxic to the biological control agents and suggested that biological control reserves should be established in systems that are not treated with herbicides and thus act as a reservoir for the agents. The integrated control programme on the Nseleni River using herbicides, collection booms and biological control has been successful, and this study has shown the contribution that biological control has made. The Nseleni River system is the only site in South Africa where six agents (*N. Eichhorniae, N. bruchi, Orthoglumna terebrantis, Niphograpta albigutallis, Eccritotarsus catarinensis* and the pathogen, *Cercospora piaropi*) have established. While they are undoubtedly having a significant impact on water hyacinth growth, there are two additional agents, the grasshopper, *Cornops aquaticum* Bruner, and the delphacid leafhopper, *Megamelus scutellaris* Berg that are also available for release in South Africa and should be

released in large numbers on the Nseleni system to improve the biological control that already exists.

CHAPTER 7

GENERAL DISCUSSION & CONCLUSION

The dispersal of non-indigenous and invasive species around the world is a consequence of globalization and has been the cause of negative impacts to indigenous species, especially within aquatic systems, (Mooney, 2005; Özdilek, 2007; van Wilgen *et al*., 2012). Van Wilgen *et al*. (2013) further suggests that due to globalization, invasions are rapidly increasing in extent and severity, leading to large-scale ecosystem degradation. Rivers have been modified across all continents, due to human activities (Cowx, 2002) such that a high percentage (>70%) of temperate, tropical, sub-tropical and arid-zone river systems are regulated assisting invasion (Dynesius and Nilsson, 1994; Arthington and Pusey, 2003; Revenga and Kura, 2003). Naylor *et al.,* (2000) suggests that alien species invasions are now recognized as an important component of global environmental change. Furthermore, that the breakdown of biogeographic barriers is changing ecosystem structure and function, and it is accelerating the decline of biodiversity on a global scale. These invasions are now proving to be economically damaging to an extent that is only in more recent times being appreciated by economists and policy makers.

Arrington *et al*., (2005) suggests that there are numerous challenges in both ecology and conservation biology when it comes to understanding the patterns of species abundance, distribution, and co-occurrence of indigenous and non-indigenous species of organisms. Therefore, considerable research has been carried out in an attempt to improve understanding of any possible "conflict" between the non-indigenous species studied here *Tarebia granifera* (see Butler, *et al*., 1980; Appelton, 2003; Miranda *et al*., 2012), *Pterygoplichthys disjunctivus* (see Bunkley-Williams *et al*., 1994; Chavez, *et al*., 2006; Capps and Flecker, 2013) and *Eichhornia crassipes* (see Akinyemiju, 1987; Hill *et al*., (1999); De Groote *et al*., (2003); Coetzee *et al*., (2014)) and indigenous flora and fauna.

In South Africa, considerable research effort has been placed on studying the effects and impacts of invasive alien species (Richardson and van Wilgen, 2004). Furthermore, they state that although South Africans are inadequately equipped to prevent the introduction of new species, to detect and eradicate potentially dangerous organisms before they become major problems (as is

the case in many countries globally), South African research has made a disproportionably large contribution to invasion ecology. The SCOPE, Global Invasive Species Programme and Working for Water programmes are good examples of these initiatives by South Africans.

Invasive alien species in KwaZulu-Natal go back as far as when the Nguni tribe started settling in the area (see Chapter 1). Although unconfirmed, they must have brought 'new' food seed with them as they travelled through East Africa, and their cattle would have introduced 'new' species of alien organisms that were collected along the way. The arrival of Europeans into the area definitely also introduced invasive alien species into the area and is well documented. By the mid- to late 1800's, the European influence had reached the area now known as the Enseleni Nature Reserve and the introduction of invasive alien species to this area had well and truly began (see Chapter 1). Today, the importance of the Enseleni Nature Reserve cannot be under estimated. Although this protected area is slightly under 300 hectares in size, from a biodiversity point of view it is extremely important, as five different distinct habitats occur within the protected area. The ecological services provided by the Enseleni Nature Reserve far outweigh some of the much larger protected areas within the Province, such as the Ukhahlamba-Drakensberg Park (UDP), Weenen Nature Reserve, Ithala Game Reserve and others, yet matched the services provided by the Hluhluwe-Imfolozi Park (HIP) which is 960 km^2 in size. One of the main reasons for the Enseleni Nature Reserve scoring so high, was due to the Nseleni River system, which forms part of the protected area's eastern boundary. Further evidence of the Enseleni Nature Reserves importance, is the fact that it scored considerably higher than all other protected area's measured in terms of dependence levels of users (see Chapter 2; Table 2.1 and 2.2).

It is therefore of utmost importance to know which terrestrial and aquatic invasive aliens species occur within this protected area, what impact they are having and if they can be controlled. From a terrestrial point of view, all invasive aliens have been identified and a management plan is in place to control them as well as to identify any possible new aliens that occur. However, it is much more difficult to continually identify new aquatic invasive alien species, as many could be submerged. From a biodiversity point of view, it is absolutely crucial to understand what invasive aliens occur and what impact they are having on the surrounding biodiversity. From this

study (and others) it has been confirmed that *Eichhornia crassipes* (see Chapter 6) is having a big impact on surrounding biodiversity and that *Tarebia granifera* and *Pterygoplichthys disjunctivus* (see Chapters 4 and 5) less so. This study was, however, carried out over a brief period in time and the present scenario concerning both *Tarebia granifera* and *Pterygoplichthys disjunctivus* may very well change in the future, as has been recorded from numerous aquatic systems around the World (see Chapters 4 and 5). It is therefore of importance that once an invasive alien species has been identified, populations must be monitored on a regular basis. In addition, as we are aware that all three of these species have spread beyond the Nseleni River system (*P. disjunctivus* was in a settling pond at Richards Bay Minerals in 2014) continual monitoring is also required in these newly invaded aquatic systems.

The question then arises: can these invasive species can be controlled in such a way that their impact is reduced, or, ultimately removed. The implementation of the Integrated Water Hyacinth Plan at the Enseleni Nature Reserve has shown that *E. crassipes* can be controlled to a satisfactory management level on the Nseleni River system with biological control contributing significantly (Chapter 6). However, the same cannot be said for *T. granifera* and *P. disjunctivus* Although chemicals have been used to control gastropods and fish in some areas of the world, including South Africa, there species have not been targeted, thus much research still needs to be done here.

The most cost effective way to combat invasive infestations, is to prevent the direct or indirect introduction into an ecological "sensitive" system (Wilson *et al*., 2009). This requires a continual and long term awareness campaigns in all forms of media. All three of the invasive species that have been introduced into the Nseleni River system are directly linked to the aquarium / horticultural trade. The recently published National Environmental Management: Biodiversity Act 10 of 2004 - Alien and Invasive Species Regulations (Government Notice R. 598 of 1 August 2014) and the National Environmental Management: Biodiversity Act 10 of 2004 - Alien and Invasive Species Lists (Government Notice R. 599 of 1 August 2014) will assist in the prevention of future invasive species introductions, but will not solve the present predicament where species have already established. If we accept that prevention is better than cure, what are

other threats to the Nseleni River system? The most immediate threat is Largemouth bass (*Micropterus salmoides* (Lacepède, 1802)).

Identified imminent threat to the Nseleni River system.

Micropterus salmoides is a North American piscivorous fish on the IUCN list of 100 of the world's worst invasive alien species (Britton *et al*., 2010). It's native range are the freshwaters of the lower great lakes in North America, central part of the Mississippi River system south to the Gulf coast, Florida and northwards along the Atlantic coast to Virginia (De Moor and Brutton 1988). They were first introduced to the Jonkershoek hatchery (Cape, South Africa) from Surrey, England and were distributed around the Cape from 1930. In 1952, the Umgeni hatchery in KwaZulu-Natal was opened and stocked with *M. salmoides* and the distributed around the province.

Largemouth bass, *M. salmoides,* were introduced into South Africa for recreational angling (Weyl *et al*., 2010). In recent times, this fish species has since been introduced illegally into many farm dams in the catchment of the Nseleni River system. In terms of the Natal Conservation Ordinance 15 of 1974, a permit was required to move / introduce any fish species within the Province of KwaZulu-Natal, prior to the proclamation of the National Environmental Management: Biodiversity Act 10 of 2004, when this species was introduced into the area. No such permits were applied for by any of the landowners in the area. The biggest threat to the Nseleni River system is the dam on an adjacent farm, that when it overflows, it flows directly into the Nseleni River. This dam has been stocked with *M. salmoides* for recreational purposes. Of concern to indigenous fish species, is the fact that *M. salmoides* can be a highly invasive predator (Lowe *et al*. 2008; Wassermann *et al*. 2011) and that it could have a negative effect on the food web efficiency thereby leading to the reduction or loss of indigenous species (Kamerath *et al*., 2008). Habitat preferences of *M. salmoides* are lakes or artificial impoundments to rivers and streams, it tolerates temperatures from 5 to 36°C, with the optimum temperature being 28°C.

To prevent another exotic invasive spp. entering the Nseleni River system, it is vitally important that a "door-to-door" awareness campaign be initiated with all landowners that have dams

stocked with largemouth bass and to advise of the negative consequences to biodiversity should this fish enter the Nseleni River system.

Recommendations

- Long-term monitoring plans are put in place for *Tarebia granifera* and *Pterygoplichthys disjunctivus*. In addition, any monitoring plan should/must include the possible invasion by other invasive non-indigenous species.

- A comprehensive awareness campaign should be initiated concerning all three of the non-indigenous species that must run indefinitely. This must be updated to include any additional invasive non-indigenous species identified.

- Further research be investigated to determine an acceptable population level of both *Tarebia granifera* and *Pterygoplichthys disjunctivus*.

- Further research be carried out to determine if biological control of *Tarebia granifera* and *Pterygoplichthys disjunctivus* would be possible to assist in reducing numbers of these species.

- Water hyacinth must not exceed the maximum allowable coverage and further biological control agents must be introduced.

- Ezemvelo KZN Wildlife, as having the Provincial mandate to ensure biodiversity integrity, must ensure that the official stationed at the Enseleni Nature Reserve has the capabilities of ensuring aquatic biodiversity within the protected area and the Nseleni River system, and

- That the Department of Environmental Affairs places pressure on other organs of State and the uMhlathuze Municipality must ensure that the effluent plant situated adjacent to the Mposa River improves the quality of effluent being pumped into the Mposa River.

The sole reason for the success in controlling an invasive species on the Nseleni River was due to the implementation of an Integrated Management approach in 1995, which consisted of: identification of the weed; mapping the extent of the weed; identification of the source of the infestation; consultation with interested and affected parties; appointment of a lead agency; ascertain an acceptable level of control; consideration of control options; implementation of control options; monitor control options and finally the evaluation of the plan and adjusting

accordingly (see Hill and Coetzee, 2008). Although the Hill and Coetzee (2008) paper referred specifically to water hyacinth, the approach is applicable to invasion in aquatic and terrestrial ecosystems.

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