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

Nelson Augusto Fernandes Miranda

Submitted in fulfillment of the requirements for the degree of

Doctor of Philosophy, in the

School of Life Sciences

University of KwaZulu-Natal, Westville

February 2012

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

Signed:__________ Name: R. PERISSINOTTO 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. granifer*a 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.

Signed:__________ Name: NAF MIRANDA Date: 20 FEB 2012

Declaration 1 - Plagiarism

I, Nelson Augusto Fernandes Miranda declare that:

1. The research reported in this thesis, except where otherwise indicated, is my original research;

2. This thesis has not been submitted for any degree or examination at any other university;

3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons;

4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
    a. Their words have been re-written but the general information attributed to them has been referenced
    b. Where their exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced

5. This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the References sections.

Signed:____________________

Declaration 2 - Publications

Details of contribution to publications that form part and/or include research presented in this thesis are as follows:

Publication 1

Miranda NAF, Perissinotto R, Appleton CC (2010) Salinity and temperature tolerance of the invasive freshwater gastropod *Tarebia granifera. South African Journal of Science* 106: 55-61.

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

Publication 2

Miranda NAF, Perissinotto R, Appleton CC (2011) Feeding dynamics of the invasive gastropod *Tarebia granifera* in coastal and estuarine lakes of northern KwaZulu Natal, South Africa. *Estuarine, Coastal and Shelf Science* 91: 442-449.

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

Publication 3

Miranda NAF, Perissinotto R (2012) Stable isotope evidence for dietary overlap between alien and native gastropods in coastal lakes of northern KwaZulu-Natal, South Africa. *PLoS ONE* 7, e31897.

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

Publication 4

Miranda NAF, Perissinotto R, Appleton CC (2011) Population structure of an invasive parthenogenetic gastropod in coastal lakes and estuaries of northern KwaZulu-Natal, South Africa. *PLoS ONE* 6: e24337.

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

Signed:__________________

Acknowledgements

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.

List of Contents

General Introduction

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

Abbott RT (1952) A study of an intermediate snail host (*Thiara granifera*) of the oriental lung fluke (*Paragonimus*). Proc U S Nat Mus 102:72-116

Appleton CC (2003) Alien and invasive freshwater gastropoda in South Africa. Afr J Aquat Sci 28:69-81

Appleton CC, Nadasan DS (2002) First report of *Tarebia granifera* (Lamarck, 1816) (Gastropoda : Thiaridae) from Africa. J Mollus Stud 68:399-401

Buckley YM (2008) The role of research for integrated management of invasive species, invaded landscapes and communities. J Appl Ecol 45:397-402

Carlton JT (1999) Molluscan invasions in marine and estuarine communities. Malacologia 41:439-454

Davis MA (2009) Invasion Biology. Oxford University Press, New York

Drake JA, Mooney HA (1989) Biological Invasions: A Global Perspective. (SCOPE) John Wiley & Sons

Elton CS (1958) The Ecology of Invasions by Animals and Plants. Methuen

Grosholz E (2002) Ecological and evolutionary consequences of coastal invasions. Trends Ecol Evol 17:22-27

Gutierrez A, Perera G, Yong M, et al. (1997) Relationships of the prosobranch snails *Pomacea paludosa, Tarebia granifera* and *Melanoides tuberculata* with the abiotic environment and freshwater snail diversity in the central region of Cuba. Malacol Rev 30:39-44

Lockwood JL, Hoopes MF, Marchetti MP (2007) Invasion Ecology. Blackwell Publishing, Malden, U.S.A.

Parker IM, Simberloff D, Lonsdale WM, et al. (1999) Impact: Toward a framework for understanding the ecological effects of invaders. Biol Invasions 1:3-19

Pillay D, Perissinotto R (2008) The benthic macrofauna of the St. Lucia Estuary during the 2005 drought year. Estuar Coast Shelf Sci 77:35-46

Pillay D, Branch GM, Steyn A (2009) Complex effects of the gastropod *Assiminea globulus* on benthic community structure in a marine-dominated lagoon. J Exp Mar Biol Ecol 380:47-52

Pointier JP, Samadi S, Jarne P, et al. (1998) Introduction and spread of *Thiara granifera* (Lamarck, 1822) in Martinique, French West Indies. Biodiv Conserv 7:1277-1290

Pointier JP (2001) Invading freshwater snails and biological control in Martinique Island, French West Indies. Mem I Oswaldo Cruz 96:67-74

Pointier JP, Facon B, Jarne P, et al. (2003) Thiarid snails, invading gastropods of tropical freshwater habitats. Xenophora 104:14-20

Roman J, Darling JA (2007) Paradox lost: Genetic diversity and the success of aquatic invasions. Trends Ecol Evol 22:454-464

Ruiz GM, Fofonoff P, Hines AH, et al. (1999) Non-indigenous species as stressors in estuarine and marine communities : Assessing invasion impacts and interactions. Limnol Oceanogr 44:950-972

Sakai AK, Allendorf FW, Holt JS, et al. (2001) The population biology of invasive species. Annu Rev Ecol Syst 32:305-332

Sher AA, Hyatt LA (1999) The Disturbed Resource-Flux Invasion Matrix: A New Framework for Patterns of Plant Invasion. Biol Invasions 1:107-114

Strayer DL (2010) Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. Freshwater Biol 55:152-174

Strayer DL, Caraco NF, Cole JJ, et al. (1999) Transformation of freshwater ecosystems by bivalves: a case study of zebra mussels in the Hudson River. BioScience 49:19-27

Whitfield A, Taylor RH (2009) A review of the importance of freshwater inflow to the future conservation of Lake St Lucia. Aquat Conserv 19:838-848

Research Article

SALINITY AND TEMPERATURE TOLERANCE OF THE INVASIVE FRESHWATER GASTROPOD *TAREBIA GRANIFERA*

Authors:
Nelson A.F. Miranda[1]
Renzo Perissinotto[1]
Christopher C. Appleton[1]

Affiliations:
[1] School of Biological and Conservation Sciences, University of KwaZulu-Natal, Westville campus, South Africa

Correspondence to:
Nelson Miranda

email:
204507499@ukzn.ac.za

Postal address:
School of Biological and Conservation Sciences, University of KwaZulu-Natal, Westville campus, Private Bag X54001, Durban 4000, South Africa

Keywords:
alien species; aquatic mollusc; lethal tolerance; South Africa, St Lucia Estuary

Dates:
Received: 17 July 2009
Accepted: 01 Feb. 2010
Published: 23 Apr. 2010

How to cite this article:
Miranda NAF, Perissinotto R, Appleton CC. Salinity and temperature tolerance of the invasive freshwater gastropod *Tarebia granifera*. S Afr J Sci. 2010;106(3/4), Art. #156, 7 pages. DOI: 10.4102/sajs.v106i3/4.156

This article is available at:
http://www.sajs.co.za

South African Journal of Science
Article #156

ABSTRACT

Invasive aquatic species, such as the gastropod *Tarebia granifera*, can cause ecological disturbances and potentially reduce biodiversity by displacing indigenous invertebrates. In South Africa, *T. granifera* was first recorded in an estuarine environment in the St Lucia Estuary. Its tolerance to salinity and temperature was investigated through the experimental manipulation of these factors. *T. granifera* can tolerate temperatures between 0 °C and 47.5 °C, allowing it to survive high temperature extremes. The species may also survive cold snaps and invade higher altitude areas. More remarkably, this snail survives high salinity for a relatively long time, as LS_{50} (lethal salinity for 50% of the population) was reached at 30 psu over 65–75 days. However, higher salinity adversely affected the *T. granifera* population. Snails acclimated to freshwater conditions and suddenly transferred to 30 psu experienced 100% mortality within 48 h. Snail activity also declined with increasing salinity. *T. granifera*'s environmental tolerance and parthenogenetic characteristics are the keys to successful introduction and establishment. Therefore, the management of *T. granifera* may prove difficult in the short to medium term. The present findings constitute a contribution to the knowledge of biological invasions in Africa and to the understanding of estuarine invasions by *T. granifera*.

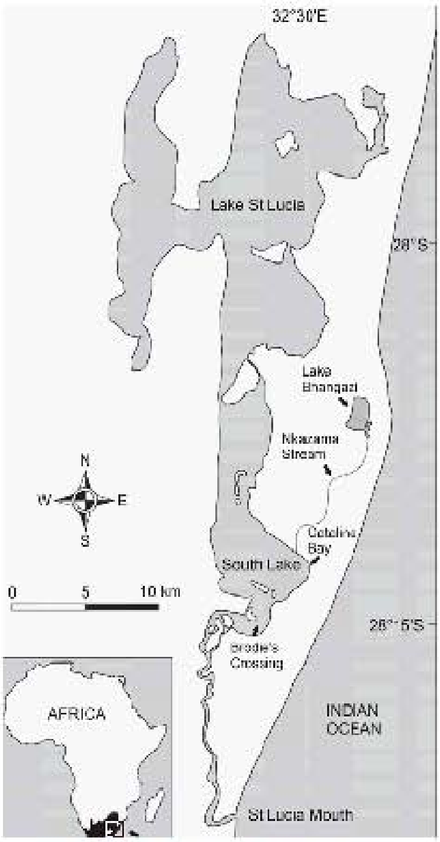
INTRODUCTION

Invasive species have been widely reported as having a negative impact on the ecology and economy of a region.[1] In South Africa, knowledge of aquatic invasive species has increased over the past two decades. So far, the focus has been on prominent invasive species, such as the water hyacinth *Eichhornia crassipes*,[2] fish such as bass and trout,[3] as well as invertebrates such as the mussel *Mytilus galloprovincialis*,[4] the crab *Carcinus maenas*[4] and the snail *Physa acuta*.[5] However, there is a lack of studies on the ecological consequences of invasive aquatic invertebrates in freshwater and estuarine systems. These consequences may include biotic homogenisation, competitive exclusion of indigenous species and changes to the properties of the invaded habitat.[5,6,7] Successful animal invaders progress beyond the introduction and establishment stages, spread in new habitats and finally have tangible ecological impacts. Management efforts should focus on preventing introduction, but, in many cases, the invader is not noticed until it is already established. Ecophysiological knowledge about the invading species is essential in considering whether, and how, to implement control measures.

Tarebia granifera is a freshwater prosobranch gastropod (family: Thiaridae), originally from South-East Asia.[8,9] The species has spread rapidly in recent years across a number of countries throughout tropical and subtropical areas of the World.[5,8,10,11,12] *T. granifera* may have been introduced into KwaZulu-Natal, South Africa, via the aquarium trade in the early 1990s.[14] It is present in an increasing number of fresh and brackish water bodies of KwaZulu Natal and Mpumalanga provinces,[5] including the Kruger National Park.[13] The first record of *T. granifera* in an estuarine environment was in South Africa at the St Lucia Estuary. It was found in December 2005 in the Nkazana Stream (a freshwater environment) which flows into the eastern shore of the South Lake, 1.2 km north of Catalina Bay (Figure 1). The St Lucia estuarine system is part of the iSimangaliso (formerly known as the Greater St Lucia) Wetland Park. This is a World Heritage Site and a Ramsar Wetland of International Importance. Recently, St Lucia has been experiencing severe freshwater starvation. The mouth closed in June 2002 and this 'closed mouth state' persisted until March 2007, leading to variable salinity and water temperature conditions.[14] Under normal conditions, the St Lucia estuarine system has a surface area of around 350 km² and an average depth of 0.9 m.[15] This high surface area to volume ratio is conducive to great evaporative water losses, causing some areas to become hypersaline (salinity greater than 40 psu). However, other areas have developed fresh or brackish conditions due to the input of fresh water from rivers, streams or seepage from sand dune aquifers.[14] The loss of water from the system has also meant a drop in water levels and the formation of large areas of very shallow depth (< 10 cm), which are quickly heated by the sun to reach temperatures over 45 °C in summer.

In January 2007, a dense population (over 5 000 individuals per square metre) of *T. granifera* was found in a very shallow freshwater seepage zone just south of the Old Jetty in Catalina Bay and, in February 2007, it was found in similarly high densities at embayments as far south as Brodie's Crossing (Figure 1). Salinity conditions in these embayments were brackish rather than fresh, but this did not prevent *T. granifera* from invading.

Salinity and temperature are among the main factors influencing growth, reproduction, survival and distribution patterns of aquatic molluscs.[16,17] A number of studies have assessed the effects of temperature and salinity on invasive aquatic invertebrates.[18,19,20] *T. granifera* is of African and global interest due to its invasive ability and the potential impact on indigenous benthic communities.[21] This study investigated the lethal tolerance limits of *T. granifera* to salinity and temperature through the experimental manipulation of these factors. It provides the first experimental data on the responses of this invader to key physicochemical factors in the saline/brackish environments of estuaries. The results reported here will contribute to an understanding of the threat that this snail poses, and possibly also to its management in Africa and elsewhere.



Source: Adapted from Vrdoljak and Hart[21] with permission of NISC (Pty) Ltd.

FIGURE 1
Map of the St Lucia estuarine system, showing Catalina Bay on the eastern shores of South Lake

MATERIALS AND METHODS

Study site

Catalina Bay is located on a limestone flat on the eastern shore of St Lucia's South Lake (Figure 1). The fringing vegetation is mainly composed of *Cyperus laevigatus*, *Juncus kraussii* and *Salicornia* spp. The water in the bay is very shallow (≈ 0.5 m) and can reach a temperature of around 40 °C during summer. From the beginning of 2005 until the breach of the St Lucia mouth in March 2007, the average salinity (± s.d.) in this area was 16 ± 9 psu, a reflection of the influence of the seepage of fresh water from dune systems located to the east of Catalina Bay. Several additional freshwater seepage zones were identified south of the Old Jetty at Catalina Bay. After the breach event, a large volume of sea water entered the system, causing an overall increase in water levels and salinity. During the open phase the average salinity in the bay rose to 30 ± 3 psu.

Temperature tolerance

T. granifera snails (mean shell height ± s.d. = 12.5 ± 2.5 mm) were collected from Catalina Bay (Figure 1) on 11 April 2007 (salinity ≈ 13 psu), transported to the laboratory, sorted into groups of 20 and put into transparent plastic bottles (900 mL). These were filled with fresh water (≈ 0 psu), placed in a controlled-environment room at 20 ± 1 °C with a photoperiod of 12 h of light followed by 12 h of darkness. Snails were left to acclimate in these conditions for 48 h prior to the experiment and fed *ad libitum* on naturally occurring benthic microalgae. Suspensions of microalgae were obtained by scooping the upper few millimetres of sediment and re-suspending them through stirring, and then harvesting the supernatant. Temperature controlled, aerated water baths were used to achieve the following treatments: 0 °C, 5 °C, 10 °C, 20 °C, 30 °C, 40 °C, 45 °C, 47.5 °C and 50 °C. Bottles reached test temperatures within 30 min. A control water bath was placed outside and its temperature was not controlled. Six replicates (900-mL bottles with 20 live individuals each) were used per water bath. Mortality, defined as a lack of response to a mechanical stimulus (gentle prodding with a fine brush), was recorded after exposure times of 2 h, 4 h, 8 h, 16 h and 32 h. At 0 °C, the water in some bottles froze due to their close proximity to the water bath's cooling element. Although these treatments were not included in the data analysis, the snails retrieved from such bottles were thawed and kept at a recovering temperature of 20 °C for 12 h, after which mortality was tested. A second stock of snails was collected from Catalina Bay on 18 July 2007 (salinity ≈ 28 psu) and the procedure was repeated at 30 ± 2 psu (diluted, sterilised sea water was used) with a freshwater control treatment. Results were expressed as per cent survival.[22]

Salinity tolerance

The experimental procedure for salinity tolerance was similar to the above, using stock animals collected in July 2007. Six replicates (900 mL bottles with 20 live individuals each) were subjected to each of the following salinities: 0 ± 0.5 psu, 10 ± 2 psu, 20 ± 2 psu, 30 ± 2 psu and 40 ± 2 psu. The 40 ± 2 psu concentration was prepared by the addition of natural sea salt and measured using a water logger (YSI 6920, YSI Incorporated, Yellow Springs, OH, USA). Mortality was monitored over a period of 85 days. Dead individuals were removed immediately. *T. granifera* is ovoviviparous and has a brood pouch from which juveniles (shell height ≈ 1 mm) are born. Hence the presence of live juveniles in a bottle was recorded as birth. Water was changed every 5–10 days and food renewed weekly. The activity of snails in each bottle was monitored and classified according to four progressive criteria: actively moving, not moving (foot anchored), head out of shell (foot not anchored) and quiescent with operculum closed. The proportions of snails in each bottle conforming to these criteria were recorded every 10 days. These data were used to calculate the distribution of snail activity for each salinity treatment over the total exposure time up to 85 days. A 'salinity shock experiment' was also conducted, in which 12 replicates were acclimated to fresh water for a week. Six replicates were then transferred directly to 30 ± 2 psu, with the remainder serving as control. Mortality was recorded after 48 h.

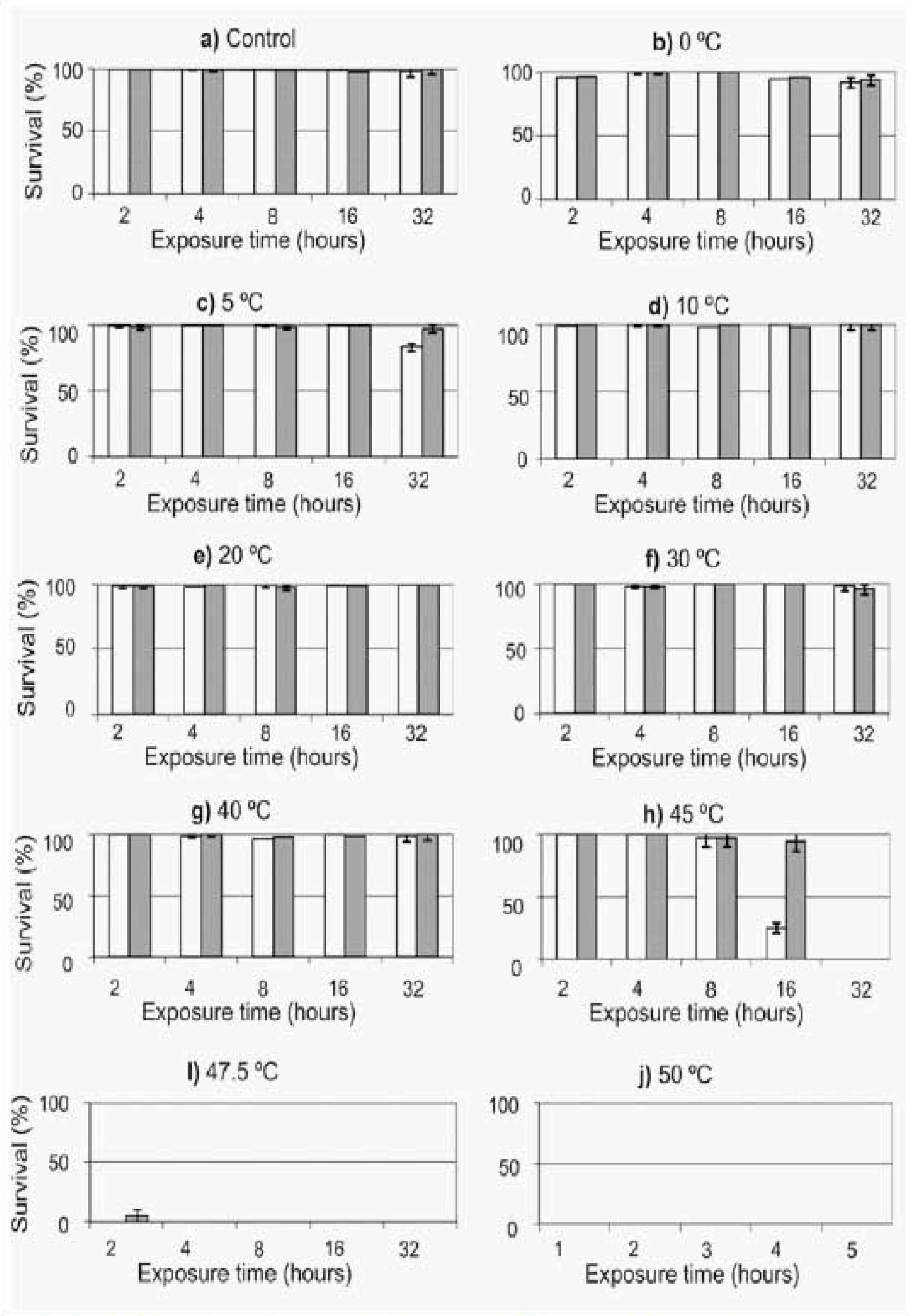
Data analysis

Data were arcsine-transformed, following a one-sample Kolmogorov-Smirnov normality test.[23] Repeated measures analyses of variance (RM-ANOVA) were used where each 900 mL bottle containing 20 live individuals was the unit of replication. Temperature tolerance data were tested using a three-way RM-ANOVA (dependent variable: survival; fixed factors: salinity, temperature, exposure time). Salinity tolerance data were tested using a two-way RM-ANOVA (dependent variable: survival; fixed factors: salinity and exposure time). Tukey post hoc analyses were done on each of these two data sets. Differences between control and experimental treatments of the 'salinity shock experiment' were tested using a two-tailed independent-samples t-test. Homogeneity and normality of residuals were tested by a Levene's test and a one-sample Kolmogorov-Smirnov test, respectively. The statistical program SPSS 15 was used in all analyses.

RESULTS

Temperature tolerance

Survival in the control and the 0 °C, 5 °C, 10 °C, 20 °C, 30 °C and 40 °C treatments, at both 0 psu and 30 psu, exceeded 75% for 32 h (Figure 2). Snails died if the water in the bottles froze. Time to LT_{50} at 45 °C was 8 h – 16 h at 0 psu and 16 h – 32 h at 30 psu (Figure 2). Mortality of 100% occurred within 2 h of exposure



Survival rates were calculated at temperatures ranging from 0 °C to 50 °C (b–j) and at salinities of 0 psu (white bars) and 30 psu (grey bars). The (a) control treatments were set in the open at 16 °C – 22 °C.

FIGURE 2
Percentage survival of *Tarebia granifera* (± s.d.) over exposure times (2 h – 32 h)

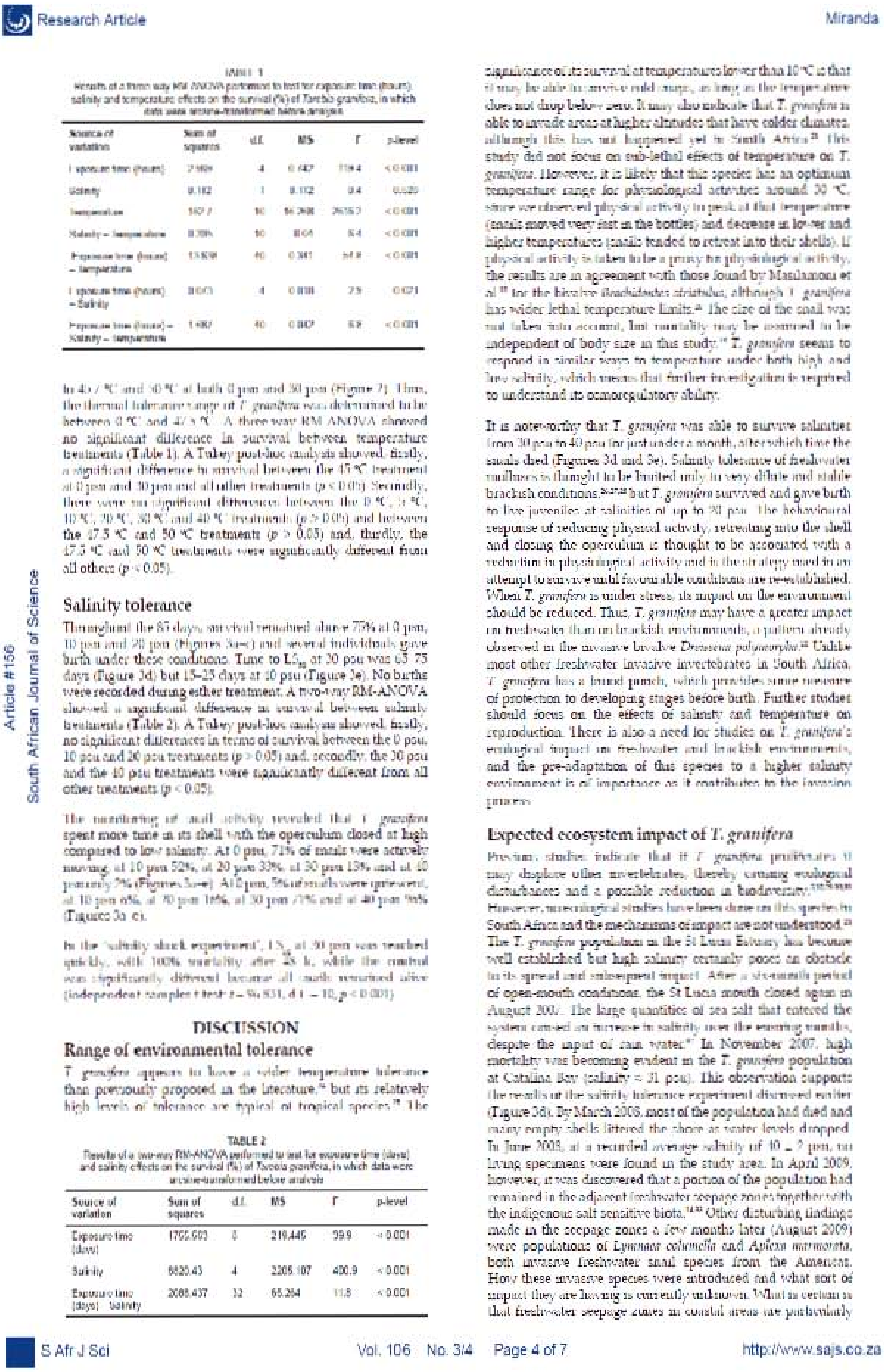


TABLE 1
Results of a three-way RM ANOVA performed to test for exposure time (hours), salinity and temperature effects on the survival (%) of *Tarebia granifera*, in which data were arcsine-transformed before analysis

Source of variation	Sum of squares	d.f.	MS	F	p-level
Exposure time (hours)	[illegible]	4	[illegible]	[illegible]	< 0.001
Salinity	0.112	1	0.112	0.4	[illegible]
Temperature	[illegible]	10	[illegible]	[illegible]	< 0.001
Salinity × Temperature	[illegible]	10	[illegible]	5.4	< 0.001
Exposure time (hours) × Temperature	[illegible]	40	[illegible]	[illegible]	< 0.001
Exposure time (hours) × Salinity	[illegible]	4	[illegible]	7.5	[illegible]
Exposure time (hours) × Salinity × Temperature	[illegible]	40	[illegible]	6.8	< 0.001

to 47.5 °C and 50 °C at both 0 psu and 30 psu (Figure 2). Thus, the thermal tolerance range of *T. granifera* was determined to be between 0 °C and 47.5 °C. A three-way RM ANOVA showed no significant difference in survival between temperature treatments (Table 1). A Tukey post-hoc analysis showed, firstly, a significant difference in survival between the 45 °C treatment at 0 psu and 30 psu and all other treatments ($p < 0.05$). Secondly, there were no significant differences between the 0 °C, 5 °C, 10 °C, 20 °C, 30 °C and 40 °C treatments ($p > 0.05$) and between the 47.5 °C and 50 °C treatments ($p > 0.05$) and, thirdly, the 47.5 °C and 50 °C treatments were significantly different from all others ($p < 0.05$).

Salinity tolerance

Throughout the 85 days, survival remained above 75% at 0 psu, 10 psu and 20 psu (Figures 3a–c) and several individuals gave birth under these conditions. Time to LS_{50} at 30 psu was 65–75 days (Figure 3d) but 15–25 days at 40 psu (Figure 3e). No births were recorded during either treatment. A two-way RM-ANOVA showed a significant difference in survival between salinity treatments (Table 2). A Tukey post-hoc analysis showed, firstly, no significant differences in terms of survival between the 0 psu, 10 psu and 20 psu treatments ($p > 0.05$) and, secondly, the 30 psu and the 40 psu treatments were significantly different from all other treatments ($p < 0.05$).

The monitoring of snail activity revealed that *T. granifera* spent more time in its shell with the operculum closed at high compared to low salinity. At 0 psu, 71% of snails were actively moving, at 10 psu 52%, at 20 psu 33%, at 30 psu 13% and at 40 psu only 7% (Figures 3a–e). At 0 psu, 5% of snails were withdrawn, at 10 psu 6%, at 20 psu 16%, at 30 psu 71% and at 40 psu 90% (Figures 3a–e).

In the 'salinity shock experiment', LS_{50} at 50 psu was reached quickly, with 100% mortality after 28 h, while the control was significantly different because all snails remained alive (independent samples t-test: $t = 96.831$, d.f. = 10, $p < 0.001$).

DISCUSSION

Range of environmental tolerance

T. granifera appears to have a wider temperature tolerance than previously proposed in the literature,[illegible] but its relatively high levels of tolerance are typical of tropical species.[illegible] The significance of its survival at temperatures lower than 10 °C is that it may be able to survive cold snaps, as long as the temperature does not drop below zero. It may also indicate that *T. granifera* is able to invade areas at higher altitudes that have colder climates, although this has not happened yet in South Africa.[23] This study did not focus on sub-lethal effects of temperature on *T. granifera*. However, it is likely that this species has an optimum temperature range for physiological activities around 30 °C, since we observed physical activity to peak at that temperature (snails moved very fast in the bottles) and decrease at lower and higher temperatures (snails tended to retreat into their shells). If physical activity is taken to be a proxy for physiological activity, the results are in agreement with those found by Masilamoni et al.[illegible] for the bivalve *Brachidontes striatulus*, although *T. granifera* has wider lethal temperature limits.[illegible] The size of the snail was not taken into account, but mortality may be assumed to be independent of body size in this study.[illegible] *T. granifera* seems to respond in similar ways to temperature under both high and low salinity, which means that further investigation is required to understand its osmoregulatory ability.

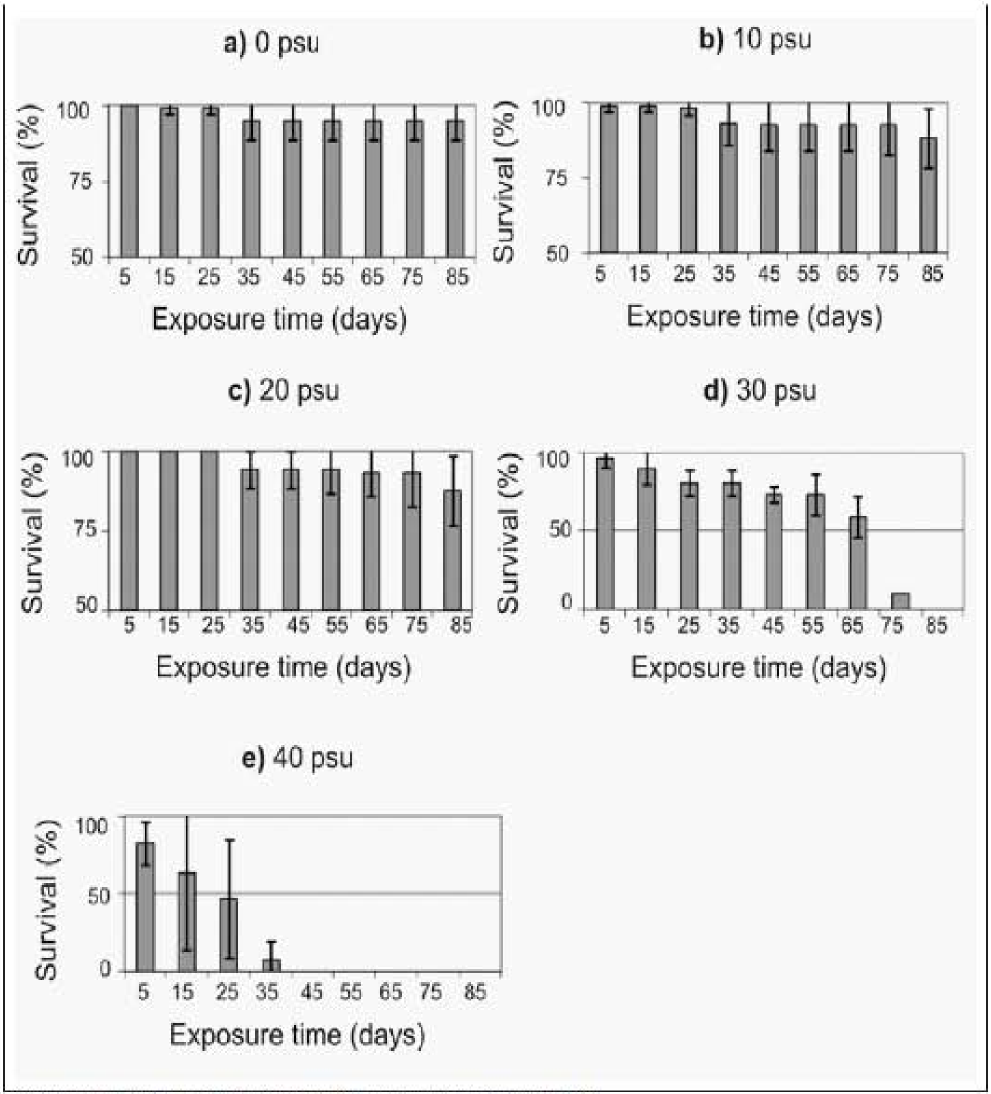
TABLE 2
Results of a two-way RM-ANOVA performed to test for exposure time (days) and salinity effects on the survival (%) of *Tarebia granifera*, in which data were arcsine-transformed before analysis

Source of variation	Sum of squares	d.f.	MS	F	p-level
Exposure time (days)	1755.603	8	219.450	39.9	< 0.001
Salinity	8820.43	4	2205.107	400.9	< 0.001
Exposure time (days) × Salinity	2088.437	32	65.264	11.8	< 0.001

It is noteworthy that *T. granifera* was able to survive salinities from 30 psu to 40 psu for just under a month, after which time the snails died (Figures 3d and 3e). Salinity tolerance of freshwater molluscs is thought to be limited only to very dilute and stable brackish conditions,[26,27,28] but *T. granifera* survived and gave birth to live juveniles at salinities of up to 20 psu. The behavioural response of reducing physical activity, retreating into the shell and closing the operculum is thought to be associated with a reduction in physiological activity and is the strategy used in an attempt to survive until favourable conditions are re-established. When *T. granifera* is under stress, its impact on the environment should be reduced. Thus, *T. granifera* may have a greater impact on freshwater than on brackish environments, a pattern already observed in the invasive bivalve *Dreissena polymorpha*.[illegible] Unlike most other freshwater invasive invertebrates in South Africa, *T. granifera* has a brood pouch, which provides some measure of protection to developing stages before birth. Further studies should focus on the effects of salinity and temperature on reproduction. There is also a need for studies on *T. granifera*'s ecological impact on freshwater and brackish environments, and the pre-adaptation of this species to a higher salinity environment is of importance as it contributes to the invasion process.

Expected ecosystem impact of *T. granifera*

Previous studies indicate that if *T. granifera* proliferates it may displace other invertebrates, thereby causing ecological disturbances and a possible reduction in biodiversity.[illegible] However, no ecological studies have been done on this species in South Africa and the mechanisms of impact are not understood.[23] The *T. granifera* population in the St Lucia Estuary has become well established but high salinity certainly poses an obstacle to its spread and subsequent impact. After a six-month period of open-mouth conditions, the St Lucia mouth closed again in August 2007. The large quantities of sea salt that entered the system caused an increase in salinity over the ensuing months, despite the input of rain water.[illegible] In November 2007, high mortality was becoming evident in the *T. granifera* population at Catalina Bay (salinity ≈ 31 psu). This observation supports the results of the salinity tolerance experiment discussed earlier (Figure 3d). By March 2008, most of the population had died and many empty shells littered the shore as water levels dropped. In June 2008, at a recorded average salinity of 40 ± 2 psu, no living specimens were found in the study area. In April 2009, however, it was discovered that a portion of the population had remained in the adjacent freshwater seepage zones together with the indigenous salt sensitive biota.[illegible] Other disturbing findings made in the seepage zones a few months later (August 2009) were populations of *Lymnaea columella* and *Aplexa marmorata*, both invasive freshwater snail species from the Americas. How these invasive species were introduced and what sort of impact they are having is currently unknown. What is certain is that freshwater seepage zones in coastal areas are particularly



Survival rates were calculated at salinities ranging from 0 psu to 40 psu (a–e). Temperature was kept constant at 20 °C.

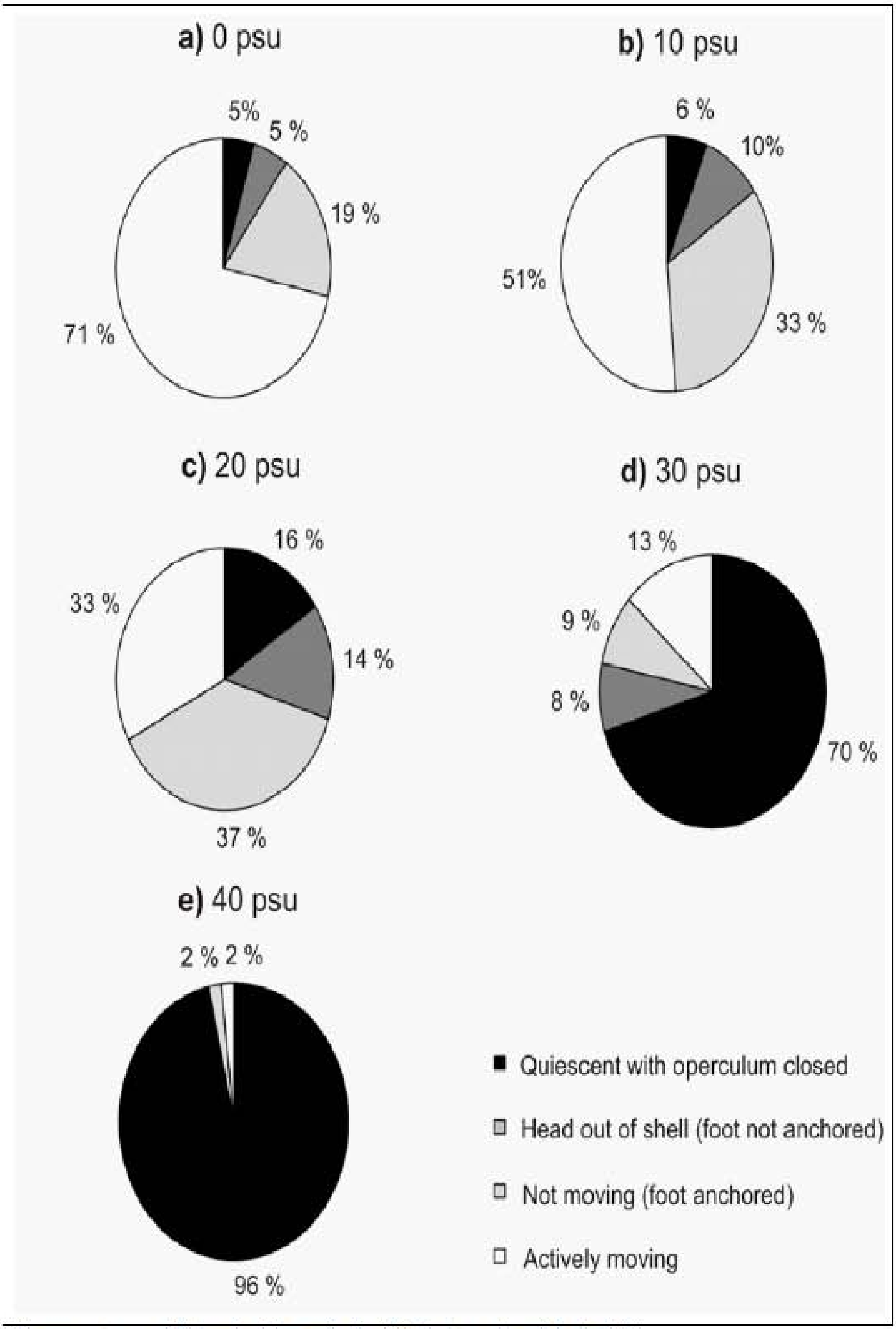
FIGURE 3
Percentage survival of *Tarebia granifera* (± s.d.) over exposure time (5–85 days)

vulnerable to invasion and may provide the opportunity for *T. granifera* populations to gradually adapt to higher salinity as they undergo a diffusion dispersal process.[7]

Prospects for the control of *T. granifera*

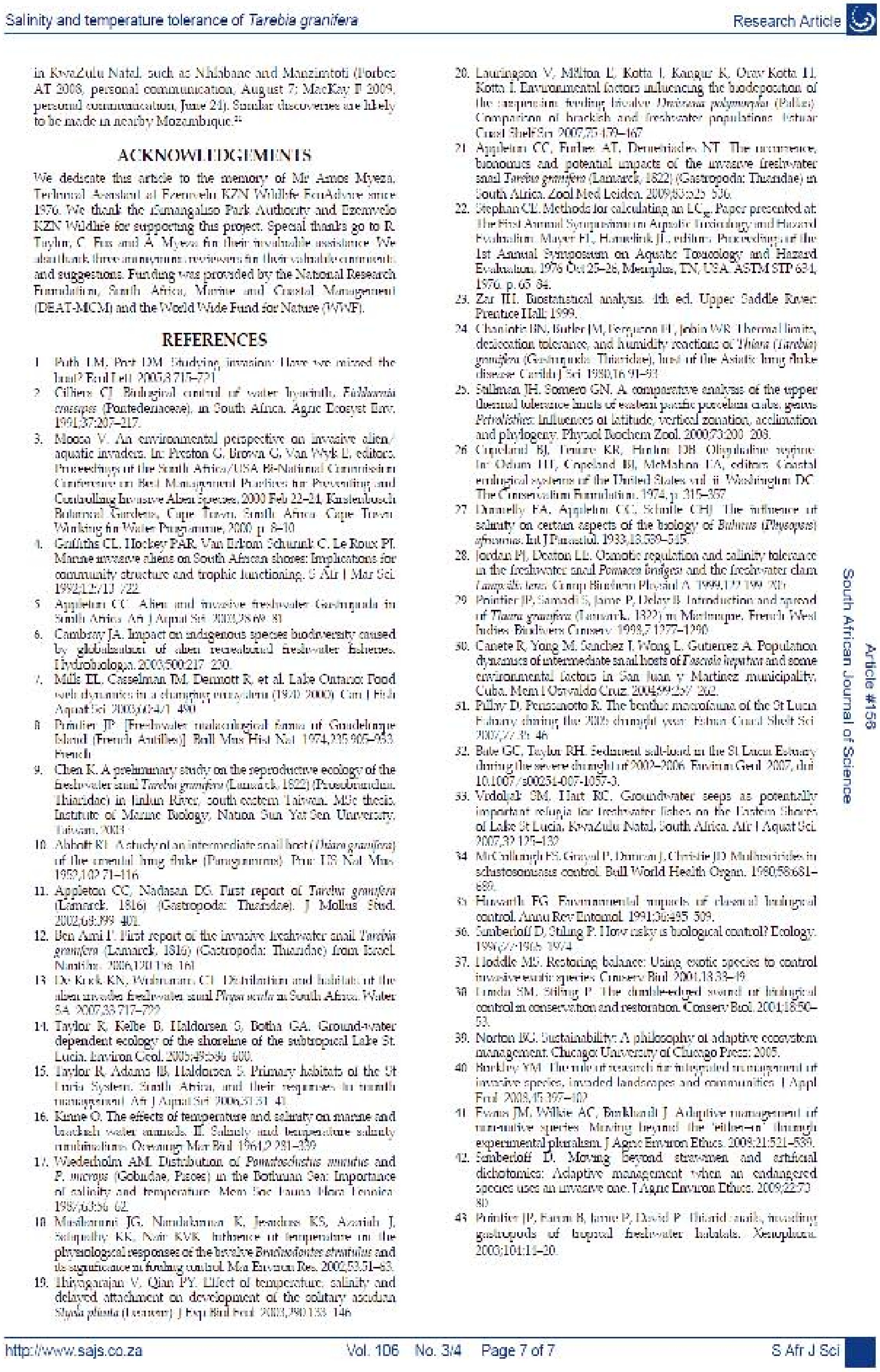
In order to completely purge *T. granifera* from an environment, 100% of the population has to be eliminated because of its parthenogenetic characteristic. The use of molluscicides in the St Lucia Estuary is not feasible at any stage, given that juveniles within the brood pouch may not be killed (personal observation) and non-target species, such as the indigenous snails *Haminoea natalensis* and *Assiminea* sp., which often dominate macrobenthic communities, may be adversely affected.[22,23] Another option would be the introduction of a biological control agent, but this has its own widely reported risks.[25,26,27,28] During this study, *T. granifera* showed a drop in physical activity as salinity increased (Figure 4) and, at salinities higher than 30 psu, snails were obviously under stress and died within a few weeks (Figure 3). The 'salinity shock experiment' showed that *T. granifera* was very vulnerable to sudden salinity increases. These findings suggest that invasions by this species could be controlled in temporarily open/closed estuaries by artificially breaching the mouth. However, such an action (effecting large scale changes in order to manage a single species) may have unintended and negative consequences, as discussed in the growing literature on adaptive management.[39,40,41,42]

In summary, *T. granifera* can be regarded as a global invader of tropical and subtropical regions.[43] The present findings suggest that tropical and subtropical freshwater and estuarine systems located in lowlands (altitude lower than 500 m) are particularly at risk in Africa, especially where the aquarium trade operates.[44] However, so far there have been no reports of this species having spread northwards from South Africa. Nevertheless, situations similar to those recorded in the St Lucia Estuary already occur elsewhere in South Africa; populations of *T. granifera* are being discovered with increasing frequency in other areas of the iSimangaliso Park, such as Kosi Bay and Lake Sibaya (Kyle R 2008, personal communication, July 20; Taylor R 2008, personal communication, February 26), and other estuaries



Activity was measured over a period of 65 days and in salinities ranging from 0 psu to 40 psu (a–e). Temperature was kept constant at 20 °C.

FIGURE 4
Average distribution of *Tarebia granifera* activity (%) classified into four successive orders.



in KwaZulu-Natal, such as Nhlabane and Manzimtoti (Forbes AT 2008, personal communication, August 7; MacKay F 2009, personal communication, June 21). Similar discoveries are likely to be made in nearby Mozambique.[21]

ACKNOWLEDGEMENTS

We dedicate this article to the memory of Mr Amos Myeza, Technical Assistant at Ezemvelo KZN Wildlife EcoAdvice since 1976. We thank the iSimangaliso Park Authority and Ezemvelo KZN Wildlife for supporting this project. Special thanks go to R. Taylor, C. Fox and A. Myeza for their invaluable assistance. We also thank three anonymous reviewers for their valuable comments and suggestions. 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

1. Puth LM, Post DM. Studying invasion: Have we missed the boat? Ecol Lett. 2005;8:715–721.
2. Cilliers CJ. Biological control of water hyacinth, *Eichhornia crassipes* (Pontederiaceae), in South Africa. Agric Ecosyst Env. 1991;37:207–217.
3. Moran V. An environmental perspective on invasive alien/aquatic invaders. In: Preston G, Brown G, Van Wyk E, editors. Proceedings of the South Africa/USA Bi-National Commission Conference on Best Management Practices for Preventing and Controlling Invasive Alien Species. 2000 Feb 22–24; Kirstenbosch Botanical Gardens, Cape Town, South Africa. Cape Town: Working for Water Programme; 2000. p. 8–10.
4. Griffiths CL, Hockey PAR, Van Erkom Schurink C, Le Roux PJ. Marine invasive aliens on South African shores: Implications for community structure and trophic functioning. S Afr J Mar Sci. 1992;12:713–722.
5. Appleton CC. Alien and invasive freshwater Gastropoda in South Africa. Afr J Aquat Sci. 2003;28:69–81.
6. Cambray JA. Impact on indigenous species biodiversity caused by globalisation of alien recreational freshwater fisheries. Hydrobiologia. 2003;500:217–230.
7. Mills EL, Casselman JM, Dermott R, et al. Lake Ontario: Food web dynamics in a changing ecosystem (1970–2000). Can J Fish Aquat Sci. 2003;60:471–490.
8. Pointier JP. [Freshwater malacological fauna of Guadeloupe Island (French Antilles)]. Bull Mus Hist Nat. 1974;235:905–933. French.
9. Chen K. A preliminary study on the reproductive ecology of the freshwater snail *Tarebia granifera* (Lamarck, 1822) (Prosobranchia: Thiaridae) in Linkun River, south eastern Taiwan. MSc thesis. Institute of Marine Biology, Nation Sun Yat Sen University, Taiwan; 2003.
10. Abbott RT. A study of an intermediate snail host (*Thiara granifera*) of the oriental lung fluke (Paragonimus). Proc US Nat Mus. 1952;102:71–116.
11. Appleton CC, Nadasan DS. First report of *Tarebia granifera* (Lamarck, 1816) (Gastropoda: Thiaridae). J Mollus Stud. 2002;68:399–401.
12. Ben-Ami F. First report of the invasive freshwater snail *Tarebia granifera* (Lamarck, 1816) (Gastropoda: Thiaridae) from Israel. Nautilus. 2006;120:156–161.
13. De Kock KN, Wolmarans CT. Distribution and habitats of the alien invader freshwater snail *Physa acuta* in South Africa. Water SA. 2007;33:717–722.
14. Taylor R, Kelbe B, Haldorsen S, Botha GA. Ground-water dependent ecology of the shoreline of the subtropical Lake St. Lucia. Environ Geol. 2005;49:586–600.
15. Taylor R, Adams JB, Haldorsen S. Primary habitats of the St Lucia System, South Africa, and their responses to mouth management. Afr J Aquat Sci. 2006;31:31–41.
16. Kinne O. The effects of temperature and salinity on marine and brackish water animals. II. Salinity and temperature salinity combinations. Oceanogr Mar Biol. 1964;2:281–339.
17. Wiederholm AM. Distribution of *Pomatoschistus minutus* and *P. microps* (Gobiidae, Pisces) in the Bothnian Sea: Importance of salinity and temperature. Mem Soc Fauna Flora Fennica. 1987;63:56–62.
18. Masilamoni JG, Nandakumar K, Jesudoss KS, Azariah J, Satapathy KK, Nair KVK. Influence of temperature on the physiological responses of the bivalve *Brachidontes striatulus* and its significance in fouling control. Mar Environ Res. 2002;53:51–63.
19. Thiyagarajan V, Qian PY. Effect of temperature, salinity and delayed attachment on development of the solitary ascidian *Styela plicata* (Lesueur). J Exp Mar Biol Ecol. 2003;290:133–146.
20. Lauringson V, Mälton E, Kotta J, Kangur K, Orav-Kotta H, Kotta I. Environmental factors influencing the biodeposition of the suspension feeding bivalve *Dreissena polymorpha* (Pallas): Comparison of brackish and freshwater populations. Estuar Coast Shelf Sci. 2007;75:459–467.
21. Appleton CC, Forbes AT, Demetriades NT. The occurrence, bionomics and potential impacts of the invasive freshwater snail *Tarebia granifera* (Lamarck, 1822) (Gastropoda: Thiaridae) in South Africa. Zool Med Leiden. 2009;83:525–536.
22. Stephan CE. Methods for calculating an LC_{50}. Paper presented at: The First Annual Symposium on Aquatic Toxicology and Hazard Evaluation. Mayer FL, Hamelink JL, editors. Proceedings of the 1st Annual Symposium on Aquatic Toxicology and Hazard Evaluation; 1976 Oct 25–26; Memphis, TN, USA. ASTM STP 634; 1976. p. 65–84.
23. Zar JH. Biostatistical analysis. 4th ed. Upper Saddle River: Prentice Hall; 1999.
24. Chaniotis BN, Butler JM, Ferguson FF, Jobin WR. Thermal limits, desiccation tolerance, and humidity reactions of *Thiara (Tarebia) granifera* (Gastropoda: Thiaridae), host of the Asiatic lung fluke disease. Caribb J Sci. 1980;16:91–93.
25. Stillman JH, Somero GN. A comparative analysis of the upper thermal tolerance limits of eastern pacific porcelain crabs, genus *Petrolisthes*: Influences of latitude, vertical zonation, acclimation and phylogeny. Physiol Biochem Zool. 2000;73:200–208.
26. Copeland BJ, Tenore KR, Horton DB. Oligohaline regime. In: Odum HT, Copeland BJ, McMahon EA, editors. Coastal ecological systems of the United States vol. ii. Washington DC: The Conservation Foundation; 1974. p. 315–357.
27. Donnelly FA, Appleton CC, Schutte CHJ. The influence of salinity on certain aspects of the biology of *Bulinus (Physopsis) africanus*. Int J Parasitol. 1983;13:539–545.
28. Jordan PJ, Deaton LE. Osmotic regulation and salinity tolerance in the freshwater snail *Pomacea bridgesi* and the freshwater clam *Lampsilis teres*. Comp Biochem Physiol A. 1999;122:199–205.
29. Pointier JP, Samadi S, Jarne P, Delay B. Introduction and spread of *Thiara granifera* (Lamarck, 1822) in Martinique, French West Indies. Biodivers Conserv. 1998;7:1277–1290.
30. Canete R, Yong M, Sanchez J, Wong L, Gutierrez A. Population dynamics of intermediate snail hosts of *Fasciola hepatica* and some environmental factors in San Juan y Martinez municipality, Cuba. Mem I Oswaldo Cruz. 2004;99:257–262.
31. Pillay D, Perissinotto R. The benthic macrofauna of the St Lucia Estuary during the 2005 drought year. Estuar Coast Shelf Sci. 2008;77:35–46.
32. Bate GC, Taylor RH. Sediment salt-load in the St Lucia Estuary during the severe drought of 2002–2006. Environ Geol. 2007; doi 10.1007/s00254-007-1057-3.
33. Vdoljak SM, Hart RC. Groundwater seeps as potentially important refugia for freshwater fishes on the Eastern Shores of Lake St Lucia, KwaZulu-Natal, South Africa. Afr J Aquat Sci. 2007;32:125–132.
34. McCullough FS, Gayral P, Duncan J, Christie JD. Molluscicides in schistosomiasis control. Bull World Health Organ. 1980;58:681–689.
35. Howarth FG. Environmental impacts of classical biological control. Annu Rev Entomol. 1991;36:485–509.
36. Simberloff D, Stiling P. How risky is biological control? Ecology. 1996;77:1965–1974.
37. Hoddle MS. Restoring balance: Using exotic species to control invasive exotic species. Conserv Biol. 2004;18:38–49.
38. Louda SM, Stiling P. The double-edged sword of biological control in conservation and restoration. Conserv Biol. 2004;18:50–53.
39. Norton BG. Sustainability: A philosophy of adaptive ecosystem management. Chicago: University of Chicago Press; 2005.
40. Buckley YM. The role of research for integrated management of invasive species, invaded landscapes and communities. J Appl Ecol. 2008;45:397–402.
41. Evans JM, Wilkie AC, Burkhardt J. Adaptive management of non-native species: Moving beyond the 'either–or' through experimental pluralism. J Agric Environ Ethics. 2008;21:521–539.
42. Simberloff D. Moving beyond strawmen and artificial dichotomies: Adaptive management when an endangered species uses an invasive one. J Agric Environ Ethics. 2009;22:73–80.
43. Pointier JP, Facon B, Jarne P, David P. Thiarid snails, invading gastropods of tropical freshwater habitats. Xenophora. 2003;104:14–20.

Estuarine, Coastal and Shelf Science 91 (2011) 442–449

Contents lists available at ScienceDirect

Estuarine, Coastal and Shelf Science

journal homepage: www.elsevier.com/locate/ecss

Feeding dynamics of the invasive gastropod *Tarebia granifera* in coastal and estuarine lakes 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, Westville Campus, Private Bag X54001, Durban 4000, South Africa

A R T I C L E I N F O

Article history:
Received 8 June 2010
Accepted 28 November 2010
Available online 3 December 2010

Keywords:
aquatic invertebrates
non-indigenous invasive species
gut fluorescence technique
carbon budget
iSimangaliso Wetland Park

A B S T R A C T

Gut fluorescence and carbon budget techniques were applied to *Tarebia granifera* (shell height 10–12 mm) at the iSimangaliso Wetland Park, a UNESCO World Heritage Site. This snail has recently invaded 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 concerns about potential top-down impacts on the ecosystem. This study shows that *T. granifera* can utilize large amounts of microphytobenthos (MPB) in addition to detritus. *In situ* total available MPB pigment concentrations ranged from 11.6 to 110.5 mg pigm. m^{-2}. *T. granifera*'s gut pigment content ranged from 54 to 1672 µg pigm. ind^{-1}. Gut evacuation rates (k) ranged from 0.36 to 0.62 h^{-1} (R^2 range: 16.2–35.2, $P < 0.05$). Individual ingestion rates ranged from 6.6 to 30.4 µg pigm. $ind.^{-1}\,d^{-1}$. *T. granifera* was estimated to consume from 0.5 to 35% of the total available MPB 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. The average carbon daily ration contributed by microalgal biomass was ≈ 16% body carbon per day. Variability in the data was attributed to the complex feeding history of snails. Further studies are needed to validate these results and provide more information on the ecological impact of *T. granifera* on this wetland and other similar invaded ecosystems, both estuarine and freshwater.

1. Introduction

Non-indigenous invasive species (NIS) have been the focus of growing research across the globe (Lockwood et al., 2007; Davis, 2009). Invasive molluscs can dominate aquatic communities and disrupt ecosystem processes (Hall et al., 2006; Riley et al., 2008; Sousa et al., 2008; Baker et al., 2010). Impacts of this kind are especially relevant when protected areas may be affected and when there are economic repercussions (Pimentel et al., 2005; Puth and Post, 2005; Barnes et al., 2009). Unfortunately, when NIS are detected it is often too late to eliminate 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/revision where amelioration of the impacts of a given invader is one of the priorities (Buckley, 2008).

Tarebia granifera is a prosobranch gastropod originally from South-East Asia that has become invasive in several countries around the world including South Africa (Abbott, 1952; Pointer et al., 2003; Appleton et al., 2009). It was first recorded in an estuarine environment at the St. Lucia Estuary (South Africa) in 2005 (Miranda et al., 2010). 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 lakes and Lake Sibaya. The St. Lucia Estuary, Kosi lakes and Lake Sibaya are Ramsar sites of international importance, part of the iSimangaliso Wetland Park, a UNESCO World Heritage Site (Fig. 1). *T. granifera* is known to reach densities of over 10,000 ind. m^{-2} and displace other snails in invaded areas (Pointer et al., 1998; Canete et al., 2004; Pillay and Perissinotto, 2008; Appleton et al., 2009). Preliminary measurements undertaken *in situ* at the St Lucia Estuary indicated that this species had relatively high gut pigment contents (pers. obs.). So although *T. granifera* was reported as being a detritivore by Larned et al. (2001), it may also significantly impact on microphytobenthos (MPB) biomass, thereby potentially competing with indigenous species for food resources. There is currently a lack of knowledge about this species' ecological impact (Appleton et al., 2009) and information on *T. granifera*'s feeding ecology is urgently needed.

Top-down effects of grazers are known to be significant in marine and freshwater ecosystems, and their underlying mechanisms are complex (Pillay et al., 2009). Estimating the grazing

---

* Corresponding author.
E-mail address: 204507499@ukzn.ac.za (N.A.F. Miranda).

0272-7714/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ecss.2010.11.007

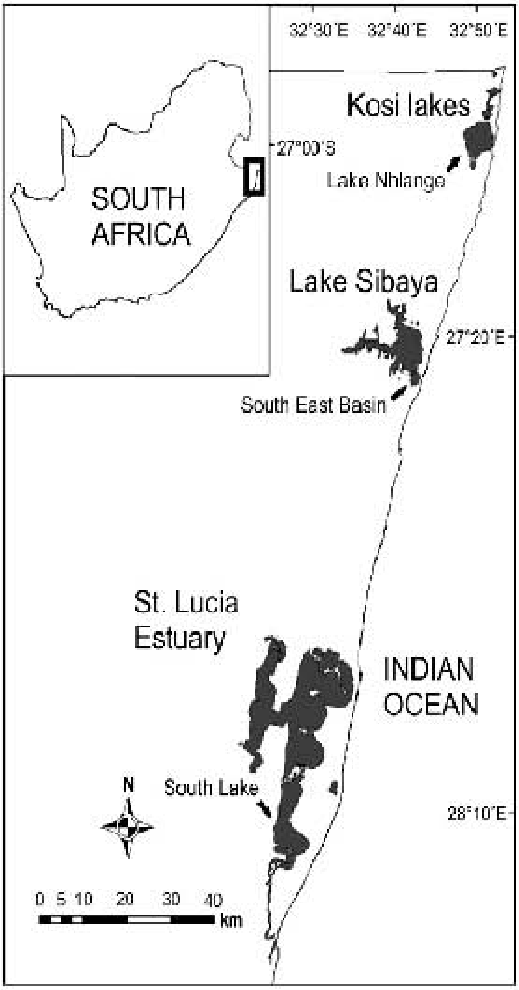


Fig. 1. Map of Maputaland. Kosi lakes, Lake Sibaya and the St. Lucia Estuary are Ramsar Wetlands of International Importance within the iSimangaliso Wetland Park in KwaZulu-Natal, South Africa.

impact of gastropods has classically been done by manipulating their densities in experimental areas or the laboratory (Range 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 microalgal biomass and primary production and to determine the contribution of carbon contributed by autotrophic microalgal biomass to the total organic carbon present in the gut of *Tarebia granifera* (Mackas and Bohrer, 1976; Perissinotto, 1992; Perissinotto et al., 2000). The aims were: (1) to develop a novel method of estimating *in situ* feeding rate and impact in a benthic invertebrate; (2) to further knowledge on the feeding ecology of the invasive gastropod *T. granifera*.

2. Materials and methods

2.1. Study site

Tarebia granifera snails of 10–12 mm shell height were collected from three areas within the iSimangaliso Wetland Park, in northern KwaZulu-Natal, South Africa (Fig. 1): St. Lucia Estuary, on 19 April 2007 and 1 December 2009; Kosi lakes, on 24 November 2008; and Lake Sibaya on 18 November 2009.

The St. Lucia Estuary is the largest estuarine lake in Africa. It has a surface area of 325 km^2 and average depth of 0.9 m (Taylor et al., 2006). 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., 2010). 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 South Lake (28°13'S, 32°29'E) (Fig. 1), where there are many freshwater seepage zones. The substrate is a limestone flat conspicuously covered with microphytobenthos.

Kosi samples were collected at Lake Nhlange (26°57'S, 32°49'E) (Fig. 1). The surface area of this lake varies from 31 to 37 km^2 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 substratum is composed of clear white sand.

Lake Sibaya has a surface area of 60–77 km^2 and an average depth of 13 m (Bruton, 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 (Bruton, 1980). Here, samples were collected on the South East Basin (27°25'S, 32°41'E) (Fig. 1). Like at Lake Nhlange, the substratum is composed of clear white sand.

2.2. Gut pigment content

Individual *Tarebia granifera* guts were excised under a dissecting microscope (40x magnification). Chlorophyll *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-acidification system (Welschmeyer, 1994). Chlorophyll *a* and phaeopigment concentrations were added and reported as chlorophyll *a* equivalents (pigment or pigm.) (Strickland and Parsons, 1968; Conover et al., 1986).

Day/night differences in *Tarebia granifera*'s gut pigments were measured in the field at Lake Nhlange in November 2009 over a 24 h period (5 replicates, removed and immediately processed at intervals of 2–4 h using the method outlined above). This was also done in the laboratory with snails collected from the St. Lucia Estuary in December 2009 aclimated to laboratory conditions for three weeks under a photoperiod of 12 h of light followed by 12 h of darkness and fed *ad libitum* with naturally occurring microphytobenthos (water temperature: 20 °C) (Miranda et al., 2010).

2.3. Feeding rates

Feeding rates were estimated by the *in situ* approach based on the gut fluorescence technique to calculate ingestion rates (J; µg pigm. ind. d^{-1}). According to Mackas and Bohrer (1976) and Perissinotto (1992):

$$I = kG/\left(1 - b^1\right)$$

where k is the gut evacuation rate constant (h^{-1}), G is the gut pigment concentration (ng pigm. ind.$^{-1}$) and b^1 is the gut pigment assimilation/destruction efficiency (%). To measure the gut evacuation rate (k), freshly caught *Tarebia granifera* individuals were isolated in 30 ml vials filled with water collected *in situ* and filtered through a GF/F 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 cornstarch particles (<100 µm) were added to the filtered water (Perissinotto, 1992; Perissinotto and Pakhomov, 1996). Only actively moving individuals were used in the experiments (Miranda et al., 2010). There were 10 replicates for time zero and 5 replicates after each subsequent exposure period. Pigment concentrations were extracted every 10 min in the first hour and then every 30 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 (Perissinotto and Pakhomov, 1996).

2.4. *Assimilation/destruction efficiency*

Gut pigment assimilation/destruction was estimated within 48 h of collection of specimens from the field. Snails were first allowed to empty their guts of pigments in filtered water collected *in situ* with cornstarch over a period of 24 h. The two compartment (water and grazer) pigment budget approach was used to estimate gut pigment assimilation/destruction (Lopez et al., 1988; Mayzaud and Razouls, 1992). The pigment budgets in experimental treatments (2 L jars with 5 snails each) and controls (jars with no snails) were compared after an incubation period of 1 h (Dagg, 1983; Kiørboe et al., 1985; Perissinotto, 1992). Significant pigment losses from experimental treatments were attributed to gut pigment assimilation/destruction only, as no fecal pellets were produced during the incubation period. Three replicates were prepared for each treatment.

2.5. *Estimation of feeding impact*

Tarebia granifera samples were collected in triplicate with a D-net (30 cm diameter, 200 µm mesh), which was pushed for 1 m so as to collect all the benthic organisms on the surface and buried in the upper 3 cm of sediment. These samples were used to calculate density (ind. m^{-2}). Salinity and temperature were measured with a Yellow Springs Instruments 6920 multiprobe. Sediment samples (upper first cm) were collected in triplicate with a Perspex corer (diameter: 20 mm). These samples were used to determine MPB biomass, as pigment concentration (mg pigm. m^{-2}). 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 (mg pigm. ind.$^{-1}$ d^{-1}) was calculated by multiplying daily ingestion rate with average density. This was then expressed as a percentage of total available MPB biomass.

2.6. *Gut organic content, carbon budget and daily ration*

The carbon budget approach of Perissinotto et al. (2000) was used to estimate the contribution of the "autotrophic carbon component" to the diet of *Tarebia granifera*, which is referred to in this study as the carbon component estimated from the gut fluorescence technique (GFT). Individuals of shell height ≈ 11 mm were collected both at Lake Nhlange 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 GF/F glass fiber filters and dried in an oven at 60 °C. Total carbon analyses were done with a Carlo Erba CHN-Analyser at the CSIR division of Natural Resources and the Environment in Stellenbosch, South Africa. Triplicates 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 (Beers, 1976; Perissinotto et al., 2000). Weight measurements were done with a Shimadzu balance with resolution at the 0.01 mg level. The carbon component estimated from GFT was estimated from data collected *in situ*, as previously outlined (10 replicates per study area). A pigment: carbon ratio of 50 was used (De Jonge, 1980). The difference in terms of the carbon component estimated from GFT between *T. granifera* populations at Lake Nhlange 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 MPB biomass data were tested with a Spearman's rank correlation. All data analyses were done with the statistical program SPSS - 15.

The daily carbon ration 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 GFT. To determine the average total carbon of *Tarebia granifera*, a triplicate of 10 pooled individuals from Lake Nhlange was analysed for total carbon. The average carbon component estimated from GFT over 24 h at Lake Nhlange was used.

3. Results

3.1. *Gut pigment content, evacuation rates and pigment assimilation/destruction efficiency*

There were no significant day/night differences in *Tarebia granifera*'s gut pigment measured at Lake Nhlange ($F_{8,44} = 0.822$, $P > 0.05$; Tukey's HSD, $P > 0.05$) (Fig. 2a). Similar results were obtained for snails kept in the laboratory ($F_{8,44} = 1.002$, $P > 0.05$; Tukey's HSD, $P > 0.05$) (Fig. 2b). *T. granifera*'s gut pigment concentrations were significantly different between sites ($F_{3,35} = 17.790$, $P < 0.05$; Tukey's HSD, $P < 0.05$). The highest gut pigment concentration (mean ± SD) in individuals of shell height 10–12 mm was measured at the St. Lucia Estuary in freshwater conditions, at 1454 ± 161 ng pigm. ind^{-1}, whereas the lowest was measured at Lake Nhlange, at 323 ± 129 ng pigm. ind. ^{1}. Gut pigment levels (mean ± SD) at Lake Sibaya and St. Lucia Estuary in brackish conditions were 700 ± 85 and 617 ± 334 ng pigm. ind.$^{-1}$, respectively. Gut pigments and MPB biomass were correlated across all sampled areas (Spearman's rank coefficient = 0.76, $P < 0.05$).

An exponential model provided the best fit for the gut evacuation rate of *Tarebia granifera* in all experiments (R^2 range: 16.2–35.2, $P < 0.05$) (Fig. 3). The highest gut evacuation rates (k) were estimated at Lake Nhlange and Lake Sibaya: 0.62 and 0.47 h ^{1}, respectively (Table 1). In the St. Lucia Estuary, the estimated gut evacuation rates (k) were lower: 0.37 and 0.36 h ^{1} (Table 1). *T. granifera*'s gut pigment assimilation/destruction efficiency (b^1) ranged from 27 to 55%. In Lake Nhlange, it was estimated (mean ± SD) to be 32 ± 5%, in Lake Sibaya 36 ± 7% and in the St. Lucia Estuary 41 ± 11%.

3.2. *Estimation of ingestion rates and feeding impact*

Tarebia granifera's ingestion rate ranged from 6.6 to 30.4 µg pigm. ind.$^{-1}$ d^{-1} (Table 2). There was a significant difference in terms of ingestion rate between sampling sites (ANOVA, $F_{3,11} = 17.519$, $P < 0.05$). Ingestion rate was significantly higher at Catalina Bay in 2009 compared with other sites (Tukey's HSD, $P < 0.05$). *T. granifera* of shell height 10–12 mm contributed up to 25% of the total *T. granifera* density over all sampled areas, making this size class the second most prominent after the class of shell

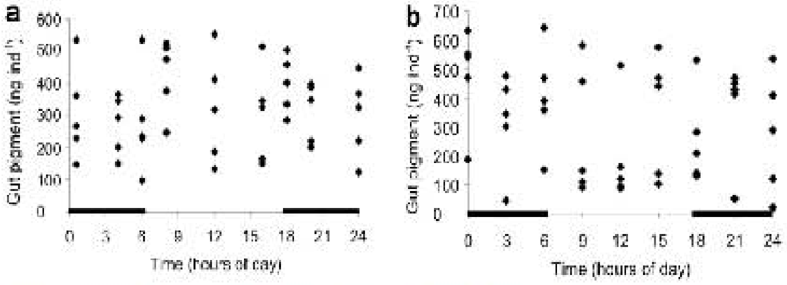


Fig. 2. Gut pigment content per *Tarebia granifera* individual (shell height: 10–12 mm): (a) in Lake Nhlange in summer 2009, water temperature (mean ± SD) was 19.6 ± 0.7 °C; (b) snails collected from the St. Lucia Estuary in summer 2009 and aclimated to laboratory conditions for three weeks, fed *ad libitum* with naturally occuring microphytobenthos, while water temperature was kept at 20 °C. Period of darkness is indicated by thicker lines on the horizontal axis.

height 1–8 mm, which contributed up to 75%. Densities were variable across all sampled areas due to *T. granifera*'s patchy distribution. Snails were not found in some parts of the sampled area whereas in other parts densities were as high as 3000 ind. m^{-2}. The highest and lowest extremes in density were recorded at Catalina Bay (Table 2). The lowest being in 2009 at a relatively isolated freshwater seepage zone after most of the population had died in November 2007, due to high salinities brought about by a mouth opening event in March 2007. By February 2010 the population had increased in numbers and spread across the entire freshwater seepage zone. The feeding impact of *T. granifera* was estimated to range from 0.2 to 11.1 mg pigm. m^{-2} d^{-1} (Table 2). Feeding impact was not significantly different between sampling sites (ANOVA, $F_{3,11} = 2.620$, $P > 0.05$; Tukey's HSD, $P > 0.05$). Total available MPB biomass ranged from 11.6 to 110.5 mg pigm. m^{-2} over all sampled areas (Table 2). The highest MPB biomass values were measured at the St. Lucia Estuary and the lowest at Lake Nhlange (Table 2). *T. granifera* was estimated to consume from 0.5 to 35% of the total available MPB biomass per day (Table 2).

3.3. *Gut organic content, carbon budget and daily ration*

The dry weight of *Tarebia granifera* gut contents ranged from 0.04 to 0.13 mg. The percentage contribution of organic material to the total gut contents ranged from 40 to 85.7% and averaged (mean ± SD) 66.4 ± 12.7%. The total gut carbon content ranged from 6.6 to 37.97 µg C ind.$^{-1}$, averaging (mean ± SD) 20.68 ± 9.54 µg C ind.$^{-1}$ at Lake Nhlange and 22.05 ± 8.42 µg C ind.$^{-1}$ at the St. Lucia Estuary. The carbon component which was estimated with the gut fluorescence technique contributed 8.7–40.9% of the total organic carbon in the gut, averaging (mean ± SD) 15.5 ± 7.2% at Lake Nhlange 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 Nhlange and St. Lucia Estuary ($t = 2.704$, $P < 0.05$). The carbon daily ration estimated from the gut fluorescence technique for *T. granifera* at Lake Nhlange was estimated to be ≈16% body carbon per day. A single individual had an average (mean ± SD) dry weight of 1.36 ± 0.06 mg, 40 ± 7.37% of which was carbon.

4. Discussion

This study is the first detailed account of *Tarebia granifera*'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 easily 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

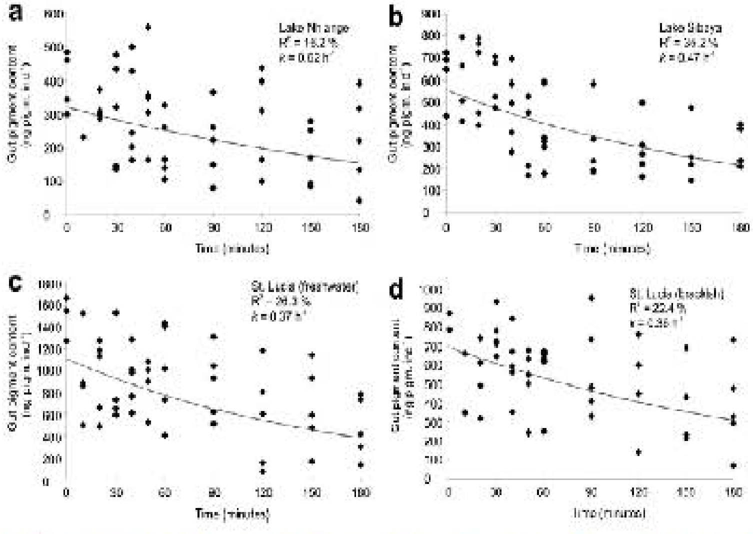


Fig. 3. Gut evacuation rates (k, h^{-1}) of *Tarebia granifera* (shell height: 10–12 mm): (a) in Lake Nhlange (November 2008); (b) in Lake Sibaya (November 2009); (c) in the St. Lucia Estuary (December 2009), in freshwater conditions; (d) in the St. Lucia Estuary (August 2007), in brackish conditions (salinity = 16).

Table 1

Estimated gut evacuation rate (*k*) of *Tarebia granifera* (shell height: 10–12 mm) in Lake Nhlange, Lake Sibaya and the St. Lucia Estuary. Temperature is expressed as mean ± SD, *G* is the gut pigment content, R^2 the adjusted coefficient of determination and *n* the number of replicates.

Area	Date	Temperature (°C)	Salinity	G (ng pigm. ind^{-1})	k (h^{-1})	R^2	n
Kosi lakes: Lake Nhlange	24 Nov 08	26 ± 1.4	Freshwater	323	0.62	16.2%	5
Lake Sibaya: South East Basin	18 Nov 09	19.6 ± 0.8	Freshwater	700	0.47	15.2%	5
St. Lucia Estuary: Catalina Bay	1 Dec 09	26.5 ± 1.2	Freshwater	1417	0.37	26.3%	5
St. Lucia Estuary: Catalina Bay (brackish)	19 Aug 07	20.9 ± 0.5	16	604	0.36	22.4%	5

to reconcile the results presented with some observations and hypotheses, in order to contribute to the knowledge of this non-indigenous invasive species.

4.1. Feeding dynamics at different sites and ecological significance

Tarebia granifera's highest gut pigment content was measured at the St. Lucia Estuary in freshwater conditions and low snail densities. At that time, *T. granifera* was recovering from a population crash due to high salinities (Miranda et al., 2010). 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 2). The high ingestion rate was probably related to high availability of food and low grazing pressure under low population density (Blancharda et al., 2000), but the low evacuation rate meant an increase in gut passage/turnover time. Changes in gut turnover time are related to nutritional quality of available food and metabolic energy requirements (Calow, 1977). Perhaps *T. granifera*'s slower gut turnover was part of a strategy to increase assimilation in order to meet its energy requirements for reproduction (Tamburi and Martin, 2008). The highest MPB biomass was also measured at the St. Lucia Estuary (Table 2). The lakes of the Kosi system (including Lake Nhlange) and Lake Sibaya have been reported to have low availability of nutrients and hence lower productivity, when compared to the St. Lucia Estuary (Allanson, 1979; Begg, 1980; Bruton, 1980; Adams and Bate, 1999). Assuming that all of the pigments detected in the guts of *T. granifera* are of microphytobenthic origin, they contribute a significantly greater portion to the total gut organic carbon in the St. Lucia Estuary, compared to Lake Nhlange (Fig. 4). The correlation between gut and food source (MPB in this case) pigment concentrations has also been widely reported for krill (Antezana et al., 1982; Ichii et al., 1998; Perissinotto et al., 2000). The highest gut evacuation rates were obtained in the more stable freshwater lakes (Table 1). Interestingly, the gut evacuation rate under brackish conditions was relatively high (salinity 16, Table 1). Although an increase in salinity is expected to decrease *T. granifera*'s feeding impact (Lauringson et al., 2007), Miranda et al. (2010) showed that this species can maintain a relatively high level of physical/metabolic activity at salinities up to 20. This goes some way to explain *T. granifera*'s establishment success in the brackish waters of estuaries.

Tarebia granifera's feeding impact can be much higher than that of other invertebrate species. It has been reported to favor MPB in its diet and significantly reduce algal standing stocks (March and Pringle, 2003). The ingestion rate of *T. granifera* can be 20 times higher than that of *Hydrobia ulvae*, which feeds primarily on MPB and influences its biomass dynamics in the intertidal mudflats of Western Europe (Blancharda et al., 2000; Haubois et al., 2005). In this study, the estimated feeding impact of *T. granifera* on MPB was relatively high, reaching values of up to 35% per day of the available biomass. According to the literature, daily primary benthic production can range from 72 to 816 mg C m^{-2} d^{-1} in Maputaland lakes (Allanson, 1979; Begg, 1980; Bruton, 1980). Given this, *T. granifera* is estimated to ingest 1.2–68% of the daily primary benthic production. Similar values have been reported for zooplankton species, which are known to affect pelagic ecosystem functioning (Perissinotto, 1992; Kibirige and Perissinotto, 2003). These results are also comparable to those obtained by Hall et al. (2003) for the freshwater non-indigenous invasive snail *Potamopyrgus antipodarum*, which was shown to have marked ecosystem-scale impacts. If *T. granifera*'s ingestion rate is converted to grams of MPB per gram of snail (dry weight) per day (g/g/d), the resulting range of 0.243–1.103 g/g/d is comparable to that of *Pomacea canaliculata*, which is listed as one of the world's worst non-indigenous invasive species (Lowe et al., 2000; Baker et al., 2010). This study revealed no significant differences in feeding impact between sites. Therefore, feeding impact can be considerable at all invaded sites, justifying concerns over the top-down ecological effects of *T. granifera* (Flint and Goldman, 1975; Hillebrand et al., 2002). Feeding impact is directly proportional to density, thus it may be severe where snail densities increase over 1000 ind. m^{-2}. The distribution of *T. granifera* was patchy despite seemingly favorable environmental conditions. Although this is common in benthic invertebrates (Covich et al., 1999), the implication is that feeding on MPB becomes focused on certain areas. In dense snail patches the feeding impact can potentially result in the depletion of available MPB. There is however an interaction between aquatic grazers and nutrients which was not explored in this study (Burkepile and Hay, 2006). The composition of MPB was also not taken into account. *T. granifera*, although suspected to be a generalist consumer, may select certain algal groups over others and thus change the relative abundance and biomass of the key algal groups that make up MPB (Armitage et al., 2009).

Trophic impact estimates are expected to be lower in benthic communities when compared to pelagic (Decho, 1988; Covich et al., 1999). This is because benthic communities tend to be more diverse and supported by resource partitioning. However, *Tarebia granifera* is a new addition to the ecosystem and its grazing activity may therefore interfere with its natural trophic dynamics. Another mechanism by which this snail may influence community structure is bioturbation or sediment disturbance (Pillay et al., 2007). Bioturbation in benthic gastropods can significantly contribute to

Table 2

Estimated feeding impact of *Tarebia granifera* (shell height: 10–12 mm) in Lake Nhlange, Lake Sibaya and the St. Lucia Estuary. Density and microphytobenthos (MPB) concentration available are expressed as mean ± SD. Ingestion rate, feeding impact and % consumption of MPB per day are expressed as a range.

Area	Density (ind m^{-2})	Ingestion rate (µg pigm. ind^{-1} d^{-1})	Feeding impact (mg pigm. m^{-2} d^{-1})	MPB concentration (mg pigm. m^{-2})	% Consumption of MPB per day
Kosi lakes: Lake Nhlange	282 ± 66	6.6–7.6	1.4–2.6	37.3 ± 25.7	2.2–27.4
Lake Sibaya: South East Basin	199 ± 90	11.2–13.9	1.2–4	27.3 ± 5.8	3.6–35
St. Lucia Estuary: Catalina Bay	16 ± 6	18.7–30.4	0.2–6.7	37.92 ± 1.6	0.5–1.8
St. Lucia Estuary: Catalina Bay (brackish)	748 ± 211	7.1–11.6	3.8–11.1	90.1 ± 20.4	3.6–23.9

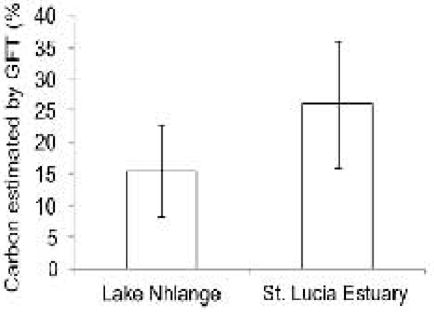


Fig. 4. Percentage contribution (mean + SD) of carbon estimated from the gut fluorescence technique (GFT) to the total gut organic carbon content of *Tarebia granifera* at Lake Nhlange and the St. Lucia Estuary.

sediment erodibility and MPB resuspension (Andersen, 2001; Orvain et al., 2004).The effects of this invader are not straightforward: there may be direct and indirect cascading effects on the ecosystem which ultimately affect community structure (Levington and Stewart, 1982; Hunt et al., 1987; Anderson, 1999; Pillay et al., 2009). For example, and in addition to affecting MPB, *T. granifera* may be affecting the density of other macrofauna as well as their larval settlement and recruitment (Jensen and Siegismund, 1980; Hunt et al., 1987; Morrisey, 1988; Kelcher et al., 2003). Indeed, *T. granifera* has been reported to displace indigenous gastropods, thus taking over their niches (Perera et al., 1990; Pointier, 1999).

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

Tarebia 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). Its gut turnover rate ($1/k$) varied from 1.6 to 2.8 h in actively feeding individuals. Although an exponential model was fitted to the gut evacuation data, the coefficients of determination (R^2) were relatively low when compared to those obtained in similar studies using pelagic crustaceans (Froneman, 2000; Kibirige and Perissinotto, 2003). There were individuals in all experiments that had ingestion rates as low as 0.12 μg pigm. ind.$^{-1}$ d^{-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 dry 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 *Tarebia granifera* can largely explain the wide variability in the data. Individual feeding variability is commonly reported for molluscs (Speiser, 2001; Hanley et al., 2003; Pardo and Johnson, 2004). It is evident that not all the individuals in the population are feeding at the same rate. Feeding rate is influenced by temperature and salinity, among other factors (Dam and Peterson, 1988; Jacobsen and Forbes, 1997). 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 where they were less abundant, compared to those found in densely populated patches (Brown et al., 1994; Baker et al., 2010). The mechanism that triggers *T. granifera*'s quiescent state is not understood but it is probably chemically mediated (Croll, 1983). Since populations often reach high densities, quiescence/activity cycles may be a strategy adopted in order to maximize resource use while maintaining high densities. Daily alternation between cycles of feeding and quiescence has been reported for *P. canaliculata* (Wada and Yoshida, 2000).

The carbon component which was estimated from GFT contributed a smaller proportion of the total organic carbon in the gut than expected (Fig. 4). However, the carbon daily ration calculated from carbon estimated from GFT is relatively high (≈16%) according to the literature (Perissinotto, 1992; Froneman, 2000). This suggests that MPB alone can meet the energetic requirements of *Tarebia granifera*. This is supported by the survival and reproduction of *T. granifera* snails kept in the laboratory and fed *ad libitum* with naturally occurring MPB for a period of over a year (pers. obs.). *T. granifera*'s variable feeding rate may indeed be related to energetic demands, one of which may be especially taxing, i.e. its reproductive energy demand (Tamburi and Martin, 2008). *T. granifera* is parthenogenetic and ovoviviparous. It has a brood pouch and gives birth to live juveniles of shell height ≈ 1 mm. This species has an extremely high reproductive rate, with some authors reporting birth rates as high as one juvenile every 12 h (Abbott, 1952; Chaniotis et al., 1980a; Appleton et al., 2009). This study focused on adult, sexually mature snails, with a shell height of 10–12 mm. All of the snails used in experiments were female and had juveniles in their brood pouch (Chaniotis et al., 1980b). It is worth keeping in mind that the size class of shell height 1–8 mm was much more abundant and therefore adds a measure of impact which was not accounted for in this study. Alarmingly, studies on other invasive freshwater snails, such as *Pomacea* spp. show that the impact of smaller/juvenile snails on food resources may be even more severe than that of the adults (Boland et al., 2008; Tamburi and Martin, 2008).

4.3. Potential sources of error

Tarebia granifera's gut pigment assimilation/destruction efficiencies (b^1) 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 however some controversy in the literature as to whether the b^1 correction should be used (Durbin and Campbell, 2007). The feeding impact in this study could thus have been overestimated by a factor of between 1.4 and 2.2. Despite this, even if the b^1 correction is not used, all the main conclusions of this study remain valid.

There are two potential sources of error in the carbon budget technique. One is the chlorophyll *a* to carbon ratio, which in this study was assumed to be 50. This ratio is however variable and should be measured directly from the microalgae assemblages (De Jonge, 1980; Faure et al., 2006). Another is that the types of measurements required for this method cannot be repeated with the same individual (Perissinotto et al., 2000).

An attempt was made to exclude quiescent snails from experiments, as they would otherwise introduce very high or 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 when compared to snails that fed continuously on cornstarch. The duration of the cycles of quiescence is not known, but it is likely that over time, the pigments in the gut may also be degraded to non-fluorescent end products, which would not be detected with a fluorometer. Actively feeding snails were selected in an attempt to avoid variation in the data and in view of maintaining continuous feeding conditions (Baars and Helling, 1985; Kiørboe and Tiselius, 1987; Perissinotto, 1992). It is however possible that snails were disturbed during collection and then mistaken for active snails as they came out of quiescence. Selecting these snails for gut evacuation measurements would result in low gut pigment values.

Finally, the origin of the proportions 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 microphytobenthic origin. So, what about the remaining component that was not estimated from the GFT? There may be a heterotrophic component to the diet, as the snail may feed on some benthic micro-organisms or even decaying animal matter (Chaniotis et al., 1980a). However, it is also possible that *Tarebia granifera* 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 photosynthetic pigments are degraded to non-fluorescent end products (Louda et al., 1998; 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 at which stage of degradation the plant detritus was when it was consumed (Bianchi et al., 1988; Louda et al., 1998). It is also suggested that *T. granifera* is not dependent on MPB for food and may therefore feed solely on detritus if MPB 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; Giménez et al., 2006). 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 larger scale impacts (Ross et al., 2003). This study presents one such field experiment as an alternative to other more classical approaches (Cowan and Peckarsky, 1990). Results show that *Tarebia granifera* can utilize large amounts of microphytobenthos in addition to detritus and can potentially cause significant top-down and cascading effects on the ecosystem. A combination of the techniques reported in this study with others, such as the gut dry mass technique (Cristo, 2001 and references therein), is needed to validate these results (Clozier et al., 2000). Simultaneous studies on stable isotopes and community structure and dynamics would provide much needed information on *T. granifera*'s impact on estuarine and freshwater ecosystems.

Acknowledgements

We are grateful to the iSimangaliso Park Authority and Ezemvelo KZN Wildlife for supporting this project. Special thanks go to R. Taylor, C. Fox and R. Kyle for their invaluable assistance and logistical support. Funding was provided by the National Research Foundation (NRF), South Africa; Department of Environmental Affairs and Tourism – Marine and Coastal Management (DEAT-MCM); and the World Wildlife Fund (WWF).

References

Abbott, R.T., 1952. A study of an intermediate snail host (*Thiara granifera*) of the oriental lung fluke (*Paragonimus*). Proceedings of the United States National Museum 102, 72–116.

Adams, J., Bate, G., 1999. Primary producers: estuarine microalgae. In: Allanson, B.R., Baird, D. (Eds.), Estuaries of South Africa. Cambridge University Press, New York, pp. 91–100.

Allanson, B.R., 1979. The phytoplankton and primary productivity of the lake. In: Allanson, B.R. (Ed.), Lake Sibaya. Dr. W. Junk Publishers, Hague, pp. 75–87.

Andersen, T.J., 2001. Seasonal variation in erodibility of two temperate, microtidal mudflats. Estuarine, Coastal and Shelf Science 53, 1–12.

Anderson, M.J., 1999. Distinguishing direct from indirect effects of grazers in intertidal estuarine assemblages. Journal of Experimental Marine Biology and Ecology 234, 199–218.

Antezana, T., Ray, K., Melo, C., 1982. Trophic behaviour of *Euphausia superba* Dana in laboratory conditions. Polar Biology 1, 77–82.

Appleton, C.C., Forbes, A.T., Demetriades, N.T., 2009. The occurrence, bionomics and potential impacts of the invasive freshwater snail *Tarebia granifera* (Lamarck, 1822) (Gastropoda: Thiaridae) in South Africa. Zoologische Mededelingen Leiden 83, 525–536.

Armitage, A.R., Gonzalez, V.L., Fong, P., 2009. Decoupling of nutrient and grazer impacts on a benthic estuarine diatom assemblage. Estuarine, Coastal and Shelf Science 84, 375–382.

Baars, M.A., Helling, G.R., 1985. Methodical problems in the emasurements of phytoplankton ingestion rate by gut fluorescence. Hydrological Bulletin 19, 81–88.

Baker, P., Zimmanck, F., Baker, S.M., 2010. Feeding rates of an introduced freshwater gastropod *Pomacea insularum* on native and nonindigenous aquatic plants. Journal of Molluscan Studies 76, 138–143.

Barnes, M.A., Fordham, R.K., Burks, R.L., Hand, J.J., 2009. Fecundity of the exotic applesnail, *Pomacea insularum*. Journal of the North American Benthological Society 27, 738–745.

Beers, J.R., 1976. Determination of zooplankton biomass. In: Steedman, H.F. (Ed.), Zooplankton Fixation and Preservation. UNESCO Press, Paris, pp. 37–84.

Begg, G.W., 1980. The Kosi system: aspects of its biology, management and research. In: Bruton, M.N., Cooper, K.H. (Eds.), Studies on the Ecology of Maputaland. Rhodes University, Grahamstown.

Bianchi, T.S., Dawson, R., Sawangwong, P., 1988. The effects of macrobenthic deposit-feeding on the degradation of chloropigments in sandy sediments. Journal of Experimental Marine Biology and Ecology 122, 243–255.

Blancharda, G.F., Guarini, J., Provot, L., Richard, P., Sauriau, P., 2000. Measurement of ingestion rate of *Hydrobia ulvae* (Pennant) on intertidal epipelic microalgae: the effect of mud snail density. Journal of Experimental Marine Biology and Ecology 255, 247–260.

Boland, B.B., Meerhoff, M., Fosalba, C., Mazzeo, N., Barnes, M.A., Burks, R.L., 2008. Juvenile snails, adult appetites: contrasting resource consumption between two species of applesnails (*Pomacea*). Journal of Molluscan Studies 74, 47–54.

Brown, K.M., Carman, K.R., Inchausty, V., 1994. Density-dependent influences on feeding and metabolism in a freshwater snail. Oecologia 99, 158–165.

Bruton, M.N., 1980. An outline of the ecology of Lake Sibaya, with emphasis on the vertebrate community. In: Bruton, M.N., Cooper, K.H. (Eds.), Studies on the Ecology of Maputaland. Rhodes University, Grahamstown.

Buckley, Y.M., 2008. The role of research for integrated management of invasive species, invaded landscapes and communities. Journal of Applied Ecology 45, 397–402.

Burkepile, D.E., Hay, M.E., 2006. Herbivore vs. nutrient control of marine primary producers: context-dependent effects. Ecology 87, 3128–3139.

Calow, P., 1977. Ecology, evolution and energetics: a study in metabolic adaptation. In: Macfadyen, A. (Ed.), Advances in Ecological Research. Academic Press, pp. 1–62.

Canete, R., Yong, M., Sanchez, J., Wong, L., Gutierrez, A., 2004. Population dynamics of intermediate snail hosts of *Fasciola hepatica* and some environmental factors in San Juan y Martinez municipality, Cuba. Memorias do Instituto Oswaldo Cruz 99, 257–262.

Chaniotis, B.N., Butler, J.M., Ferguson, F., Jobin, W.R., 1980a. Bionomics of *Tarebia granifera* (Gastropoda:Thiaridae) in Puerto Rico, an Asian vector of *Paragonimiasis westermani*. Caribbean Journal of Science 16, 81–89.

Chaniotis, B.N., Butler, J.M., Ferguson, F., Jobin, W.R., 1980b. Presence of males in Puerto Rican *Thiara (Tarebia) granifera* (Gastropoda: Thiaridae), a snail thought to be parthenogenetic. Caribbean Journal of Science 16, 95–97.

Conover, R.J., Durvasula, R., Roy, S., Wang, R., 1986. Probable loss of chlorophyll-derived pigments during the passage through the gut of zooplankton and some of the consequences. Limnology and Oceanography 31, 878–887.

Covich, A.P., Palmer, M.A., Crowl, T.A., 1999. The role of benthic invertebrate species in freshwater ecosystems. BioScience 49, 119–127.

Cowan, C.A., Peckarsky, B.L., 1990. Feeding by a lotic mayfly grazer as quantified by gut fluorescence. Journal of the North American Benthological Society 9, 368–378.

Cristo, M., 2001. Gut evacuation rates in *Nephrops norvegicus* (L., 1758): laboratory and field estimates. Scientia Marina 65, 341–346.

Croll, R.P., 1983. Gastropod chemoreception. Biological Reviews 58, 293–319.

Cuny, P., Marty, J., Chiavérini, J., Vescovali, I., Raphel, D., Rontani, J., 2002. One-year seasonal survey of the chlorophyll photodegradation process in the north-western Mediterranean Sea. Deep-Sea Research 49, 1987–2005.

Dagg, M.J., 1983. A method for determination of copepod feeding rates during short intervals. Marine Biology 75, 63–67.

Dam, H.G., Peterson, W.T., 1988. The effect of temperature on the gut clearance rate constant on planktonic copepods. Journal of Experimental Marine Biology and Ecology 123, 1–14.

Davis, M.A., 2009. Invasion Biology. Oxford University Press, New York.

De Jonge, V.N., 1980. Fluctuations in the organic carbon to chlorophyll *a* ratios for estuarine benthic diatom populations. Marine Ecology - Progress Series 2, 345–353.

Decho, A.W., 1988. How do harpacticoid grazing rates differ over a tidal cycle? Field verification using chlorophyll-pigment analyses. Marine Ecology - Progress Series 45, 263–270.

Durbin, E.G., Campbell, R.G., 2007. Reassessment of the gut pigment method for estimating *in situ* zooplankton ingestion. Marine Ecology - Progress Series 331, 305–307.

N.A.F. Miranda et al. / Estuarine, Coastal and Shelf Science 91 (2011) 442–449 449

Faure, V., Pinazo, C., Torréton, J., Douillet, P., 2006. Relevance of various formulations of phytoplankton chlorophyll a:carbon ratio in a 3D marine ecosystem model. Comptes Rendus Biologies 329, 813–822.

Flint, R.W., Goldman, C.R., 1975. The effects of a benthic grazer on the primary productivity of the littoral zone of Lake Tahoe. Limnology and Oceanography 20, 935–944.

Froneman, P.W., 2000. Feeding studies on selected zooplankton in a temperate estuary, South Africa. Estuarine. Coastal and Shelf Science 51, 543–552.

Glozier. N.E.. Culp, J.M.. Scrimgeour, G.J., Halliwell, D.B., 2000. Comparison of gut fluorescence and gut dry mass techniques for determining feeding periodicity in lotic mayflies. Journal of the North American Benthological Society 19, 169–175.

Giménez, L., Dimitriadis, C., Carranza, A., Borthagaray, A.I., Rodríguez, M., 2006. Unravelling the complex structure of a benthic community: a multiscale-multianalytical approach to an estuarine sandflat. Estuarine, Coastal and Shelf Science 68, 462–472.

Hall, R.O., Dybdahl, M.F., Vanderloop, M.C., 2006. Extremely high secondary production of introduced snails in rivers. Ecological Applications 16, 1121–1131.

Hall, R.O., Tank, J.L.. Dybdahl, M.F.. 2003. Exotic snails dominate nitrogen and carbon cycling in a highly productive stream. Frontiers in Ecology and the Environment 1, 407–411.

Hanley, M.E.. Bulling, M.T.. Fenner. M., 2003. Quantifying individual feeding variability: implications for mollusc feeding experiments. Functional Ecology 17, 673–679.

Haubois. A.G., Guarini. J.M.. Richard. P.. Fichet, D.. Radenac, G.. Blanchard, G.F., 2005. Ingestion rate of the deposit-feeder *Hydrobia ulvae* (Gastropoda) on epipelic diatoms: effect of cell size and algal biomass. Journal of Experimental Marine Biology and Ecology 317, 1–12.

Hillebrand, H., Kahlert, M., Haglund, A., Berninger, U.G., Nagel, S., Wickham, S., 2002. Control of microbenthic communities by grazing and nutrient supply. Ecology 83, 2205–2219.

Hunt, J.H., Ambrose Jr., W.G., Peterson, C.H., 1987. Effects of the gastropod, *Ilyanassa obsoleta* (Say), and the bivalve, *Mercenaria mercenaria* (L.) on larval settlement and juvenile recruitment of infauna. Journal of Experimental Marine Biology and Ecology 108, 229–240.

Ichii, T., Katayama, K., Obitsu, N., Ishii, H., Naganobu, M., 1998. Occurrence of antarctic krill (*Euphausia superba*) concentrations in the vicinity of the South Shetland Islands: relationship to environmental parameters. Deep-sea Research 45, 1235–1262.

Jacobsen, R., Forbes, V.E., 1997. Clonal variation in life-history traits and feeding rates in the gastropod, *Potamopyrgus antipodarum*: performance across a salinity gradient. Functional Ecology 11, 260–267.

Jensen, K.T., Siegismund, H.R., 1980. The importance of diatoms and bacteria in the diet of *Hydrobia* species. Ophelia 17, 193–199.

Kelaher, B.P., Levinton, J.S., Hoch, J.M., 2003. Foraging by the mudsnail, *Ilyanassa obsoleta* (Say), modulates spatial variation in benthic community structure. Journal of Experimental Marine Biology and Ecology 292, 139–157.

Kibirige, I., Perissinotto, R., 2003. *In situ* feeding rates and grazing impact of zooplankton in a South African temporarily open estuary. Marine Biology 142, 357–367.

Kiørboe, T., Møhlenberg, F., Riisgård, H.U., 1985. *In situ* feeding rates of planktonic copepods: a comparison of four methods. Journal of Experimental Marine Biology and Ecology 88, 67–81.

Kiørboe, T., Tiselius, P., 1987. Gut clearance and pigment destruction in a herbivorous copepod, *Acartia tonsa*, and the determination of in situ grazing rates. Journal of Plankton Research 9, 592–534.

Larned, S.T., Chong, C.T., Punewai, N., 2001. Detrital fruit processing in a Hawaiian stream ecosystem. Biotropica 33, 241–248.

Lauringson, V., Mälton, E., Kotta, J., Kangur, K., Orav-Kotta, H., Kotta, I., 2007. Environmental factors influencing the biodeposition of the suspension feeding bivalve *Dreissena polymorpha* (Pallas): comparison of brackish and freshwater populations. Estuarine, Coastal and Shelf Science 75, 459–467.

Levington, J.S., Stewart, S., 1982. Marine succession: the effect of two deposit-feeding gastropod species on the population growth of *Paranais litoralis* Muller 1784 (Oligochaeta). Journal of Experimental Marine Biology and Ecology 59, 231–241.

Lockwood, J.L., Hoopes, M.F., Marchetti, M.P., 2007. Invasion Ecology. Blackwell Publishing, Malden, USA.

Lopez, M.D.G., Huntley, M.E., Sykes, P.F., 1988. Pigment destruction by *Calanus pacificus*: impact on the estimation of water column fluxes. Journal of Plankton Research 10, 715–734.

Louda, J.W., Li, J., Liu, L., Winfree, N., Baker, E.W., 1998. Chlorophyll-a degradation during cellular senescence and death. Organic Geochemistry 29, 1233–1251.

Lowe, S., Browne, M., Boudjelas, S., De Poorter, M., 2000. 100 of the world's worst invasive alien species. A selection from the global invasive species database. The invasive species specialist group. Hollands Printing Ltd., New Zealand.

Mackas, D., Bohrer, R., 1976. Fluorescence analysis of zooplankton gut contents and an investigation of diel feeding patterns. Journal of Experimental Marine Biology and Ecology 25, 77–85.

MacKay, F., Cyrus, D., Russell, K.L., 2010. Macrobenthic invertebrate responses to prolonged drought in South Africa's largest estuarine lake complex. Estuarine. Coastal and Shelf Science 86, 553–567.

March, J.G., Pringle, C.M., 2003. Food web structure and basal resource utilization along a tropical island stream continuum, Puerto Rico. Biotropica 35, 84–93.

Mayzaud, P., Razouls, S., 1992. Degradation of gut pigment during feeding of a subantarctic copepod: importance of feeding history and digestive acclimation. Limnology and Oceanography 37, 393–404.

Miranda, N.A.F., Perissinotto, R., Appleton, C.C., 2010. Salinity and temperature tolerance of the invasive freshwater gastropod *Tarebia granifera*. South African Journal of Science 106, 55–61.

Morrisey, D.Y., 1988. Differences in effects of grazing by deposit-feeders *Hydrobia ulvae* (Pennant) (Gastropoda: Prosobranchia) and *Corophium arenarium* (Crawford) (Amphipoda) on sediment microalgal populations. II. Quantitative effects. Journal of Experimental Marine Biology and Ecology 118, 43–53.

Orvain, F., Sauriau, P.G., Sygut, A., Jossard, L., Le Hir, P., 2004. Interacting effects of *Hydrobia ulvae* bioturbation and microphytobenthos on the erodability of mudflat sediments. Marine Ecology - Progress Series 278, 205–223.

Pardo, L.M., Johnson, L.E., 2004. Activity and shelter use of an intertidal snail: effects of sex, reproductive condition and tidal cycle. Journal of Experimental Marine Biology and Ecology 301, 175–191.

Pasternak, A.F., 1994. Gut fluorescence in herbivorous copepods - an attempt to justify the method. Hydrobiologia 293, 241–248.

Perera, G., Yong, M., Ferrer, J.R., Arrinda, C., Amador, O., 1990. Effectiveness of three biological control agents against intermediate hosts of snail-mediated parasites in Cuba. Malacological Review 23, 47–52.

Perissinotto, R., 1992. Mesozooplankton size-selectivity and grazing impact on the phytoplankton community of the Prince Edward Archipelago (Southern Ocean). Marine Ecology - Progress Series 79, 243–258.

Perissinotto, R., Gurney, L., Pakhomov, E.A., 2000. Contribution of heterotrophic material to diet and energy budget of Antarctic krill, *Euphausia superba*. Marine Biology 136, 129–135.

Perissinotto, R., Pakhomov, E.A., 1996. Gut evacuation and pigment distruction in the Antarctic krill *Euphausida superba*. Marine Biology 125, 47–54.

Pillay, D., Branch, G.M., Forbes, A.T., 2007. Experimental evidence for the effects of the thalassinidean sandprawn *Callianassa kraussi* on macrobenthic communities. Marine Biology 152, 611–618.

Pillay, D., Branch, G.M., Steyn, A., 2009. Complex effects of the gastropod *Assiminea globulus* on benthic community structure in a marine-dominated lagoon. Journal of Experimental Marine Biology and Ecology 380, 47–52.

Pillay, D., Perissinotto, R., 2008. The benthic macrofauna of the St. Lucia Estuary during the 2005 drought year. Estuarine, Coastal and Shelf Science 77, 35–46.

Pimentel, D., Zuniga, R., Morrison, D., 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics 52, 273–288.

Pointier, J.P., Facon, B., Jarne, P., David, P., 2003. Thiarid snails, invading gastropods of tropical freshwater habitats. Xenophora 104, 14–20.

Pointier, J.-P., Samadi, S., Jarne, P., Delay, B., 1998. Introduction and spread of *Thiara granifera* (Lamarck, 1822) in Martinique, French West Indies. Biodiversity and Conservation 7, 1277–1290.

Pointier, J., 1999. Invading freshwater gastropods: some conflicting aspects for public health. Malacologia 41, 403–411.

Puth, L.M., Post, D.M., 2005. Studying invasion: have we missed the boat? Ecology Letters 8, 715–721.

Range, P., Chapman, M.G., Underwood, A.J., 2008. Field experiments with "cageless" methods to manipulate grazing gastropods on intertidal rocky shores. Journal of Experimental Marine Biology and Ecology 365, 23–30.

Riley, L.A., Dybdahl, M.F., Hall, R.O., 2008. Invasive species impact: asymmetric interactions between invasive and endemic freshwater snails. Journal of the North American Benthological Society 27, 509–520.

Ross, D.J., Johnson, C.R., Hewitt, C.L., 2003. Assessing the ecological impacts of an introduced seastar: the importance of multiple methods. Biological Invasions 5, 3–21.

Sousa, R., Nogueira, A.J.A., Gaspar, M.B., Antunes, C., Guilhermino, L., 2008. Growth and extremely high production of the non-indigenous invasive species *Corbicula fluminea* (Müller, 1774): possible implications for ecosystem functioning. Estuarine. Coastal and Shelf Science 80, 289–295.

Speiser, B., 2001. Food and feeding behaviour. In: Barker, G.M. (Ed.), The Biology of Terrestrial Molluscs. CABI, Wallingford, pp. 259–288.

Strickland, J.D.H., Parsons, T.R., 1968. A practical handbook for sewater analysis. Bulletin of the Fisheries Research Board of Canada 167, 1–311.

Tamburi, N.E., Martin, P.R., 2008. Feeding rates and food conversion efficiencies in the apple snail *Pomacea canaliculata* (Caenogastropoda: ampullariidae). Malacologia 51, 221–232.

Taylor, R., Adams, J.B., Haldorsen, S., 2006. Primary habitats of the St Lucia estuarine system, South Africa, and their responses to mouth management. African Journal of Aquatic Science 31, 31–41.

Taylor, R., Kelbe, B., Haldorsen, S., Botha, G.A., 2005. Ground-water dependent ecology of the shoreline of the sub-tropical Lake St. Lucia. Environmental Geology 49, 586–600.

Wada, T., Yoshida, K., 2000. Burrowing by the apple snail, *Pomacea canaliculata* (Lamarck): daily periodicity and factors affecting burrowing. Proceedings of the Association for Plant Protection of Kyushu 46, 88–93.

Welschmeyer, N.A., 1994. Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. Lymnology and Oceanography 39, 1985–1992.

OPEN ACCESS Freely available online

PLoS one

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 Perissinotto**

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 (*Cladophora* sp.), detritus and sedimentary organic matter. IDO was significant ($>60\%$) between *T. granifera* and each of the following gastropods: *Haminoea natalensis* (Krauss, 1848), *Bulinus natalensis* (Küster, 1841) and *Melanoides tuberculata* (Müller, 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. *ovata* (Krauss, 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.

Citation: Miranda NAF, Perissinotto R (2012) Stable Isotope Evidence for Dietary Overlap between Alien and Native Gastropods in Coastal Lakes of Northern KwaZulu-Natal, South Africa. PLoS ONE 7(2): e31897. doi:10.1371/journal.pone.0031897

Editor: Dirk Steinke, Biodiversity Insitute of Ontario - University of Guelph, Canada

Received September 8, 2011; **Accepted** January 16, 2012; **Published** February 21, 2012

Funding: Funding was provided by the National Research Foundation, South Africa, Marine and Coastal Management (DEAT-MCM) and the World Wide Fund for Nature (WWF). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

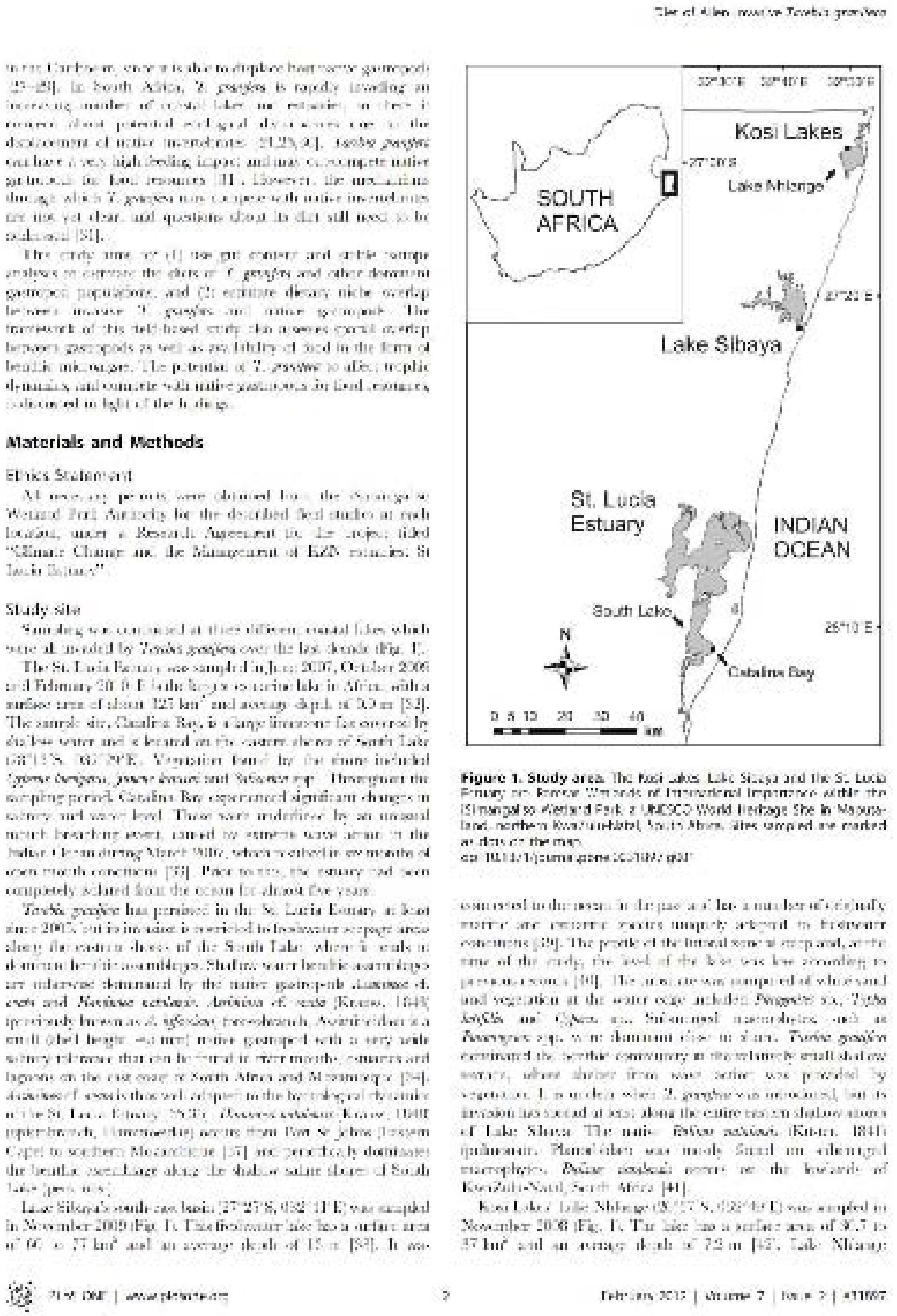
Competing Interests: The authors have declared that no competing interests exist.

* E-mail: 204507499@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, provide 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 [8–12]. Although comparable studies on gastropods are lacking, numerous studies have been published on invasive gastropods such as *Pomacea canaliculata* (Lamarck, 1822) (prosobranch, Ampullariidae) [13–15] and *Potamopyrgus antipodarum* (Gray, 1843) (prosobranch, Hydrobiidae) [16,17], some of which involved stable isotope techniques [18,19].

Tarebia granifera (Lamarck, 1822) (prosobranch, Thiaridae) 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]. *Tarebia granifera* was accidentally introduced in South Africa, probably in the 1990s 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 [24,25]. *Tarebia 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

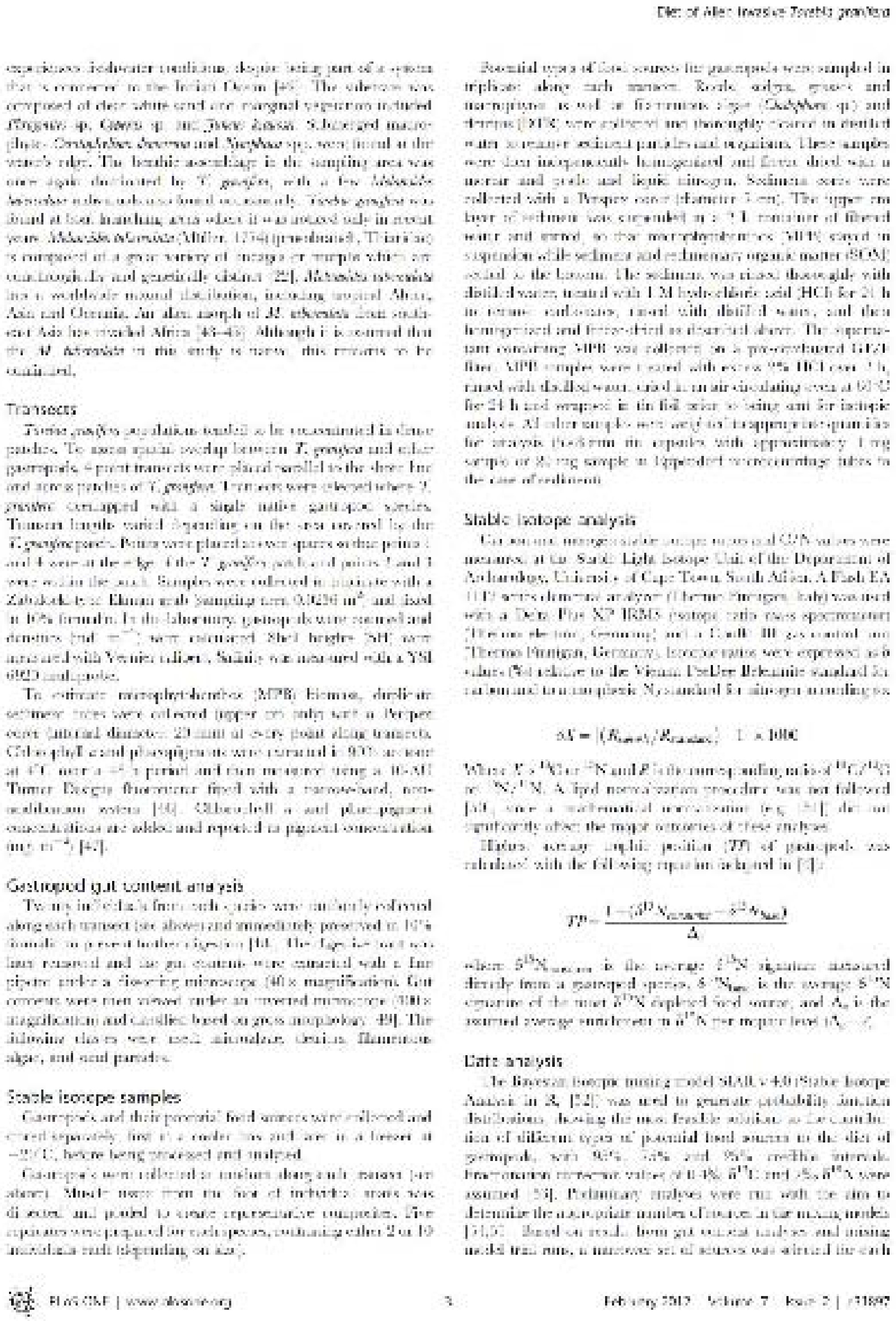


Materials and Methods

Ethics Statement

Study site

Figure 1. Study area. The Kosi Lakes, Lake Sibaya and the St. Lucia Estuary are Ramsar Wetlands of International Importance within the iSimangaliso Wetland Park, a UNESCO World Heritage Site in Maputaland, northern KwaZulu-Natal, South Africa. Sites sampled are marked as dots on the map.
doi:10.1371/journal.pone.0031897.g001



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]:

$$IDO_{jk} = \left[\sum_{1}^{n} \min p_{ij}\, p_{ik}\right] \times 100$$

where *p* is the SIAR mean contribution of source *i* resulting from the final mixing models run for subjects *j* and *k* respectively, and *n* 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 availability of food, gastropod abundance and diet. A t-test was used to compare trophic position between *T. 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.

Results

Spatial overlap and resource availability

Tarebia granifera exhibited a patchy distribution and tended to be concentrated in shallow water (depth <1 m). Transects revealed spatial overlap between *T. granifera* and *Haminoea natalensis* (Fig. 2A), *Assiminea* cf. *ovata* (Fig. 3A and 4A), *Bulinus natalensis* (Fig. 5A) and *Melanoides tuberculata* (Fig. 6A) at respective sampling sites in the three lakes of Maputaland (Fig. 1). *Tarebia granifera* was found together with native gastropods in a total of 10 of the 20 transect points sampled. Of the 10 transect points where these gastropods were found together, 7 were at the edge of the *T. granifera* patch (transect points 1 and 4). *Haminoea natalensis* was the only native gastropod not to be found in the middle (transect points 2 and 3) of a *T. granifera* patch.

Gastropod densities as well as available microphytobenthic (MPB) biomass varied within transects (Fig. 2A–6A; Table S1).Generally, native gastropods had lower population densities when compared to *T. granifera* (Table S1). However, *Assiminea* cf. *ovata* was found in densities greater than *T. granifera* at Catalina Bay in 2009 (Table S1). MPB biomass was available as a food source to gastropods at all sites, although its biomass was variable (Table S1). MPB biomass was not significantly correlated with gastropod densities (Table 1), despite being positively correlated with gastropod MPB diets (Table 1). Native gastropod MPB diet was strongly positively correlated with *T. granifera* MPB diet; however, it was negatively correlated with *T. granifera* density (Table 1).

Gastropod diets

Gut content analysis revealed that microalgae and detritus were ingested by all gastropods (Table 2). Filamentous algae were also found in the guts of most 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. natalensis*, *B. natalensis*, and in smaller amounts in *T. granifera* adults (shell height ≥10 mm) and *M. tuberculata* (Table 2). Distinctive remains of meiofauna were not found in gut contents. Macrophytes and fringing vegetation such as reeds, sedges 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

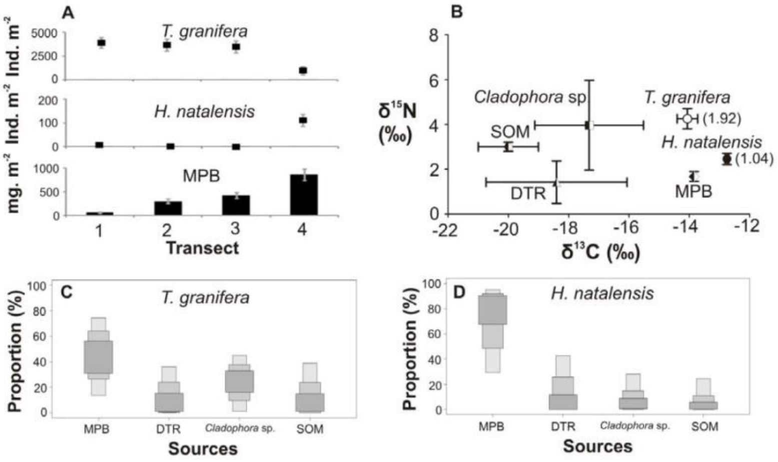


Figure 2. Gastropods, food sources and diets in Catalina Bay, 2007. (A) Four-point 6 m transect showing gastropod densities and microphytobenthic (MPB) biomass (as chl-*a* concentration). (B) Carbon and nitrogen stable isotope signatures of gastropods (number in brackets: trophic position) and their potential food sources; such as detritus (DTR) and sedimentary organic matter (SOM). SIAR boxplots show the proportional contribution (%) of different food sources to the diet of (C) *Tarebia granifera* and (D) *Haminoea natalensis*. 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 breach, after which the freshwater ponds associated with seepage areas along the eastern shores were flooded by seawater.
doi:10.1371/journal.pone.0031897.g002

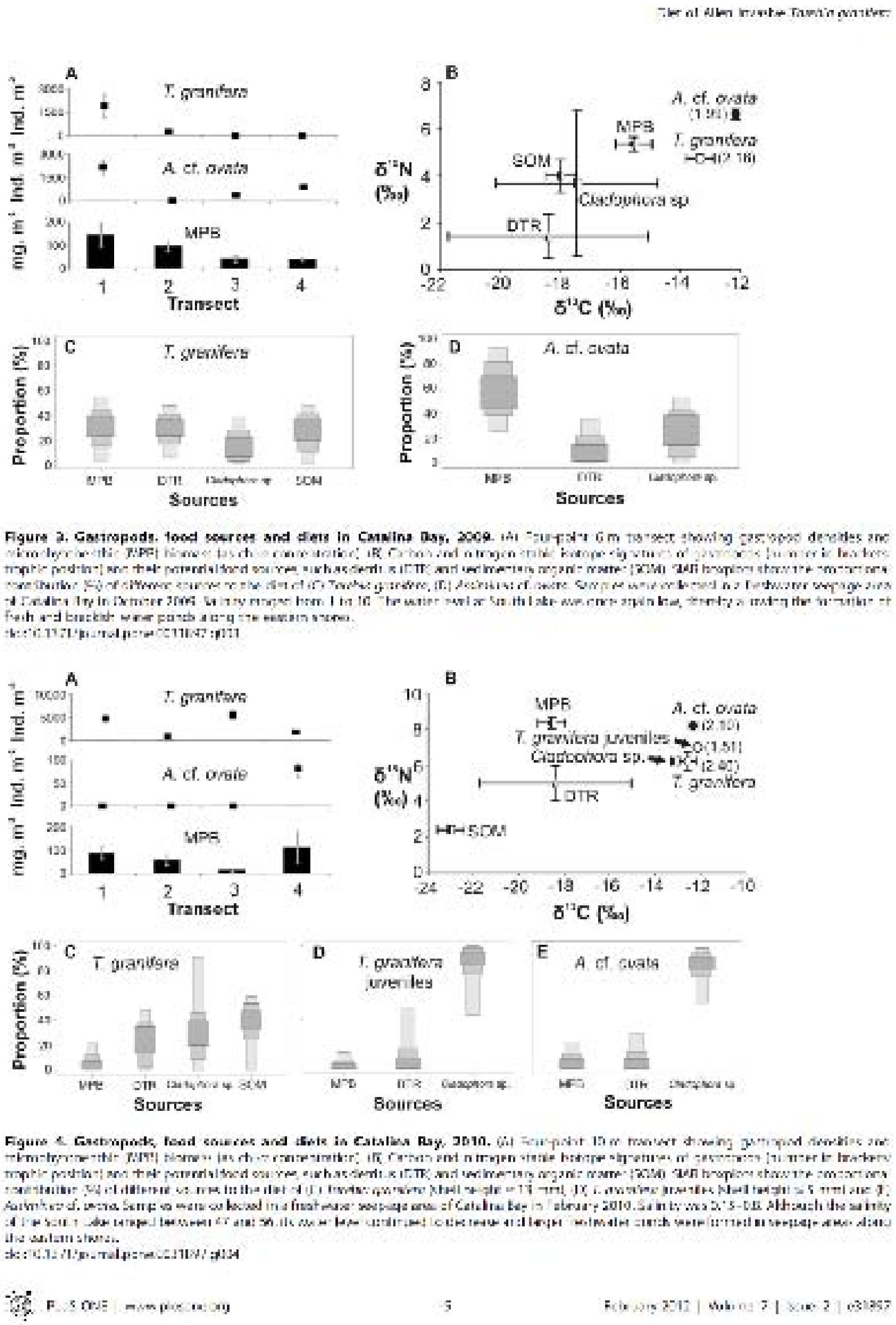


Figure 3. Gastropods, food sources and diets in Catalina Bay, 2009. (A) Four-point 6 m transect showing gastropod densities and microphytobenthic (MPB) biomass (as chl-a concentration); (B) Carbon and nitrogen stable isotope signatures of gastropods (number in brackets: trophic position) and their potential food sources, such as detritus (DTR) and sedimentary organic matter (SOM). SIAR boxplots show the proportional contribution (%) of different sources to the diet of (C) *Tarebia granifera*, (D) *Assiminea* cf. *ovata*. Samples were collected in a freshwater seepage area of Catalina Bay in October 2009. Salinity ranged from 1 to 10. The water level at South Lake was once again low, thereby allowing the formation of fresh and brackish water ponds along the eastern shores.
doi:10.1371/journal.pone.0031897.g003

Figure 4. Gastropods, food sources and diets in Catalina Bay, 2010. (A) Four-point 10 m transect showing gastropod densities and microphytobenthic (MPB) biomass (as chl-a concentration); (B) Carbon 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). SIAR boxplots show the proportional contribution (%) of different sources to the diet of (C) *Tarebia granifera* (shell height ≥ 15 mm), (D) *T. granifera* juveniles (shell height ≤ 5 mm) and (E) *Assiminea* cf. *ovata*. Samples were collected in a freshwater seepage area of Catalina Bay in February 2010. Salinity was 0.15–0.8. Although the salinity of the South Lake ranged between 47 and 56, its water level continued to decrease and larger freshwater ponds were formed in seepage areas along the eastern shores.
doi:10.1371/journal.pone.0031897.g004

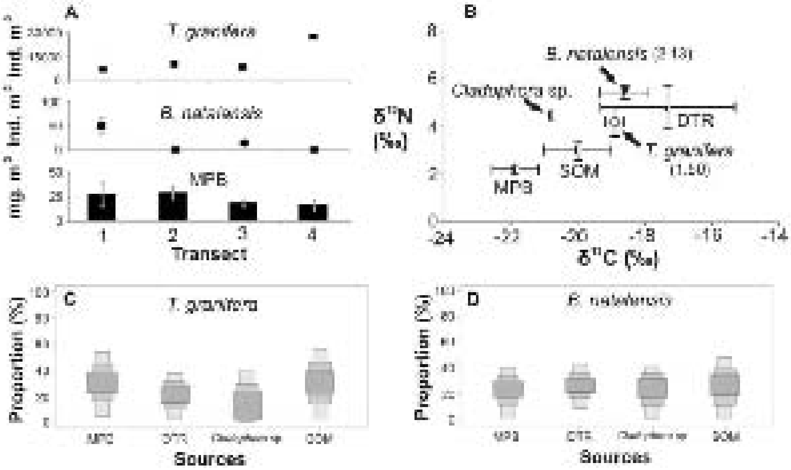


Figure 5. Gastropods, food types and diets in Lake Sibaya, 2009. (A) Four point 5 m transect showing gastropod densities and microphytobenthic (MPB) biomass (as chl-*a* concentration). (B) Carbon and nitrogen stable isotope signatures of gastropods (number in brackets: trophic position) and their potential food sources such as detritus (DTR) and sedimentary organic matter (SOM). SIAR boxplots show the proportional contribution (%) of different sources to the diet of (C) *Tarebia granifera* and (D) *Bulinus natalensis*. Samples were collected in November 2009. This lake has no connection to the sea and contains pure freshwater.
doi:10.1371/journal.pone.0031897.g005

times at Catalina Bay (Table 3, Fig. 2B–6B). The $\delta^{13}C$ signature of food source types also differed significantly between locations and between times (Table 4, Fig. 2B–6B). The $\delta^{15}N$ signature of food source types did not differ significantly between locations (Table 4), although it differed significantly over time at Catalina Bay (Table 4). There was no significant difference between the trophic position of *T. granifera* and that of native gastropods (t-test, df = 8, t = 0.496, p>0.05).

Stable isotope mixing model results indicated the largest proportional contributions of food sources to the diets of gastropods (Fig. 2C–6C, Table S1). At Catalina Bay in 2008, microphytobenthos (MPB) made the greatest contribution to the diet of *T. granifera* (15–74% of total); in 2009 it was MPB (2–52%), detritus (1–49%) and sedimentary organic matter (SOM) (0–47%), and in 2010 *Cladophora* sp. (1–67%) and SOM (0–50%). At Lake Sibaya, MPB (1–51%) and SOM (1–55%) made the greatest contribution to the diet of *T. granifera*, but at Lake Nhlange it was MPB (0–37%) and detritus (0–50%) (Fig. 2C–3C, Table S1). *Cladophora* sp. (13–95%) made the greatest contribution to the diet of *T. granifera* juveniles at Catalina Bay in 2010 (Fig. 4D, Table S1). *Haminoea natalensis* fed on MPB (20–95%) (Fig. 2D, Table S1), *Assiminea* cf. *ovata* fed on MPB in 2009 (23–63%) and *Cladophora* sp. in 2010 (15–88%) (Fig. 3D, Fig. 4E, Table S1). At Lake Sibaya, the native *B. natalensis* appeared to feed on a combination of all available food sources (Fig. 5D, Table S1). At Lake Nhlange, *M. tuberculata* diet consisted for the most part of MPB (0–55%), detritus (DTR) (0–50%) and SOM (1–63%) (Fig. 6D, Table S1).

Assuming that the microalgae found in the gut content analysis correspond to the mixing model MPB food source, detritus to DTR and filamentous algae to *Cladophora* sp., there was a significant correlation between gut contents and diet estimated from stable isotope mixing models (n = 3, $r^2 = 0.69$, p = 0.159).

Isotopic (stable isotope) dietary overlap (IDO) was significant (over 60%) between *T. granifera* and native gastropods in most cases (Table 5). The IDO between *T. granifera* adults and *A.* cf. *ovata* was not significant (below 60%) at Catalina Bay in 2010 (Table 5). The IDO between *T. granifera* adults and juveniles was also not significant (Table 5). However, the IDO of 91% between *T. granifera* juveniles (shell height ≤5 mm) and *A.* cf. *ovata* was the highest recorded in the study (Table 5).

Discussion

According to the competitive exclusion principle, species with identical ecological niche compete with each other for resources and, if these are limiting, there is a tendency towards the exclusion of the weaker competitor or the development of resource partitioning or niche differentiation [57–61]. Although recent research indicates that competition involving invasive species only rarely causes local extinction [62], there is still a need to better understand the mechanisms of competitive interactions and how the ecosystem is affected.

This study focuses only on situations where spatial overlap existed between the invasive gastropod *Tarebia granifera* and a single other native gastropod species. Yet the spatial distribution of *T. granifera* can simultaneously overlap with that of several species at a time [26]. However, this study does not address the greater complexity of interactions between multiple gastropod species in the same area.

Tarebia granifera invaded the shallow waters (less than 2 m depth) at Kosi Bay's Lake Nhlange, Lake Sibaya and the eastern shores of the South Lake of the St. Lucia Estuary (Fig. 1). The *T. granifera* populations tend to form dense patches (often >1000 ind. m^{-2}) of varying dimensions. *Tarebia granifera* patches such as the ones sampled in this study are often found in very shallow embayments with clear sandy substrate where particulate organic matter settles or where there is freshwater seepage. In contrast, the native species in this study have much lower population densities and their distribution is more extensive and much less patchy [26]. E.g. *Assiminea* cf. *ovata* at the St. Lucia Estuary is a historically

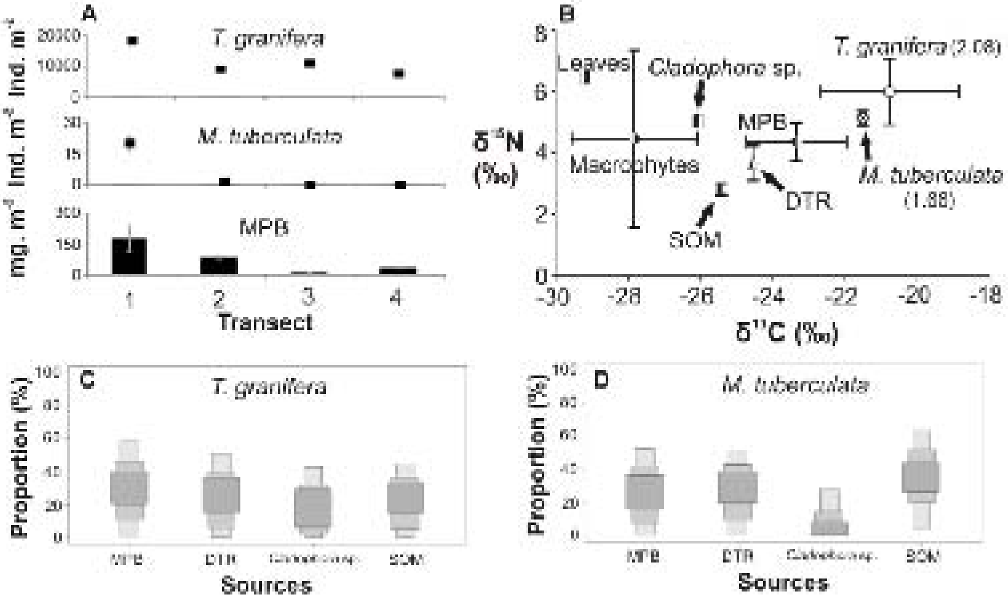


Figure 6. Gastropods, food sources and diets in Lake Nhlange, 2008. (A) Four-point 3 m transect showing gastropod densities and microphytobenthic (MPB) biomass (as chl-a concentration). (B) Carbon and nitrogen stable isotope signatures of gastropods (number in brackets: trophic position) and their potential food sources, such as detritus (DTR) and sedimentary organic matter (SOM). (C–D) Boxplots show the proportional contribution (%) of different sources to the diet of (C) *Tarebia granifera* and (D) *Melanoides tuberculata*. Samples were collected at Lake Nhlange in Kosi Bay in November 2008. At the time, the site was dominated by freshwater conditions.
doi:10.1371/journal.pone.0031897.g006

dominant species found throughout the system [35]. *Tarebia granifera* also appears to be displacing native gastropods from certain areas in the iSimangaliso Wetland Park (Fig. 1), whereas the native species do not appear to be mutually exclusive.

Tarebia granifera has previously been classified as both detritivore and grazer [31,43]. This study shows that *T. granifera* has a generalist diet which includes a wide variety of food sources. *Tarebia granifera* is established in all major coastal lakes of Maputaland (Fig. 1), where in particular cases its diet was either largely composed of a single or a combination of general food sources (Fig. 2C–6C, Table S1). Native gastropods such as and *Haminoea natalensis* tended to favor a single food source in Catalina Bay, whereas *Bellamya capillata* and *Melanoides tuberculata* had a more varied diet in Lake Sibaya and Lake Nhlange respectively.

Table 1. Correlations between microphytobenthic biomass and gastropod densities and diets.

		MPB	*T. granifera* density	Native density	*T. granifera* MPB diet	Native MPB diet
MPB	r	1	−0.211	−0.021	0.815	0.49
	p		ns	ns	<0.001	<0.05
***T. granifera* density**	r	−0.211	1	−0.252	0.035	−0.430
	p	ns		ns	ns	<0.05
Native density	r	−0.021	−0.252	1	−0.114	0.594
	p	ns	ns		ns	<0.05
***T. granifera* MPB diet**	r	0.815	0.035	−0.114	1	0.649
	p	<0.001	ns	ns		<0.001
Native MPB diet	r	0.49	−0.430	0.594	0.649	1
	p	<0.05	<0.05	<0.05	<0.001	

Pearson correlations (r is the correlation coefficient, n = 10 for every case) between microphytobenthic (MPB) biomass, *Tarebia granifera*, native gastropod densities and corresponding contribution of MPB to their diets. Diets were estimated by a stable isotope mixing model. Samples were collected at three coastal lakes in Maputaland. Native gastropods were *Haminoea natalensis*, *Bellamya capillata* and *Melanoides tuberculata*.
doi:10.1371/journal.pone.0031897.t001

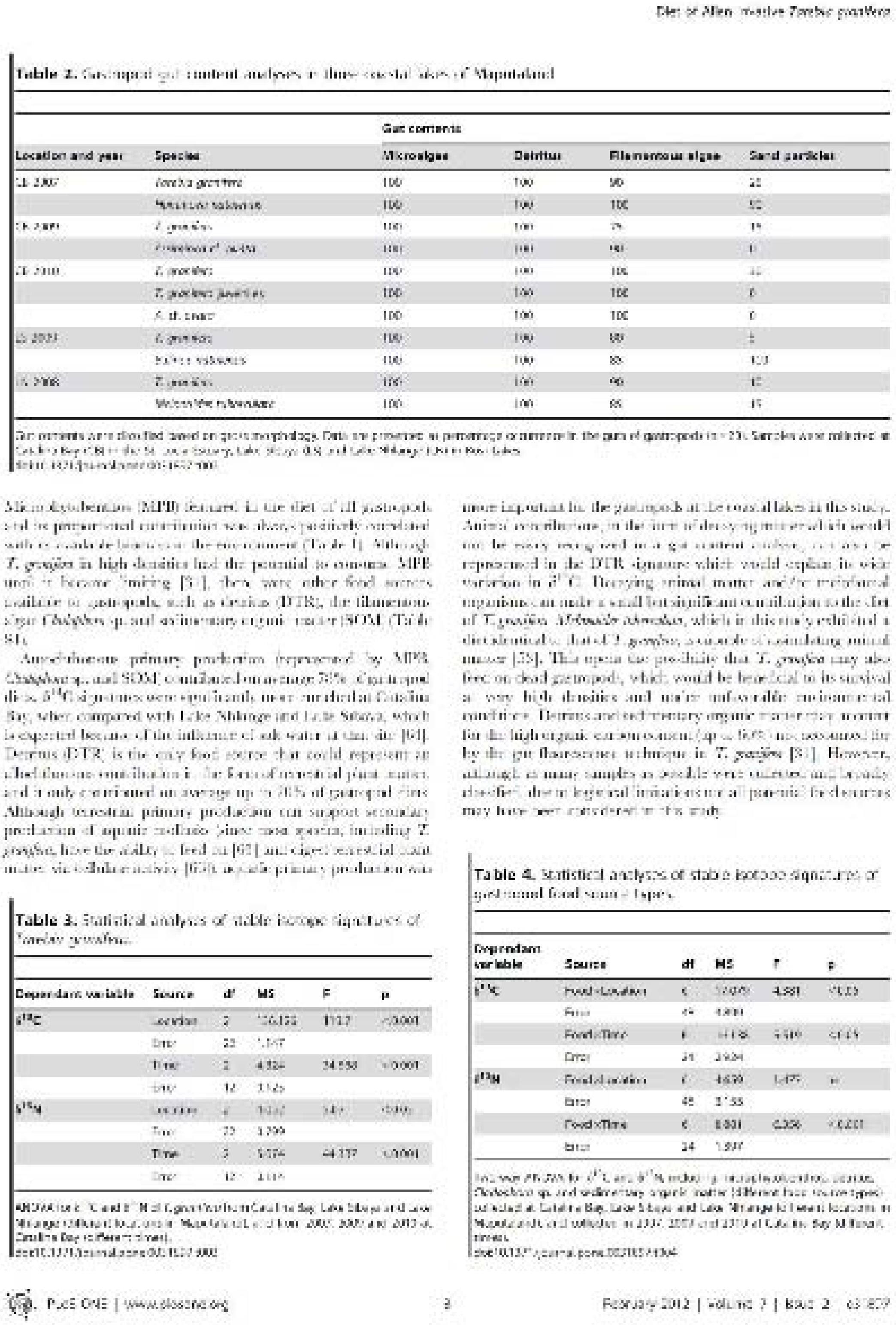


Table 5. Isotopic (stable isotopes) dietary overlap between gastropods in three coastal lakes of Maputaland.

Gastropod species	Location and year	Salinity	IDO (%)
*Tarebia granifera** and *Haminoea natalensis*	CB 2009	16–52	73
T. granifera and *Assiminea* cf. *ovata*	CB 2009	1–10	62
T. granifera and *A.* cf. *ovata*	CB 2010	0.15–0.8	50
T. granifera juveniles* and *A.* cf. *ovata*	CB 2010	0.15–0.8	9
T. granifera and *T. granifera* juveniles	CB 2010	0.15–0.8	49
T. granifera and *Bellamya* [illegible]	LS 2008	0.18–0.45	87
T. granifera and *Melanoides tuberculata*	LN 2008	0.91–1.36	83

**T. granifera* = shell height ≥10 mm; *T. granifera* juveniles = shell height ≤5 mm; all other gastropods were adults.
Locations are Catalina Bay (CB) in the St Lucia Estuary, Lake Sibaya (LS) and Lake Nhlange (LN) in Kosi Lakes. Isotopic dietary overlap (IDO %) values over 60% indicate a significant overlap and a value of 100% indicates absolute overlap.
doi:10.1371/journal.pone.0031897.t005

Assiminea cf. *ovata* has an affinity for MPB, as do other assimineid species [66]. However, the increase in abundance of *T. granifera* at St. Lucia from 2009 to 2010 may have influenced a dietary shift in *A.* cf. *ovata*, which started to feed less on MPB and more on *Cladophora* sp. (Fig. 3D, 4E and Table S1). This is not surprising as dietary shifts in native species tend to be caused by introduced species, as they disrupt trophic connections [15]. If resource partitioning between *T. granifera* and *A.* cf. *ovata* took place, it was effective in reducing IDO from 62% in 2009 to 50% in 2010. However, it is very likely that dietary shifts occurred in response to changing environmental conditions, which affected food availability and quality at Catalina Bay.

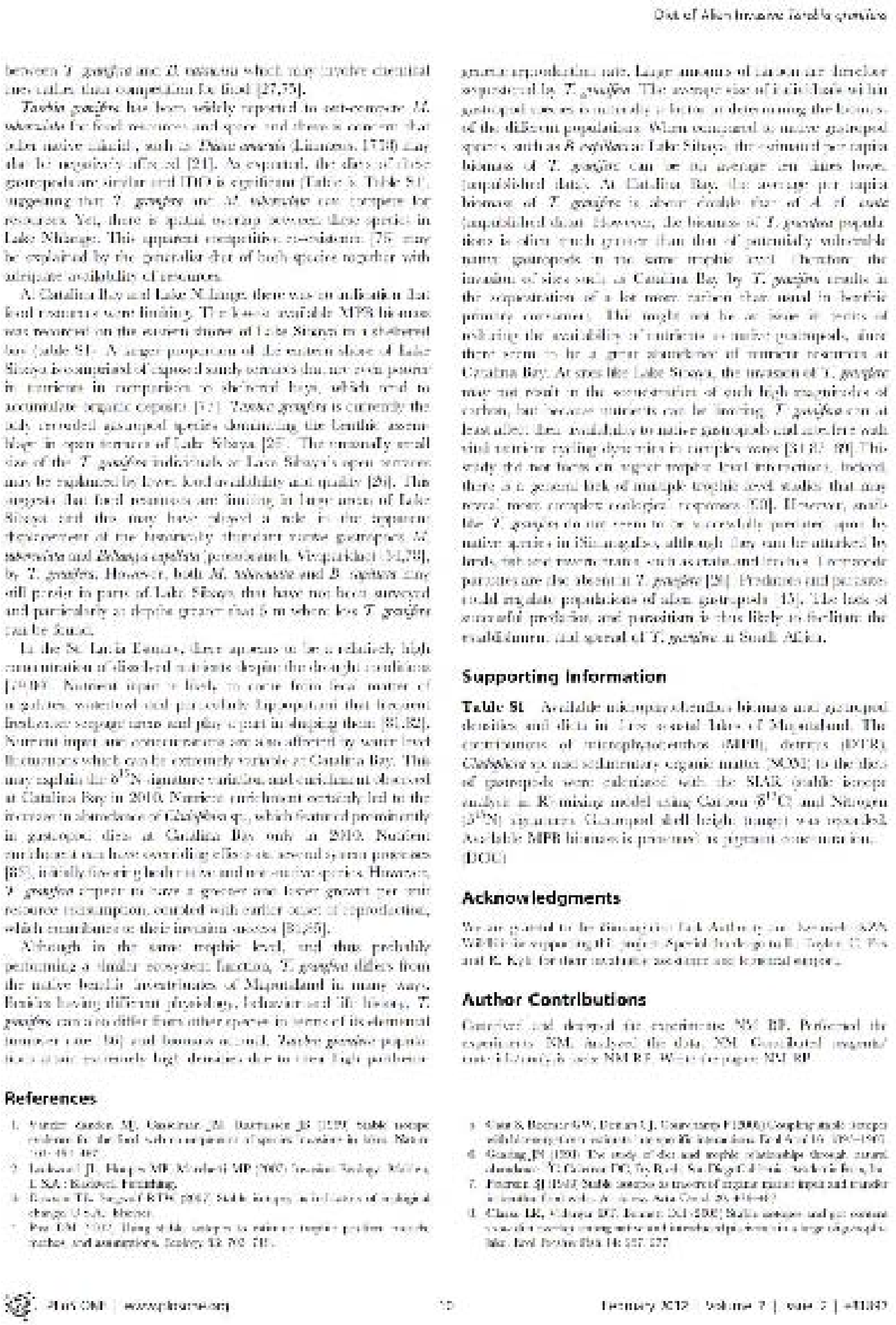
Due to its remarkable salinity tolerance, *T. granifera* managed to survive the unusual 2007 marine breaching event when seawater entered the St. Lucia Estuary and flooded the freshwater seepage areas of Catalina Bay (Fig. 1) [30]. However, *A.* cf. *ovata* was abundant in most of the seepage areas only in 2008, while the salinity level was high (around 30) [26]. When fresh water conditions became re-established in the seepage areas in 2009, *T. granifera* increased in numbers and spread until 2010, when it once again dominated benthic assemblages while evidently displacing *A.* cf. *ovata* [26]. Along the eastern shores of South Lake (Fig. 1), *A.* cf. *ovata* continued to be abundant in areas with high salinity and was even found in a few freshwater seepage areas where *T. granifera* was not present (e.g. Hand Tree Bay: 28°5'2.83"S, 32°27'35.75"E). At least over the last six years, *A.* cf. *ovata* and *H. natalensis* have consistently been found together in shallow-water habitats on the western shores of South Lake (Fig. 1), where there are fewer freshwater seepage areas, with only one being found to harbor *T. granifera*, i.e. Makakatana Bay (28°14'10.82"S, 32°25'1.61"E). In contrast to *T. granifera*, *H. natalensis* does not appear to displace *A.* cf. *ovata*.

Salinity is indeed one of the most important factors influencing the distribution of gastropods in coastal lakes of Maputaland [26]. Although there was a significant IDO between *T. granifera* and *H. natalensis*, there was very little spatial overlap between the two, since *H. natalensis* preferred the saltier conditions outside the seepage area. High salinity conditions were also associated with *A.* cf. *ovata* [26]. When salinity dropped below 20, *T. granifera* expanded its range and density. *Assiminea* cf. *ovata* was recorded in the St. Lucia Estuary at low salinities, but only when *T. granifera* was either absent or present in relatively low densities. This suggests that *T. granifera* may displace *A.* cf. *ovata* only under specific salinity conditions. This also supports the theory that euhaline native species can resist invasion by non-native species under certain conditions [8].

Interestingly, at Catalina Bay in 2010, it was observed that both *A.* cf. *ovata* and *T. granifera* juveniles tended to concentrate in extremely shallow areas (depth <0.1 meter), whereas *T. granifera* adults tended to concentrate in adjacent deeper waters (depth >0.1 meter). In this environment where water level fluctuation is commonplace, it is conceivable that smaller snails would continue to feed in extremely shallow areas whereas larger snails tend to move away and/or burrow to avoid desiccation. This behavior reduces the potential for competitive interactions involving *T. granifera* adults and can be interpreted in terms of spatial niche differentiation. However, the spatial overlap between *A.* cf. *ovata* and *T. granifera* juveniles remains unchanged and becomes effectively greater than that between *A.* cf. *ovata* and *T. granifera* adults. *Tarebia granifera* juveniles are often numerous, have fast growth rates and may even grow faster and larger in the presence of other snails [24,51,69]. They are voracious feeders and may have greater impacts on food stocks than adults [31,70]. It is therefore suggested that *T. granifera* juveniles played a role in the displacement of *A.* cf. *ovata*. A high degree of spatial overlap between *T. granifera* adults and juveniles has been recorded [26]. However, there is also evidence of an ontogenetic dietary shift in *T. granifera*, since SOM was only used by adults and IDO was not significant (Table 5, Table S1, Fig. 4C–D). Resource partitioning certainly minimizes potential competition between *T. granifera* adults and juveniles.

Food resource use within populations at the microhabitat scale can be a significant factor in gastropod interactions [21]. Some of the large variations in isotopic signatures recorded for gastropods in this study may be due to individual variation, which in turn may be primarily affected by habitat heterogeneity and resource availability [71]. This adds to the complexity of addressing dietary overlap and potential competition for food resources between gastropods.

Tarebia granifera and *H. natalensis* appear to have very similar and overlapping diets (Table S1, Table 5). Thiarids and temperate pulmonates can have similar diets [72]. The lack of competitive exclusion may be explained by selective ingestion and behavioral differences [73], as well as selection of different microhabitats, such as macrophyte fronds where *H. natalensis* positions itself close to the water surface [71], thus avoiding physical interactions with *T. granifera* which tend to stay on the substrate. However, in the Caribbean, *T. granifera* is known to displace *Biomphalaria glabrata* (Say, 1818) (pulmonata, Planorbidae), which like *H. natalensis* is associated with submerged vegetation rather than the substrate. Further empirical studies are thus needed to address interactions



between *T. granifera* and *B. natalensis* which may involve chemical cues rather than competition for food [27,73].

Tarebia granifera has been widely reported to out-compete *M. tuberculata* for food resources and space and there is concern that other native thiarids, such as *Thiara amarula* (Linnaeus, 1758) may also be negatively affected [21]. As expected, the diets of these gastropods are similar and DO is significant (Table 3, Table S1), suggesting that *T. granifera* and *M. tuberculata* can compete for resources. Yet, there is spatial overlap between these species in Lake Nhlange. This apparent competitive co-existence [75] may be explained by the generalist diet of both species together with adequate availability of resources.

At Catalina Bay and Lake Nhlange there was an indication that food resources were limiting. The lowest available MPB biomass was recorded on the eastern shores of Lake Sibaya in a sheltered bay (Table S1). A larger proportion of the eastern shore of Lake Sibaya is comprised of exposed sandy terraces that are even poorer in nutrients in comparison to sheltered bays, which tend to accumulate organic deposits [77]. *Tarebia granifera* is currently the only recorded gastropod species dominating the benthic assemblage in open terraces of Lake Sibaya [27]. The unusually small size of the *T. granifera* individuals in Lake Sibaya's open terraces may be explained by lower food availability and quality [26]. This suggests that food resources are limiting in large areas of Lake Sibaya and this may have played a role in the apparent displacement of the historically abundant native gastropods *M. tuberculata* and *Bellamya capillata* ([illegible]) [34,78], by *T. granifera*. However, both *M. tuberculata* and *B. capillata* may still persist in parts of Lake Sibaya that have not been surveyed and particularly at depths greater than 5 m where few *T. granifera* can be found.

In the St. Lucia Estuary, there appears to be a relatively high concentration of dissolved nutrients despite the drought conditions [79,80]. Nutrient input is likely to come from fecal matter of ungulates, waterfowl and particularly hippopotami that frequent freshwater seepage areas and play a part in shaping them [81,82]. Nutrient input and concentrations are also affected by water level fluctuations which can be extremely variable at Catalina Bay. This may explain the $\delta^{15}N$ signature variation and enrichment observed at Catalina Bay in 2010. Nutrient enrichment certainly led to the increase in abundance of *Cladophora* sp., which featured prominently in gastropod diets at Catalina Bay only in 2010. Nutrient enrichment can have overriding effects on several system processes [83], initially favoring both native and non-native species. However, *T. granifera* appear to have a greater and faster growth per unit resource consumption, coupled with earlier onset of reproduction, which contributes to their invasion success [84,85].

Although in the same trophic level, and thus probably performing a similar ecosystem function, *T. granifera* differs from the native benthic invertebrates of Maputaland in many ways. Besides having different physiology, behavior and life history, *T. granifera* can also differ from other species in terms of its elemental turnover rate [86] and biomass accrual. *Tarebia granifera* populations attain extremely high densities due to their high parthenogenetic reproduction rate. Large amounts of carbon are therefore sequestered by *T. granifera*. The average size of individuals within gastropod species is naturally a factor in determining the biomass of the different populations. When compared to native gastropod species such as *B. capillata* at Lake Sibaya, the estimated per capita biomass of *T. granifera* can be on average ten times lower (unpublished data). At Catalina Bay, the average per capita biomass of *T. granifera* is about double that of *A.* cf. *ovata* (unpublished data). However, the biomass of *T. granifera* populations is often much greater than that of potentially vulnerable native gastropods in the same trophic level. Therefore, the invasion of sites such as Catalina Bay by *T. granifera* results in the sequestration of a lot more carbon than usual in benthic primary consumers. This might not be an issue in terms of reducing the availability of nutrients to native gastropods, since there seems to be a great abundance of nutrient resources at Catalina Bay. At sites like Lake Sibaya, the invasion of *T. granifera* may not result in the sequestration of such high magnitudes of carbon, but because nutrients can be limiting, *T. granifera* can at least affect their availability to native gastropods and interfere with vital nutrient cycling dynamics in complex ways [31,87–89]. This study did not focus on higher trophic level interactions. Indeed, there is a general lack of multiple trophic level studies that may reveal more complex ecological responses [90]. However, snails like *T. granifera* do not seem to be successfully predated upon by native species in iSimangaliso, although they can be attacked by birds, fish and invertebrates such as crabs and leeches. Trematode parasites are also absent in *T. granifera* [26]. Predators and parasites could regulate populations of alien gastropods [15]. The lack of successful predation and parasitism is thus likely to facilitate the establishment and spread of *T. granifera* in South Africa.

Supporting Information

Table S1 Available microphytobenthic biomass and gastropod densities and diets in three coastal lakes of Maputaland. The contributions of microphytobenthos (MPB), detritus (DTR), *Cladophora* sp. and sedimentary organic matter (SOM) to the diets of gastropods were calculated with the SIAR (stable isotope analysis in R) mixing model using Carbon ($\delta^{13}C$) and Nitrogen ($\delta^{15}N$) signatures. Gastropod shell height (range) was recorded. Available MPB biomass is presented as pigment concentration. (DOC)

Acknowledgments

We are grateful to the iSimangaliso Park Authority and Ezemvelo KZN Wildlife for supporting this project. Special thanks go to R. Taylor, C. Fox and R. Kyle for their invaluable assistance and technical support.

Author Contributions

Conceived and designed the experiments: NM RP. Performed the experiments: NM. Analyzed the data: NM. Contributed reagents/materials/analysis tools: NM RP. Wrote the paper: NM RP.

References

1. Vander Zanden MJ, Casselman JM, Rasmussen JB (1999) Stable isotope evidence for the food web consequences of species invasions in lakes. Nature 401: 464–467.
2. Lockwood JL, Hoopes MF, Marchetti MP (2007) Invasion Ecology. Malden, USA: Blackwell Publishing.
3. Dawson TE, Siegwolf RTW (2007) Stable isotopes as indicators of ecological change. USA: Elsevier.
4. Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83: 703–718.
5. Caut S, Roemer GW, Donlan CJ, Courchamp F (2006) Coupling stable isotopes with bioenergetics to estimate interspecific interactions. Ecol Appl 16: 1893–1900.
6. Gearing JN (1991) The study of diet and trophic relationships through natural abundance [illegible]. In: Coleman DC, Fry B, eds. San Diego, California: Academic Press, Inc.
7. Peterson BJ (1999) Stable isotopes as tracers of organic matter input and transfer in benthic food webs: A review. Acta Oecol 20: 479–487.
8. Claus LR, [illegible] (2008) Stable isotopes and gut content [illegible] in a large oligotrophic lake. [illegible] 14: 257–277.

OPEN ACCESS Freely available online
PLoS one

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, Westville 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. *Tarebia granifera* is a prosobranch 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 influences gastropod communities, and specifically how the population density and size structure of *T. granifera* were influenced by environmental change in estuaries and coastal lakes in southern Africa.

Methodology/Principal Findings: *T. granifera*'s density, number of brooded juveniles and size structure were measured at the St. Lucia Estuary, Mgobozeleni Estuary, Lake Sibaya and Lake Nhlange. Size structure was classified according to shell height (SH). All dissected 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. granifera* often reached densities well over 1000 ind. m^{-2}, displacing indigenous gastropods and becoming a dominant component of the benthic community. *T. granifera* successfully invaded estuaries despite frequent exposure to high salinity and desiccation, which could together eliminate >97% of the population. The persistence of *T. granifera* was ensured due to its high fecundity and the environmental tolerance of large adults (20–30 mm SH) which carried an average of 158±12.8 SD brooded 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. granifera* (including the previously overlooked "brood pouch ecology"), which affects population dynamics and may be relevant to other parthenogenetic NIS, such as *Melanoides tuberculata* and *Potamopyrgus antipodarum*.

Citation: Miranda NAF, Perissinotto R, Appleton CC (2011) Population Structure of an Invasive Parthenogenetic Gastropod in Coastal Lakes and Estuaries of Northern KwaZulu-Natal, South Africa. PLoS ONE 6(8): e24337. doi:10.1371/journal.pone.0024337

Editor: Carles Lalueza-Fox, Institut de Biologia Evolutiva - Universitat Pompeu Fabra, Spain

Received May 27, 2011; **Accepted** August 4, 2011; **Published** August 31, 2011

Funding: This project was funded by the National Research Foundation, South Africa (www.nrf.ac.za); Marine and Coastal Management (DEAT-MCM, www.mcm-deat.gov.za); and the World Wide Fund (WWF, www.wwf.org.za). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: 204507499@ukzn.ac.za

Introduction

Although asexual reproduction can be found in numerous organisms, it is rare 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 asexual populations occupy different habitats to populations that reproduce sexually [2,3]. Parthenogenetic populations may have several advantages for establishment in new and variable habitats: a single parthenogenetic organism can start a population [3–6]; the genotype is isolated from gene flow and adaptations to the new habitat, especially in "general purpose genotypes", can thus not be broken by recombination (see frozen - niche variation [7]); in habitats where populations undergo frequent local extinction and recolonization events, genetic bottleneck and drift effects will have less negative fitness consequences for asexual populations [8]; also, according to the Red Queen Hypothesis, reduced biotic interactions with parasites and predators favor asexual populations (see [9], but also [10]).

The geographic patterns and rates of species' invasion are changing on an unprecedented scale due to direct and indirect anthropogenic action [11]. Non-indigenous invasive species (NIS) are a serious threat to biodiversity [12], particularly in estuarine and coastal environments [13,14]. Fortunately, relatively few introduced species are successful in establishing populations, fewer go on to spread and fewer still become pests [15]. Chance plays a role in the invasion success of all NIS and there can be many repeated attempts before an invasion is successful [12,16]. However, certain biological and ecological characteristics are thought to increase the probability of invasion [17]. In this context, it is not surprising that an introduced parthenogenetic species pre-adapted to colonize marginal habitats with wide physiological tolerance, high fecundity and very high population densities would also make a successful NIS [18].

Tarebia granifera is a prosobranch gastropod (Thiaridae) originally from south-east Asia. This parthenogenetic species has a brood pouch and gives birth to fully developed juveniles. *T. granifera* has high fecundity and has been reported to reach densities over 20 000 ind. m^{-2} [19]. It has invaded several subtropical parts of the world, including Texas, Hawaii, Caribbean islands, Mexico and Israel [20–23]. In South Africa, *T. granifera* has invaded an increasing number of estuaries and coastal lakes over the past decade [19]. The species is regarded as a freshwater dweller, but its recent invasion patterns [19], a physiological tolerance study [24] and a strontium isotope ($^{87}Sr/^{86}Sr$) study of fossils dating back 1.5 million years [25] suggest that this species is pre-adapted to brackish environments. The shallow marginal habitats of coastal lakes and estuaries can be extremely variable environments [26,27]. Stochastic events involving changes in water level and salinity have been observed to repeatedly wipe out most of the *T. granifera* population and yet, this NIS not only persists but often becomes a dominant component of the shallow-water benthos (pers. obs.).

This study aimed to address two questions: 1) is gastropod community structure influenced by a small number of key environmental factors? 2) What are the longer term effects of environmental change on *T. granifera* within a variable estuarine setting? The size structure of *T. granifera* (in terms of shell height size classes) has previously only been described for freshwater bodies and in the laboratory. Differences in population density and size structure over time and under different environmental conditions revealed how *T. granifera* populations persisted during unfavorable periods and then recovered. *T. granifera*'s reproductive output was tentatively assessed in terms of number of unborn juveniles in the brood pouch.

Materials and Methods

Ethics Statement

Permission for this study was granted under a Research Agreement with the iSimangaliso 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 ~325 km^2 and average depth of 0.9 m [28]. Recently, this estuary has been experiencing unprecedented low water levels and the mouth has been closed for the most part from 2002 to present. There is a reversed salinity gradient and salinities over five times higher than seawater were recorded in the most northerly parts of the system [29]. However, areas such as the eastern shores of South Lake, receive a considerable input of freshwater from sand dune aquifers [30]. Samples were collected at Catalina Bay (28°13′S, 32°29′E), on the eastern shores of South Lake (Fig. 1). In March 2007, the St. Lucia Estuary mouth breached and seawater from the Indian Ocean entered the system [29], increasing water levels and introducing a number of marine species, including the sea hare *Stylocheilus striatus* (Fig. 2).

Mgobozeleni Estuary (27°32′S, 32°40′E; Fig. 1) has a surface area of 0.014 km^2, maximum width of 25 m and average depth of 0.3 m [31]. This estuary is supplied with freshwater from Lake Mgobozeleni and is strongly influenced by tidal regimes [32]. It is a typical Temporarily Open/Closed Estuary (TOCE, Perissinotto et al. 2010) and a combination of low rainfall and spring tide may result in the periodical closure of the mouth [33].

Lake Sibaya has a surface area of 60 to 77 km^2 and an average depth of 13 m [34]. This land-locked freshwater lake currently undergoes wide fluctuations in water level [34]. Samples were collected on the eastern shores (27°22′S, 32°42′E; Fig. 1).

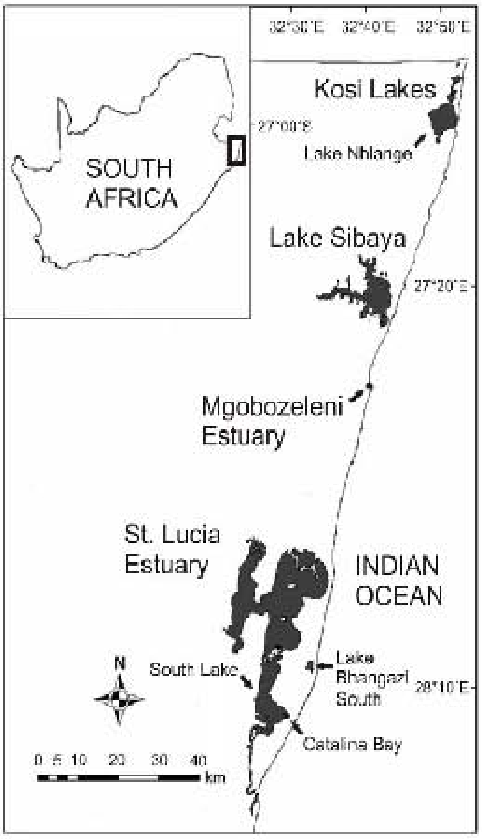


Figure 1. Map of Maputaland. The Kosi Lakes, Lake Sibaya and the St. Lucia Estuary are Ramsar Wetlands of International Importance within the iSimangaliso Wetland Park, a UNESCO World Heritage Site in northern KwaZulu-Natal, South Africa.
doi:10.1371/journal.pone.0024337.g001

Lake Nhlange (26°57′S, 32°19′E) is the largest of the Kosi Lakes (Fig. 1). The surface area of this lake varies from 30.7 to 37 km^2 and the average depth is 7.2 m [35]. Lake Nhlange is connected to the ocean via channels and other lakes but its 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; Mgobozeleni Estuary, Lake Sibaya and Lake Nhlange were surveyed during the wet seasons in 2009 and 2010 (Table S1).

Physical and chemical parameters

All samples were taken in shallow marginal habitats (<2 m depth). Salinity, dissolved oxygen, pH and temperature were measured with a YSI 6920 multiprobe. Sub-surface water samples were sieved through a GF/F filter and the supernatant was analysed for nitrates and phosphates with a Skalar SAN^{++} continuous flow nutrient analyzer. Sediment samples were dried and weighed before being sieved through a 2000 µm sieve. The

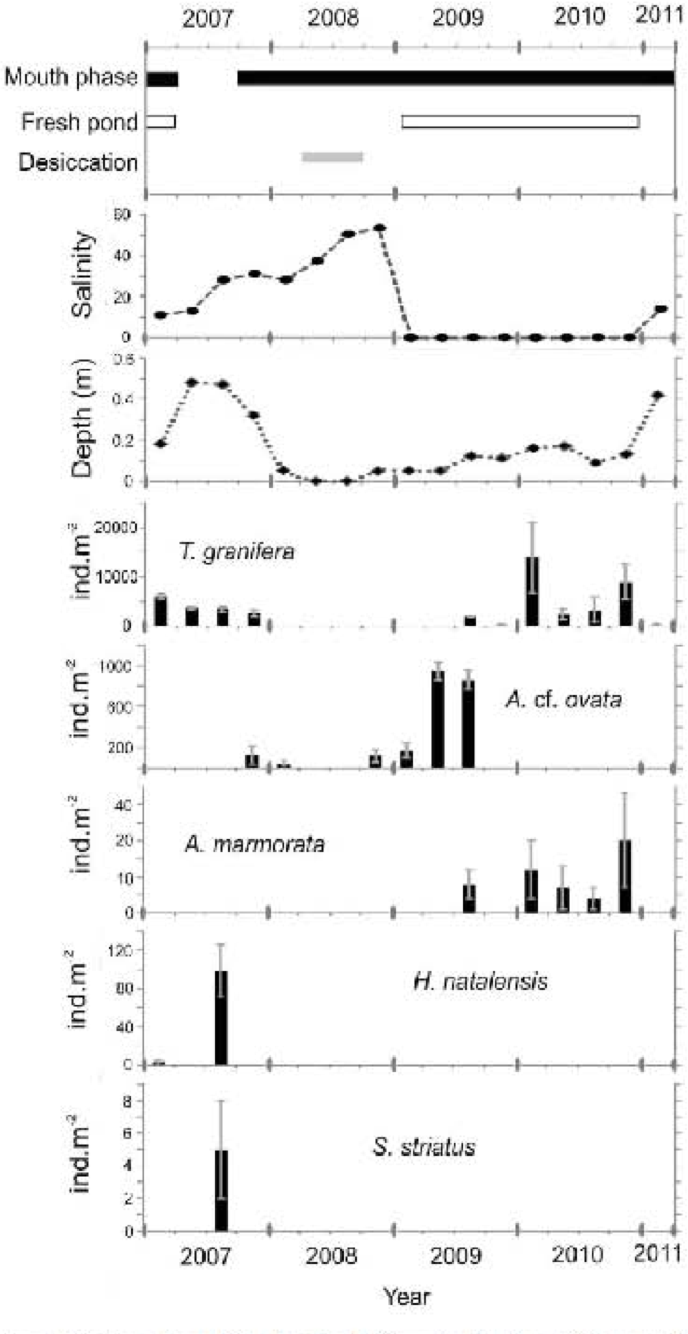


Figure 2. Gastropod population densities (± SD) under changing salinities and depths. Data were collected at Catalina Bay, in the St. Lucia Estuary from 2007 to 2011 at quarterly intervals. The black horizontal bar represents a closed mouth phase, the white bar represents the presence of a freshwater pond, and the grey bar represents the desiccation of most of the sampling area. Species: *Tarebia granifera*, *Assiminea* cf. ovata, *Aplexa marmorata*, *Haminoea natalensis* and *Stylocheilus striatus*.
doi:10.1371/journal.pone.0024337.g002

finer sediment was analysed by a Malvern Analyser. The median sediment particle size was then calculated by taking into account the weighted sediment retained on the sieve.

Gastropods

Triplicate macrofauna samples were taken with a Zabalocki-type Ekman grab (area = 0.0236 m^2). Immediately after collection, samples were washed through a 500 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). *Tarebia granifera* 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, its (damaged) shell height was multiplied by the ratio of average SH/average height of the last whorl of unaffected (undamaged) specimens belonging to that population and appropriate size class [36]. Thus, the corrected SH was used in spacial and temporal comparisons. The gastropods were sorted into one of the following 10 size classes according to (corrected) SH: <1 mm; 1–5.99 mm; 6–7.99 mm; 8–9.99 mm; 10–11.99 mm; 12–13.99 mm; 14–15.99 mm; 16–17.99 mm; 18–19.99 mm; 20–30 mm. No less than five individuals belonging to each size class were dissected. The gonads were inspected to determine sex and presence of trematode parasites. The brood pouch of adult specimens (6–30 mm SH) was carefully dissected and the shelled juveniles contained inside were counted.

Data analysis

Analysis of covariance (ANCOVA) using size class as a covariate was used to assess differences in average number of unborn juveniles per brood pouch between sampling events, and between locations. A two-way analysis of variance (ANOVA) was used to determine if *T. granifera* densities differed temporally (in terms of year×season) at Catalina Bay, St. Lucia Estuary. An ANOVA and Tukey's Honestly Significant Difference (HSD) multiple comparisons were conducted to determine if *T. granifera* densities differed between locations. The relationship between shell height and number of juveniles in brood pouch was analysed with Pearson's correlation. All data were log-transformed to meet normality requirements. The statistical package PASW version 18 for Windows was used.

A canonical correspondence analysis was conducted between log-transformed gastropod (8 species, Fig. 3) abundance data and standardized environmental data (depth, salinity, temperature, dissolved oxygen, pH, median sediment particle size, turbidity, nitrates and phosphates) collected at the study site between 2007 and 2010. A forward selection model was used to determine the four environmental variables which best explained the variation in density data of gastropod species. The CANOCO software version 4.5 was used for this purpose.

Results

Canonical correspondence analysis

The canonical correspondence analysis showed that the combination of salinity, depth, temperature and pH explains 78% of the variation in the abundance data 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 *Haminoea natalensis* and *Assiminea* cf. *ovata* and the decline of *Tarebia granifera*

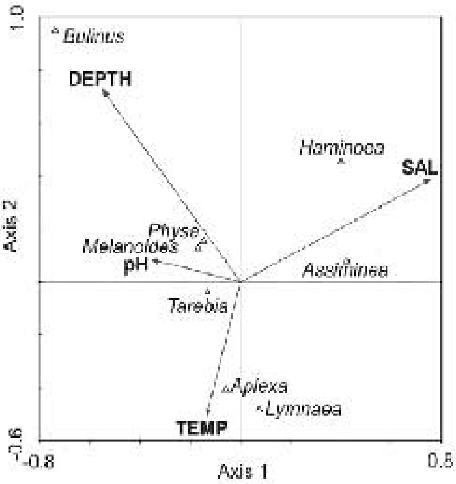


Figure 3. Maputaland gastropod population densities correlated with physical and chemical parameters. First and second axes of a canonical correspondence analysis performed on gastropod population densities and physical and chemical parameters measured at Lake Nhlange, Lake Sibaya, Mgobozeleni Estuary and St. Lucia Estuary in northern KwaZulu-Natal, South Africa. Data were collected from 2007 to 2011at quarterly intervals. Physical and chemical parameter vectors: salinity (SAL), depth, water temperature (TEMP) and pH. Species: *Tarebia granifera* (*Tarebia*), *Melanoides tuberculata* (*Melanoides*), *Physa acuta* (*Physa*), *Assiminea* cf. *ovata* (*Assiminea*), *Haminoea natalensis* (*Haminoea*), *Aplexa marmorata* (*Aplexa*), *Lymnaea natalensis* (*Lymnaea*) and *Bulinus natalensis* (*Bulinus*).
doi:10.1371/journal.pone.0024337.g003

(Fig. 2). The mouth was only open for 6 months, and in 2008 water levels dropped dramatically, leaving vast areas dry. In 2009, freshwater pooled on the eastern shores of the South Lake where non-indigenous *T. granifera* as well as *Aplexa marmorata* were found (Fig. 2).

T. granifera population density was not significantly different in terms of year×season at Catalina Bay (Two-way ANOVA: $F_{21,13} = 1.051$, $P>0.05$). *T. granifera* density was however signifi-

Table 1. Canonical correspondence analysis performed on abundances of Maputaland gastropods and physical and chemical parameters.

		Axis 1	Axis 2	Axis 3
Eigenvalues		0.397	0.191	0.146
Cumulative percentage:	**of species data**	20.1	29.9	37.3
	species-environment relation	50.3	74.8	93.5
	Species-environment correlation	0.87	0.75	0.656
Component loadings:	**Depth**	−0.628	0.737	−0.223
	Salinity	0.803	0.384	−0.298
	Temperature	−0.190	−0.501	−0.262
	pH	−0.343	0.085	0.636

doi:10.1371/journal.pone.0024337.t001

cantly different between locations (Catalina Bay, Mgobozeleni Estuary, Lake Sibaya and Lake Nhlange, 2007–2010 wet seasons only) (ANOVA: $F_{3,31} = 30.359$, $P<0.05$). *T. granifera* densities at the freshwater Sibaya and Nhlange lakes were significantly different from densities at the brackish St. Lucia and Mgobozeleni Estuaries (Tukey's HSD: $P<0.05$).

Size classes and juveniles in brood pouch

All *T. granifera* found in this study were female. An inspection of the gonads and digestive gland also revealed no trematode infections. Specimens with shell height smaller than 6 mm had under-developed brood pouches, which did not contain shelled juveniles. Variable sizes of juveniles were found within the brood pouch.

The shells of *T. granifera* were most severely damaged at Catalina Bay, where in 2009 and 2010 on average 78% of the population showed signs of shell erosion and SH was reduced by an average of 12.3%. The damage was concentrated in the size classes 6–13.99 mm SH.

At Catalina Bay in February 2007, the distribution of the *T. granifera* population was unimodal, with size class 6–7.99 mm SH being the most common and contributing 32.7%±11 SD of the total (Fig. 4A). Each adult of 6–7.99 mm SH carried on average 1.3±0.6 SD juveniles, whereas each adult of 18–19.99 mm SH carried 48.6±12.1 SD juveniles in its brood pouch (Fig. 4a). In May 2007, after mouth breach and under salinity>28 (Fig. 2, Table S1), the *T. granifera* population appeared to have a bimodal distribution and the size class 6–7.99 mm SH was poorly represented (Fig. 4B). The largest size class represented was 16–17.9 mm SH and each snail in this class carried an average of 18±13.4 SD juveniles in its brood pouch. In 2008 there were no *T. granifera* recorded at the seepage area of Catalina Bay, which dried out completely resulting in the *T. granifera* population being reduced by >97%. In June 2009, seepage water accumulated to form a freshwater pond independent of the South Lake. Large *T. granifera* (14–30 mm SH) were found making conspicuous trails on the sediment surface and the largest (20–30 mm SH) carried on average 158±12.8 SD juveniles in the brood pouch (Fig. 4C). 77.3%±22.8 SD of the total population was composed of juveniles (Fig. 4C, Table S1). In November 2010, 50.5%±18.1 SD of the total population was composed of juveniles at Catalina Bay (Fig. 4D).

At the Mgobozeleni Estuary, juveniles contributed 30.9%±40.3 SD and adults ranged from 6 to 19.99 mm SH (Fig. 5A). At Lake Sibaya, the largest adults found on the open terraces of the eastern shores were 10–11.9 mm SH and carried on average 4±1.8 SD

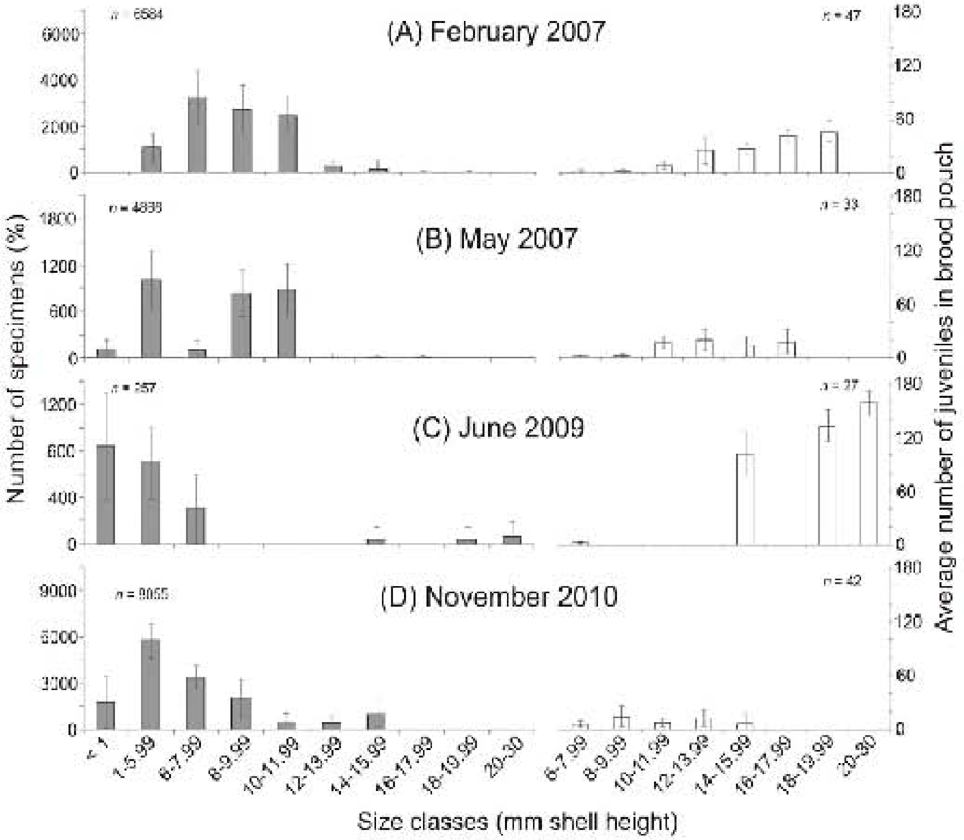


Figure 4. *Tarebia granifera* size structure over time. Size class (mm shell height) distribution of *T. granifera* specimens (percentages ± SD) and average number of unborn juveniles (± SD) per adult size classes (6–30 mm shell height) collected by triplicate Ekman grab at Catalina Bay, in the St. Lucia Estuary during (A) February 2007, (B) May 2007, (C) June 2009 and (D) November 2010.
doi:10.1371/journal.pone.0024337.g004

juveniles in the brood pouch (Fig. 5B). At Lake Nhlange, 59%±10.3 SD of the population was composed of size classes 10–13.99 mm SH in November 2010 (Fig. 5C). The largest adults (20–30 mm SH) carried 35±24 SD juveniles in the brood pouch (Fig. 5C).

T. granifera shell height and number of unborn juveniles in brood pouch were positively correlated (Pearson's correlation coefficient: $r = 0.806$, $P < 0.01$, $n = 186$). There was no significant difference in average number of unborn juveniles per brood pouch between sampling events at Catalina Bay (ANCOVA: $F_{11,30} = 1.626$, $P = 0.115$). There was also no significant difference in average number of unborn juveniles per brood pouch between locations (ANCOVA: $F_{3,37} = 1.174$, $P = 0.328$).

Discussion

Tarebia granifera population densities were often well over 1000 ind. m^{-2} regardless of location, making this NIS a dominant component of the local invertebrate macrofauna. However, at the St. Lucia and Mgobozeleni estuaries, population densities were variable, whereas at the Sibaya and Nhlange coastal lakes the *T. granifera* populations appeared relatively stable (Table S1). South African estuaries are particularly variable environments in terms of salinity and water depth and these factors can change unpredictably and directly influence macrofauna [27]. As expected for any benthic invertebrate, the spatial distribution of *T. granifera* was heterogeneous [37,38,39]. In general, shallow water (depth<2 m) freshwater sources and sheltered bays with organic deposits seemed to be favored. Extreme 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. granifera* population densities were recorded at Catalina Bay (Table S1). Indeed, it has been suggested that the high densities of *T. granifera* may minimise the risk of extirpation 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, *Bulinus natalensis* was associated with deeper water, whereas *Aplexa marmorata* and *Lymnaea natalensis* were associated with very shallow and warm waters (Fig. 3). *Haminoea natalensis* and *Assiminea* cf. *ovata* were the only species associated with high salinity (Fig. 3). However, in comparison to other gastropods, *T. granifera* tended to be least associated with any one environmental factor (Fig. 3) and was the most widespread and abundant gastropod in all study areas.

The *T. granifera* population density did not appear to undergo seasonal patterns and year-round births were recorded. The average number of juveniles per brood pouch, which was assumed to indicate reproductive output, did appear to increase when the salinity suddenly increased in the St. Lucia Estuary in 2007 and also when the population was recovering in 2009 (Table S1). An increase in average number of juveniles per brood pouch was also measured during *T. granifera*'s recovery at the Mgobozeleni Estuary. *T. granifera* may increase its reproductive output in response to disturbances, such as sudden salinity increases that negatively affects the population, therefore accelerating its recovery. However, size classes were not taken into account in this interpretation.

The structure of the *T. granifera* population was defined in terms of its proportional contribution to different shell height size classes.

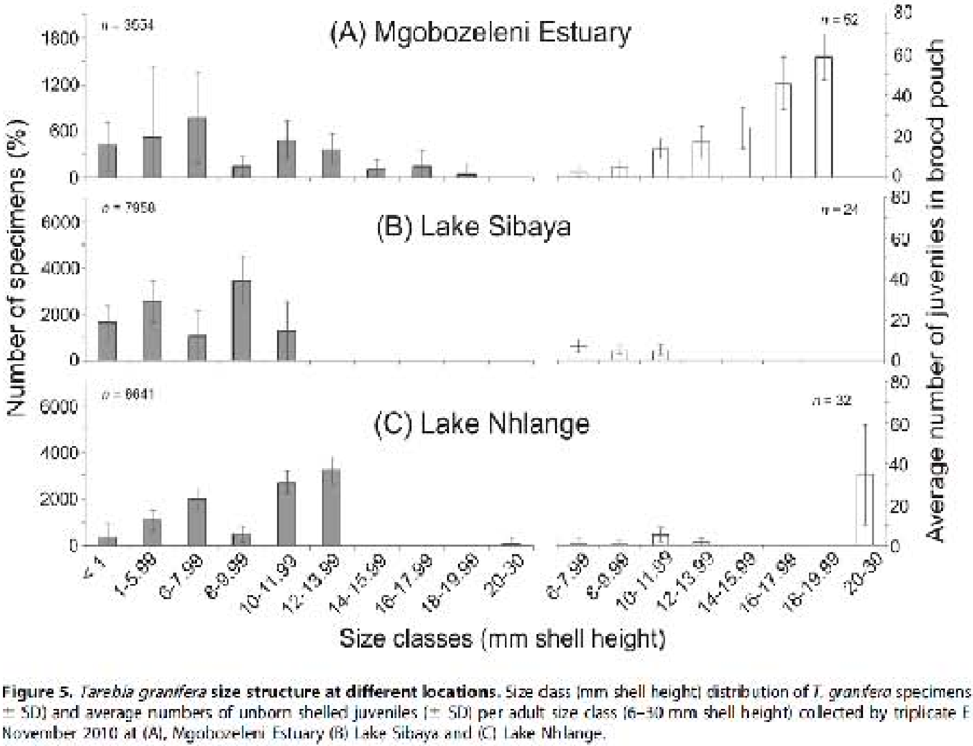


Figure 5. *Tarebia granifera* size structure at different locations. Size class (mm shell height) distribution of *T. granifera* specimens (percentages ± SD) and average numbers of unborn shelled juveniles (± SD) per adult size class (6–30 mm shell height) collected by triplicate Ekman grab in November 2010 at (A), Mgobozeleni Estuary (B) Lake Sibaya and (C) Lake Nhlange.
doi:10.1371/journal.pone.0024337.g005

T. granifera's shell height (SH) ranged from >1 mm to 28.75 mm. The largest specimens were recorded at the St. Lucia Estuary. *T. granifera* adults collected in Lake Nhlange and particularly at Lake Sibaya tended to be small (Fig. 5B), although larger specimens were found in sheltered and eutrophic bays. The size of specimens may have been influenced by food availability and quality [36,40]. Both Lake Nhlange and Sibaya are nutrient poor and other species have been reported to have unusually smaller sizes [41]. In contrast, the St. Lucia Estuary has been reported to have very high levels of accumulated microphytobenthos that *T. granifera* can feed on [42,43]. *T. granifera* maturity in this study was reached between 6 and 7.99 mm SH [36]. The erosion of *T. granifera* shells at Catalina Bay was most likely caused by low pH and abrasive effect of sand particles. Interstitial pore water at Catalina Bay, which seeped from sand dune aquifers, had a pH≈6.3. *T. granifera* populations are sensitive to low pH [36,44]. Lake Bhangazi South [45] (Fig. 1) and surrounding small streams tend to have low pH and this may partially explain why the *T. granifera* invasion has not taken hold in these habitats (pers. obs.).

T. granifera size classes were clearly affected by stochastic events in different ways. Size classes between >1 and 7.99 mm SH seemed particularly vulnerable to the sudden increase in salinity during the 2007 mouth breach of St. Lucia (Fig. 4A B). Yet a higher proportion of juveniles was recorded during that period (Fig. 4A–B) and the juvenile to adult ratio also increased (Table S1), indicating that birth rate did not slow down. The ensuing desiccation in 2008 killed most of the population, with only the largest adults able to survive such extreme conditions. This was at least in part due to their tolerance to desiccation [46], ability to burrow and undergo periods of quiescence [20], but also because large *T. granifera* probably took shelter in freshwater seeps. In comparison, smaller and juvenile snails are less tolerant to desiccation [46] and also move over smaller spatial ranges [37], therefore they were not able to reach freshwater seeps and survive. Once favourable conditions were re-established in 2009, adults returned and gave birth to large numbers of juveniles, thus quickly increasing the juvenile to adult ratio. These larger adults carried a great number of juveniles in their brood pouch, thus contributing to the perceived high reproductive output during the recovery period of 2009 (Fig. 4C, Table S1). By the end of 2010, the Catalina Bay population had recovered and its structure and density was comparable to those of other *T. granifera* populations in Maputaland.

The evolutionary significance of brooding may be unclear [47]. However, in *T. granifera*, brooding is associated with increased parental care, which minimizes mortality of vulnerable early life stages. The anatomy of the brood pouch of *T. granifera* is similar to that of *M. tuberculata* [36,48] and the larger the animal, the greater the number of juveniles it can carry. The current study also found a variety of sizes of juveniles in the brood pouch. This suggests that juveniles may be retained during adverse conditions and raises the question of whether the number of juveniles present in a brood pouch is an adequate indicator of fecundity. Under salinities higher than 20, an adult can shift its energy from reproduction to survival [19,50], which involves entering a quiescent state [21]. Therefore, an increase in number of juveniles in brood immediately after an increase in salinity may be due to retention, rather than increase in reproduction rate. The increase in the ratio of juveniles to adults during adverse conditions could then be explained by the release of brooded juveniles after the death of adults and/or births by large and more tolerant adults. An empirical study is needed to address this hypothesis since many factors affect birth [20].

Propagule pressure can be described in terms of quantity of released propagules, the quality of the propagules and the quantity of release events [51]. *T. granifera* reaches high population densities and a single adult with several brooded juveniles may start a new population. The brood pouch plays an important role in the internal cycle of propagule production and dispersal [11], which ensures *T. granifera* persistence and spread even if there is no further input from an external propagule pool. Juveniles grow within the protection of the brood pouch, thus increasing their chance of survival and reducing the time to maturity after birth. This may ensure year-round continuity in *T. granifera* reproductive output, despite unfavorable and unpredictable events.

T. granifera is known to displace indigenous gastropods in freshwater [19,22,23] but its ecological impacts in brackish water are largely unknown. However, ecological impact on estuaries and coastal lakes is difficult to assess because of unprecedented overlaps and interactions between NIS and other stressors [52] such as drought intensification. It would have been very useful to make a comparison of *T. granifera* size structure with that of indigenous *Melanoides tuberculata* and *Bellamya capillata* at Lake Sibaya, since all three species reproduce via parthenogenesis [53]. Unfortunately though, the historically abundant native snails were not found during this study, having possibly been displaced by *T. granifera*. However, it is likely that they persist in deeper water or in parts of Lake Sibaya that were not surveyed. *T. granifera* has been reported to displace *M. tuberculata* under natural settings [23,54]. However, at Lake Nhlange, *T. granifera* and *M. tuberculata* have been found together in a small sheltered and eutrophic bay, but *T. granifera* was more numerous.

An understanding of the population dynamics of NIS is important for predicting their interaction with the environment and determining the best control strategy [55]. Already invaded habitats with reduced biotic influences can present an opportunity to gain insights into how NIS interact with the environment. This study has revealed how *T. granifera*'s population density and structure can change in variable estuarine and coastal environments. High densities, fecundity, and particularly the environmental tolerance of adults with brooded juveniles, ensure the persistence of *T. granifera* despite frequent mouth breaching events and desiccation. The mode of reproduction and the type of embryo development affect population dynamics. However, the ecology of the post-larval and pre-birth stage in the life history of *T. granifera*, which takes place within its brood pouch, needs further consideration. It is suggested that this brood pouch ecology plays a very significant role in the establishment and spread of *T. granifera*, thus also affecting population dynamics. These findings are also relevant to invasions by other parthenogenetic NIS, such as *M. tuberculata* and *P. antipodarum*, across the world.

Supporting Information

Table S1 Summary of physical and chemical parameters, and *Tarebia granifera* populations in Maputaland estuaries and coastal lakes.
(DOC)

Acknowledgments

We are grateful to the iSimangaliso Park Authority and Ezemvelo KZN Wildlife for supporting this project. Special thanks go to R. Taylor, C. Fox and R. Kyle for their invaluable assistance and logistical support. We are also grateful to two anonymous reviewers for their constructive suggestions and contribution to the final manuscript.

Author Contributions

Conceived and designed the experiments: NM GA. Performed the experiments: NM. Analyzed the data: NM. Contributed reagents/materials/analysis tools: NM RP. Wrote the paper: NM RP GA.

References

1. Vrijenhoek RC (1998) Animal clones and diversity. Bio Science 48: 617–628.
2. Jacobsen R, Forbes VE (1997) Clonal variation in life-history traits and feeding rates in the gastropod, *Potamopyrgus antipodarum*: performance across a salinity gradient. Funct Ecol 11: 260–267.
3. Ben-Ami F, Heller J (2007) Temporal patterns of geographic parthenogenesis in a freshwater snail. Biol J Linn Soc 91: 711–718.
4. Maynard-Smith J (1971) What use is sex? J Theor Biol 30: 319–335.
5. Gerritsen J (1980) Sex and parthenogenesis in sparse populations. Amer Nat 115: 718–742.
6. Jokela J, Lively CM, Dybdahl MF, Fox JA (1997) Evidence for a cost of sex in the freshwater snail *Potamopyrgus antipodarum*. Ecology 78: 452–460.
7. Vrijenhoek RC (1979) Factors affecting clonal diversity and coexistence. Amer Zool 19: 787–797.
8. Haag CR, Ebert D (2004) A new hypothesis to explain geographic parthenogenesis. Ann Zool Fenn 41: 539–544.
9. Lively CM (2001) Trematode infection and the distribution and dynamics of parthenogenetic snail populations. Parasitology 123: 19–26.
10. Ben-Ami F, Heller J (2005) Spatial and temporal patterns of parthenogenesis and parasitism in the freshwater snail *Melanoides tuberculata*. J Evolution Biol 18: 138–146.
11. Davis MA (2009) Invasion Biology. New York: Oxford University Press.
12. Williamson M (1996) Biological Invasions. London: Chapman & Hall.
13. Grosholz E (2002) Ecological and evolutionary consequences of coastal invasions. Trends Ecol Evol 17: 22–27.
14. Williams S, Grosholz E (2008) The invasive species challenge in estuarine and coastal environments: marrying management and science. Estuar Coast 31: 3–20.
15. Miller AW, Ruiz GM, Minton MS, Ambrose RF (2007) Differentiating successful and failed molluscan invaders in estuarine ecosystems. Mar Ecol Prog Ser 332: 41–51.
16. Caley P, Lonsdale WM, Pheloung PC (2006) Quantifying uncertainty in predictions of invasiveness, with emphasis on weed risk assessment. Biol Invasions 8: 1595–1604.
17. Kolar CS, Lodge DM (2001) Progress in invasion biology: predicting invaders. Trends Ecol Evol 16: 199–204.
18. Jones JPG, Rasamy JR, Harvey A, Toon A, Oidtmann B, et al. (2009) The perfect invader: a parthenogenic crayfish poses a new threat to Madagascar's freshwater biodiversity. Biol Invasions 11: 1475–1482.
19. Appleton CC, Forbes AT, Demetriades NT (2009) The occurrence, bionomics and potential impacts of the invasive freshwater snail *Tarebia granifera* (Lamarck, 1822) (Gastropoda: Thiaridae) in South Africa. Zool Med Leiden 83: 525–536.
20. Chaniotis BN, Butler JM, Ferguson F, Jobin WR (1980) Bionomics of *Tarebia granifera* (Gastropoda:Thiaridae) in Puerto Rico, an Asian vector of *Paragonimiasis westermani*. Caribbean J Sci 16: 81–89.
21. Pointier JP, Facon B, Jarne P, David P (2003) Thiarid snails, invading gastropods of tropical freshwater habitats. Xenophora 104: 14–20.
22. Karatayev AY, Burlakova LE, Karatayev VA, Padilla DK (2009) Introduction, distribution, spread, and impacts of exotic freshwater gastropods in Texas. Hydrobiologia 619: 181–194.
23. Lopez-Lopez E, Sedeño-Díaz JE, Vega PT, Oliveros E (2009) Invasive mollusks *Tarebia granifera* Lamarck, 1822 and *Corbicula fluminea* Müller, 1774 in the Tuxpam and Tecolutla rivers, Mexico: spatial and seasonal distribution patterns. Aquat Invasions 4: 435–450.
24. Miranda NAF, Perissinotto R, Appleton CC (2010) Salinity and temperature tolerance of the invasive freshwater gastropod *Tarebia granifera*. S Afr J Sci 106: 55–61.
25. Joordens JCA, Wesselingh FP, de Vos J, Vonhof HB, Kroon D (2009) Relevance of aquatic environments for hominins: a case study from Trinil (Java, Indonesia). J Hum Evol 57: 656–671.
26. Hofmann H, Lorke A, Peeters F (2008) Temporal scales of water-level fluctuations in lakes and their ecological implications. Hydrobiologia 613: 85–96.
27. Perissinotto R, Stretch DD, Whitfield AK, Adams JB, Forbes AT, et al. (2010) Temporarily Open/Closed Estuaries in South Africa. New York: Nova Publishers.
28. Taylor R, Adams JB, Haldorsen S (2006) Primary habitats of the St Lucia Estuarine System, South Africa, and their responses to mouth management. Afr J Aquat Sci 31: 31–41.
29. Bate GC, Taylor RH (2007) Sediment salt-load in the St Lucia Estuary during the severe drought of 2002–2006. Environ Geol 55: 1089–1098.
30. Vrieet L, Kelbe B, Haldorsen S, Taylor R (2009) A modelling study of the effects of land management and climatic variations on groundwater inflow to Lake St Lucia, South Africa. Hydrogeol J 17: 1949–1967.
31. Begg G (1978) The Estuaries of Natal. Natal Town and Regional Planning report 41.
32. Bruton MN, Appleton CC (1975) Survey of Mgobezeleni Lake-System in Zululand, with a Note on the Effect of a Bridge on the Mangrove Swamp. T Roy Soc S Afr 41: 283–293.
33. Bruton MN (1980) An outline of the ecology of the Mgobezeleni Lake System at Sodwana, with emphasis on the mangrove community. In: Bruton MN, Cooper KH, eds. Studies on the ecology of Maputaland. Grahamstown: Rhodes University.
34. Bruton MN (1980) An outline of the ecology of Lake Sibaya, with emphasis on the vertebrate community. In: Bruton MN, Cooper KH, eds. Studies on the ecology of Maputaland. Grahamstown: Rhodes University.
35. Begg GW (1980) The Kosi system: aspects of its biology, management and research. In: Bruton MN, Cooper KH, eds. Studies on the ecology of Maputaland. Grahamstown: Rhodes University.
36. Abbott RT (1952) A study of an intermediate snail host (*Thiara granifera*) of the oriental lung fluke (*Paragonimus*). Proc U S Nat Mus 102: 72–116.
37. De la Vega RAR, Fernández LD, Espinosa AQ, García AMH (2003) Change of the weight/foot area coefficient in relation to aggregation in *Tarebia granifera*. Rev Saude Publica 37: 297–300.
38. Facon B, David P (2006) Metapopulation dynamics and biological invasions: A spatially explicit model applied to a freshwater snail. Amer Nat 168: 769–783.
39. Snider SB, Gilliam JF (2008) Movement ecology: Size-specific behavioral response of an invasive snail to food availability. Ecology 89: 1961–1971.
40. Yong M, Sanchez R, Perera G (1987) Seasonal Studies of Two Populations of *Tarebia granifera*. Walkerana Trans POETS Soc 2: 159–163.
41. Bruton MN (1979) The fishes of Lake Sibaya. In: Allanson BR, ed. Lake Sibaya. Hague: Dr. W. Junk Publishers. pp 162–245.
42. Miranda NAF, Perissinotto R, Appleton CC (2011) Feeding dynamics of the invasive gastropod *Tarebia granifera* in coastal and estuarine lakes of northern KwaZulu Natal, South Africa. Estuar Coast Shelf Sci e 91: 442–449.
43. Van der Mollen J, Perissinotto R (2011) Microalgal productivity in an estuarine lake during a drought cycle: The St. Lucia Estuary, South Africa. Estuar Coast Shelf Sci 92: 1–9.
44. Perera G, Yong M (1984) The influence of some abiotic factors on the distribution of freshwater mollusks on the Isle of Youth (Isle of Pines), Cuba. Walkerana Trans POETS Soc 2: 131–139.
45. Hart RC, Appleton CC (1997) A limnological synopsis of Bhangazi South, a dystrophic coastal lake in the Greater St Lucia Wetland Park (KwaZulu/Natal), with comments on its conserva- tion value. South Afr J Aquat Sci 23: 20–41.
46. Facon B, Machline E, Pointier JP, David P (2004) Variation in desiccation tolerance in freshwater snails and its consequences for invasion ability. Biol Invasions 6: 283–293.
47. Strong EE, Glaubrecht M (2007) The morphology and independent origin of ovoviviparity in *Tiphobia* and *Lavigeria* (Caenogastropoda: Cerithioidea: Paludomidae) from Lake Tanganyika. Org Divers Evol 7: 81–105.
48. Ben-Ami F, Hodgson AN (2005) Ovoviviparity and the structure of the brood pouch in *Melanoides tuberculata* (Gastropoda: Prosobranchia: Thiaridae). J Morphol 263: 322–329.
49. Arking R, Buck S, Hwangbo D, Lane M (2002) Metabolic alterations and shifts in energy allocations are corequisites for the expression of extended longevity genes in *Drosophila*. Ann NY Acad Sci 959: 251–262.
50. Núñez V (2010) Differences on allocation of available resources, in growth, reproduction, and survival, in an exotic gastropod of Physidae compared to an endemic one. Iheringia, Sér Zool, Porto Alegre 100: 275–279.
51. Lockwood JL, Hoopes MF, Marchetti MP (2007) Invasion Ecology. Malden, U.S.A.: Blackwell Publishing.
52. Ruiz GM, Fofonoff P, Hines AH, Grosholz ED (1999) Non indigenous species as stressors in estuarine and marine communities: Assessing invasion impacts and interactions. Limnol Oceanogr 44: 950–972.
53. Appleton CC (1980) Non-marine molluscs and schistosomiasis in Maputaland. In: Bruton MN, Cooper KH, eds. Studies on the ecology of Maputaland. Grahamstown: Rhodes University.
54. Ben-Ami F (2006) First report of the invasive freshwater snail *Tarebia granifera* (Lamarck, 1816) (Gastropoda : Thiaridae) from Israel. Nautilus 120: 156–161.
55. Burlakova L, Padilla D, Karatayev A, Hollas D, Cartwright L, et al. (2010) Differences in population dynamics and potential impacts of a freshwater invader driven by temporal habitat stability. Biol Invasions 12: 927–941.

PLOS ONE: MANUSCRIPT IN REVIEW

Analysis of Environmental and Invasion Impact in Shallow Water Benthic Assemblages of North-Eastern South Africa.

Nelson A. F. Miranda[1,]*, Renzo Perissinotto[1]

[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.

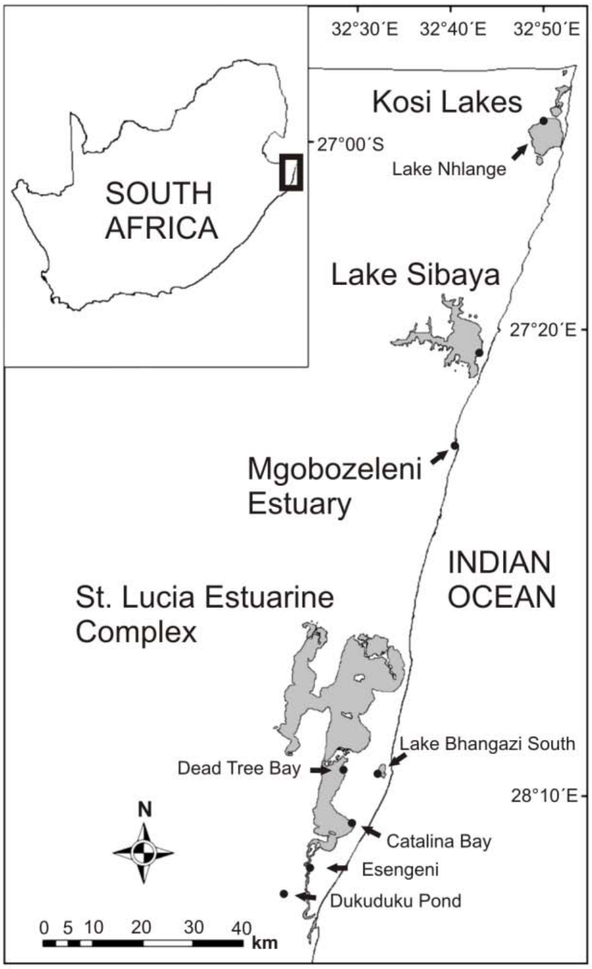


Figure 1. Map of the study area. Sampling locations within the iSimangaliso Wetland Park are indicated by arrow and dot.

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 Nhlange (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 m^2, 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

[5]. 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 Nhlange (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 Mgobozeleni (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_{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.

Sources of variation	**df**	**SS**	**MS**	***F***	***P***	**F_{denom}**	**Permutable units**
Before vs. After = BA	1	53522.66	53522.66				
Location = L	5	134473.7	26894.74				
Controls vs. Impacts = CI	1	57942.2	57942.2				
Among Controls = C	1	42536.14	42536.14				
Among Impacts = I	3	33995.38	11331.79				
BA×L	5	88695.22	17739.04				
BA×CI	1	23504.12	23504.12	10.3	0.0152	Time (BA×L)	72 Time (BA×L) cells
BA×C	1	25888.41	25888.41	2.8	*0.0278*	Time (BA×C)	24 Time (BA×C) cells
BA×I	3	41685.87	13895.29	2.5	*0.0054*	Time (BA×I)	48 Time (BA×I) cells
Time (BA×L)	32	72831.15	2275.97				
Time (BA×C)	10	93888.29	9388.83				
Time (BA×I)	20	112403.6	5620.18				
Residual	58	107490.1	1853.28				

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.

Sources of variation	**df**	**SS**	**MS**	F	P	F_{denom}	**Permutable units**
Before vs. After = BA	1	32190.59	32190.59				
Location = L	5	105556.3	21111.26				
Controls vs. Impacts = CI	1	43426.82	43426.82				
Among Controls = C	3	83234.6	27744.87				
Among Impacts = I	1	21501.91	21501.91				
BA×L	5	115140.1	23028.02				
BA×CI	1	31381.56	31381.56	6.8	0.0086	Time (BA×L)	72 Time (BA×L) cells
BA×C	3	39301.22	13100.41	2.8	0.4052	Time (BA×C)	60 Time (BA×C) cells
BA×I	1	16766.79	16766.79	3.2	*0.0124*	Time (BA×I)	36 Time (BA×I) cells
Time (BA×L)	32	147771.1	4617.85				
Time (BA×C)	20	93670.77	4683.54				
Time (BA×I)	10	51619.23	5161.92				
Residual	58	151196.2	2606.83				

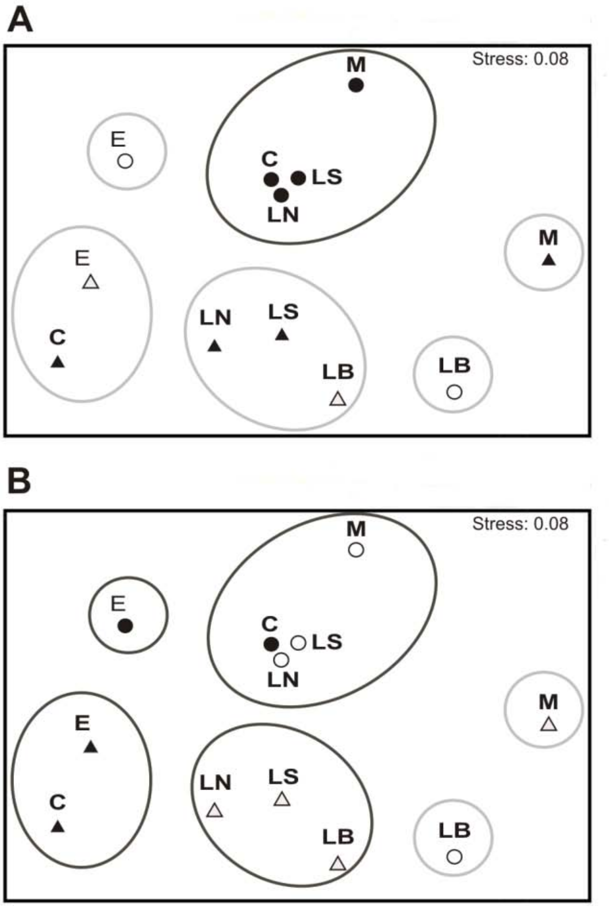


Figure 2. Non-metric multidimensional scaling (nMDS) of BACI design data. nMDS plots are based on a Bray-Curtis dissimilarity measure of centroids representing each location sampled in the context of a putative *Tarebia granifera* invasion impact (**A**) and a putative drought intensification impact (**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).

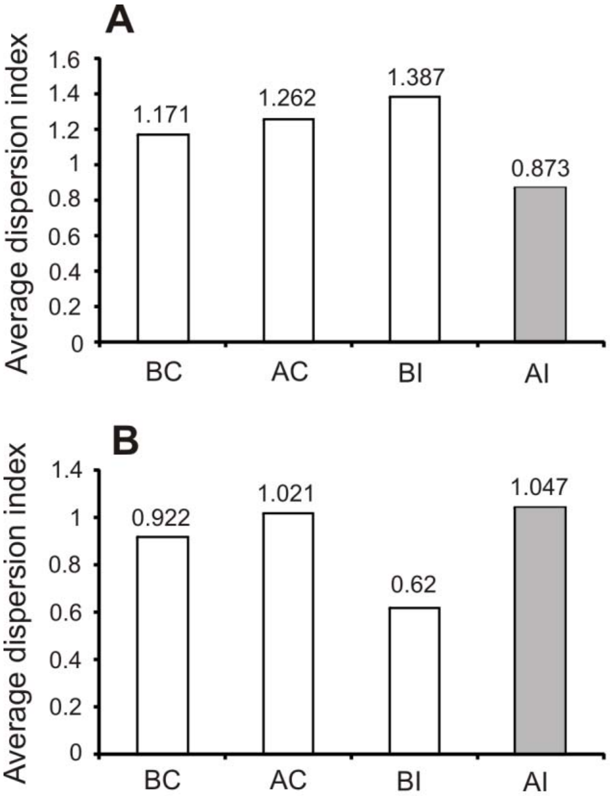


Figure 3. Average multivariate dispersion index of BACI design. Represented are data from groups of control and impact locations before (BC, BI) and after (AC, AI) the putative impact of *Tarebia granifera* invasion (**A**) and drought intensification (**B**). Shaded columns indicate actual putatively 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.

Group	**Taxa**	**Av. Abund**	**Contrib %**	**Cum. %**
BC				
	Chironomid larvae	178.9	29.9	29.9
	Assiminea cf. *ovata*	9568.5	21.6	51.6
	Copepods	10200.6	19.5	71.1
	Oligochaetes	64.4	10.9	82
	Melanoides tuberculata	42.5	5.9	88.1
	Caridina nilotica	34.4	5.8	93.9
BI				
	Assiminea cf. *ovata*	35566.6	38.1	38.1
	Oligochaetes	196.1	19.1	57.2
	Chironomid larvae	107.4	13.7	70.9
	Polychaetes	231.3	6.8	77.7
	Palaemon concinnus	0.8	6.2	83.9
	Apseudes digitalis	594.3	4.4	88.3
	Amphipods	92.3	3.8	92.1
AC				
	Caridina nilotica	39.9	46.2	46.2
	Polychaetes	25.3	42.9	89.1
	Coleoptera	5.2	5.3	94.4
AI				
	Tarebia granifera	5889.8	98.5	98.5

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.

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

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 M_1) 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_{denom} column.

Sources of variation	df		SS	MS	*F*	*P*	F_{denom}	Permutable units
Location = L	7		189321.8	27045.97	9.1	0.0002	Site (L)	24 Site (L) cells
Controls vs. Impacts = CI		1	96415.28	96415.28	32.5	0.0002	Site (L)	24 Site (L) cells
Among Controls = C		3	68076.49	22692.16	20.4	0.0002	Site (C)	12 Site (C) cells
Among Impacts = I		3	24830	8276.67	1.7	0.1102	Site (I)	12 Site (I) cells
Site (L)	16		47530.2	2970.63	9.5	0.0002	Residual	72 raw data units
Site (C)		8	8917.1	1114.64	3.9	0.0002	Res C	36 raw data units
Site (I)		8	38613.14	4826.64	14.4	0.0002	Res I	36 raw data units
Residual	48		14975.72	311.99				
Res C		24	6934.41	288.93				
Res I		24	8041.30	335.05				

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_{denom} column.

Sources of variation	df	SS	MS	F	P	F_{denom}	Permutable units
Location = L	7	189321.8	27045.97	9.1	0.0002	Site (L)	24 Site (L) cells
Controls vs. Impacts = CI	1	39022.78	39022.78	13.1	0.0002	Site (L)	24 Site (L) cells
Among Controls = C	4	112421.3	28105.33	10.9	0.0002	Site (C)	15 Site (C) cells
Among Impacts = I	2	37877.65	18938.82	5.2	0.0036	Site (I)	9 Site (I) cells
Site (L)	16	47530.2	2970.64	9.5	0.0002	Residual	72 raw data units
Site (C)	10	25836.43	2583.64	8.3	0.0002	Res Cs	45 raw data units
Site (I)	6	21693.75	3615.63	11.5	0.0002	Res I	27 raw data units
Residual	48	14975.72	311.99				
Res C	30	9301.16	310.04				
Res I	18	5674.56	315.25				

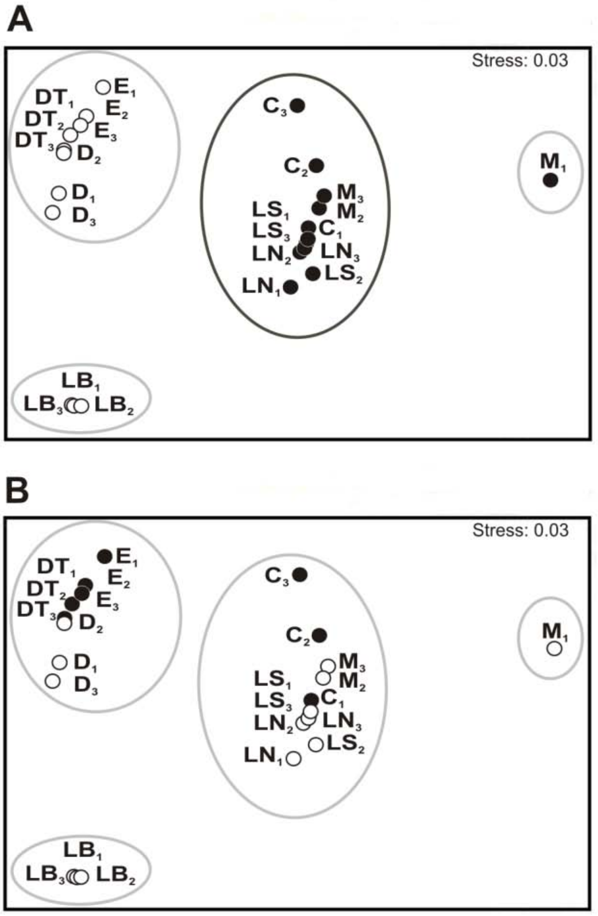


Figure 4. Non-metric multidimensional scaling (nMDS) of ACI design data. nMDS plots are based on a Bray-Curtis dissimilarity measure of centroids representing sites within locations sampled in the context of a putative *Tarebia granifera* invasion impact (**A**) and a putative drought intensification 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 Nhlange (LN), Lake Sibaya (LS), Mgobozeleni Estuary (M), Lake Bhangazi (LB), Dead Tree Bay (DT), Catalina Bay (C), Esengeni (E) and Dukuduku Pond (D).

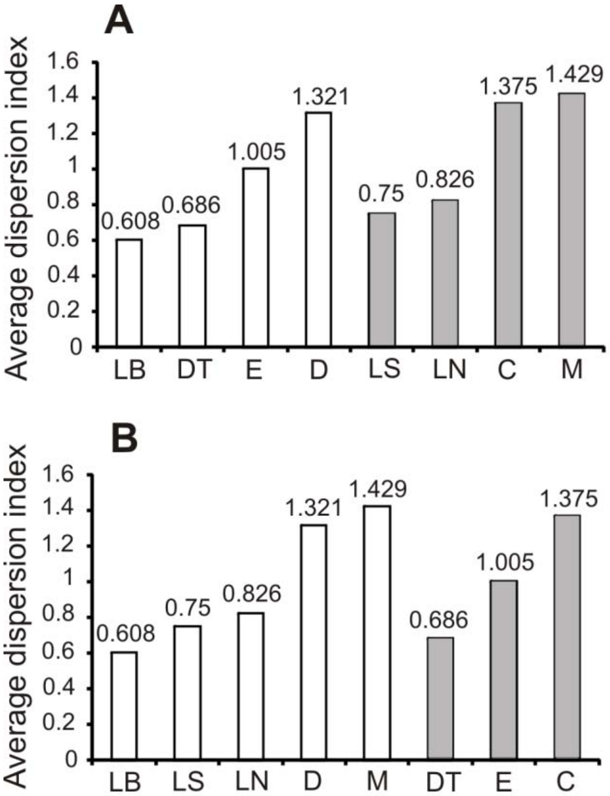


Figure 5. Average multivariate dispersion index of ACI design. Represented are data collected at impact locations (shaded columns) and control locations (unshaded columns) in the context of the putative impact of *Tarebia granifera* invasion (**A**) and drought intensification (**B**). Locations are 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.

Group	Taxa	Av. Abund	Contrib %	Cum. %
Putative *T. granifera* invasion:				
C				
	Polychaetes	56.4	81.4	81.4
	Caridina nilotica	30.2	13.6	94.9
I				
	Tarebia granifera	6087.1	98.2	98.2
Putative Drought intensification:				
C				
	T. granifera	4293.5	74.9	74.9
	Caridina nilotica	31.34	13.4	88.3
	Polychaetes	37.9	6.5	94.9
I				
	Polychaetes	159.3	86.3	86.3
	T. granifera	960.3	12.3	98.6

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 priory* 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

1. Lockwood JL, Hoopes MF, Marchetti MP (2007) Invasion Ecology. Malden, U.S.A.: Blackwell Publishing.
2. Parker IM, Simberloff D, Lonsdale WM, Goodell K, Wonham M, et al. (1999) Impact: Toward a framework for understanding the ecological effects of invaders. Biol Invasions 1: 3-19.
3. Ruiz GM, Fofonoff P, Hines AH, Grosholz ED (1999) Non-indigenous species as stressors in estuarine and marine communities : Assessing invasion impacts and interactions. Limnol Oceanogr 44: 950-972.
4. Fraterrigo JM, Rusak JA (2008) Disturbance-driven changes in the variability of ecological patterns and processes. Ecol Lett 11: 756-770.
5. Underwood AJ (1992) Beyond BACI: The detection of environmental impacts on populations in the real, but variable, world. J Exp Mar Biol Ecol 161: 145-178.
6. Underwood AJ (1996) On Beyond BACI: Sampling designs that might reliably detect environmental disturbances. In: Russell JS, Craig WO, editors. Detecting Ecological Impacts. San Diego: Academic Press. pp. 151-175.
7. Underwood AJ (1997) Experiments in ecology: Their logic design and interpretation using analysis of variance. Cambridge: Cambridge University Press.
8. Forrest BM, Taylor MD (2002) Assessing invasion impact: Survey design considerations and implications for management of an invasive marine plant. Biol Invasions 4: 375-386.
9. Glasby TM (1997) Analysing data from post-impact studies using asymmetrical analyses of variance: A case study of epibiota on marinas. Aust J Ecol 22: 448-459.
10. Lardicci C, Rossi F, Maltagliati F (1999) Detection of thermal pollution: Variability of benthic communities at two different spatial scales in an area influenced by a coastal power station. Mar Pollut Bull 38: 296-303.

11. Guidetti P, Fanelli G, Fraschetti S, Terlizzi A, Boero F (2002) Coastal fish indicate human-induced changes in the Mediterranean littoral. Mar Environ Res 53: 77-94.
12. Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. Aust J Ecol: 117-143.
13. Legendre P, Anderson MJ (1999) Distance-Based Redundancy Analysis: Testing Multispecies Responses in Multifactorial Ecological Experiments. Ecol Monogr 69: 1-24.
14. Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecol 26: 32-46.
15. Terlizzi A, Benedetti-Cecchi L, Bevilacqua S, Fraschetti S, Guidetti P, et al. (2005) Multivariate and univariate asymmetrical analyses in environmental impact assessment: A case study of Mediterranean subtidal sessile assemblages. Mar Ecol Prog Ser 289: 24-42.
16. Terlizzi A, Scuderi D, Fraschetti S, Anderson M (2005) Quantifying effects of pollution on biodiversity: A case study of highly diverse molluscan assemblages in the Mediterranean. Mar Biol 148: 293-305.
17. Miranda NAF, Perissinotto R, Appleton CC (2011) Population structure of an invasive parthenogenetic gastropod in coastal lakes and estuaries of Northern KwaZulu-Natal, South Africa. PLoS ONE 6: e24337.
18. Appleton CC, Forbes AT, Demetriades NT (2009) The occurrence, bionomics and potential impacts of the invasive freshwater snail *Tarebia granifera* (Lamarck, 1822) (Gastropoda: Thiaridae) in South Africa. Zool Med Leiden 83: 525-536.
19. Miranda NAF, Perissinotto R, Appleton CC (2010) Salinity and temperature tolerance of the invasive freshwater gastropod *Tarebia granifera*. S Afr J Sci 106: 55-61.
20. Appleton CC (2003) Alien and invasive freshwater gastropoda in South Africa. Afr J Aquat Sci 28: 69-81.
21. Taylor R, Adams JB, Haldorsen S (2006) Primary habitats of the St Lucia Estuarine System, South Africa, and their responses to mouth management. Afr J Aquat Sci 31: 31-41.
22. Whitfield A, Taylor RH (2009) A review of the importance of freshwater inflow to the future conservation of Lake St Lucia. Aquat Conserv 19: 838-848.
23. Taylor RH (2006) Ecological responses to changes in the physical environment of the St Lucia estuary. Doctor Scientiarum thesis. Norwegian University of Life Sciences. 2006:7. ISSN: 0802-3220, ISBN: 82-575-0706-7.

24. Boltt RE (1975) The benthos of some southern African lakes Part V: The recovery of the benthic fauna of St Lucia Lake following a period of excessively high salinity. Trans Roy Soc South Afr 41: 295-323.
25. Pillay D, Perissinotto R (2008) The benthic macrofauna of the St. Lucia Estuary during the 2005 drought year. Estuar Coast Shelf Sci 77: 35-46.
26. MacKay F, Cyrus D, Russell KL (2010) Macrobenthic invertebrate responses to prolonged drought in South Africa's largest estuarine lake complex. Estuar Coast Shelf Sci 86: 553-567.
27. Cyrus D, Jerling H, MacKay F, Vivier L (2011) Lake St Lucia, Africa's largest estuarine lake in crisis: Combined effects of mouth closure, low levels and hypersalinity. S Afr J Mar Sci 107: 1-13.
28. Boltt RE (1969) The benthos of some southern African lakes. Part II: The epifauna and infauna of Lake Sibaya. Trans Roy Soc South Afr 38: 249-269.
29. Boltt RE, Allanson BR (1975) The benthos of some southern African Lakes Part III: The benthic fauna of Lake Nhlange, KwaZulu, South Africa. Trans Roy Soc South Afr 41: 241-262.
30. Hart RC (1979) The invertebrate comunities: Zooplankton, zoobenthos and littoral fauna. In: Allanson BR, editor. Lake Sibaya. Hague: Dr. W. Junk Publishers. pp. 108-161.
31. Ellis D (1985) Taxonomic sufficiency in pollution assessment. Mar Pollut Bull 16: 459.
32. Terlizzi A, Bevilacqua S, Fraschetti S, Boero F (2003) Taxonomic sufficiency and the increasing insufficiency of taxonomic expertise. Mar Pollut Bull 46: 556-561.
33. Anderson MJ (2003) XMATRIX: A FORTRAN computer program for calculating design matrices for terms in ANOVA designs in a linear model. Department of Statistics, University of Auckland, Auckland.
34. Anderson MJ (2004) DISTLM v.5: A FORTRAN computer program to calculate a distance-based multivariate analysis for a linear model. Department of Statistics, University of Auckland, Auckland.
35. Anderson MJ (2001) Permutation tests for univariate or multivariate analysis of variance and regression. Can J Fish Aquat Sci 58: 626-639.
36. Anderson MJ, Robinson J (2003) Generalised discriminant analysis based on distances. Aust NZ J Stat 45: 301-318.
37. Gray JS (1990) Statistics and the precautionary principle. Mar Pollut Bull 21: 174-176.
38. Clarke KR, Warwick RM (2001) Change in marine Communities: An approach to statistical analysis and interpretation, 2nd edition. PRIMER-E: Plymouth.

39. Warwick RM, Clarke KR (1993) Increased variability as a symptom of stress in marine communities. J Exp Mar Biol Ecol 172: 215-226.

40. Vrdoljak SM, Hart RC (2007) Groundwater seeps as potentially important refugia for freshwater fishes on the Eastern Shores of Lake St Lucia, KwaZulu-Natal, South Africa. Afr J Aquat Sci 32: 125-132.

41. Taylor R, Kelbe B, Haldorsen S, Botha G, Wejden B, et al. (2006) Groundwater-dependent ecology of the shoreline of the subtropical Lake St Lucia estuary. Environ Geol 49: 586-600.

42. Perissinotto R, Stretch DD, Whitfield AK, Adams JB, Forbes AT, et al. (2010) Temporarily Open/Closed Estuaries in South Africa. New York: Nova Publishers.

43. Miranda NAF, Perissinotto R, Appleton CC (2011) Feeding dynamics of the invasive gastropod *Tarebia granifera* in coastal and estuarine lakes of northern KwaZulu Natal, South Africa. Estuar Coast SHelf Sci 91: 442-449.

44. Byers JE, Reichard S, Randall JM, Parker IM, Smith CS, et al. (2002) Directing research to reduce the impacts of nonindigenous species. Conserv Biol 16: 630-640.

45. Buckley YM (2008) The role of research for integrated management of invasive species, invaded landscapes and communities. J Appl Ecol 45: 397-402.

46. West EJ, Davis AR, Barnes PB, Wright JT (2009) The role of recreational activities in creating fragments of invasive *Caulerpa taxifolia*. J Exp Mar Biol Ecol 376: 17-25.

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).

References

Andersen M (2005) Potential applications of population viability analysis to risk assessments for invasive species. Hum Ecol Risk Assess 11:1083-1095

Anderson MJ (2001) Permutation tests for univariate or multivariate analysis of variance and regression. Can J Fish Aquat Sci 58:626-639

Appleton CC (1980) Non-marine molluscs and shistosomiasis in Maputaland. In: Bruton MN and Cooper KH (eds) Studies on the ecology of Maputaland. Rhodes University, Grahamstown

Buckley YM (2008) The role of research for integrated management of invasive species, invaded landscapes and communities. J Appl Ecol 45:397-402

Chaniotis BN, Butler JM, Ferguson F, Jobin WR (1980) Presence of males in Puerto Rican *Thiara* (*Tarebia*) *granifera* (Gastropoda: Thiaridae), a snail thought to be parthenogenetic. Caribb J Sci 16:95-97

Cohen AN, Carlton JT (1998) Accelerating invasion rate in a highly invaded estuary. Science 279:555-558

Cyrus D, Jerling H, MacKay F, Vivier L (2011) Lake St Lucia, Africa's largest estuarine lake in crisis: Combined effects of mouth closure, low levels and hypersalinity. S Afr J Mar Sci 107:1-13

Davis MA (2009) Invasion Biology. Oxford University Press, New York

Davis MA, Grime JP, Thompson K (2000) Fluctuationg resources in plant communities: a general theory of invasibility. J Ecol 88:528-536

Drown DM, Levri EP, Dybdahl MF (2011) Invasive genotypes are opportunistic specialists not general purpose genotypes. Evol Appl 4:132-143

Facon B, Pointier JP, Jarne P, Sarda V, David P (2008) High genetic variance in life-history strategies within invasive populations by way of multiple introductions. Curr Biol 18:363-367

Genner MJ, Todd JA, Michel E, Erpenbeck D, Jimoh A, Joyce DA, Piechocki A, Pointier JP (2007) Amassing diversity in an ancient lake: evolution of a morphologically diverse parthenogenetic gastropod assemblage in Lake Malawi. Mol Ecol 16:517-530

Giovanelli A, Vieira MV, da Silva C (2002) Interaction between the intermediate host of schistosomiasis in Brazil *Biomphalaria glabrata* (Planorbidae) and a possible competitor *Melanoides tuberculata* (Thiaridae): I. Laboratory experiments. Mem I Oswaldo Cruz 97:363-369

Giovanelli A, Vieira MV, da Silva C (2003) Apparent competition through facilitation between Melanoides tuberculata and *Biomphalaria glabrata* and the control of schistosomiasis. Mem I Oswaldo Cruz 98:429-431

Gomez JD, Vargas M, Malek EA (1990) Biological control of *Biomphalaria glabrata* by *Thiara granifera* under Laboratory Conditions. Trop Med Parasitol 41:43-45

Griffiths HI, Butlin RK (1995) A timescale for sex versus parthenogenesis: evidence from subfossil Ostracods. Proc Biol Sci 260:65-71

Grosholz E (2002) Ecological and evolutionary consequences of coastal invasions. Trend Ecol Evol 17:22-27

Heger T, Trepl L (2003) Predicting Biological Invasions. Biol Invasions 5:313-321

Herbert DG (1998) Molluscan conservation in South Africa: Diversity, issues and priorities. J Conchol Spec Publ 2:61-76

Lodge DM (1993) Biological invasions: lessons for ecology. Trends Ecol Evol 8:133-137

Lockwood JL, Hoopes MF, Marchetti MP (2007) Invasion Ecology. Blackwell Publishing, Malden, U.S.A.

Madsen H (1979) Further laboratory studies on the interspecific competition between *Helisoma duryi* (Wetherby) and the intermediate hosts of *Schistosoma mansoni* (Sambon), *Biomphalaria alexandrina* (Ehrenberg) and *B. camerunensis* (Boettger). Hydrobiologia 66:181-192

Miller AW, Ruiz GM, Minton MS, et al. (2007) Differentiating successful and failed molluscan invaders in estuarine ecosystems. Mar Ecol Prog Ser 332:41-51

Miller PS, Lacy RC (2005) VORTEX: A stochastic simulation of the extinction process. Version 9.50 User’s Manual., Apple Valley, MN: Conservation Breeding Specialist Group (SSC/IUCN).

Moosa V (2000) An environmental perspective on invasive alien/aquatic invaders. In: Preston G, Brown G and Van Wyk E (eds) Proceedings of the South Africa/USA Bi-National Commission Conference on Best Management Practices for Preventing and Controlling Invasive Alien Species., pp. 8-10

Myers MJ, Meyer CP, Resh VH (2000) Neritid and thiarid gastropods from French Polynesian streams: how reproduction (sexual, parthenogenetic) and dispersal (active, passive) affect population structure. Freshwater Biol 44:535-545

Rocha-Miranda F, Martins-Silva MJ (2006) First record of the invasive snail *Melanoides tuberculatus* (Gastropoda: Prosobranchia: Thiaridae) in the Paranã River basin, GO, Brazil. Braz J Biol 66

Roman J, Darling JA (2007) Paradox lost: genetic diversity and the success of aquatic invasions. Trends Ecol Evol 22:454-464

Ruiz GM, Fofonoff P, Hines AH, et al. (1999) Non-indigenous species as stressors in estuarine and marine communities: Assessing invasion impacts and interactions. Limnol Oceanogr 44:950-972

Samadi S, Mavárez J, Pointier JP, Delay B, Jarne P (1999) Microsatellite and morphological analysis of population structure in the parthenogenetic freshwater snail *Melanoides tuberculata*: insights into the creation of clonal variability. Mol Ecol 8:1141-1153

Schreiber ESG, Quinn GP, Lake PS (2003) Distribution of an alien aquatic snail in relation to flow variability, human activities and water quality : Invasive species in freshwater: impacts and distributions. Freshwater Biol 48:951-961

Schreiber ESG, Lake PS, Quinn GP (2002) Facilitation of native stream fauna by an invading species? Experimental investigations of the interaction of the snail, *Potamopyrgus antipodarum* (Hydrobiidae) with native benthic fauna. Biol Invasions 4:317-325

Shea K, Chesson P (2002) Community ecology theory as a framework for biological invasions. Trends in Ecology and Evolution 17:170-176

Taylor R, Kelbe B, Haldorsen S, Botha G, Wejden B, Været L, Simonsen M (2006) Groundwater-dependent ecology of the shoreline of the subtropical Lake St Lucia estuary. Environ Geol 49, 586-600

Terlizzi A, Benedetti-Cecchi L, Bevilacqua S, et al. (2005a) Multivariate and univariate asymmetrical analyses in environmental impact assessment: A case study of Mediterranean subtidal sessile assemblages. Mar Ecol Prog Ser 289:24-42

Terlizzi A, Scuderi D, Fraschetti S, et al. (2005b) Quantifying effects of pollution on biodiversity: A case study of highly diverse molluscan assemblages in the Mediterranean. Mar Biol 148:293-305

Thuiller W, Gallien L, Boulangeat I, et al. (2010) Resolving Darwin's naturalization conundrum: a quest for evidence. Diversity Distrib:1-15

Underwood AJ (1978) An experimental evaluation of competition between three species of intertidal prosobranch gastropods. Oecologia 33:185-202

Whitfield A, Taylor RH (2009) A review of the importance of freshwater inflow to the future conservation of Lake St Lucia. Aquat Conserv 19:838-848

Winsome T, Epstein L, Hendrix PF, et al. (2006) Competitive interactions between native and exotic earthworm species as influenced by habitat quality in a California grassland. Appl Soil Ecol 32:38-53