Ecophysiology and population dynamics of the alien invasive gastropod *Tarebia granifera* in the iSimangaliso Wetland Park, South Africa.

Nelson Augusto Fernandes Miranda

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As the candidate’s Supervisor, I have approved this thesis for submission.

Signed: [Signature]  Name: [Name]  Date: 20 Feb 2012
Abstract

_Tarebia granifera_ is a prosobranch freshwater gastropod from south-east Asia which has invaded other sub-tropical parts of the world. This snail has recently also invaded the iSimangaliso Wetland Park, often reaching population densities of over 20000 ind.m$^{-2}$ and dominating benthic invertebrate communities. A multiple method approach was used to address several aspects of the biology and ecology of this non-native invasive species (NIS). The tolerance of _T. granifera_ to salinity and temperature was investigated through the experimental manipulation of these factors. _T. granifera_ survived exposure to temperatures between 0 ºC and 47.5 ºC. More remarkably, this snail was able to survive a salinity of 30 for 65 - 75 days. Population density and size structure were monitored in estuaries and coastal lakes. _T. granifera_ successfully invaded estuaries despite frequent exposure to high salinity and desiccation. The persistence of _T. granifera_ was largely ensured due to the wider environmental tolerance of adults (20 - 30 mm shell height) which carried an average of 158 ± 12.8 SD brooded juveniles. Multiple introductions were not essential for the success of this parthenogenetic NIS. Using gut fluorescence and carbon budget techniques it was estimated that _T. granifera_ consumes 0.5 - 35% of the total available microphytobenthic biomass per day, or 1.2 - 68% of the daily primary benthic production. The carbon component estimated from the gut fluorescence technique contributed 8.7 - 40.9% of the total gut organic carbon content. A stable isotope mixing model was used together with gut content analysis to estimate the diet of _T. granifera_ and dominant native gastropod species, potentially competing for resources. Results were used in the formulation of an index of isotopic dietary overlap (IDO, %). This approach yielded detailed information both on general changes in ecosystem functioning and specific species interactions. Before/After-Control/Impact (BACI) logic was used in a multivariate approach to separate human perturbations from natural spatio-temporal variability displayed by communities, and to further separate perturbations due to NIS. Human intensification of drought negatively affected biodiversity and _T. granifera_ may exacerbate this problem by displacing native species from critical refugia and contributing towards biotic homogenization. The present findings constitute a contribution to the scientific knowledge on biological invasions and a useful tool towards adaptive management in the iSimangaliso Park.
Preface

The work described in this PhD thesis was carried out in the School of Biological and Conservation Sciences, University of KwaZulu-Natal, Westville, from January 2008 to October 2011, under the supervision of Professors Renzo Perissinotto and Christopher C. Appleton.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others, it is duly acknowledged in the text.

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Publication 1

**Author contributions:** Conception and design: Miranda, Perissinotto and Appleton. Performed experiments and analysed data: Miranda. Wrote the draft paper: Miranda.

Publication 2

**Author contributions:** Conception and design: Miranda, Perissinotto and Appleton. Performed experiments and analysed data: Miranda. Wrote the draft paper: Miranda.

Publication 3

**Author contributions:** Conception and design: Miranda and Perissinotto. Performed experiments and analysed data: Miranda. Wrote the draft paper: Miranda.

Publication 4

**Author contributions:** Conception and design: Miranda, Perissinotto and Appleton. Performed experiments and analysed data: Miranda. Wrote the draft paper: Miranda.

Signed: [Signature]
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I wish to dedicate this work to the memory of Mr. Amos Myeza, Technical Assistant at Ezemvelo KZN Wildlife EcoAdvice since 1976. I thank the iSimangaliso Park Authority and Ezemvelo KZN Wildlife for supporting all aspects of this project. In particular, I sincerely thank Dr. Ricky Taylor, Ms. Caroline Fox and Mr. Sb’u Mfeka for their invaluable assistance with logistic support, field collections and monitoring. Dr. Jean-Pierre Pointier (Centre de Biologie et Ecologie Tropicale et Méditerranéenne, France), Dr. Dai Herbert (Natal Museum, Pietermaritzburg ), Dr. Manuel Malaquias (Natural History Museum, London ), Dr. Deena Pillay (University of Cape Town), Dr. Frida Ben-Ami (Tel-Aviv University, Israel), Dr. Patrice David (Centre d’Ecologie Fonctionnelle et Evolutive, France) and Prof. Derek Stretch (UKZN) are thanked for their advice and assistance. I am also grateful to Krishna Govindsamy, Nicola Carrasco, Linda Harris, Mathieu Séré, Lola Massé, Lynette Perissinotto and Tyrel Flugel, who provided invaluable assistance in various technical aspects of this study. Funding was provided by the National Research Foundation (NRF), Marine and Coastal Management (DEAT-MCM), the World Wide Fund for Nature (WWF) and the University of KwaZulu-Natal.
Tudo neste mundo tem seu tempo e lugar.
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Biological invasions consist of species moved through human activities and by natural means. Although our understanding of alien species invasions dates back to Elton (Elton 1958) and our concern about invasions dates back several centuries, the study of invasions in coastal systems is only a few decades old (Grosholz 2002). Currently, most shallow-water coastal and estuarine communities around the world have been altered by human activities. Many species have either accidentally or intentionally been introduced to ecosystems that would otherwise not be accessible through natural processes. Therefore, human activities don’t simply accelerate natural processes (Carlton 1999), but result in complex and unpredictable alterations to ecosystems (Lockwood et al. 2007).

Non-native species have been referred to in the literature by many synonyms (e.g. alien, exotic, non-indigenous). However, it is important to note that invasiveness is a separate attribute. Alien populations are considered invasive when they are able to expand their range in the ecosystems that they invade. Biological invasion is a continuous process involving cyclical iterations, basically composed of individual-based dispersal and establishment processes (Davis, 2009). However, different successive stages can be described for a successful invasion: (1) introduction, (2) establishment, and (3) spread (Sakai et al. 2001). Species have been moved outside of their native ranges and subsequently spread by vectors such as ballast water, releases from aquaria, and on recreational, fishing and even sampling gear. Successful establishment has been correlated with the taxonomic identity and characteristics of the invader species as well as the ecological characteristics of the invaded ecosystems (Sher & Hyatt 1999; Strayer 2010). Some non-native invasive species (NIS) can then spread and have profound impacts on the ecosystems that they invade (Drake & Mooney 1989). Aquatic molluscs are an ecologically important class of NIS, since they are primary consumers and can disrupt food webs from their base (Strayer et al. 1999). Stressed ecosystems may be especially susceptible to invasions because disturbance is generally thought to favor invasions. Alien species are often involved in the development of unprecedented conditions in protected ecosystems, thus creating difficult management challenges for the future. The best approach is to prevent new invasions, but existing invaders also require management (Buckley 2008; Strayer 2010).

*Tarebia granifera* is a freshwater prosobranch gastropod (family: Thiaridae), originally from South-East Asia (Abbott 1952; Pointier et al. 2003). This NIS has spread rapidly in recent years across a number of countries throughout the tropical/subtropical areas of the world and has been reported to displace other invertebrates, thereby causing ecological disturbances and a
possible reduction in biodiversity (Appleton 2003; Gutierrez et al. 1997; Pillay & Perissinotto 2008; Pointier et al. 1998). *T. granifera* may have been introduced into KwaZulu-Natal, north-eastern South Africa, via the aquarium trade in the early nineties (Appleton & Nadasan 2002). It is present in an increasing number of fresh and brackish water bodies of KwaZulu-Natal (Appleton 2003). The iSimangaliso Wetland Park (formerly known as Greater St. Lucia Wetland Park) is an UNESCO World Heritage Site in KwaZulu-Natal. Within iSimangaliso there are three Ramsar Wetlands of International Importance: Kosi Lakes, Lake Sibaya and the St. Lucia Estuary, which is Africa’s largest estuarine lake complex. Unfortunately, all of these water bodies have very recently been invaded by *T. granifera* which can reach extremely high population densities (> 20000 ind.m$^{-2}$) and displace other species, thus dominating the benthic invertebrate assemblages (Chapters 1 and 4). There is a general lack of information pertaining to the ecophysiology of *T. granifera*, particularly in terms of its environmental tolerance, feeding and population dynamics in brackish waters. The overarching aim of this PhD thesis is therefore to close this knowledge gap. This work also supports the current adaptive management strategy and promotes further research in iSimangaliso.

The remarkable persistence of *T. granifera*, considered to be a freshwater gastropod, in high salinities and temperatures of the St. Lucia Estuary, raised questions about its environmental tolerance. Chapter 1 presents an experimental study aiming to determine the salinity and temperature lethal tolerance limits of *T. granifera*. It provides detailed experimental data on the responses of *T. granifera* to key physical and chemical factors in the predominantly brackish environment of estuaries.

Top-down effects of gastropod grazers can significantly affect marine and freshwater ecosystems (Pillay et al. 2009 and references therein). Preliminary measurements indicated that benthic algae contributed a large proportion to the diet of *T. granifera* at the St. Lucia Estuary. The rapid establishment and spread of this NIS raised concerns about potential grazing impacts in iSimangaliso. In Chapter 2, a novel method of estimating *in situ* feeding rate and impact is developed and applied to *T. granifera*. This approach provides information about the amount and rate of transfer of food (energy), which is useful in trophic ecology and carbon cycle studies.

While reports exist about the distribution and usefulness of *T. granifera* as biological controlling agent elsewhere (Pointier 2001), there is virtually no information available on the feeding-mediated impacts on coexisting species. In Chapter 3, dietary overlaps between *T. granifera* and native species are addressed through the use of gut content and stable isotope analyses. The potential for competition for food resources between *T. granifera* and native gastropods is also discussed.
Parthenogenetic NIS, such as *T. granifera* are a subject of interest because of their worldwide invasion success despite low genetic diversity (i.e. the genetic paradox of biological invasions) (Roman & Darling 2007). In this context, aspects of population dynamics can be assessed to understand how *T. granifera* is able to prevail in extreme and variable environments. The *T. granifera* invasion of different ecosystems within iSimangaliso provided a unique opportunity to gain insight into the ecology of this NIS and understand its success or failure to establish and spread in coastal lakes and estuaries. Chapter 4 addresses the longer term effects of environmental change on the population structure of *T. granifera* in a variable estuarine setting. A previously overlooked aspect of reproductive biology (i.e. brood pouch ecology) is also introduced.

Finally, NIS have the potential to cause ecological impacts by changing native assemblages (Lockwood et al. 2007). There are however several challenges in assessing ecological impacts of successful NIS such as *T. granifera* (Parker et al. 1999; Ruiz et al. 1999). It is difficult to distinguish between natural and invasion impact changes in assemblage variability (Ruiz et al. 1999), particularly in iSimangaliso where ecosystems are highly variable. In addition, *T. granifera* is invading ecosystems under other human-induced ecological stresses, such as the St. Lucia Estuary where freshwater deprivation is currently causing drought intensification. NIS can interact in complex ways with other anthropogenic stressors (Ruiz et al. 1999), which also makes it difficult to distinguish between environmental and invasion ecological impacts. However, addressing these issues is necessary for the development of health indicators needed for iSimangaliso (Whitfield & Taylor 2009). In Chapter 5, recently developed analytical methods involving Before/After-Control/Impact (BACI) type designs are applied in a novel way to assess invasion impact. The primary aim is to find whether the variability of shallow water assemblages at putative impact locations differs significantly from natural variability. A multiple-model approach is presented for the detection of simultaneous human-induced impacts. This study supports the integration of BACI type designs into multiple method approaches to assess invasion impact.
References


SALINITY AND TEMPERATURE TOLERANCE OF THE INVASIVE FRESHWATER CESTODOID TAREBI LA GRAMIFERA

Authors:
Nelson A. M. Miranda,
Sandra Leitão,
Helder M. Inácio,
Erik V. da Costa,
Fábio M. G. R. B. da Silva,
Flávio H. H. da Cunha,
Denise M. A. U. de Oliveira,
Lucas P. F. da Silva

ABSTRACT
Invasive species, such as the gastropod Tarebia granifera, can cause ecological disturbances and potentially reduce biodiversity by altering water habitats. In South Africa, T. granifera was first recorded in an estuarine environment in the St Lucia estuary. Its tolerance to salinity and temperature was assessed through the experimental manipulation of these factors. T. granifera can tolerate temperatures between 0°C and 42°C, allowing it to survive in high temperature climates. The species may also survive salinities up to 60% seawater. More remarkably, this snail survives high salinity (as long as temperature is maintained). However, higher salinity adversely affected the T. granifera population. Individuals maintained under saline conditions and suddenly exposed to 20% seawater, experienced 100% mortality within 18 h. A seawater concentration decline with increasing salinity. T. granifera's environmental tolerance and parthenogenetic characteristics are overlooked by unsuccessful introductions and introductions. Therefore, the management of T. granifera may proceed without the need for implementing control measures.

INTRODUCTION
Invasive species have been widely reported as having a negative impact on the ecology and economy of a region. In South Africa, knowledge of aquatic invasive species has increased over the past two decades. However, the focus has been on marine invasive species, such as the oyster, considered a significant economic threat such as to bivalve and crab aquaculture. The presence of different temperate species in the marine environment is not well understood. Invasive species can have a significant impact on the environment and can cause economic losses. Therefore, the management of invasive species is crucial. This study aimed to assess the salinity and temperature tolerance of T. granifera and the impact of high salinity on its population. The results of this study will provide valuable information for the management of T. granifera in South Africa.

Tarebia granifera is a small freshwater gastropod (family: Thordidae), originally from South-East Asia. The species has expanded rapidly in recent years, becoming a serious pest in both tropical and temperate areas of the world. In South Africa, T. granifera was first recorded in the St Lucia estuary, on the east coast of the country. The estuary is a shallow, estuarine system with a high concentration of freshwater input. The estuary is connected to the ocean, and the water temperature varies from 18°C to 28°C. The salinity varies from 0 to 60% seawater, depending on the season. The water depth is shallow, ranging from 0 to 5 m. The estuary is a important for the local economy, providing habitat for a variety of fish and invertebrates. The estuary is also home to a large population of T. granifera.

In January 2007, a dense population of T. granifera was found in a very shallow freshwater area connected to the ocean. The estuary is bordered by a sandy beach and a mixture of freshwater and seawater. The estuary is also home to a variety of fish and invertebrates. The water temperature varies from 18°C to 28°C. The salinity varies from 0 to 60% seawater, depending on the season. The water depth is shallow, ranging from 0 to 5 m. The estuary is a important for the local economy, providing habitat for a variety of fish and invertebrates. The estuary is also home to a large population of T. granifera.

In this study, the salinity and temperature tolerance of T. granifera were assessed. The results of this study will provide valuable information for the management of T. granifera in South Africa.
Chapter 1

MATERIALS AND METHODS

Study site

Catalina Bay is located on the east side of the entrance of St. Leon's South Harbor (Figure 1). The fishing vessels are usually equipped with at least one engine and one winch. The port is well-protected from the prevailing winds that come from the east. The bay is accessible from the north through a narrow channel and from the south through a wider channel. The depth of the water varies from 1 to 3 m, allowing an overall increase in water flow and salinity. During the open phase, the average salinity is in the range of 30-35 ppt.

Temperature tolerance

Gracilaria salicornia used in this study were collected from the seashore of Catalina Bay. The seaweed was sampled at different times of the day and was used as experimental material. Seaweeds were kept in the laboratory under a controlled environment and were placed in a climate-controlled room at 20°C with a photoperiod of 12 h of light followed by 12 h of darkness. Results were expressed as mean ± SEM.

Salinity tolerance

The experimental procedures for salinity tolerance were similar to the above with algal cells inoculated in 0.5 L of modified seawater media containing 2% NaCl, 2% MgCl₂, 2% CaCl₂, 0.2% KCl, 0.05% NaHCO₃, 0.05% Ca(OH)₂, and 0.05% Na₂SO₄. The seaweed was cut into small pieces and was incubated in 0.5 L of the medium containing 2% NaCl, 2% MgCl₂, 2% CaCl₂, 0.2% KCl, 0.05% NaHCO₃, 0.05% Ca(OH)₂, and 0.05% Na₂SO₄. The seaweed was kept in the laboratory under a controlled environment and was placed in a climate-controlled room at 20°C with a photoperiod of 12 h of light followed by 12 h of darkness. Results were expressed as mean ± SEM.

RESULTS

Temperature tolerance

Survival in the control and the 0°C, 5°C, 10°C, 15°C, 20°C, 25°C, and 30°C treatments of 7 days and 20 days was recorded. At 10°C, 15°C, 20°C, 25°C, and 30°C, the seaweed survived for a period of 7 days, while at 0°C, 5°C, and 15°C, the seaweed survived for a period of 20 days. Seaweed was cut into small pieces and was incubated in 0.5 L of the medium containing 2% NaCl, 2% MgCl₂, 2% CaCl₂, 0.2% KCl, 0.05% NaHCO₃, 0.05% Ca(OH)₂, and 0.05% Na₂SO₄. The seaweed was kept in the laboratory under a controlled environment and was placed in a climate-controlled room at 20°C with a photoperiod of 12 h of light followed by 12 h of darkness. Results were expressed as mean ± SEM.
Survival data were collected at temperatures ranging from 0°C to 50°C (optimal and sublethal temperatures for the species and 20°C control). The lower limit (optimal) was set at 10°C, 15°C and 20°C. The survival time of exposure to each temperature range is as follows:

- **a) Control**: Survival at 0°C.
- **b) 0°C**: Survival at 10°C.
- **c) 5°C**: Survival at 20°C.
- **d) 10°C**: Survival at 30°C.
- **e) 20°C**: Survival at 40°C.
- **f) 30°C**: Survival at 45°C.
- **g) 40°C**: Survival at 47.5°C.
- **h) 45°C**: Survival at 50°C.

*Note: Survival was calculated as a percentage of the initial number of animals (N) after exposure.*
in the 63°C and 75°C treatments did not survive even at 10°C, while only 20% of the survivors were in the 0°C treatment. The mean survival time for the 63°C and 75°C treatments was 10 days, while for 0°C it was 20 days. The 10°C treatment had the highest survival rate, with 90% of the animals surviving after 10 days.

To further investigate the effect of different temperatures on the survival of T. granifera, an ANOVA was conducted. The results showed that there was a significant difference in survival rate among the different temperature treatments (p < 0.05). The mean survival time was highest at 10°C, followed by 0°C, 30°C, and 63°C, respectively.

**Discussion**

The results of this study suggest that T. granifera is highly tolerant of temperature fluctuations. The animals were able to survive for up to 10 days in 63°C and 75°C treatments, while in 0°C, the survival rate was significantly higher. This finding is important for understanding the potential distribution of T. granifera in different environments, as temperature fluctuations are common in many habitats. The ability of T. granifera to survive in high temperatures may also be important for its role in the food web, as it can provide a food source for other species even during periods of high temperature. Further studies are needed to investigate the impact of temperature on the growth and reproduction of T. granifera, as well as the potential for it to adapt to changing environmental conditions.
vulnerable in terms of size and may provide the opportunity for T. granifera populations to gradually adapt to higher salinity as they undergo a gradual period of exposure.

Prospects for the control of T. granifera

In order for complete purging of populations to be economically feasible, the population has to be eliminated because of its pathogenic characteristics. The use of selective agents in the SARS experiment is achievable at lower average growth rates without the benefit of a screening system. However, there are already indications that the parasite population may be affected by exposure to salinity. Another option would be the introduction of biological control agents that are less costly and more sustainable. This could have significant implications for the local environment and economic sustainability.

In summary, T. granifera can be regarded as a global model for the study of marine and estuarine ecosystems. The present findings suggest that the effects of salinity on marine and estuarine ecosystems can be predicted, and that these effects can be mitigated by appropriate management strategies. However, it is clear that there is a need for further research on this subject, especially with regard to the impact of salinity on the survival of T. granifera, and the potential for the development of effective control strategies.
Figure 1. Distribution of species at different salinities in the study area. Percentages are based on samples per salinity with quiescent with operculum closed, head out of shell (foot not anchored), not moving (foot anchored), and actively moving as the response categories.
Chapter 1

Salinity and temperature tolerance of Tapesia granifera

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We dedicate this article to the memory of Dr. Ann M. Yeager, Technical Assistant at PIAP in Fiji. She was a valued member of the team and her contributions were essential to the success of the project. We also acknowledge the support of the East New Britain Provincial Government and the New Britain Agriculture Development Authority for their contributions to the project. Finally, we thank the New Britain Provincial Government for their support and the East New Britain Provincial Government for their assistance.

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Feeding dynamics of the invasive gastropod *Tarebia granifera* in coastal and estuarine lakes of northern KwaZulu-Natal, South Africa

Nelson A.E. Miranda*, Renzo Perissinotto, Christopher C. Appleton

School of Biological and Conservation Sciences, University of KwaZulu-Natal, Pietermaritzburg Campus, Pietermaritzburg, South Africa

**Abstract**

Geochemistry and carbon budget techniques were applied to invasive *T. granifera* (shell height 10–12 mm) at the iSimangaliso Wetland Park, a UNESCO World Heritage Site. This study has recently identified a number of estuaries in northern KwaZulu-Natal where it reaches densities of over 1000 ind. m⁻² and becomes a dominant component of the benthic community. Its rapid establishment and spread have raised concern about potential top-down impacts on the ecosystem. This study shows that *T. granifera* can utilize large amounts of macrophytobenthos (MPH) in addition to detritus. In situ total available MPH carbon concentrations ranged from 16.8 to 113 kg C per ha in *T. granifera* gut pigment carbon content ranging from 54 to 167 mg C per ind.¹. Gut excretion rates (R) ranged from 0.18 to 0.92 mg C per ind. per day (r = 0.51) on the basis of total MPH carbon content to determine MPH carbon flux. *T. granifera* was estimated to consume 2.0 to 30.8% of the total available MPH biomass per day, or 0.2–8.8% of the daily primary production. These estimates are based on the gut flux technique which contributed 27–50% of the total gut organic carbon content. The average carbon daily assimilation by *T. granifera* was 0.066 body carbon per day. Variability in the data was attributed to the complex feeding history of *T. granifera*. Further studies are needed to validate these results and provide more information on the ecological impact of *T. granifera* on the wetland and other similar invaded ecosystems, both estuarine and freshwater.

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1. Introduction

Non-indigenous invasive species (NIS) have been the focus of growing research across the globe (Lincoln et al., 2007; Davis, 2009). Invasive molluscs can dominate aquatic communities and disrupt ecosystem processes (Hall, 2005; Rhy, 2005; South, 2005; Potter, 2005; Baran, 2008). Impacts of this kind are especially relevant when protected areas may be affected and when there are economic repercussions (Parentel et al., 2005; South and Potter, 2005; Baran, 2008). Unfortunately, where NIS species are detected it is often not feasible to eradicate them. However, measuring the impact of NIS is still important from an adaptive management point of view. NIS research contributes to the process of management goal setting and determination where mitigation of the impacts of a given invader is one of the priorities (Bullock, 2006).

*Tarebia granifera* is a deep-sea branch gastropod originally from South East Asia that has become invasive in several countries around the world including South Africa (Abrahm, 1993; Potter et al., 2005; Appleton et al., 2009). It was first recorded in an estuarine environment at the St Lucia Estuary (South Africa) in 2005 (Miranda et al., 2005). Since then, well-established populations have been found in an increasing number of estuaries in northern KwaZulu-Natal, as well as coastal lakes such as the Kosi Lake and Lake Sibaya. The St Lucia Estuary, Kosi Lakes and Lake Sibaya are Ramsar sites of international importance, part of the iSimangaliso Wetland Park, UNESCO World Heritage Site.¹

¹ *T. granifera* is known to reach densities of over 1000 ind. m⁻² and displace other native invertebrates (Potter et al., 1998; Carrere et al., 2008; Pistorius and Potissinotto, 2008; Appleton et al., 2009). Preliminary measurements undertaken at the iSimangaliso Wetland Park indicated that this species had relatively high gut pigment contents (i.e., algal). Therefore, although *T. granifera* was reported to be a dominant species for food resources, there is currently a lack of knowledge of this species' ecological impact (Appleton et al., 2009) and information on *T. granifera* feeding ecology is urgently needed.

The effects of grazers are known to be significant in marine and freshwater ecosystems, and their underlying mechanisms are complex (Hay, 2009). Estimating the grazing...
impact of gastropods has classically been done by manipulating their densities in experimental arenas in the laboratory (Jange et al., 2008 and references therein). The present study has taken a different approach, providing information about the amount and rate of transfer of food (energy) which is useful in trophic ecology and carbon cycle studies. The techniques in this study were used to estimate feeding impact on the benthic microbial biomass and primary production and to determine the contribution of carbon contributed by autotrophic microbial biomass to the total organic carbon present in the gut of Taraba granifera. Mackas and Behren (1976), Persiannoto (1993), and Persiannoto et al. (2003). The aims were: (1) to develop a novel method of estimating in situ feeding rate and impact in a benthic intermaree; (2) to further knowledge on the feeding ecology of the invasive gastropod T. granifera.

2. Materials and methods

2.1. Study site

Taraba granifera snails of (10–12 mm shell length) were collected from three areas within the St. Lucia estuarine and Park in northern Kosi-Nganja Basin, South Africa (Fig. 1); St. Lucia Estuary, on 18 April 2007 and 1 December 2002; Kosi lakes, on 24 November 2001; and Lake Sibaya, on 16 November 2002.

The St. Lucia Estuary is the largest estuarine lake in Africa. It has a surface area of 325 km² and average depth of 0.5 m (Taylor et al., 2000). The St. Lucia Estuary has been experiencing unprecedented low water levels and its mouth has remained closed for the most part since 2002 (Mackay et al., 2003). Its high surface area to volume ratio leads to high evaporative water loss, causing a reversed salinity gradient and wide water level fluctuations. Particularly in the northern parts of the system, large areas tend to dry up or become hypersaline, but there are also areas which have brackish and freshwater conditions because of inputs from rivers, streams or seepage from sand dune aquifers (Taylor et al., 2005). Samples were collected on the eastern shores of Lake Sibaya at 27°20′ S, 32°22′ E (Fig. 1), where there are many freshwater streams and where the vegetation is composed of mangroves.

Kosi lakes were sampled at Lake Nhlangeni (27°17′5, 32°49′ E) (Fig. 1). The surface area of this lake varies from 31 to 37 km² and the average depth is 7.2 m (Begg, 1980). This lake is connected to the ocean and is affected by tides (Begg, 1980), however, at the time of this study, salinity levels were consistent with freshwater. The vegetation is composed of clear white sand.

Lake Sibaya has a surface area of 60–74 km² and an average depth of 13 m (Bruten, 1980). Evidence of the geological evolution of this freshwater lake, and the presence of many organisms of estuarine and marine origin in its waters, show that it was once connected with the ocean (Bruten, 1980). Here, samples were collected on the South East Bank (27°25′, 32°45′ E) (Fig. 1). Lake at Lake Nhlangeni, the substratum is composed of clear white sand.

2.2. Gut element content

Individual Taraba granifera guts were excised under a dissecting microscope (10× magnification). Chlophyll a and phaeopigments were extracted in 90% acetone at 4 °C over a 48 h period and measured using a 10-AU Turner Designs fluorometer fitted with a narrow-band, non-attenuation system (Welshofer, 1994). Chlophyll a and phaeopigment concentrations were added and reported as chlophyll a equivalents (pg/gm) (Smith and Purcell, 1986; Connolly et al., 1986).

Daylight differences in Taraba granifera's gut pigments were measured in the field at Lake Nhlangeni in November 2002 over a 24 h period (25°C) by measuring, removing and immediately processing at intervals of 2–4 h using the method outlined above. This was also done in the laboratory with samples collected from the St. Lucia Estuary in December 2002 utilizing laboratory conditions for three weeks under a photoperiod of 12 h light followed by 12 h darkness and fed at ambient with naturally occurring microphytoplankton (water temperature: 20°C) (Munro et al., 2014).

2.2. Feeding rates

Feeding rates were estimated by the in situ approach based on the gut fluorescence technique to calculate ingestion rates (µg pnm², h⁻¹). According to Mackas and Behren (1976) and Persiannoto (1992):

\[ f = K + \left( \frac{1}{h} \right) \]

where \( f \) is the gut evacuation rate constant (h⁻¹); \( K \) is the gut pigments concentration (µg pnm⁻¹); \( C \) is the gut pigments assimilation destructeur efficiency (a); to measure the gut evacuation rate (h), freshly caught Taraba granifera individuals were incubated in 10 mL vials filled with water collected in site and observed...
through a GFF glass fiber filter to remove all naturally available food particles. In order to promote continuous feeding conditions and to displace previously ingested food, non-fluorescent control particles (< 160 μm) were added to the filtered water (Perssino et al., 1998). Only actively moving individuals were used in the experiments (Milanta et al., 2010). There were 10 replicates for time zero and 5 replicates after each subsequent exposure period. Pigment concentrations were calculated every 10 min in the first hour and then every 10 min for another 2 h (total duration: 3 h). Gut pigment concentrations were measured using a 10 AU Turner Designs fluorometer as outlined previously. The results were assumed to follow an exponential model and 10% outliers were excluded (Perssino and Pullin, 1996).

2.4. Assimilation/digestion efficiency

Gut pigment assimilation/digestion was estimated within 48 h of collection of specimens from the field. Snails were first allowed to empty their guts of particles in filtered water collected in the field with pumps every period of 24 h. The two compartments (water and gut) pigment budgets approach was used to estimate gut pigment assimilation/digestion (Lopez et al., 1988; Mazzucco and Kozlowski, 1982). The pigment budgets in experimental treatments (2 Labs with 3 samples each) and controls (1 Lab with 3 samples) were calculated after an incubation period of 1 h (Dagg, 1989; Kanboe et al., 1989). Significant pigment losses from experimental treatments were attributed to gut pigment assimilation/digestion only as no fecal pellets were produced during the incubation period. Three replicates were prepared for each treatment.

2.5. Estimation of feeding impact

Tenebrio gut samples were collected in triplicate with an 18-mm (60 μm diameter) probe, which was pushed for 5 min to collect all the gut contents on the surface and buried in the upper 3 cm of sediment. These samples were used to calculate densities (ind. cm−2). Solidity and temperature were measured with a Yellow Springs Instrument 8200 pH meter. Sediment samples (upper 1 cm) were collected in triplicate with a Perspex corer (diameter: 20 mm). These samples were used to determine MBH biomass, as pigment concentration (μg mg−1). Pigment concentrations were measured using a 10 AU Turner Designs fluorometer as previously outlined, with the exception that extractions were done in 30 ml of acetone. The feeding impact (%) of MBH was determined as pigment assimilation/digestion rate with average density. This was then expressed as a percentage of total available MBH biomass.

2.6. Gut particle content: carbon budget and daily ration

The carbon budget approach of Perssino et al. (2000a) was used to estimate the contribution of the "isotrophic carbon component" to the diet of Tenebrio gramineus, which is referred to in this study as the carbon component estimated from the gut biomass carbon budget (GCB). Individuals of shell height = 11 mm were collected both at Lake Hlinage and St. Lucia Estuary and preserved at 20 °C. The stomach and intestines were later excised and gut contents were suspended in water, filtered onto pre-combusted GFF glass fiber filters and dried in an oven at 60 °C. Total carbon analyses were done with a Carlo Erba CHN Analyzer at the CSIR Division of Natural Resources and the Environment in Stellenbosch, South Africa. Triplicate consisted of the pooled gut contents of 15 individuals to determine the organic proportion or ash-free dry weight (AFDW) of the gut contents. A further 10 snails were dissected (Beets, 1986; Perssino et al., 2000). Weight measurements were done with a Shimadzu balance with resolution at 0.01 mg level. The carbon component estimated from GCB was estimated from data collected in 1998, as previously outlined (10 replicates per study area). A pigment:carbon ratio of 50 was used (De Jonge, 1880). The difference in the carbon component estimated from GCB between T. gramineus populations at Lake Hlinage and St. Lucia Estuary was tested with an independent-samples t-test (two-tailed). Analyses of variance (one way ANOVA) and Tukey's Honestly Significant Difference (HSD) post hoc tests were used to assess differences in terms of gut pigment content, ingestion rate and feeding impact, at different sites or times. Non-parametric gut pigment and MBH biomass data were tested with a Spearman rank correlation. All data analyses were done with the statistical program SPSS 13.

The daily carbon budget is the percentage contribution of the total average carbon that passes through the gut in 24 h to the total average carbon of the target animal. In this study, it was estimated using only the carbon component estimated from GCB to determine the average total carbon of Tenebrio gramineus, a population of 10 pooled individuals from Lake Hlinage was analyzed for total carbon. The average carbon component estimated from GCB for 24 h at Lake Hlinage was used.

3. Results

3.1. Gut particle content, evacuation rates and pigment assimilation/digestion efficiency

There were no significant day/night differences in Tenebrio gramineus' gut particle content measured at Lake Hlinage (n = 8, 0.66 ± 0.05 μg mg−1, Tukey's HSD, P = 0.05; Table 1). Similar results were obtained from analysis kept in the laboratory (n = 10, 0.66 ± 0.05 μg mg−1, Tukey's HSD, P = 0.05). The highest gut particle content mean ± SD in individuals of shell height 10–12 mm was measured at the St. Lucia Estuary in freshwater conditions at 1854 ± 161 mg pg−1 ind−1, whereas the lowest was measured at Lake Hlinage of 521 ± 23 mg pg−1 ind−1. Out particle levels (mean ± SD) at Lake Hlinage and St. Lucia Estuary in brackish conditions were 740 ± 85 and 167 ± 34 mg pg−1 ind−1, respectively. Gut pigments and MBH biomass were correlated across all sampling areas (Spearman rank coefficient = 0.78, P < 0.05).

An exponential model provided the best fit for the gut evacuation rate of Tenebrio gramineus in all experiments o0 in 10 as ranging from 3.2 to 10.2, P < 0.05 (Fig. 3). The highest gut evacuation rates (F) were estimated at Lake Hlinage and Lake Shaiya (0.2 and 0.43 h−1, respectively (Table 1). In the St. Lucia Estuary, the estimated gut evacuation rates were lower; 0.27 and 0.36 h−1 (Table 1). T. gramineus gut pigment assimilation/digestion efficiency (β) ranged from 26 to 57% in Lake Hlinage, it was increased (mean ± SD) to 32 ± 5% in Lake Shaiya 56 ± 7% and in the St. Lucia Estuary 41 ± 11%.

3.2. Estimation of ingestion rates and feeding impact

Tenebrio gramineus' ingestion rate ranged from 8.8 to 20.4 mg pg−1 ind−1 (n = 2). There was a significant difference in terms of ingestion rate between sampling sites (ANOVA, F = 12.50, P < 0.05). Ingestion rate was significantly higher at Calatma Bay in 2009 compared with other sites (Tukey's HSD, P < 0.05). T. gramineus' shell height 10–12 mm contributed to 26% of the total T. gramineus density over all sampled areas, making this size the second most prominent after the size of shell
height 1–8 mm, which contributed up to 75%. Densities were variable across all sampled areas due to T. prensis's patchy distribution. Seals were not found in some parts of the sampled area, whereas in other parts densities were as high as 3,000 ind. m$^{-2}$. The highest and lowest extremes in density were recorded at Catalina Bay (Table 2). The lowest being in 2006 at a relatively isolated freshwater seapage zone after most of the population had died in November 2007, due to high salinities brought about by a month-long event in March 2007. By February 2008 the population had increased in numbers and spread across the entire freshwater seapage zone. The feeding impact of T. prensis was estimated to range from 6.8 to 11.1 mg piper. cm$^{-2}$ d$^{-1}$ (Table 2). Feeding impact was not significantly different between sampling sites (ANOVA, F$_{3,12}$ = 2.55, P = 0.09; Tukey's HSD, P = 0.05). Total available MPR biomass ranged from 11.6 to 110.5 mg piper. m$^{-2}$ (Table 2). The highest MPR biomass values were measured at the St. Lucia Estuary and the lowest at Lake Niñango (Table 2). T. prensis was estimated to consume from 6.5 to 15% of the total available MPR biomass per day (Table 2).

3.3. Gut organic content, carbon budget and daily ration

The dry weight of Terebralia pretremsis gut contents ranged from 0.14 to 0.33 mg. The percentage contribution of organic material to the total gut contents ranged from 80.6 to 85.3% and averaged (mean ± SD) 84.4 ± 12.7%. The total gut carbon content ranged from 6.6 to 37.97 mg C ind$^{-1}$, averaging (mean ± SD) 20.68 ± 9.34 mg C ind$^{-1}$ at Lake Niñango and 22.05 ± 9.41 mg C ind$^{-1}$ at the St. Lucia Estuary. The carbon component which was estimated with the gut fluorescence technique contributed 97–40% of the total organic carbon in the gut, averaging (mean ± SD) 15.5 ± 7.2% at Lake Niñango and 26 ± 10% at the St. Lucia Estuary (Fig. 4). There was a significant difference, in terms of this contribution to the total gut organic carbon, between Lake Niñango and St. Lucia Estuary (t = 2.704, P < 0.05). The carbon daily ration estimated from the gut fluorescence technique for T. prensis at Lake Niñango was estimated to be 16% body carbon per day. A single individual had an average (mean ± SD) dry weight of 1.36 ± 0.05 mg, 40 ± 7.5% of which was carbon.

4. Discussion

This study is the first detailed account of Terebralia pretremsis's feeding ecology. To our knowledge, it is also the first time the gut fluorescence and carbon budget techniques have been used together on a benthic invertebrate. Although mainly adapted to this benthic gastropod, these techniques have been used more extensively on pelagic invertebrates and, unfortunately, the information available in the literature may not be entirely comparable to that obtained in this study. Despite this, some of the key generalizations and potential sources of error are discussed and there is an attempt...
to reconcile the results presented with some observations and hypotheses, in order to contribute to the knowledge of this non-indigenous invasive species.

41. Feeding dynamics at different sites and ecological significance

Tambaqui’s highest gut pigmentation content was measured at the St. Lucía Estuary in freshwater conditions and low sand densities. At that time, T. tambaqui was recovering from a population crash due to high salinities (Miranda et al., 2011). Although the gut evacuation rate was not very high when compared to other sites, the ingestion rate was the highest estimated in this study (Table 1). The high ingestion rate is probably related to high availability of food and low grazing pressure under low population density (Miranda et al., 2011). For low gut evacuation rate, it is important to notice that the gut evacuation rate was observed to increase in gut post-feeding time. Changes in gut turnover time are related to nutritional quality of available food and metabolic energy requirements (Calow, 1977). Therefore, T. tambaqui’s highest gut pigmentation rate could be the result of a strategy to increase assimilation in order to meet its energy requirements for reproduction (Tamayo and Martin, 2008). The highest MFB biomass was also measured at the St. Lucía Estuary (Table 2). The lakes of the Kosi system (including Lake Nhlanga) and Lake Shilale have been reported to have a low availability of nutrients and hence lower productivity when compared to the St. Lucía Estuary (Allanson, 1979; Dagg, 1980; Britton, 1980; Adams and Britton, 1990). Assuming that all of the pigments detected in the gut of T. tambaqui are of microphytoplankton origin, they constitute a significant protein portion of the total gut organic carbon in the St. Lucía Estuary compared to Lake Nhlanga. Fig. 4. The correlation between gut and food source (MFB in this case) pigmentation indicates that both have been widely reported for T. tambaqui (Antzani et al., 1992; Ichii et al., 1998; Anderson et al., 2000). The highest gut evacuation rate was obtained in the more stable freshwater sites (Table 1). Interestingly, the gut evacuation rate under brackish conditions was relatively high (Table 1). Although an increase in salinity is expected to decrease T. tambaqui’s feeding impact (Lawrence et al., 2007), Miranda et al. (2011) showed that this species can maintain a relatively high level of physical/physiological activity at salinities up to 30. This may be relevant to explain T. tambaqui’s establishment success in the brackish waters of estuaries.

Tambaqui’s feeding impact can be much higher than that of other teleost species. It has been reported to favor MFB in its diet and significantly reduce algal standing stocks (March and Pringle, 2003). The ingestion rate of T. tambaqui can be 20 times higher than that of Heteropneustes fossilis, which feeds primarily on MFB and influences its biomass dynamics in the inshore mixed zone of Western Europe (Marchand et al., 2006; Eklöf et al., 2005). In this study, the estimated feeding impact of T. tambaqui on MFB was relatively high, reaching values of up to 23% per day of the available biomass. According to the literature, daily primary benthic production can range from 72 to 600 mg C m⁻² d⁻¹ in the Mzimba lakes (Allanson, 1979; Dagg, 1980; Britton, 1980). Given this, T. tambaqui is estimated to ingest 12–50% of the daily primary benthic production. Similar values have been reported for a variety of species, which are known to affect pelagic ecosystem functioning (Price and Patten, 1989; Britton and Patten, 1993). These results are also comparable to those obtained by Håkansson et al. (2003) for the freshwater non-indigenous invasive cladoceran Daphnia galeata, which is known to have marked ecosystem-scale impacts. The estimated feeding rate on MFB per gram of dry weight per day (gDW/d) was calculated as the resulting value of 124.3 ± 16.3 gDW/d is comparable to that of Decapods crustacea, which is based on the world’s most non-indigenous invasive species (Love et al., 2000; Baker et al., 2003). This study revealed significant differences in feeding impact between sites. Therefore, feeding impact is a variable that can be considered at all invaded sites, justifying concerns over the top-down ecological effects of T. tambaqui (Håkansson and Golden, 1979; Hollebrandt et al., 2002). Feeding impact is directly proportional to density, that is, it may be severe where small densities increase over 1000 ind. m⁻². The distribution of T. tambaqui was patchy despite seemingly favorable environmental conditions. Although this is common in bentho-invertebrates (Gotelli et al., 1999), the implication is that feeding on MFB becomes focused on certain areas. In dense small patches the feeding impact can potentially result in the depletion of available MFB. This is however an interaction between aquatic grazers and nutrients which was not explored in this study (Bartolome and Bay, 2000). The composition of MFB was also not taken into account. T. tambaqui, although suspected to be a grazer consumer, may select certain algal groups over others and thus change the relative abundance and biomass of the key algal groups that make up MFB (Arroyo et al., 2009).

Table 2. Feeding impact of Tambaqui (mean ± SE, n = 3) in Lake Nhlanga, Lake Shilale and for the St. Lucía Estuary. Density and microphytoplankton (MFB) concentration are expressed as means. MFB biomass, mean feeding impact and microphytoplankton (MFB) density are expressed as a range.

<table>
<thead>
<tr>
<th>Area</th>
<th>Density (ind. m⁻²)</th>
<th>Ingestion rate (gDW/d, mean ± SE)</th>
<th>Feeding impact (gDW/d, mean ± SE)</th>
<th>MFB concentration (mg m⁻³, mean ± SE)</th>
<th>T. tambaqui consumption of MFB per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake Nhlanga</td>
<td>38 ± 6</td>
<td>65 ± 7.8</td>
<td>14 ± 2.8</td>
<td>137 ± 10.2</td>
<td>2.2 ± 0.4</td>
</tr>
<tr>
<td>Lake Shilale, Matlala</td>
<td>159 ± 10</td>
<td>112 ± 11.9</td>
<td>2.4 ± 0.3</td>
<td>277 ± 15.7</td>
<td>2.9 ± 0.9</td>
</tr>
<tr>
<td>St. Lucía Estuary, Goba</td>
<td>92 ± 5</td>
<td>112 ± 26.4</td>
<td>62 ± 17</td>
<td>610 ± 38.4</td>
<td>5.1 ± 1.8</td>
</tr>
<tr>
<td>St. Lucía Estuary, Constancia Bay</td>
<td>241 ± 7</td>
<td>71 ± 19.5</td>
<td>56 ± 11.1</td>
<td>901 ± 36.4</td>
<td>7.6 ± 3.5</td>
</tr>
</tbody>
</table>
sediment variability and MPR transgression (Anderson, 2004; O'Connell et al., 2004). The effects of this variance are not straightforward: there may be direct and indirect cascading effects on the ecosystem which ultimately affects community structure (Levinson and Stewart, 1982; Hunt et al., 1987; Anderson, 1999; Hiller et al., 2000). For example, and in addition to affecting MPR, T. granifera may be affecting the density of other macroalgae as well as their local settlement and recruitment (Jensen and Stensgaard, 1980; Hunt et al., 1987; Mansley, 1988; Kitchen et al., 2003). Indeed, T. granifera has been reported to displace indigenous gastropods, thus taking over their niche (Perera et al., 1999; Painter, 1990).

4.2. Variability in the data: complex feeding ecology and energetic demands

T. granifera appeared to feed continuously throughout the day and night. There was, however, a wide variation in the level of its gut pigments measured both in situ and in the laboratory (Fig. 2). Gut turnover rates (K) varied from 1.6 to 2.8 in actively feeding individuals. Although an exponential model was fitted to the gut evacuation data, the coefficients of determination (R²) were relatively low when compared to those obtained in similar studies using pelagic crustaceans (Brommer et al., 2000; Kebirge and Pistorius, 2006). There were individuals in all experiments that had ingestion rates as low as 0.57 gut pigments nd -1. These individuals made up to 15% of the total subjects although their influence in the estimation of gut evacuation rates (K) was minimized by the removal of 10% outliers. The range in daily weight of the gut contents suggests that there was a wide range of gut fullness. Alternatively, snails may have been feeding on material with different volumes.

It is proposed that the complex feeding history of T. granifera can largely explain the wide variability in the data. Individual feeding variability is commonly reported for molluscs (Spitzer, 2001; Haynie et al., 2003; Ford and Johnson, 2004). It is evident that not all the individuals in the population are feeding at the same time. Feeding rate is influenced by temperature and salinity, among other factors (Hann and Peterson, 1981; Jackson and Forbes, 1983; Hanlon and Branch, 1983). However, some snails have been observed in a quiescent state in the field, despite favorable conditions of temperature, salinity and even food availability. This behavior may be density-dependent, in that snails observed in the field appeared to be more active and engaged in feeding when they were less abundant, compared to those found in dense populations (Brown et al., 1994; Eckert et al., 2013). The mechanism that triggers T. granifera's quiescent state is not understood, but it is probably chemically mediated (Cook, 1980). Since populations often reach high densities, quiescence/activity cycles may be a strategy adopted in order to minimize resource use while maintaining high densities. When unpopulated between cycles of feeding and quiescence has been reported for P. carnea (Wada and Yasuda, 2000).

The carbon component which was estimated from GFF contributed a much smaller proportion of the total organic carbon in the gut than expected (Fig. 4). However, the carbon daily ration calculated from carbon estimated from GFF is relatively high (~10%) according to the literature (Petrisimons, 1992; Bellgrand 2006). This suggests that MPR alone can meet the energetic requirements of T. granifera. It is supported by the survival and reproduction of T. granifera snails kept in the laboratory and fed ad libitum with naturally occurring MPR for a period of over one year (pers. obs.). T. granifera's variable feeding rate may indeed be related to energetic demands, one of which may be reproductive energy demand (Teaney and Martin, 2008). T. granifera is a hermaphroditic snail with a long reproductive season, which has been shown to produce abundant viable eggs and sperm (Teaney and Martin, 2008). It is not surprising to find that the size class of shell height 1–3 mm was much more abundant and therefore it is assumed that the impact of smaller juvenile snails on food resources may be even more severe than that of the adults (Bobalinski et al., 2008; Tomlinson and Paul, 2006).

4.3. Potential sources of error

T. granifera's gut pigment assimilation/destruction efficiencies (b') were estimated in order to account for a loss of detectable pigment during the digestive process, which would result in the underestimation of ingestion rate (Pasternak, 1994). There is evidence that the assimilation rate is lower in the digestive tract of the snail compared to the gut evacuation rate, as shown in the work of Barbe et al. (2006). The feeding impact of this study could be underestimated by a factor of between 1.4 and 2.3. However, this is a conservative estimate, and the actual underestimation could be higher. There is some evidence that the assimilation rate is lower in the digestive tract of the snail compared to the gut evacuation rate, as shown in the work of Barbe et al. (2006). The feeding impact of this study could be underestimated by a factor of between 1.4 and 2.3. However, this is a conservative estimate, and the actual underestimation could be higher.

A plot was made to include all recent studies from experiments, as they would otherwise introduce very high or even low gut pigment concentration values. If snails went into quiescence with a full gut and were selected for the gut evacuation experiments, the gut pigment signal may have remained high even when compared to snails that fed continuously. The duration of the quiescent state is not known, but it is likely that over time, the pigment in the gut may also be degraded to non-fluorescent red products, which would not be detected with a fluorimeter. Active feeding snails were selected in an attempt to avoid variation in the data and in view of maintaining continuous feeding conditions (Babcock and Helling, 1982; Nobbe and Hellberg, 1987; Petrisimons, 1992). It is possible that snails were disturbed during collection and thus may be active snails as they came out of quiescence. Seemingly active snails for gut evaluation measurements would result in low gut pigment values.
Finally, the origin of the proportion of total gut organic carbon cannot be determined with the techniques used in this study. It was assumed that the gut pigments detected were entirely of microphytoplankton origin. So, what about the remaining component that was not estimated from the GPP? There may be a heterotrophic component to the diet, as the small fish may feed on some benthic microorganisms or even decaying animal material (Charlton et al., 1984). However, it is also possible that Dunlin granulans feeds on a high proportion of plant detritus. This component, although of autotrophic origin, may not be detected with available fluorometric techniques, as plant material decomposes rapidly in water and phytoplankton pigments are degraded to non-fluorescent products (Lauder et al., 1988; Cuny et al., 2002). This process is influenced by multiple factors and may take between one and three days. Unfortunately, it was not possible to establish the proportion of detritus in the plant diet when it was consumed (Lauder et al., 1988: Leal et al., 1988). It is also suggested that this granulans is not dependent on MPW for food and may therefore be highly on detritus if MPW resources are low. Stable isotope studies are needed to bridge this gap in our knowledge.

5. Conclusion

Estimating the impact of invasive species on ecosystem resources requires a multiple method approach, in order to obtain information on its nature, pattern and magnitude (Ross et al., 2003; Guttman, 1998). Field experiments with small spatial and temporal scales which provide quantitative estimates of impact, can simply be repeated at several sites and several times in order to estimate large scale impacts (Ross et al., 2003). This study presents one such field experiment as a alternative to other more classical approaches (Conway and Peckarsky, 1995). Results show that Dinella granulans can utilize large amounts of microphytoplankton in addition to Dinella nitrogen, and are allocated to non-Dinella and detritus products (Lauder et al., 1988; Cuny et al., 2002). If this continues to occupy a high proportion of plant detritus when it is consumed (Lauder et al., 1988: Leal et al., 1988). It is also suggested that this granulans is not dependent on MPW for food and may therefore be highly on detritus if MPW resources are low. Stable isotope studies are needed to bridge this gap in our knowledge.

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References

Stable Isotope Evidence for Dietary Overlap between Alien and Native Gastropods in Coastal Lakes of Northern KwaZulu-Natal, South Africa

Nelson A. F. Miranda*, Renzo Perissinutto
School of Biological and Conservation Sciences, University of KwaZulu-Natal, Durban, South Africa

Abstract

Background: *Tarebia granifera* (Lamarck, 1822) is originally from South-East Asia, but has been introduced and become invasive in many tropical and subtropical parts of the world. In South Africa, *T. granifera* is rapidly invading an increasing number of coastal lakes and estuaries, often reaching very high population densities and dominating shallow water benthic invertebrate assemblages. An assessment of the feeding dynamics of *T. granifera* has raised questions about potential ecological impacts, specifically in terms of its dietary overlap with native gastropods.

Methodology/Principal Findings: A stable isotope mixing model was used together with gut content analysis to estimate the diet of *T. granifera* and native gastropod populations in three different coastal lakes. Population density, available biomass of food and salinity were measured along transects placed over *T. granifera* patches. An index of isotopic (stable isotopes) dietary overlap (IDO, %) aided in interpreting interactions between gastropods. The diet of *T. granifera* was variable, including contributions from microphytobenthos, filamentous algae (*Codiophore* sp.), detritus and sedimentary organic matter. IDO was significant (>60%) between *T. granifera* and each of the following gastropods: *Haminoea natalensis* (Kraus, 1848), *Balanus natatorius* (Körner, 1841) and *Melanoides tuberculata* (Mueller, 1774). However, food did not appear to be limiting. Salinity influenced gastropod spatial overlap. *Tarebia granifera* may only displace native gastropods, such as *Assiminea cf. avatara* (Kraus, 1848), under salinity conditions below 20. Ecosystem-level impacts are also discussed.

Conclusion/Significance: The generalist diet of *T. granifera* may certainly contribute to its successful establishment. However, although competition for resources may take place under certain salinity conditions and if food is limiting, there appear to be other mechanisms at work, through which *T. granifera* displaces native gastropods. Complementary stable isotope and gut content analysis can provide helpful ecological insights, contributing to monitoring efforts and guiding further invasive species research.

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* E-mail: 204507899@ukzn.ac.za

Introduction

Alien invasive species (AIS) can cause disruptions to ecosystems. However, quantifying their impacts is problematic due to the complexity of ecological interactions [1,2]. Stable isotope analysis is a powerful quantitative tool for detecting and tracking changes in trophic structure and ecosystem processes (energy flows) caused by abiotic and biotic interactions [3]. The combination of stable isotopes with other techniques is very useful for assessing interactions between several organisms and contributes towards a better understanding of how an ecosystem can be affected by native and non-native species [4,5]. Stable isotope analysis provides a time-integrated view of ecological processes, whereas gut content analysis reveals snapshots of feeding activity [6]. Stable isotope and gut content analyses are complementary, providing insight into diets and trophic dynamics in ecosystems [7,8] and have successfully been used in several studies addressing the impacts of AIS on aquatic ecosystems [9-12]. Although comparable studies on gastropods are lacking, numerous studies have been published on invasive gastropods such as *Pomacea canaliculata* (Lamarck, 1822) (prosobranch, Amphaliidae) [13-15] and *Pomatusugia microcephala* (Gray, 1849) (prosobranch, Haliotidae) [16,17], some of which involved stable isotope techniques [18,19].

*Tarbesia granifera* (Lamarck, 1822) (prosobranch, Thoracidae) is a non-native invasive gastropod originally from South-East Asia. This AIS is reported from many tropical and sub-tropical areas of North and South America and Africa [20-24]. *Tarbesia granifera* was accidentally introduced in South Africa, probably in the 1950s via the aquarium trade [24]. This is reported as one of the most recent introductions of non-native gastropod into South African natural environments, where it has quickly become invasive and widespread, particularly in the KwaZulu-Natal and Mpumalanga provinces [21,25]. *Tarbesia granifera* is parthenogenetic and ovoviviparous, giving birth to live juveniles and often reaching population densities of over 1000 ind. m⁻² [26]. *Tarebia granifera* has been successfully used as a biological control of schistosomiasis...
in the Cretaceous, events such as the replacement between marine grass beds (25-26). In South Africa, 9 species is today invaded by a growing number of coastal lands. This invites us to think about potential and good ecologists, in the discovery of the micro-habitat (24-26). Some species on land have fast developing habitat and new connections with this habitat (24-26). However, the mechanism behind this change remains unclear and questions about its effect will need to be made (24-26).

The study was carried out on three research articles to determine the effects of different grasses and other dominant grassland populations, and to determine whether the development from these grasses and other grasses that are occupying this study area. The treatment for this study is only the presence or absence of grasses as well as the types of grasses in the form of ducks and hogs. A portion of the grasses to be studied are shown in Figure 1. The results are indicated in light of the high diversity.

Materials and Methods

Study sites

Swartkops was considered in three different coastal lakes which were studied, namely, the Kosi grasslands over the last decade (Fig. 1).

The St. Lucia Estuary was sampled in October 2005, March 2005 and February 2005. The St. Lucia is a brackish lake in South Africa with a surface area of about 125 km² and average depth of 0.02 m (24).

The sample site, Catalina Bay, is a large inshore area covered by the lake water and located on the eastern shores of South Lake (39°1°54′N, 27°3°10′E). The vegetation forms the main feature included green algae, seaweed, and Seaweed spp. Throughout the sampling period, Catalina Bay experienced significant changes in water quality and stability. These were influenced by an annual spring bloom of green algae, caused by nutrient input from the inland areas during March (25). Water temperatures are not a significant concern in the coastal ecosystems (25). Prior to the study, the coast was never completely studied from the ocean for almost 20 years.

Zebra grass has been found in the St. Lucia Estuary since 2005, but its abundance is not considered to be a major concern area east of the South Lake where it has a small presence. The grasses are usually dominated by the native grasses that are common in the area, based on preliminary observations (25). However, the grasses are not very common and they are not considered to be a major concern area east of the South Lake where it has a small presence (25).

Lake Sibaya in the south east basin (25°25′S, 33°24′E) was sampled in November 2005 (Fig. 1). The lake is considered a shallow area of 60 to 77 km² and an average depth of 15 m (25). It was

![Figure 1. Study areas. The Kosi River, Lake Sibaya and the St. Lucia Estuary for pioneering work on the effects of different grasses and other dominant grassland populations.](image-url)
Chapter 3

Chapter 3

Fruit acidity, ripening, and yield of the fruit are influenced by several environmental factors, including temperature, humidity, and soil conditions. The fruit acidity is measured using a pH meter, which indicates the level of acidity or alkalinity of a solution. The pH scale ranges from 0 to 14, where 0 is the most acidic and 14 is the most basic. A pH of 7 is considered neutral.

Fruit ripening is a complex process that involves changes in the fruit's color, texture, and flavor. The ripening process is influenced by several factors, including temperature, light exposure, and the presence of ethylene gas. Ethylene gas is produced by the fruit itself and is a natural hormone that triggers ripening.

Yield of the fruit is also influenced by several factors, including the care and management of the orchard, the variety of the fruit, and the climate. Proper management of the orchard, including pruning and fertilization, can help increase the yield of the fruit.

In addition to these factors, the fruit yield can also be affected by pests and diseases. Regular monitoring of the orchard and the use of appropriate pesticides can help control these issues.

Overall, the fruit industry is an important part of the economy, providing employment and income for farmers and workers. The fruit industry also plays a role in global trade, with many countries exporting their fruit to other countries around the world.
gastropod species, which provided a better resolution of results \[56\]. Sources that were not found in the gut content analysis and/or had minor contributions (<5%) in the trial mixing models were omitted from the final mixing models. Isotopic dietary overlap (IDO, %) between species j and k was then calculated with the following equation based on Schoener’s Index \[57,58\]:

\[
\text{IDO}_{jk} = \left( \sum_{i} \min(p_i/p_a) \right) \times 100
\]

where \( p \) is the SIM mean contribution of source \( i \) resulting from the final mixing models run for subjects \( j \) and \( k \) respectively, and \( a \) is the total number of different resources used by both \( j \) and \( k \). IDO values have an absolute limit of 100%, which indicates complete overlap, and values exceeding 60% were considered to indicate significant dietary overlap \[57\].

Normality and equality of variance of stable isotope data were confirmed, and analysis of variance (ANOVA) was used for comparisons between locations and times. Pearson correlation was used to assess relationships between gut content and stable isotope results. Analyses were done with the statistical package SPSS version 19 for Windows.

**Results**

**Spatial overlap and resource availability**

*Tanais granifera* and native gastropods. A linear regression was used to assess the relationship between gut content and stable isotope results. Analyses were done with the statistical package SPSS version 19 for Windows.

**Gastropod diets**

Gut content analysis revealed that macroalgae and detritus were ingested by all gastropods (Table 2). Filamentous algae were also found in the guts of host gastropods (Table 2); their occurrence was 89.17% in *T. granifera* and 92% in native gastropods. Sand particles were only found in the guts of *H. natator, B. natator*, and its smaller amounts in *T. granifera* adults (shell height >10 mm) and *H. natator* (Table 2). Distinctive remains of microalgae were not found in gut contents. Macroalgae and detrital vegetation such as reds, seagrasses, and grasses were excluded as potential food sources as their presence was not detected in the gut.

Statistical analyses revealed that *T. granifera* δ^{13}C and δ^{15}N signatures differed significantly between locations and between

---

**Figure 2.** Gastropods, food sources and diets in Catalina Bay, 2007. (A) Four-point 6 m transect showing gastropod densities and microphytobenthic (MPB) biomass (as chl-a concentration). (B) Nitrogen and nitrogen stable isotope signatures of gastropods (number in brackets: trophic position) and their potential food sources, such as detritus (DTR) and sedimentary organic matter (SOM). (C) Proportional contribution (%) of different food sources to the diet of *Tanais granifera* and (D) *Hieminoe natator*.

Samples were collected in a freshwater seepage area of Catalina Bay in June 2007. Salinity ranged from 16 to 32. The water level of the South Lake rose due to the March 2007 mouth break, after which the freshwater ponds associated with seepage areas along the eastern shores were flooded by seawater.

doi:10.1371/journal.pone.0031109.g002
Figure 3. Gastropods, food sources and diets in Catalina Bay, 2006. (A) Transect 6 m transect showing gastropod densities and chlorophyll-aa (CHL) biomass levels (n = 10, 15, 20, 15, 15, 15). (B) T. granifera (NGM) and T. ovata (NGM) levels in the transect. (C) T. granifera (NGM) and T. ovata (NGM) levels in the transect. (D) T. granifera (NGM) and T. ovata (NGM) levels in the transect. (E) T. granifera (NGM) and T. ovata (NGM) levels in the transect.
Discussion

Among the three species found in the present analysis, none of the species showed a significant difference between the two stations (Fig. 3, Table S1). The RSD between the three species was not significant (Fig. 3). The RSD between the three species was not significant (Table S1). The RSD between the two species was also not significant (Table S1). However, the RSD between the two species was not significant (Table S1). The RSD between the two species was not significant (Table S1).
Table 2. Gut microbial content analysis in three coastal sites of Mozambique

<table>
<thead>
<tr>
<th>Location and year</th>
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<th>Filamentous algae</th>
<th>Sand particles</th>
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<tr>
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<tr>
<td>Sand particles</td>
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Table 3. Statistical analysis of stable isotopic signatures of gastropod tissues

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<th>MS</th>
<th>F</th>
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<td>&lt;0.001</td>
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<tr>
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<td>S. jenni</td>
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Table 4. Statistical analysis of stable isotopic signatures of gastropod food stained types

<table>
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<td>1.33</td>
<td>&lt;0.001</td>
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ACKNOWLEDGMENTS (MBR) thanks to the Late Mr. Neves and Late
Mr. Dhlakama for identifying Late Mr. Neves after he passed away in 2001.
Late Mr. Dhlakama was identified at the University of Chemistry,
Department of Molecular and Applied Chemistry, University of
Maputo, Mozambique, and his work is acknowledged in this paper.
Table 3. Selection clause for bayou vs. geyser as places to visit in three coastal areas of Louisiana.

<table>
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<th>Visitor satisfaction (%)</th>
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<tr>
<td>Galveston Island, Texas</td>
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...
Population Structure of an Invasive Parthenogenetic Gastropod in Coastal Lakes and Estuaries of Northern KwaZulu-Natal, South Africa

Nelson A. F. Miranda, Renzo Perissinotto, Christopher C. Appleton
School of Biological and Conservation Sciences, University of KwaZulu-Natal, Pietermaritzburg Campus, Durban, South Africa

Abstract

Background: Estuaries and coastal lakes receive little attention despite being heavily invaded by non-indigenous invasive species (NIS). In these situations, studies of population dynamics in invaded habitats can provide valuable insights into how NIS interact with new environments. Tenticola granulata is a parthenogenetic gastropod from South-East Asia which has invaded other sub-tropical parts of the world. This study addresses whether a small number of key environmental factors influence gastropod communities, and specifically how the population density and size structure of T. granulata are influenced by environmental change in estuaries and coastal lakes in southern Africa.

Methodology/Principal Findings: T. granulata density, number of brouched juveniles and size structure were measured at the St Lucia Estuary, Hibberdene Estuary, Lake Sibaya and Lake Hludzini. Site structure was classified according to shell height (SH). All observed individuals were found to be female and free from trematode infection. Salinity, water depth, temperature, and pH were the main factors correlated with population density of gastropod communities. T. granulata often reached densities well over 1000 ind m$^{-2}$, displacing indigenous gastropods and becoming a dominant component of the benthic community. T. granulata successfully invaded estuaries despite frequent exposure to high salinity and desiccation, which could together eliminate >99% of the population. The persistence of T. granulata was ensured due to its high fecundity and the environmental tolerance of large adults (20-30 mm SL), which carried on average of 159-128.5-50 brouched juveniles. Repeat introductions were not essential for the success of this parthenogenetic NIS.

Conclusion/Significance: There is a need for a broader study on the reproductive biology of T. granulata (including the previously overlooked "brood pouch ecology"), which affects population dynamics and may be relevant to other parthenogenetic NIS, such as Abaladae subulata and Pometrigyris amphigadenus.

Keywords: NIS, Tenticola granulata, population density, estuarine invaded habitats

Introduction

Although sexual reproduction is found in numerous organisms, it is now to find obligate parthenogenetic taxa, in which single organisms can only reproduce by producing genetically identical offspring [1]. Some gastropods show patterns of geographic parthenogenesis, where annual populations remain different from populations that reproduce sexually [2,3]. Some populations may have several advantages for establishment, lower and variable birth rate, as parthenogenetic organisms can use a population [3,4]. The system is isolated from gene flow and adaptation to the new habitat, especially in "general purpose gastropods," since they are not broken by recombination [5]. Some taxa are able to produce viable offspring from heterospecific crosses [6]. In habitats where populations undergo frequent local extinction and colonization events, genetic bottlenecks and demographic options are used to increase fitness consequences for sexual populations [7]. Also, due to the Red Queen Hypothesis, reduced mate interactions with parasites and pathogens reduce sexual populations [8], thus leaving [9].

The geographic patterns and sizes of species' invasive are changing on an unprecedented scale due to their rapid invasion and anthropogenic activity [10]. Non-indigenous invasive species (NIS) and non-indigenous invasive species (NIS) are a serious threat to biodiversity [11], particularly in estuarine and coastal environments [12]. Fortunately, relatively few introduced species are successful in establishing populations, fewer than 5% of species ever become invasive [13]. Changes in the invasive success of NIS and their ability to spread and colonize will only become possible [14]. Changes in the invasive success of introduced species are not only a concern for maintaining biodiversity [15], but also for the Red Queen Hypothesis, reduced mate interactions with parasites and pathogens reduce sexual populations [16], thus leaving [17].

However, recent studies have shown that invasive parthenogenetic species are adapted to invade marginal habitats with wide ecological tolerance, high productivity and very high population densities would make a successful NIS [18].
Talapia galilaea is a postbenthic gasteropod (Thaumatomyia) originally from south-east Asia. This parthenogenetic species has a brood pouch and gives birth to fully developed juveniles. *T. galilaea* has high fecundity and has been reported to reach densities over 20 009 in m² [19]. It has invaded several sub-tropical parts of the world, including Texas, Hawaii, Caribbean islands, Mexico and Israel [20-22]. In South Africa, *T. galilaea* has invaded on increasing number of continents and coastal lakes over the past decade [30]. The species is regarded as a freshwater invader, but its recent invasion patterns [19], a physiological tolerance study [24] and a minimum accepted 1.5 million years [25] suggest that this species is pre-adapted to brackish environments. The shallow marginal habitat of coastal lakes and rivers can be currently variable due to runoff events involving changes in water level and salinity have been observed to repeatedly wipe out most of the *T. galilaea* population and yet, this NIS not only persists but often becomes a dominant component of the shallow-water benthic community [pts 00].

This study aimed to address two questions: 1) is gasteropod community structure influenced by a small number of key environmental factors? 2) Are the longer term effects of environmental change on *T. galilaea* within a variable estuarine setting? The size structure of *T. galilaea* from one of the largest colonies has previously only been described for freshwater bodies and in the literature. Differences in population density and structure over time and under different environmental condition revealed how *T. galilaea* populations persisted during variable periods and then declined. The gasteropod reproduction output was relatively assessed in terms of number of embryos juveniles in the brood pouch.

**Materials and Methods**

**Ethics Statement**

Permission for this study was granted under a Research Agreement with the Shimangalo Wetland Park Authority for the project titled "Climate Change and the Management of KZN Estuaries: St Lucia Estuary".

**Study site**

The St Lucia Estuary is the largest estuarine lake in Africa, with a surface area of 423.5 km² and average depth of 2.3 m [38]. Recently, deposits have been discovered representing previous low-water levels and the mouth has been closed for the first time in 2002 to prevent. There is a reverse salinity gradient and high activities over five times that seawater were recorded in the most northern parts of the system [28]. However, due to the extensive opening of South Lake, receive a considerable input of freshwater and saltwater are the estuaries [30]. Samples were collected at Catocala Bay (33° 30', 32° 39'), on the eastern shores of South Lake (Fig. 1). In March 2002, the St Lucia Estuary was breached seawater from the Indian Ocean entered the system [29], increasing water levels and introducing a large number of marine species, including the sea hare *Aplysia californica* (Fig. 2).

Mapouzeli Estuary (37° 39' S, 39° 40'E; Fig. 1) has a surface area of 95,014 km², maximum width of 25 km and average depth of 0.3 m [32]. This estuary, although small, has a long history of salinity in its estuaries [32]. It is a typical Estuary Opened Closed System (TOLS: P. Possidonio et al. 2010) and a combination of low rainfall and high impact may result in the periodical closure of the mouth [31].

Lake Sibaya has a surface area of 58.7 km² and an average depth of 13 m [34]. This limnetic freshwater lake currently undergoes wide variations in water level [34]. Samples were collected at the eastern shores (32° 25', 32° 45'; Fig. 1).

**Lake Valume (2003):** The area of the Kosi Lakes (Fig. 1). The surface area of this lake varies from 50 to 30 km² and the average depth is 7.2 m [31]. Lake Nkhlume is connected to the ocean, a number of rivers and other lakes, but salinity is low [35]. Samples were collected on the western shores.

**Sampling procedure**

The St Lucia Estuary was surveyed at quarterly intervals between February 2007 and March 2011. Mapouzeli Estuary, Lake Sibaya and Lake Nkhlume were surveyed during the wet seasons in 2007 and 2010 (Table 1).

**Physical and chemical parameters**

All samples were taken to shallow marginal habitats (35° 25' S, 35° 45' E). Salinity, dissolved oxygen, pH and temperature were measured with a YSI 6920 multiprobe. Sub-surface water samples were stored using a GF/F filter and the ammonia was analysed for nitrate and nitrite with a Technic SpectroGard continuous flow analysis. Sediment samples were dried and weighted before being sent through a 200-mum sieve. The
Figure 2. Gastropod population densities (± SD) under changing salinities and depths. Data were collected at Catalina Bay in the St. Lucie Estuary from 2007 to 2011 at quarterly intervals. The black horizontal bar represents a closed mouth phase, the white bar represents the presence of a freshwater pond, and the grey bar represents the desiccation of most of the sampling area. Species: A. cf. cavata, A. marmorata, A. reticulata, and S. striatus. doi:10.1371/journal.pone.0043437.g002
Gastropods

Trichobranchia samples were taken with a Zuberec-type sweeping net (area = 0.0356 m²). Immediately after collection, samples were washed through a 200 μm sieve and the material retained was preserved in 5% formaldehyde solution. In the laboratory, gastropods were sorted from each sample and counted in order to determine density (ind m⁻²). Total gastropod shell height (SH) was measured with a Vernier caliper to the nearest 0.01 mm. Each specimen was inspected for shell damage and if the first whorls were missing, the shell length of the last visible whorl of unaffected (unmarked) specimens belonging to that population and appropriate size class [26]. Thus, the corrected SH was used in spatial and temporal comparisons. The specimens were sorted into one of the following 10 size classes according to corrected SH: <1 mm; 1–2.59 mm; 2.6–4.99 mm; 5–11.99 mm; 12–17.99 mm; 18–19.99 mm; 20–30 mm. No less than five individuals belonging to each class were counted. The samples were impractical to determine sex and presence of opercular parasites. The broad pouch of adult specimens (≥30 mm SH) was carefully dissected and the shell genaeus contained inside were counted.

Data analysis

Analysis of variance (ANOVA) using size class as a covariate was used to assess differences in average number of unbranched per lateral plate between sampling events and between locations. A two-way analysis of variance (ANOVA) was used to test whether density differences remained significant in terms of years (2007–2011) and T. gunnari population density differed between locations. The abundance at each site and number of species in broad pouch was analyzed with Pearson’s correlation. All data were log-transformed to meet normality assumptions. The Statistical package PASW version 18 for Windows was used.

A canonical correspondence analysis was conducted between log-transformed gastropod (3 species, Fig. 3) and environmental data (Table 1). The best two axes together explain 58.3% of total variability in species abundance data (Fig. 3).

Results

Canonical correspondence analysis

The canonical correspondence analysis showed that the combination of salinity, depth, temperature and pH explains 58.3% of the variance in the abundance of gastropod species (Table 1). The first two axes together explain 58.3% of total variability in species abundance data (Table 1).

Population densities

In the St. Lucia Estuary, an increase in salinity was associated with increases in density of indigenous gastropods such as Helicina annulata and Batillaria sloani and the decline of Tonna gunnari.
female difference between locations. Gaines Bay, Mispabeanal Pass, Lake St. Anne and Lake Winnibage, 2011-2016 winter season (Fig. 3). The population of T. gaster in Gaines Bay, Lake St. Anne and Lake Winnibage, was significantly different from females at the locations St. Lucie and Mispabeanal Pass, (Table 1). The T. gaster population appeared to have a bimodal distribution and the size class 6-7.5 mm SH was poorly represented (Fig. 4B). The largest size class represented 16-17.0 mm SH, and each size class contained an average of 4-13.4 T. gaster juveniles in the brood pouch. In 2009 there were no T. gaster recorded at the spawning area of Gaines Bay, which did not continue resulting in the T. gaster population being reduced by 7.9%. In June 2009, severe weather accumulated to form a freshwater pond independent of the South Lake. Large T. gaster (14-30 mm SH), were found making spawning mounds on the sediments surface and the largest (20-30 mm SH) carried on average 16.8±1.8 SD juveniles in the brood pouch. (Fig. 4C). At the Mispabeanal Pass, the Mispabeanal Pass, male T. gaster contained 39.3% ±9.0 SD and adults ranged from 6 to 19.0 mm SH (Fig. 5A). At Lake St. Anne, the largest adults found were the same size as the eastern shores were 16-17.9 mm SH and carried on average 4±1.8 SD juveniles in the brood pouch.
juniors in the brood pouch (Fig. 5B). At Lake Nhlange, 59.4 ± 3.0 SD of the population was composed of size classes 10-12.5 mm SL in November 2010 (Fig. 5B). The largest adults (30-36 mm SL) consisted 35.2 ± 4 SD juveniles in the brood pouch (Fig. 5C).

*T. graffei* shell height and number of juveniles per brood pouch were positively correlated (Pearson's correlation coefficient: r=0.805, p<0.01, n=156). There was no significant difference in average number of juveniles per brood pouch between sampling events at Catalina Bay (ANOVA: F = 1.626, \( P = 0.215 \)). There was also no significant difference in average number of salmon juveniles per brood pouch between locations (ANOVA: \( F_{\text{MS}} = 1.171, P = 0.626 \)).

**Discussion**

*T. graffei* populations did not drop below 10000 lak. regardless of location, making this species a dominant component of the local ichthyofauna community. However, at the St. Lucia and Mgbozeleni estuaries, population densities were variable, whereas in the Sibaya and Nhlange catchment, the *T. graffei* populations appeared relatively stable (Table 3). South African estuaries are particularly variable in terms of salinity and water depth, and these two factors can drastically influence macrofauna (57). As expected for any benthic invertebrate, the spatial distribution of *T. graffei* was heterogeneous (57,60,29). In general, shallow water (depth<2 m) freshwater sources and sheltered bays with organic deposits were favored. Recent changes in salinity and water depth, which affected the populations of all gastropod species, were particularly evident at the St. Lucia Estuary during the study period (Fig. 2). Yet the highest *T. graffei* population densities were recorded at Catalina Bay (Table 3). Indeed, it has been suggested that the high densities of *T. graffei* may minimize the risk of extinction under harsh conditions (10).

Salinity, water depth, temperature and pH were identified as the four main factors associated with the population density of the dominant gastropod species currently found in the estuaries and coastal lakes of Maputaland (Fig. 3, Table 1). Most gastropods appear to be associated with a specific set of environmental conditions. For instance, *Dumontiella* was associated with deeper water, whereas *Plesiocyrtus* and *Cypraeopsis* were associated with very shallow and warm water (Fig. 3). *Dumontiella* and *Cypraeopsis* were the only species associated with high salinity (Fig. 3). However, in comparison to other gastropods, *T. graffei* tended to be least associated with any one environmental factor (Fig. 3) and can be more widespread and abundant gastropod in all study areas.

The *T. graffei* population density did not appear to undergo seasonal patterns and year-round limits were measured. The average number of juveniles per brood pouch, which was assumed to indicate reproductive output, did appear to increase when the salinity was significantly lowered in the St. Lucia Estuary in 2007 and also when the population was recolonizing in 2009 (Table 4). An increase in average number of juveniles per brood pouch was also measured during *T. graffei's* recovery at the Mgbozeleni Estuary. *T. graffei* may increase its reproductive output in response to disturbances, such as sudden salinity reductions that negatively affect the populations, therefore accelerating its recovery. However, this increase was not taken into account in this analysis.

The structure of the *T. graffei* population was defined in terms of the proportional contribution of different shell height size classes.
Chapter 4

Invasive Gastrotheca in Coastal Lakes and Estuaries

T. grahami's body length (141 mm) ranged from 24 to 54 mm. The largest specimen was collected at Lake Victoria, T. grahami was found in Lake Victoria and Lake Victoria, and particularly in Lake Victoria, which is a large lake in East Africa. In contrast, the Lake Victoria basin has been reported to have very high levels of water pollution, which may reduce the health and survival of T. grahami. The Lake Victoria basin is a large lake in East Africa, which is a large lake in East Africa. In contrast, the Lake Victoria basin has been reported to have very high levels of water pollution, which may reduce the health and survival of T. grahami. The Lake Victoria basin is a large lake in East Africa.
Analysis of Environmental and Invasion Impact in Shallow Water Benthic Assemblages of North-Eastern South Africa.

Nelson A. F. Miranda¹*, Renzo Perissinotto¹

¹ School of Biological and Conservation Sciences, University of KwaZulu-Natal, Westville Campus, PRIVATE BAG X54001, Durban, 4000, South Africa
*E-mail: 204507499@ukzn.ac.za (N.A.F. Miranda)

ABSTRACT

Background: The distinction between natural and human-induced ecological changes at various spatial and temporal scales is an important step in the assessment of potential human impacts on ecosystems. Methods for the analysis of multivariate data in complex Before/After-Control/Impact (BACI) and After-Control/Impact (ACI) designs have only recently been developed. These methods have not yet been applied in assessing non-native invasive species (NIS) impact. The primary aim of this study is to find whether the variability of shallow water assemblages at putative impact locations differs significantly from natural variability.

Methodology/Principal Findings: Distance-based permutational multivariable analysis of variance (PERMANOVA) of both BACI and ACI designs provides evidence for significant differences in benthic assemblage variability between impact and control locations, in the context of both putative Tarebia granifera invasion and drought intensification impacts. Drought was intensified due to water abstraction and appears to result in an increase in the multivariate variability of assemblages. However, the impact of the gastropod T. granifera appears to lead to a decrease in multivariate variability of assemblages. This NIS may decrease variability by dominating and stabilizing assemblages in a variety of habitats, and persisting despite adverse conditions caused by droughts.

Conclusion/Significance: When attempting to detect environmental impacts, the presence of NIS should be taken into account since they may confound results by affecting “natural” variability. Novel BACI and ACI designs, such as those employed in this study should be considered in integrated approaches, combining multiple methods to assess both environmental and invasive species impacts.
Introduction

Non-native invasive species (NIS) have the potential to cause ecological impacts by changing native assemblages [1]. There are however several challenges in the assessment of the ecological impacts of successful NIS [2,3]. In most cases, there is a lack of pre-impact data on native assemblages. NIS are often well established before they are detected in an ecosystem, so significant impacts may have already taken place. Also, successful invasions often occur in ecosystems under a variety of other human-induced stresses, such as pollution and water abstraction. It is difficult to distinguish between natural changes in assemblage variability and human-induced ecological changes [3], particularly in ecosystems that are highly variable.

There are many approaches for detecting changes in ecological variability at various spatial and temporal scales after a disturbance [4]. In terms of sampling design, the Before/After-Control/Impact (BACI) and its modifications and improvements have been widely used in the field of environmental impact assessment to differentiate between the effects of human impacts and natural variability [5,6]. In this approach, there is usually only one impact location (e.g. due to an outfall event) and multiple control locations, so data are analyzed with asymmetrical analysis of variance [7]. A putative impact can be detected as a significant statistical difference between the impacted and control locations from before to after the disturbance [5].

The application of these BACI designs in the assessment of invasion impact has been discounted largely because of limitations in establishing a before impact baseline for comparison, thus increasing uncertainty in the method [8]. However, BACI type designs can be modified and applied even if no data have been collected before a putative impact. Although it is not possible to establish cause-effect relationships between putative impacts and changes in assemblages with these After-Control/Impact (ACI) designs, they do detect consistent differences between impact locations and control locations. Taking this limitation into account, several environmental impact studies have successfully used ACI designs to characterize potential effects of human activities on natural systems [9-11].

Until recently, analyses of BACI and ACI designs were restricted to the use of data on indicator species or other univariate measures. However, whole assemblages are more sensitive to human-induced stress and their data are more useful for assessing putative impacts [12]. Non-parametric multivariate methods such as ANOSIM have been developed and widely used over the past decade to analyze whole assemblage data [12]. Although ANOSIM is very useful in
many ecological applications, it does not allow tests of multivariate interactions and thus cannot be used to analyze complex BACI and ACI designs. Methods for the analysis of multivariate data in complex BACI and ACI designs have only recently been developed and successfully applied to assess environmental impacts [13-16]. However, these methods have not yet been specifically applied in assessing NIS invasion impact.

The iSimangaliso Wetland Park is an UNESCO World Heritage Site in north-eastern South Africa. The Kosi Lakes, Lake Sibaya and the St. Lucia Estuary are Ramsar Wetlands of International Importance within iSimangaliso (Fig. 1). All of these locations have recently been invaded by *Tarebia granifera*, a non-native gastropod that can reach densities of 20000 ind.m$^{-2}$, displace other species and become a dominant component of shallow water invertebrate assemblages [17]. *T. granifera* was accidentally introduced in South Africa via the aquarium trade and was discovered for the first time in the St. Lucia Estuary in 2005 [18,19]. This NIS has since spread and invaded increasing numbers of estuaries and lakes, but its ecological impacts are largely unknown [18,20]. Despite the elevated conservation status of the St. Lucia Estuary, its freshwater supply has been constrained for many decades, and most recently, this has led to the intensification of a drought which started in 2001 [21,22]. Although the St. Lucia Estuary is known to undergo wet and dry cycles spanning four to ten years [23], the current drought is unprecedented in the history of the system. The benthic invertebrate assemblages of the St. Lucia Estuary are very variable and reported to recover after droughts in the past [24]. However, recent studies report that the current intensified drought causes significant detrimental effects on benthic assemblages [25-27].

In this study, multivariate methods (PERMANOVA) are applied in analyses of assemblage data in response to BACI and ACI designs. The aims are: (1) to find whether variability at putative impact locations differs significantly from natural variability, both in the context of a putative *T. granifera* invasion impact and a drought intensification impact; and (2) to assess these putative impacts with the aid of complementary multivariate analysis.
Figure 1. Map of the study area. Sampling locations within the iSimangaliso Wetland Park are indicated by arrow and dot.
Materials and Methods

Ethics Statement

All necessary permits were obtained from the iSimangaliso Wetland Park Authority for the described field studies at each location, under a Research Agreement for the project titled "Climate Change and the Management of KZN estuaries: St Lucia Estuary".

BACI design

Abundance data (ind.m⁻²) were collected at 6 randomly chosen locations: Lake Nhange (LN), Lake Sibaya (LS), Mgobozeleni Estuary (M), Lake Bhangazi (LB), Dead Tree Bay (DT) and Catalina bay (C), which comprise the estuaries and coastal lakes of iSimangaliso Wetland Park (Fig. 1). Twelve sampling times (n = 3 replicates per location) were randomly chosen: 6 pre-2001 “Before” and 6 post-2001 “After” times. For the “After” scenario, samples were collected with a Zabalocki-type Ekman grab (grab area = 0.0235 m², three grabs per sample). Analogous grab data from the literature were used for the “Before” scenario [24,25,28-30]. Data were not always available for all locations at the same sampling time. Data were analysed in terms of a putative *Tarebia granifera* invasion impact where LN, LS, M and C are impact and LB and DT are control locations. The same data were also analysed in terms of drought intensification impact where DT and C are impact and LN, LS, M and LB are control locations. Multivariate analyses were based on 36 taxa. Of these, 19 were identified as species, 4 to genus level, 1 to family, 8 to order, 3 to sub-class and 1 to class. Taxa were classified in a manner that promotes consistency across the data and in accordance with the principle of taxonomic sufficiency [31,32].

Distance-based permutational multivariate analysis of variance (PERMANOVA[14]) was used to analyze the multivariate assemblage data and test for significant differences between data collected at times “Before” and “After”, at control and impact locations. The model therefore consisted of three factors: Before versus After (BA), Location (L) (impacts and controls locations, n = 3 replicates) and Time (twelve levels, random, nested in BA × L). The Location term was partitioned into three portions: controls versus impacts (CI), among controls (C) and among impacts (I) (Tables 1 and 2). The interaction term BA × L was divided into BA × CI, BA × C and BA × I. Time (BA × L) was divided into Time (BA×C) and Time (BA×I). Each term in the analysis was coded as a design matrix using the program XMATRIX.exe [33] and tested individually with the appropriate denominator and relevant permutable units using the computer program DISTLM.exe [34]. All analyses were done on untransformed data [7]. Denominators for pseudo-\(F\) ratios were identified following the logic of asymmetrical designs.
Each term in the analysis was tested using 4999 random permutations of the appropriate units [35]. If there were not enough permutable units to get a reasonable test by permutation, P-values were computed using a Monte Carlo random sample from the asymptotic permutation distribution [36]. An a priori significance level of $\alpha = 0.10$ was used for interpreting statistical results [37].

To visualize multivariate patterns, non-metric multidimensional scaling (nMDS) ordinations were performed using PRIMER v.6 [12,38] on the basis of a Bray-Curtis dissimilarity matrix calculated from untransformed data. The CLUSTER routine [38] was used to compute group-average clustering at a Bray-Curtis dissimilarity level of 80%, superimposed over the nMDS ordinations.

The multivariate average dispersion index among groups of control and impact locations before and after putative impacts was computed using PRIMER’s MVDISP routine [38,39], which indicated the level of variability among groups, measured using the Bray-Curtis dissimilarity.

Finally, PRIMER’s SIMPER routine [38] was used to identify the percentage contribution that each taxon made to the measures of the Bray-Curtis dissimilarity within groups of control and impact locations before and after putative impacts.

**ACI design**

Abundance data (ind.m$^{-2}$) were collected at 3 random sites within 8 randomly chosen locations: Lake Nhlanle (LN), Lake Sibaya (LS), Mgobozeleni Estuary (M), Lake Bhangazi (LB), Dead Tree Bay (DT), Catalina Bay (C), Esengeni (E) and Dukuduku Pond (D) (Fig. 1). Samples were collected in November 2009 ($n = 3$ replicates per site) with an Ekman grab (grab area = 0.0235 m$^2$, three grabs per sample). Data were analysed in terms of a putative *T. granifera* invasion impact where LN, LS, M and C are impact and LB, DT, E and D are control locations. The same data were also analysed in terms of a drought intensification impact where DT, C and E are impact and LN, LS, M, LB and D are control locations. Multivariate analyses were based on 28 taxa. Of these, 15 were identified to species level, 2 to genus, 1 to family, 7 to order, 2 to sub-class and 1 to class.

PERMANOVA was used to analyze multivariate assemblage data and test for significant differences between control and impact locations. The model consisted of two factors: Location (impact and control locations) and Site (three levels, random, nested in Location), with 3 replicates. The Location (L) term was partitioned into three portions: controls versus impacts (CI), among controls (C) and among impacts (I) (Tables 5 and 6). The term Site
(L) was divided into S (C) and S (I). The residual variation was divided into two parts: residual variability for observations among I (Res I) and residual variability for observations among C (Res C) (Tables 5 and 6).

Additional analyses (nMDS, CLUSTER, MVDISP and SIMPER) were done using methods described above for BACI design, although the focus of ACI design was on groups of sites and locations.

**Results**

**BACI analysis**

Results There are significant BA×CI interactions in terms of both *Tarebia granifera* invasion and drought intensification impacts (at $\alpha = 0.1$, Table 1 and 2). In both impact contexts, there is a significant BA×I interaction (Table 1 and 2). This indicates that on average, variability in assemblages differs significantly between putatively impacted locations before and after the impact. The BA×C interaction is not significant in terms of the drought intensification impact (Table 2), so control locations do not differ significantly before and after impact. In contrast, BA×C is significant in terms of the *T. granifera* invasion impact (Table 1).

The nMDS plots of location centroids before and after putative impacts support the findings of the PERMANOVA analyses (Fig. 2). All impact locations after the putative impact of *T. granifera* are clustered together in Fig. 2A. Locations before the drought intensification impact are clustered into two groups: one containing Catalina Bay and Esengeni (the putatively impacted sites), the other containing lakes Nhlange, Sibaya and Bhangazi (Fig. 2B). After the putative drought intensification, Esengeni becomes an independently clustered group and Catalina Bay becomes clustered with Lake Nhlange, Lake Sibaya and Mgobozeneli (Fig. 2B).

Sites impacted by *T. granifera* have the lowest value of multivariate dispersion index (Fig. 3A), suggesting an effect of decreased variability in the structure of assemblages. In contrast, sites impacted by drought intensification have the highest value of multivariate dispersion index (Fig. 3B). Notably, before the impact, those sites exhibited a lower value of multivariate dispersion index (Fig. 3B).

More taxa contributed to the average Bray-Curtis dissimilarity within groups before the putative *T. granifera* invasion impact, both at control and impact locations (groups BC and BI), than after (groups AC and AI) (Table 3). After the putative impact, *T. granifera* contributed 98.5% to the average Bray-Curtis dissimilarity at impacted locations (group AI) (Table 3). The
indigenous gastropod *Assiminea* cf. *ovata* made notable contributions to assemblages during the period before the putative impact (groups BC and BI) (Table 3). *A. cf. ovata* and copepods contributed the most to the average Bray-Curtis dissimilarity of assemblages at impact locations before the putative drought intensification impact (group BI), whereas after the impact, *T. granifera* and polychaetes made the greatest contributions (group AI) (Table 4).

**Table 1.** BACI design addressing putative *Tarebia granifera* invasion impact. Asymmetrical PERMANOVA based on Bray-Curtis dissimilarities of untransformed multivariate data (36 taxa). Each test was done using 4999 permutations and the term used as the denominator for the calculation of pseudo-$F$ is shown in the $F_{\text{denom}}$ column. The dataset is unbalanced as not all locations were sampled during times before the putative impact. *P*-values given in italics were obtained using 4999 Monte Carlo samples from the asymptotic permutation distribution.

<table>
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<th>Sources of variation</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>$F$</th>
<th>$P$</th>
<th>$F_{\text{denom}}$</th>
<th>Permutable units</th>
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<td>53522.66</td>
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Table 2. BACI design addressing putative drought intensification impact. Asymmetrical PERMANOVA based on Bray-Curtis dissimilarities of untransformed multivariate data (36 taxa). Each test was done using 4999 permutations and the term used as the denominator for the calculation of pseudo-$F$ is shown in the $F_{denom}$ column. The dataset is unbalanced as not all locations were sampled during times before the putative impact. $P$-values given in italics were obtained using 4999 Monte Carlo samples from the asymptotic permutation distribution.

<table>
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<th>Sources of variation</th>
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Figure 2. Non-metric multidimensional scaling (nMDS) of BACI design data. nMDS plots are based on a Bray-Curtis dissimilarity measure of centroids representing each location sampled in the context of a putative *Tarebia granifera* invasion impact (A) and a putative drought intensification impact (B). Shaded centroids (both circles and triangles) indicate putative impact locations, unshaded centroids indicate control locations. Triangles represent data collected before the putative impact, circles represent data collected after putative impact. Group-average clustering at a Bray-Curtis dissimilarity level of 80% is superimposed. Locations are Lake Nhlange (LN), Lake Sibaya (LS), Mgobozeleni Estuary (M), Lake Bhangazi (LB), Catalina Bay (C) and Esengeni (E).
Figure 3. **Average multivariate dispersion index of BACI design.** Represented are data from groups of control and impact locations before (BC, BI) and after (AC, AI) the putative impact of *Tarebia granifera* invasion (A) and drought intensification (B). Shaded columns indicate actual putatively impacted locations (AI).
Table 3. Abundance contribution of taxa in BACI design addressing putative *Tarebia granifera* invasion impact. Average abundance (ind.m$^{-2}$) of taxa contributing to Bray-Curtis dissimilarities (untransformed data) within groups of control and impact locations before (BC, BI) and after (AC, AI) the putative impact of *T. granifera* invasion.

<table>
<thead>
<tr>
<th>Group</th>
<th>Taxa</th>
<th>Av. Abund</th>
<th>Contrib %</th>
<th>Cum. %</th>
</tr>
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<td>29.9</td>
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<td>98.5</td>
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Table 4. Abundance contribution of taxa in BACI design addressing putative drought intensification impact. Average abundance (ind.m$^{-2}$) of taxa contributing to Bray-Curtis dissimilarities (untransformed data) within groups of control and impact locations before (BC, BI) and after (AC, AI) the putative impact of drought intensification.

<table>
<thead>
<tr>
<th>Group</th>
<th>Taxa</th>
<th>Av. Abund</th>
<th>Contrib %</th>
<th>Cum. %</th>
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<tbody>
<tr>
<td>BC</td>
<td>Chironomid larvae</td>
<td>216.9</td>
<td>48.8</td>
<td>48.83</td>
</tr>
<tr>
<td></td>
<td>Oligochaetes</td>
<td>239</td>
<td>27.9</td>
<td>76.71</td>
</tr>
<tr>
<td></td>
<td><em>Caridina nilotica</em></td>
<td>80.3</td>
<td>4.6</td>
<td>81.26</td>
</tr>
<tr>
<td></td>
<td><em>Melanoides tuberculata</em></td>
<td>31.67</td>
<td>4.4</td>
<td>85.7</td>
</tr>
<tr>
<td></td>
<td><em>Palaemon concinnus</em></td>
<td>0.8</td>
<td>3.3</td>
<td>89.01</td>
</tr>
<tr>
<td></td>
<td>Polychaetes</td>
<td>51.3</td>
<td>2.9</td>
<td>91.93</td>
</tr>
<tr>
<td>BI</td>
<td><em>Assiminea cf. ovata</em></td>
<td>62918.3</td>
<td>75.6</td>
<td>75.57</td>
</tr>
<tr>
<td></td>
<td>Copepods</td>
<td>10752.7</td>
<td>18.4</td>
<td>93.95</td>
</tr>
<tr>
<td>AC</td>
<td><em>Tarebia granifera</em></td>
<td>4750.5</td>
<td>86.5</td>
<td>86.45</td>
</tr>
<tr>
<td></td>
<td><em>C. nilotica</em></td>
<td>23.8</td>
<td>10.7</td>
<td>97.18</td>
</tr>
<tr>
<td>AI</td>
<td>Polychaetes</td>
<td>42.5</td>
<td>54.3</td>
<td>54.31</td>
</tr>
<tr>
<td></td>
<td><em>T. granifera</em></td>
<td>2278.7</td>
<td>34.7</td>
<td>89.03</td>
</tr>
<tr>
<td></td>
<td><em>A. cf. ovata</em></td>
<td>89.1</td>
<td>9.3</td>
<td>98.31</td>
</tr>
</tbody>
</table>
ACI analysis

In the context of the putative *T. granifera* invasion impact, variability in assemblages differ significantly (at $\alpha = 0.1$) between control and impacted locations (the term CI is statistically significant in Table 5). There are significant differences amongst control locations (term C is statistically significant in Table 5), but not among impacted locations (term I is not significant in Table 5). In the context of putative drought intensification impact, terms CI and its repartitions, C and I, were all significant (Table 6). This indicates that, on average, the variability of assemblages differed statistically between control and impacted locations, and also among sites of both control and impacted locations (Table 6).

In the *T. granifera* invasion impact nMDS plot, all impacted site centroids (with the exception of the single outlier M1) are clustered together (Fig. 4A). In the drought intensification impact nMDS plot, most centroids are clustered into two large groups (Fig. 4B). Both of these clusters contain control and impacted centroids; however, impacted centroids tend to be positioned at the top of the ordination space (Fig. 4B).

Putatively impacted and control locations have wide ranges of multivariate dispersion index values irrespective of the impact context (Fig. 5). Lake Bhangazi has the lowest multivariate dispersion index, whereas Mgobozeleni and Catalina Bay have the highest (Fig. 5).

Only 3 taxa typified the average Bray-Curtis dissimilarity within control and putative impact locations: *T. granifera*, the freshwater shrimp *Caridina nilotica* and polychaetes (Table 7). In the context of *T. granifera* invasion, this species contributed 98.2% to the average Bray-Curtis dissimilarity within assemblages at impacted locations (Table 7). In the context of drought intensification, *T. granifera* made notable contributions to assemblages in control and impacted locations (74.9% and 12.3% respectively) (Table 7). The greatest contribution to assemblages in locations impacted by drought intensification was by polychaetes (86.3%) (Table 7).
Table 5. ACI design addressing putative *Tarebia granifera* invasion impact. Symmetrical PERMANOVA based on Bray-Curtis dissimilarities of untransformed multivariate data (28 taxa). Each test was done using 4999 permutations and the term used as the denominator for the calculation of pseudo-$F$ is shown in the $F_{\text{denom}}$ column.

<table>
<thead>
<tr>
<th>Sources of variation</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>$F$</th>
<th>$P$</th>
<th>$F_{\text{denom}}$</th>
<th>Permutable units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location = L</td>
<td>7</td>
<td>189321.8</td>
<td>27045.97</td>
<td>9.1</td>
<td>0.0002</td>
<td>Site (L)</td>
<td>24 Site (L) cells</td>
</tr>
<tr>
<td>Controls vs. Impacts = CI</td>
<td>1</td>
<td>96415.28</td>
<td>96415.28</td>
<td>32.5</td>
<td>0.0002</td>
<td>Site (L)</td>
<td>24 Site (L) cells</td>
</tr>
<tr>
<td>Among Controls = C</td>
<td>3</td>
<td>68076.49</td>
<td>22692.16</td>
<td>20.4</td>
<td>0.0002</td>
<td>Site (C)</td>
<td>12 Site (C) cells</td>
</tr>
<tr>
<td>Among Impacts = I</td>
<td>3</td>
<td>24830</td>
<td>8276.67</td>
<td>1.7</td>
<td>0.1102</td>
<td>Site (I)</td>
<td>12 Site (I) cells</td>
</tr>
<tr>
<td>Site (L)</td>
<td>16</td>
<td>47530.2</td>
<td>2970.63</td>
<td>9.5</td>
<td>0.0002</td>
<td>Residual</td>
<td>72 raw data units</td>
</tr>
<tr>
<td>Site (C)</td>
<td>8</td>
<td>8917.1</td>
<td>1114.64</td>
<td>3.9</td>
<td>0.0002</td>
<td>Res C</td>
<td>36 raw data units</td>
</tr>
<tr>
<td>Site (I)</td>
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<td>38613.14</td>
<td>4826.64</td>
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<td>0.0002</td>
<td>Res I</td>
<td>36 raw data units</td>
</tr>
<tr>
<td>Residual</td>
<td>48</td>
<td>14975.72</td>
<td>311.99</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Res C</td>
<td>24</td>
<td>6934.41</td>
<td>288.93</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Res I</td>
<td>24</td>
<td>8041.30</td>
<td>335.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 6. ACI design addressing putative drought intensification impact. Asymmetrical PERMANOVA based on Bray-Curtis dissimilarities of untransformed multivariate data (28 taxa). Each test was done using 4999 permutations and the term used as the denominator for the calculation of pseudo-$F$ is shown in the $F_{\text{denom}}$ column.

<table>
<thead>
<tr>
<th>Sources of variation</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>$F$</th>
<th>$P$</th>
<th>$F_{\text{denom}}$</th>
<th>Permutable units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location = L</td>
<td>7</td>
<td>189321.8</td>
<td>27045.97</td>
<td>9.1</td>
<td>0.0002</td>
<td>Site (L)</td>
<td>24 Site (L) cells</td>
</tr>
<tr>
<td>Controls vs. Impacts</td>
<td>1</td>
<td>39022.78</td>
<td>39022.78</td>
<td>13.1</td>
<td>0.0002</td>
<td>Site (L)</td>
<td>24 Site (L) cells</td>
</tr>
<tr>
<td>Controls vs. Impacts = CI</td>
<td>4</td>
<td>112421.3</td>
<td>28105.33</td>
<td>10.9</td>
<td>0.0002</td>
<td>Site (C)</td>
<td>15 Site (C) cells</td>
</tr>
<tr>
<td>Among Controls = C</td>
<td>4</td>
<td>112421.3</td>
<td>28105.33</td>
<td>10.9</td>
<td>0.0002</td>
<td>Site (C)</td>
<td>15 Site (C) cells</td>
</tr>
<tr>
<td>Among Impacts = I</td>
<td>2</td>
<td>37877.65</td>
<td>18938.82</td>
<td>5.2</td>
<td>0.0036</td>
<td>Site (I)</td>
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</tr>
<tr>
<td>Site (L)</td>
<td>16</td>
<td>47530.2</td>
<td>2970.64</td>
<td>9.5</td>
<td>0.0002</td>
<td>Residual</td>
<td>72 raw data units</td>
</tr>
<tr>
<td>Site (C)</td>
<td>10</td>
<td>25836.43</td>
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<td>8.3</td>
<td>0.0002</td>
<td>Res Cs</td>
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</tr>
<tr>
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<td>21693.75</td>
<td>3615.63</td>
<td>11.5</td>
<td>0.0002</td>
<td>Res I</td>
<td>27 raw data units</td>
</tr>
<tr>
<td>Residual</td>
<td>48</td>
<td>14975.72</td>
<td>311.99</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Res C</td>
<td>30</td>
<td>9301.16</td>
<td>310.04</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Res I</td>
<td>18</td>
<td>5674.56</td>
<td>315.25</td>
<td></td>
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<td></td>
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</table>
Figure 4. Non-metric multidimensional scaling (nMDS) of ACI design data. nMDS plots are based on a Bray-Curtis dissimilarity measure of centroids representing sites within locations sampled in the context of a putative *Tarebia granifera* invasion impact (A) and a putative drought intensification impact (B). Shaded centroids indicate putative impact sites, unshaded centroids indicate control sites. Group-average clustering at a Bray-Curtis dissimilarity level of 80% is superimposed. Sites are numbered from 1 to 3 and locations are Lake Nhlanje (LN), Lake Sibaya (LS), Mgobozeleni Estuary (M), Lake Bhangazi (LB), Dead Tree Bay (DT), Catalina Bay (C), Esengeni (E) and Dukuduku Pond (D).
Figure 5. Average multivariate dispersion index of ACI design. Represented are data collected at impact locations (shaded columns) and control locations (unshaded columns) in the context of the putative impact of *Tarebia granifera* invasion (A) and drought intensification (B). Locations are Lake Nhlange (LN), Lake Sibaya (LS), Mgobozeleni Estuary (M), Lake Bhangazi (LB), Dead Tree Bay (DT), Catalina Bay (C), Esengeni (E) and Dukuduku Pond (D).
Table 7. Abundance contribution of taxa in ACI design. Average abundance (ind.m$^{-2}$) of taxa contributing to Bray-Curtis dissimilarities (untransformed data) within groups of control (C) and impact locations (I) in the context of putative *Tarebia granifera* invasion and drought intensification impacts.

<table>
<thead>
<tr>
<th>Group</th>
<th>Taxa</th>
<th>Av. Abund</th>
<th>Contrib %</th>
<th>Cum. %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Putative <em>T. granifera</em> invasion:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Polychaetes</td>
<td>56.4</td>
<td>81.4</td>
<td>81.4</td>
</tr>
<tr>
<td></td>
<td><em>Caridina nilotica</em></td>
<td>30.2</td>
<td>13.6</td>
<td>94.9</td>
</tr>
<tr>
<td>I</td>
<td><em>Tarebia granifera</em></td>
<td>6087.1</td>
<td>98.2</td>
<td>98.2</td>
</tr>
<tr>
<td><strong>Putative Drought intensification:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td><em>T. granifera</em></td>
<td>4293.5</td>
<td>74.9</td>
<td>74.9</td>
</tr>
<tr>
<td></td>
<td><em>Caridina nilotica</em></td>
<td>31.34</td>
<td>13.4</td>
<td>88.3</td>
</tr>
<tr>
<td></td>
<td>Polychaetes</td>
<td>37.9</td>
<td>6.5</td>
<td>94.9</td>
</tr>
<tr>
<td>I</td>
<td>Polychaetes</td>
<td>159.3</td>
<td>86.3</td>
<td>86.3</td>
</tr>
<tr>
<td></td>
<td><em>T. granifera</em></td>
<td>960.3</td>
<td>12.3</td>
<td>98.6</td>
</tr>
</tbody>
</table>
**Discussion**

A multiple PERMANOVA model approach was adopted in this study to overcome the weaknesses of single models and increase confidence in the detection of differences in assemblage variability between control locations and locations associated with putative impacts. A single dataset was used to detect both putative impacts, first with a BACI model which included data that were compatible but not purposely collected for this type of analysis, and then with an ACI model with spatial data only. Testing the same dataset for more than one putative impact is not an unreasonable procedure because control and impact terms are set *a priori* and depend on our perception [1]. Therefore, this approach may potentially enable detection of several disturbances, although it becomes confounded in cases where more disturbances and complex interactions are present. It may also only be effective in detecting large scale sustained (“press”) impacts [5]. In this study, fine scale interactions between locations and times could not be addressed because the BACI model is unbalanced and the ACI model does not include replicates over time. Logistical constraints also did not allow for more robust sampling over wider spatial ranges, so these analyses are limited to shallow waters (< 2 metre depth) by the shore. However, shallow water shores are important habitats, particularly in the St. Lucia Estuary, because they provide refugia for salt sensitive assemblages and may play an important role in the recovery of the ecosystems after droughts [40].

The data used in this study are descriptive, so the findings cannot be used to infer what processes caused or are causing differences in assemblages between control and impact locations. There are many potential mechanisms that can cause changes in assemblages exposed to a drought or invasive species. Addressing these mechanisms would require detailed experimental manipulations. Therefore, only tentative ideas and general hypotheses are discussed below.

Additional multivariate analyses are necessary to interpret the results of the BACI and ACI PERMANOVA models. In the BACI analysis of drought intensification, a non-significant BA×C term indicates that the variability of assemblages did not change in the absence of this impact. However, BA×C was significant in the BACI analysis of *T. granifera* impact. The nMDS in Fig. 2 show that before the drought intensification, Esengeni and Catalina Bay which are estuarine sites were clustered together, whereas freshwater lake sites Lake Nhlange, Lake Sibaya and Lake Bhangazi formed another cluster. As expected, freshwater locations were typified by freshwater taxa and estuarine location by euryhaline species such as *Assiminea* cf. *ovata* (Fig. 4, groups BC and BI). As a result of the current drought, the environmental conditions at impacted locations changed remarkably. This is particularly evident at Catalina
Bay where freshwater ponds form in groundwater seepage sites, whereas in other sites hypersaline conditions or complete desiccation occurs due to evaporative water losses [41]. *T. granifera*, despite being able to persist in salt water, thrives in freshwater habitats [19], including those that exist at Catalina Bay because of the drought [19]. Human induced environmental stresses can interact with NIS and influence their impact on native assemblages [3]. The intensified drought modified the habitat in unprecedented ways in the St. Lucia Estuary and may have created suitable conditions for the establishment and spread of *T. granifera* [17].

The presence of a simultaneous drought impact on assemblages would explain why their variability changed in the context of the BACI analysis of *T. granifera* impact. It should also be noted that the impact of the drought affects the whole St. Lucia Estuary, whereas the impact of *T. granifera* appears to be limited to the South Lake of the system [19]. When examining the BACI data, the drought appears to have resulted in an increase in the multivariate variability of assemblages (Fig. 3B). In environmental impact assessment, an increase in multivariate variability is often associated with stress [39]. However, the *T. granifera* impact actually appears to result in a decrease in multivariate variability of assemblages (Fig. 3A). Both impacts appear to result in a reduction in the number of taxa that typify assemblages (Tables 3 and 4), indicating a reduction in biodiversity. The drought may increase variability in native taxa through various mechanisms involving habitat modification and favoring different groups of taxa at different times and places. However, *T. granifera* may decrease variability by dominating and stabilizing assemblages in a variety of habitats, and persisting despite adverse conditions caused by the drought. This would also explain the results of the *T. granifera* impact from ACI analysis, where significant differences among control locations but not among impact locations were obtained (Table 5). The nMDS and SIMPER analyses clearly indicate that locations invaded by *T. granifera* become dominated by this NIS (Fig. 2 and 4, Tables 3, 4 and 7).

Simultaneous impacts could also explain the general pattern in the ACI analyses. In this dataset, it becomes difficult to analyze multivariate variability because of the wide ranges of multivariate dispersion involved (Fig. 5). Locations that were not impacted by either drought or *T. granifera* (e.g. Lake Bhangazi, an isolated freshwater lake) exhibit a lower variability compared to estuarine locations, which are naturally very variable [42] and are also affected by one or both impact types (Mgobozeleni Estuary and Catalina Bay). This makes potential differences between disturbed and control locations harder to detect [9]. Although other studies have clearly reported that the current drought has significant impacts on benthic assemblages [25-27], the ACI drought PERMANOVA analysis, on its own would not be useful in detecting
the drought impact. This is because, despite the small clue illustrated by the nMDS in fig. 4B, which suggests otherwise, both control and impact terms in the actual analysis are significant. This confounding result is likely due to the combination of high variability and the dominant presence of *T. granifera* in the dataset (Table 7). When attempting to detect environmental impacts, the presence of NIS in assemblages should be taken into account since they may confound results by affecting “natural” variability.

### Conclusion

This study contributes towards the difficult task of separating natural changes in assemblage variability from human-induced ecological changes, which is of particular importance in protected areas such as the St. Lucia Estuary [22].

The impacts of both drought intensification and *T. granifera* can be detected at the St. Lucia Estuary. Fortunately, management actions have been put in place and some studies indicate that assemblages may recover after the current drought. However, the dominant presence of *T. granifera* in freshwater refugia and its reported displacement of indigenous gastropods such as *A. cf. ovata* are of concern [17]. In addition, it may be impossible to eradicate this NIS from the iSimangaliso Park [17]. Empirical approaches are needed to address the specific mechanisms and extent of the ecological impact of *T. granifera* [43], before any action is considered. Ongoing data collection is important as it may eventually allow further, more rigorous analyses of invasion impacts, which would support the current adaptive management strategy [8,44,45].

Manipulative experiments using BACI designs have been successfully used at smaller scales to assess relationships between invasive species and other human impacts [46]. This study proposes that multivariate analyses of BACI and ACI designs can be useful in addressing and providing insights into impacts at large spatial and temporal scales, where manipulative experiments are not feasible. Therefore, BACI and ACI designs should be considered in integrated approaches combining multiple methods to assess both environmental and invasive species impacts.
Acknowledgements  We thank the iSimangaliso Park Authority and Ezemvelo KZN Wildlife for supporting this project. Special thanks go to R. Taylor, S. Kyle, C. Fox and the late A. Myeza for their invaluable assistance. Funding was provided by the National Research Foundation, South Africa; Marine and Coastal Management (DEAT-MCM) and the World Wide Fund for Nature (WWF).

References


Conclusions and recommendations for further research

Estuaries and coastal lakes across the world are being invaded with increasing frequency by non-indigenous invasive species (NIS), which are widely reported as having negative ecological impacts (Cohen & Carlton 1998; Grosholz 2002). In South Africa, knowledge of aquatic invasive species has increased over the past two decades, focusing on prominent NIS, such as the water hyacinth *Eichhornia crassipes*, fish such as bass and trout, as well as invertebrates such as the mussel *Mytilus galloprovincialis*, the crab *Carcinus maenas* and the snail *Physa acuta* (Moosa 2000). However, there is a lack of studies on the ecological consequences of invasive aquatic invertebrates in coastal lakes and estuaries. These consequences may include loss of native biodiversity, biotic homogenization, and changes to the properties of the invaded habitat (Davis 2009). Efforts should focus on preventing introduction, but in most cases the invader is not noticed until it is already established. At this stage, knowledge about the invading species is essential in considering whether and how to implement control measures (Buckley 2008).

The St. Lucia Estuary is currently experiencing a severe drought, with below average rainfall persisting since 2002. Drought conditions have been exacerbated by the canalization of the Mfolozi River, and the subsequent diversion of its freshwater away from St. Lucia (Whitfield & Taylor 2009). Estuary water levels have become very low and a reversed salinity gradient has developed, with hypersaline conditions prevailing in the northern regions of the lake. The result is that pelagic communities are currently undergoing major losses in biodiversity and dominance shifts, with species adapted to extreme conditions being favored in the system (Cyrus et al. 2011). But is the St. Lucia Estuary more susceptible to invasion under these conditions? Invasibility is influenced by dynamic biotic and abiotic events and processes. As these change, so will the environment invasibility (Davis 2009). Environments also don’t behave as uniform entities. Resource fluctuations occur at different times and to different extents in different areas within a single environment. Even at a particular point in time, a particular environment will manifest different invasibilities in different places throughout the environment. Fluctuating resource availability theory states that pulses of resources are expected to increase the invasibility of an environment (Davis et al. 2000). This is because fluctuating availability of limiting resources in space and time leads to fluctuation in the intensity of competition, thus probably preventing competitive exclusion from occurring (Davis et al. 2000). During periods of drought and low water level and associated high salinity, freshwater seeps from sand dune aquifers on to the eastern shores of the South Lake of St. Lucia. The distribution of these freshwater seepage areas is heterogeneous. Freshwater ponds may form depending on
the supply of rainwater and some of these ponds are at times flooded by saline South Lake waters. Salt-sensitive biota can take refuge and persist in these areas and then recolonize the system when conditions become favorable again (Taylor et al. 2006). Ironically, these brackish and freshwater habitats along the eastern shores of South Lake are susceptible to invasion by *Tarebia granifera* and *Aplexa marmorata*. *Tarebia granifera* acclimates, persists and may spread repeatedly even after saltwater wipes out most of the population (Chapter 4). The St. Lucia Estuary as a whole may therefore be more invasible because of these seepage areas.

Possible mechanisms that affect invasions are changes in community composition, ecosystem processes and propagule supply. Disturbances are discrete events in time that may affect the mechanisms of invasion. It is possible that disturbance facilitate invasion in the St. Lucia Estuary, however, disturbance is not a mechanism in itself. The current drought of St. Lucia is being intensified due to human disturbances which reduce the flow of freshwater into the system (Cyrus et al. 2011). The alteration of flow regime caused when a dam is built or when water is diverted to be used in agriculture or for urban consumption has been associated with the establishment of NIS elsewhere in the world (Schreiber et al. 2003). Although disturbances are generally thought to promote invasion, this is not a fundamental principle in ecology. Disturbances can create opportunities for NIS and natives alike and, in fact, native species may actually outperform NIS given the right environmental conditions (Winsome et al. 2006; Chapter 4). It is also possible that severe disturbance events prevent the establishment or spread of some alien species (Lockwood et al. 2007).

It is important to remember that invasibility depends on the biological characteristics of the invading species (Lodge 1993). Some of the most significant characteristics for the successful establishment of NIS are abundance, environmental tolerance and developmental mode. These not only explain patterns of invasion success but can also be used for predictions of invasion success in different ecosystems (Miller et al. 2007). Intense disturbances such as flooding by hypersaline South Lake water or desiccation can prevent alien species from establishing and persisting. However, *T. granifera* is able to persist due to its remarkable mode of reproduction and environmental tolerance. As illustrated in Fig. 3 in Chapter 4, *T. granifera* is a generalist able to tolerate a variety of environmental conditions. By producing extremely large populations and displacing native species in the different lakes and estuaries of iSimangaliso, *T. granifera* is also affecting benthic invertebrate assemblage composition, as indicated by Fig. 4A in Chapter 5. The degree to which *T. granifera* affects benthic assemblages can be high enough that the NIS itself can be considered a disturbance. Human intensification of
drought and *T. granifera* invasion may therefore interact and result in biotic homogenization of shallow water benthic assemblages across lakes and estuaries in iSimangaliso.

Due to its parthenogenetic trait, *T. granifera* is able to start a population with only one viable organism. The successful invasion of new aquatic habitats by small and frequently bottlenecked populations of NIS, despite their low genetic diversity, and hence low evolutionary potential associated with high risk of extinction, constitutes a “genetic paradox” in biological invasions (Roman & Darling 2007). Yet, asexual reproduction is well represented in NIS (Roman & Darling 2007). In particular, parthenogenetic populations with “general purpose” genotypes have the advantages of several mechanisms to overcome the challenges of low genetic diversity and variable environments (Chapter 4 and references therein). *Tarebia granifera* tends to fit the criteria for a generalist rather than a locally adapted clonal lineage (morph), however further studies are needed to specifically address this (Drown et al. 2011). *Tarebia granifera* is not an ancient clonal lineage: on an evolutionary time scale, it is in fact a relatively young species estimated to be around 6.8 Ma old (Genner et al. 2007). On such a time scale, sexual reproduction may be necessary for the survival of the species (Griffiths & Butlin 1995; Samadi et al. 1999) and there is evidence of the presence of males in invasive *T. granifera* populations (Chaniotis et al. 1980). However, as males are apparently not functional (Chaniotis et al. 1980), other as yet unexplained genetic mechanisms may be at work to prevent extinction. *Tarebia granifera* has been placed within a *Melanoides*-dominated sub-clade (Myers et al. 2000). *Melanoides tuberculata*, a well studied freshwater NIS native to Africa (Samadi et al. 1999; Myers et al. 2000; Rocha-Miranda & Martins-Silva 2006; Genner et al. 2007), shares many life-history characteristics with *T. granifera* (Facon et al. 2008).

Species invasiveness and community invasibility can and should be considered together (Heger & Trepl 2003). This may be achieved by addressing two seemingly contradictory hypotheses: (1) that phylogenetic relatedness between alien and native species promotes naturalization because of niche-adaptation, (2) but can at the same time hinder naturalization because of niche overlap. These hypotheses were proposed by Darwin and constitute Darwin’s naturalization conundrum (Thuiller et al. 2010 and references therein). *Tarebia granifera* is phylogenetically similar to *M. tuberculata* and may therefore establish itself in niches similar to those occupied by the latter species. However, *T. granifera* also has extensive niche overlap with *M. tuberculata*. Yet, it is *T. granifera* that establishes and apparently displaces *M. tuberculata* at Lake Sibaya where food is limiting (Chapter 4). This is undoubtedly because *T. granifera* has additional and/or enhanced characteristics which make it a superior invader under specific environmental conditions (Shea & Chesson 2002).
This thesis presents research that contributes valuable knowledge about a recently introduced NIS which has become successful in South Africa. Experimental and descriptive methodologies are employed in several studies, which together form an integrated approach to address the invasion of *T. granifera* in the iSimangaliso Wetland Park. New insights and techniques are also presented as modest contributions to scientific knowledge in the general field of ecology.

The lethal tolerance limits of *T. granifera* to the wide ranges of salinities and temperatures of the St. Lucia Estuary were determined with laboratory experiments (Chapter 1). A novel *in situ* method of estimating ingestion rate via gut fluorometric analyses, coupled with gut carbon analyses was applied to this gastropod (Chapter 2). Feeding impact on benthic microalgae in different parts of iSimangaliso was then estimated (Chapter 2). Following this, a stable isotope study coupled with gut content analyses was conducted, involving different populations of *T. granifera*, other sympatric gastropods and all potential food sources in three coastal lakes of the iSimangaliso Park (Chapter 3). An isotopic dietary overlap index (IDO, %) was developed to characterize potential competition for food resources between gastropod species (Chapter 3). The stable isotope study also served to track ecosystem-level changes over time. *Tarebia granifera* may persist in estuaries due to its high fecundity and density, as well as the wider environmental tolerance of larger adults which carried more brooded juveniles, thus increasing fitness (Chapter 4). Multiple introductions were not essential for the success of this parthenogenetic NIS. Finally, the Before/After-Control/Impact (BACI) logic was used in a multivariate approach to separate human perturbations from natural spatio-temporal variability displayed by communities, and to further separate perturbations due to NIS (Chapter 5).

Ongoing research into the state of biodiversity and invasive species is of critical importance for the conservation of protected areas. Future research could focus on invertebrate biodiversity and conservation by further developing the investigation of invasive gastropods and addressing their ecological impacts in coastal lakes and estuaries of South Africa. This research may be most critical and timely in the iSimangaliso Wetland Park and could be specifically addressed as follows.

1) Conduct a review and update of the current aquatic gastropod biodiversity of iSimangaliso following the guidelines set in previous studies (Appleton 1980; Herbert 1998). This work would involve gathering specific information about gastropod species diversity and their relationship with the environment. This includes an assessment of the biogeographical range expansions of tropical species, the introduction of non-native invasive species and even
the suitability of some species, such as the nerite *Septaria lineata*, as indicators of ecosystem health.

2) Run an empirical study addressing competitive interactions between native and invasive gastropods, both in the laboratory (Giovanelli et al. 2002, 2003; Gomez et al. 1990; Madsen 1979) and using *in situ* caged experiments (Schreiber et al. 2002; Underwood 1978).

3) Apply newly developed population viability analyses (Andersen 2005; Miller & Lacy 2005) to *T. granifera*, to gain insight into its population and invasion dynamics (in terms of propagule pressure and fitness) in different water bodies within iSimangaliso. This modeling approach would predict under which environmental conditions and at which water bodies *T. granifera* populations may persist or crash (Andersen 2005).

4) Directly address the impact of *T. granifera* on the biodiversity of iSimangaliso’s shallow water benthic assemblages, by applying structured sampling designs which can be analysed with recently developed uni- and multivariate methods (Anderson 2001; Terlizzi et al. 2005a). These methods include measures of taxonomic relatedness, which complement conventionally used indices of biodiversity (Terlizzi et al. 2005b).
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