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Feeding dynamics of invasive (*Tarebia granifera*) and native (*Melanoides tuberculata*) freshwater gastropods.

By

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A research dissertation submitted in fulfilment of the academic requirements for the degree of
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in Biological Sciences

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PREFACE

The fieldwork mentioned in this study was conducted in the iSimangaliso Wetland Park, St Lucia, KwaZulu-Natal, South Africa. The Gastropoda sampled in this study were collected under an iSimangaliso Wetland Park Authority Research Agreement together with the Environmental and Fisheries Research Permits (RES 2017/71 and RES 2019/93) issued by the South African Department of Agriculture, Forestry and Fisheries). The research contained in this MSc thesis was completed by the candidate while based in the School of Life Science, University of KwaZulu-Natal, Westville, South Africa, under the supervision of Dr Nicola K. Carrasco and Dr Nelson A.F. Miranda. This project was funded by the National Research Foundation (NRF, unique grant number 109523).

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

[redacted]

Signed: Dr Nicola K. Carrasco
Date: 9 Aug 2021

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Signed: Dr Nelson A.F. Miranda
Date: 9 Aug 2021

DECLARATION: PLAGIARISM

I, **Senine Naidoo**, declare that:

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v) Where I have used material for which publications followed, I have indicated in detail my role in the work;

vi) This dissertation is primarily a collection of material, that was prepared by myself, published as journal articles or presented as a poster and oral presentations at conferences. In some cases, additional material has been included;

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ABSTRACT

Tarebia granifera has invaded several South African estuaries, outnumbering native gastropods such as *Melanoides tuberculata*. Comparative feeding dynamics can aid in estimating and understanding invasion impacts. This study aimed to explore the feeding dynamics of these gastropods with the use of two different approaches. Stable Isotope Analyses (SIAs) were used to investigate the dietary preferences and niche overlaps in the St Lucia Estuary, while Functional Response Experiments measured the relationship between the consumption rate of a food resource and its availability. These experiments were run both with and without the presence of heterospecific chemical cues (secondary metabolites secreted by either species) to determine whether chemical cues influence the functional responses of *T. granifera* and *M. tuberculata.* Mixed species experiments were also conducted to observe individual behaviour (active, inactive and feeding) over time and measure resource consumption when both species are placed together under high and low food availability. The SIA showed that both gastropods exhibited generalist diets, however, due to niche partitioning, there was no significant difference in their dietary niche overlap. In the FR experiments, both species exhibited Type II FRs and had similar feeding rates. However, in the presence of heterospecific chemical cues, *M. tuberculata* exhibited a higher feeding rate at high food availabilities, whereas *T. granifera* showed efficient feeding dynamics under limited food resources. In the mixed species experiments, *T. granifera* spent more time feeding under low food availability while *M. tuberculata* spent more time feeding under high food availability. This study showed that in certain conditions (e.g. high resource availability) the alien species did not always have a feeding advantage. A multiple method approach is recommended when assessing ecological impacts of invasive species.

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LIST OF ABBREVIATIONS AND SYMBOLS

Δ or δ - Change

°C - Degrees Celsius

‰ - Per Mille

a - Attack Rate

AIS - Alien and Invasive Species Regulations

ANOVA - Analysis of Variances

C - Carbon

CC - Chemical Cue

CaCO3 - Calcium Carbonate (Inorganic Carbonate)

df - Degrees of Freedom

F_{max} - Maximum Feeding Rate

FR - Functional Response

Frair - Functional Response Analysis in R

FRR - Functional Response Ratio

GEF - Global Environment Facility

h - Handling Time

HCl - Hydrochloric Acid

IDO - Isotopic Dietary Overlap

KZN - KwaZulu-Natal

MANOVA - Multivariate Analysis of Variance

MCMC – Markov Chain Monte Carlo

MPB - Microphytobenthos

N - Nitrogen

NEM:BA - National Environmental Management: Biodiversity Act

NRF - National Research Foundation

PERMANOVA - Permutational Multivariate Analysis of Variances

POM - Particulate Organic Matter

PPG - Potential Plant Growth RGF- Reinforced Glass Fibre

SS - Single Species

SD - Standard Deviation

SEA - Standard Ellipse Area

SEA_C - Standard Ellipse Area Corrected

SH – Shell Height

SIA - Stable Isotope Analysis

SIBER - Stable Isotope Bayesian Ellipses in R
SIMMR - Stable Isotope Mixing Models in R
SOM - Sedimentary Organic Matter
sp. - Species
TEFs -Trophic Enrichment Factors
UNESCO - United Nations Educational, Scientific and Cultural Organization
VOCs - Volatile Organic Compounds
WHO - World Health Organisation

CHAPTER 1

General Introduction

Biological invasions: introduction and dispersal of invasive species

An alien species can be described as a species that relocates and establishes its population beyond its native geographic range (Richardson et al. 2000; Lockwood et al. 2007; Wilson 2009; Blackburn et al. 2011; Robinson et al. 2016). The expansion of an alien species beyond biogeographical barriers can be human-mediated (Blackburn et al. 2011, Robinson et al. 2016) via purposeful introduction (e.g. as biocontrol agents) or accidental introduction (e.g. via aquarium industries or incorrect disposal of organisms) (Pointier et al. 1994; Appleton et al. 2009; Miranda et al. 2010). However, an alien species that spreads from the point of introduction and affects ecosystem functioning (i.e. influencing the native abiotic and biotic factors) is characterized as an invasive species. (Lockwood et al. 2007; Miranda et al. 2011a, Robinson et al. 2016). Other factors that influence biological invasions include 1) the density of species that were introduced, 2) the reproductive rate of the native species, 3) the predator abundance, 4) the functional response of the non-native to the prey (Twardochleb et al. 2012) and 5) resource availability (Catford et al. 2009). The dispersal of an invasive species within surrounding areas can occur passively via birds such as waterfowl (ingested then excreted), through pumping of water from one waterbody to another (e.g. dredging) and biofouling (Appleton et al. 2009). Biological invasions can have significant ecological impacts on multiple levels of organisation, i.e. from individuals to populations and ecosystems (Parker et al. 1999). The introduction of invasive species into an ecosystem can have many evolutionary impacts whereby the native species are subjected to selective pressure/s that influence factors such as reproduction, growth, feeding activity, distribution and behaviour (Trussell 1996; van der Velde et al. 2006). Therefore, determining the effects of invasive species on an ecosystem is vital in ecosystem ecology, ecosystem management and invasion biology (Hill et al. 2015).

Aquatic ecosystems are challenging environments to investigate due to the complexity of ecological interactions (Alexander et al. 2012; Miranda and Perissinotto, 2012; Heinrichs et al. 2020). There has been an increased focus on aquatic invasive species in South Africa in order to address the knowledge gaps regarding the ecological consequences of invasive species (Miranda et al. 2010, Weyl et al. 2020). These ecological consequences include competitive exclusion of native species (Griffiths et al. 1992), trophic restructure (Vander Zanden et al. 1999) and biotic homogenization (McKinney and Lockwood, 1999; Cambray et al. 2003). Feeding dynamics determine the trophic structure of food webs and thus influence the complexities of ecosystems (Williams and Martinez, 2004). Aquatic invasive species can occur at any trophic level and have similar ecological impacts on native ecosystems. Macrophytes such as *Eichhornia crassipes* (Water Hyacinth), *Myriophyllum aquaticum*

(Parrot’s Feather), *Sagittaria platyphylla* (Delta Arrowhead) and *Egeria densa* (Brazilian Waterweed) are just a few examples of crucial non-native plant species that have successfully invaded South African aquatic ecosystems (Hill et al. 2020). These invasive plant species are known to cause significant impacts (e.g. decreasing oxygen levels) on aquatic ecosystems due to their rapid vegetative and sexual reproduction (resulting in high densities), efficient dispersal mechanisms, high phenotypic plasticity and the ability to redevelop from fragments (Hill and Coetzee, 2017). Although biological control measures have been implemented, they have not been sufficient to prevent the dispersal of these invasive species. The addition of chemical and mechanical control measures has been more effective (Hill and Coetzee, 2017). Invasive aquatic fauna such as the *Cherax quadricarinatus* (Redclaw crayfish) has also raised concern in South African aquatic systems (Nunes et al. 2017). This non-native species was introduced in aquaculture research in the 1980s, however, this species escaped from facilities in Swaziland and invaded numerous river systems within South Africa (e.g. Phongolo River, Mbuluzi River and Usutu River) (Nunes et al. 2017). Although the impacts of this invasive species are not well understood, commercial industries have been affected as *C. quadricarinatus* gets entangled in the gill nets, which subsequently affects the quality of catch and the profits in small-scale fisheries (Weyl et al. 2017). This invader has also caused concern for other native trophically analogous predators, invertebrate populations and disrupted substrate-spawning fishes' reproductive activity (Nunes et al. 2017). South African legislation, the Alien and Invasive Species (AIS) Regulation in the National Environmental Management: Biodiversity Act (Act no 10 of 2004), prohibits the movement, release and importation of invasive species. However, it is difficult to enforce these regulations due to the lack of resources (such as finances and workforce) and many unintentionally relocated/introduced species (NEM:BA 2014; Nunes et al. 2017).

Tarebia granifera: a prominent threat in South African aquatic systems

Tarebia granifera is a freshwater gastropod, also known as “Quilted Melania”, which originated from the Indo-Malayan and Oceania realms (Madhyastha and Dutta, 2012) (Figure 1.1). *Tarebia granifera* is a successful invader in Mexico (López-López et al. 2009), United States of America, Venezuela, Hawaii and South Africa (Abbott 1952; Appleton and Nadasan, 2002; Appleton et al. 2009). *Tarebia granifera* was introduced to South African estuaries in the 1990s via the aquarium trade industries (Cowie 1998; Appleton and Nadasan, 2002; Miranda et al. 2010) and was first reported in a reservoir located in Mandeni, northern KwaZulu-Natal (Appleton and Nadasan, 2002). However, since the 1990s, this invasive species has expanded approximately five degrees of latitude (25 - 30°S) (Appleton et al. 2009; Jones et al. 2017) and has become widespread along areas such as the eastern coast of South Africa (particularly KwaZulu-Natal), Kruger National Park and Mpumalanga (Appleton et al. 2009).

Figure 1.1: Adult (21 mm) *Tarebia granifera* (invasive) from Catalina Bay, KwaZulu-Natal, South Africa (Image supplied by Dr N.A.F. Miranda).

Tarebia granifera has disrupted many benthic communities of South African estuaries (Miranda et al. 2011a) (Figure 1.2). In Mgobozeleni Estuary, *T. granifera* invaded to such an extent that no other macrobenthic invertebrate existed (Miranda and Perissinotto, 2014). Within the St Lucia Estuary, *T. granifera* has outnumbered and displaced native species such as *Assiminea* cf. *capensis* (Miranda and Perissinotto, 2012; Raw et al. 2013). Blackburn et al. (2011) proposed a cohesive framework to categorize invasive populations. Within this framework, *T. granifera* can be classified as a category E invader, i.e. a "fully invasive species, with individuals dispersing, surviving and reproducing at multiple sites across a greater or lesser spectrum of habitats and extent of occurrence" (Blackburn et al. 2011). In 2014, the Alien and Invasive Species Regulations were published in terms of the National Environmental Management: Biodiversity Act (Act no 10 of 2004) in the Government Gazette, which classified *T. granifera* as a category 1b invader (NEM:BA 2014; van Wilgen et al. 2020). A category 1b invader is regarded as a species that "must be controlled as part of a national management programme and cannot be traded or otherwise allowed to spread" (NEM:BA 2014).

Figure 1.2: A rout of *Tarebia granifera* at Umdloti Estuary (-29° 39' 0" S, 31° 07' 0" E), KwaZulu-Natal, South Africa.

The St Lucia Estuary: a case study for Tarebia granifera invasion

Tarebia granifera was first recorded in the St Lucia Estuary in 2005, occurring in densities of over 6000 ind.m^{-2} (Figure 1.2) and outnumbering native species (Appleton et al. 2009; Raw et al. 2015). The St Lucia Estuary is the largest estuary in Africa and is situated on the northeast coast of South Africa and forms part of the iSimangaliso Wetland Park, a UNESCO World Heritage Site (Taylor et al. 2006). The St Lucia Estuary is connected to six river systems i.e. the Mkuze River, Mzinene River, Hluhluwe River, Nyalazi River, Mpate River and Mfolozi River (Whitfield et al. 2013). During the 1930s to 1950s, the St Lucia Estuary was artificially separated from the Mfolozi River to avoid the high silt deposition from the sugarcane farming along the Mfolozi River floodplain (Taylor 2006; Whitfield and Taylor, 2009; Taylor 2013). There have been several attempts to relink the Mfolozi River to the St Lucia Estuary in order to alleviate freshwater deprivation, especially during drought conditions (Whitfield and Taylor, 2009). In the late 1960s, a back channel was dredged from the Mfolozi River through the mangroves of the St Lucia Narrows to allow for the reconnection of the two systems (Whitfield and Taylor, 2009; Taylor 2013). A link channel was later created in the early 1980s (Jones et al. 2020) to combat the drought conditions within the St Lucia Estuary. Due to severe drought conditions from 2002 until 2007/08, the iSimangaliso Wetland Park authority attempted to reopen and widen the back channel again to allow for a brief relinkage of the two systems (Whitfield and Taylor, 2009; Taylor and Adams, 2013; Cyrus et al. 2020; Schutte et al. 2020). However, this was still not enough to decrease the St Lucia Estuary's hypersaline conditions during 2009 and 2010. In 2012, a new channel called the Mfolozi spillway or beach channel was dredged, allowing for freshwater input from the Mfolozi River (Cyrus et al. 2020). In late 2015/early 2016, the St Lucia Estuary had become highly fragmented due to low water levels and decreased rainfall (Jones et al. 2020). However, in June 2016, the iSimangaliso Wetland Park Authority initiated a Global Environment Facility (GEF) restoration project (Jones et al. 2020). This project aided in removing artificial dredger spoil at the mouth area that was historically placed to prevent the two systems from reconnecting (Whitfield et al. 2013; The World Bank 2017). The GEF project ended in June of 2017 and increased freshwater input from the Mfolozi River to the St Lucia Estuary. Increased rainfall combined with the freshwater input ultimately resulted in the estuary shifting state from dry hypersaline to a predominantly freshwater system in 2017 (Whitfield et al. 2013; Miranda et al. 2017; Jones et al. 2020).

Droughts, floods and mouth breaching events may cause systems to shift state (transition from one stable state to another, mainly depending on perturbations), which may cause those systems to become unstable and susceptible to invasion (Diez et al. 2012; Miranda and Adams, 2013; Wilson et al. 2020). State shifts can create unfavourable conditions that affect the biotic tolerance of the native species, thereby allowing non-native species (that have efficient adaptive skills to cope with these drastic environmental changes) to thrive (Diez et al. 2012). During 2016/2017, the state shift (high rainfall and the increased Mfolozi River input) at the St Lucia Estuary likely facilitated the range expansion of *T. granifera* (Miranda et al. 2011b; Raw et al. 2013). A high impact invader such as *T. granifera* could cause local extinctions of trophically analogous species and food resources (van Leeuwen et al. 2007; Alexander et al. 2012; Dick et al. 2014; Xu et al. 2016), therefore, potentially disrupting how an ecosystem can function.

Interactions with Melanoides tuberculata

Melanoides tuberculata is typically located in temporary or perennial rivers, freshwater or brackish waters (including streams, estuaries, coastal lakes and wetlands) (Appleton et al. 2009; Perissinotto et al. 2014). The home range of *Melanoides tuberculata* extends from East Africa, through the Middle East and to South East Asia (Facon et al. 2003; Raw et al. 2015, 2016a). *Melanoides tuberculata* have genetically and phenotypically distinct morphs within each clade (Figures 1.3), which have either evolutionally originated from Asia or Africa (Samadi et al. 1999; Facon et al. 2003). There are various indigenous morphs of *M. tuberculata* that exist on the African continent, however, in the 1980s, an Asian lineage invaded Lake Malawi (van Bocxlaer et al. 2015). The Asian lineage remained unnoticed for an extended period amongst the indigenous morphs due to their similar reproductive capacity, shell morphology, ecological traits and feeding dynamics (van Bocxlaer et al. 2015; Raw et al. 2016b). The distinction between clades was determined by phylogenetics which revealed the Asian morph in Africa (van Bocxlaer et al. 2015). The Asian morph was able to camouflage invasion due to the high similarity between the invasive and native morphs. Therefore, more research should be conducted on the phylogeny and morphology of the invasive and native morphs (Genner et al. 2008; van Bocxlaer et al. 2015). There were two distinct morphs (Figure 1.3c) located at the St Lucia Estuary Mouth. The native was identified using images and shell morphological data obtained from previous literature (Appleton 1996; Facon et al. 2003; Genner et al. 2007; Miranda et al. 2011b).

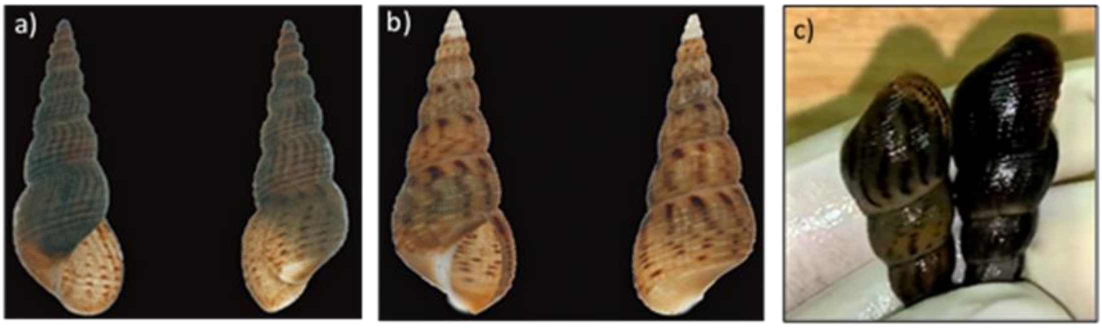


Figure 1.3: a) *Melanoides tuberculata* (14 mm) from the St Lucia Estuary Mouth, b) *Melanoides tuberculata* (23 mm) from False Bay, St Lucia Estuary (Images supplied by Dr N.A.F. Miranda) and c) *Melanoides tuberculata* (30 mm, left) and a *Melanoides tuberculata* distinct morph (36 mm, right) sampled from St Lucia Estuary Mouth, KwaZulu-Natal, South Africa.

Reproduction in invasive (*T. granifera*) and native (*M. tuberculata*) gastropods are parthenogenic, however, occasionally, sexual reproduction can occur (Abbott 1952; Samadi et al. 1999; Fofonoff et al. 2003; Facon et al. 2008). Sexual reproduction is ovoviviparous, where the embryos develop in brood pouches until hatching occurs (Abbott 1952; Samadi et al. 1999; Fofonoff et al. 2003; Facon et al. 2008). *Tarebia granifera* can give birth to one juvenile every 12 hours (Abbott 1952; WHO 1981), whereas *M. tuberculata* is estimated to give birth to one juvenile every day (Keller et al. 2007), therefore, the invasive species has a higher fecundity compared to the native species. Juveniles at birth can range from 0.7 - 2.1 mm SH (shell height) for *T. granifera* (Appleton et al. 2009) and 1.2 - 4.5 mm for *M. tuberculata* (Fofonoff et al. 2003; Appleton et al. 2009). These juveniles can grow up to 40 mm for both species (Abbott 1952; Pointier 1989), however, this is dependent on the environmental conditions and the type of morph (Pointier 1989). The invasive and native gastropod can tolerate salinities ranging from 0 - 30 (Roessler et al. 1977; Miranda et al. 2010), however, the invasive gastropod can tolerate a wider range of temperatures (0 - 47.5°C) (Miranda et al. 2010) compared to the native (18 - 35°C) (Murray 1971). *Tarebia granifera* has a higher shell crushing resistance than the native shell, therefore, native birds are unable to break the shell to feed on this invader (Miranda et al. 2016). Both gastropods are considered herbivores/detritivores and deposit feeders with generalist diets (Miranda et al. 2011a; Perissinotto et al. 2014; Raw et al. 2016b). *Tarebia granifera* and *M. tuberculata* are known to consume resources such as detritus, sedimentary organic matter (SOM), filamentous algae (e.g. *Cladophora* sp.) and microphytobenthos (MPB) (Miranda and Perissinotto, 2012; Raw et al. 2016a). In summary, *T. granifera* is successful in invading the South African estuaries due to their high reproductive rates, high feeding rates, their ability to adapt to a wide range in physicochemical factors (e.g. salinity and temperature) as well as lack of predation and parasitism (Appleton et al. 2009; Miranda et al. 2010, 2011a; Raw et al. 2016a).

Tools to investigate feeding ecology

Feeding plays a role in transferring energy, growth, distribution, densities and overall survival of individual consumers (Brodeur et al. 2017). Therefore, feeding ecology may aid in understanding aspects such as diet composition, habitat preferences, resource partitioning, competition, and trophic structures (Braga et al. 2012). Feeding ecology is useful in observing energy transfers within and between ecosystems as well as predicting possible changes in food webs (Nakano and Murakami 2001; Baxter et al. 2004). The information provided by feeding ecology can aid in explaining the interactions between species, including whether potential competitive interactions exist among these species (Williams 1981; Jaksić et al. 1993). Conservation strategies can thus be constructed from information provided to protect native species and ecosystems from the impacts of invasions (Braga et al. 2012). There are various tools used to investigate the feeding ecology of species, including 1) gut content analysis (e.g. Miranda et al. 2011a; Raw et al. 2016a), 2) direct observations (e.g. Manko, 2016), 3) fatty acid analysis (e.g. Descroix et al. 2010; Jardine et al. 2020), 4) stable isotope analysis (SIA) (e.g. Miranda and Perissinotto, 2012; Raw et al. 2016b) and 5) functional responses (FR) (e.g. Xu et al. 2016; Cuthbert et al. 2019; South et al. 2019). Due to the complexity of ecosystems, studies that use multiple approaches (e.g. Miranda and Perissinotto, 2012; Raw et al. 2015; Chapter 2, 3) have been found to increase the reliability and robustness of predicting the implications of invasion. Gut content analysis is an accurate method for determining actual dietary composition over a short period, however, this method provides a temporally subjective view on consumption (Hyslop 1980). Direct observations are conducted using videography to assess foraging behaviour, however, this type of approach is not appropriate when observing minute changes in feeding (Manko 2016). Fatty acid analysis is used as biomarker resources of allochthonous and autochthonous origin (Descroix et al. 2010), however, this method has many uncertainties in consumer biochemical modifications due to selective metabolism and retention (Jardine et al. 2020). Stable isotope analysis and functional responses are frequently used methods to assess feeding ecology as they provide a more detailed view (spatially and temporally) of diet composition (Miranda and Perissinotto, 2012; Hill et al. 2015) and assess the feeding strategies of consumers (Dick et al. 2014; Rosenbaum and Rall, 2018). This study focused on the stable isotope analysis and functional response approaches in order to assess the feeding ecology of the gastropods.

Stable isotope analysis is useful in tracing changes in energy flows and trophic structures in an ecosystem (Hill et al. 2015; Cuthbert et al. 2019). Stable isotopes analyses are used to determine the assimilated diet of organisms, thereby identifying which resources within a specific niche are consumed (Bearhop et al. 1999; Votier et al. 2003). Examining interactions between organisms within the system using stable isotope analyses assists in understanding how an ecosystem is affected by invasive species and native species (Caut et al. 2006; Hill et al. 2015). An isotopic niche of a species was initially referred to as the n-dimensional hypervolume that defines all biological and environmental factors the species needs to exist and reproduce (Hutchinson 1957). However, Hutchinson (1959) improved this definition by separating a habitat into the multiple parameters it embodied. These parameters include food requirements, interactions between species as well as seasonal biotic and abiotic (temperature and moisture) conditions that a habitat may experience (Hutchinson 1957, 1959; Kearney 2006; Moore 2013). These parameters can be thought of as an n-dimension in space and are graphically represented as independent axes (Kearney 2006; Moore 2013). The fundamental niche is defined by the basic parameters a species requires to exist within a habitat (Kearney 2006; Moore 2013). However, interactions with antagonist organisms can reduce the size of the fundamental niche (Kearney 2006; Moore 2013). Feeding niches developed by stable isotope analyses can also assist in understanding the type of competitive effect that invasive populations can potentially have on the native populations by determining the degree of niche overlap. (Schmidt et al. 2007). The degree of niche overlap shows the similarities or differences in diet, where larger overlap between feeding niches can imply potential competition between species. Niche width is the proportion of the fixed resource space a species uses, and niche overlap is the proportion of shared area of a particular food resource (van Valen 1965). Stable isotope ratios (such as $\delta^{13}C$ and $\delta^{15}N$) can also be used in quantifying isotopic niches and niche widths of consumers, which helps in identifying overlaps in niches of an invasive and native species (Middleburg 2014; Swanson et al. 2015). The changes in stable isotope ratios are primarily produced by naturally occurring processes (e.g. physical, chemical and biological processes established during primary production or subsequent heterotrophic processing) are known as isotope fractionation (Craig 1953; Middleburg 2014). Factors such as diet quality, feeding rate, body size and nutritional stress can affect the magnitude of this stepwise isotopic fractionation (ΔC or ΔN) (Hobson and Welch, 1995; Pinnegar et al. 2001; Mill et al. 2007). Stable carbon isotopes ratios ($\delta^{13}C$) of the primary producers, found within herbivorous consumer tissues, have been known to show very little fractionation between energy transfers and has been used to quantify energy flows and types of food sources (De Niro and Epstein, 1978; Peterson and Fry, 1987; Middleburg 2014). Stable nitrogen isotope ratios ($\delta^{15}N$) fractionate much more than the stable carbon isotope ratios, allowing for the estimation of the trophic position of organisms (Minagawa and Wada, 1984; Middleburg 2014). The trophic enrichment of $\delta^{13}C$ during trophic transfers is smaller ($\Delta C = 0\text{-}1‰$) than the $\delta^{15}N$ ($\Delta N = 2\text{-}4‰$), but the higher $\delta^{15}N$ trophic enrichment makes $\delta^{15}N$ favourable for identifying and quantifying trophic positions and trophic transfers within a system (De Niro and Epstein, 1978; Middleburg 2014; Post 2002). Chapter 2 of this thesis focuses on the dietary preferences and niche widths of invasive (*Tarebia granifera*) and native (*Melanoides tuberculata*) gastropods, using stable isotope analysis (SIA).

Other factors facilitating species interactions include the functional response relationships with the food resources, temporal variations and varying densities in resources (Kneitel 2013). Functional responses determine the relationship between food density and the rate of food consumed. This approach aids in identifying the feeding impact a consumer has on an ecosystem, the feeding efficiency of a consumer with limited food resources and if potential competition occurs between these consumers (Rogers 1972; Jeschke et al. 2002; Kalinkat et al. 2013). Functional responses of consumers are represented as FR curves, where the magnitude of the curve is determined by feeding parameters such as attack rate (a), handling time (h) and maximum feeding rate (F_{max}) (Dick et al. 2014; Rosenbaum and Rall, 2018). There are three types of FR curves: Type I FR indicates that food consumed rises linearly with food density (Holling 1959a, b; Alexander et al. 2013); Type II FR indicates food consumed rises with food density, but gradually decreases until a plateau is reached (Holling 1959a, b; van Leeuwen et al. 2007; Alexander et al. 2012; Rosenbaum and Rall, 2018) and a Type III FR indicates an increasing rate in consumption, followed by a decreasing rate in consumption and subsequently a plateau is reached (Holling 1959a, b; Hassell 1978; Alexander et al. 2013). The possibility of coexistence between two consumers feeding on a single resource can be reflected in the curvature of functional responses (Koch 1974; Abrams and Holt, 2002; Kneitel 2013; Klauschies and Gaedke, 2020).

Functional responses have been used for decades to assess feeding ecology. Dick et al. (2014) proposed that functional responses can be used to predict feeding impacts of invaders by measuring the relationship between the food consumed per capita and the food density (Abrams 1990; Jeschke et al. 2004; Alexander et al. 2012, 2014; Rosenbaum and Rall, 2018). The feeding parameters can be used to estimate the type of feeding impact an invader may exhibit: 1) low a and high h, indicating a low impact invader; 2) low h and high a, indicating a high impact invader (Cuthbert et al. 2019; South et al. 2019). However, in certain circumstances, the consumer may exhibit either a low a and h or a high a and h, this is indicative of an intermediate impact invader (Cuthbert et al. 2019; South et al. 2019). The functional response approach has been debated by Vonesh et al. (2017), who argued that this approach does not account for invasion history (i.e. long-term data on invasion success) or the potential invader impact indices. Vonesh et al. (2017) suggested that other parameters such as duration of invasion, conversion efficiency and mortality are neglected when considering consumer-resource dynamics and that it is not practical to assume that all species share the same parameters. Dick et al. (2017) also argued that invasion histories are essential and that it is difficult to predict the ecological impacts of emerging and potential future invaders with no information on invasion history. However, Dick et al. (2017) supported the use of FRs, provided that new metrics (e.g. Relative Impact Potential and Functional Response Ratios) are used to increase the reliability of predicting invasive impact. Functional Response Ratio (FRR) is a relatively recent concept which resolves contradictions of individual FR parameters and has been used to distinguish ecologically damaging invaders from various trophic groups, ecological systems, taxa and habitats (Cuthbert et al. 2019; South et al. 2019). A combination of the feeding parameters using the FRR metric may increase the robustness and reliability of predicting the impacts of an invader and the degree of “invasiveness” (Cuthbert et al. 2019). Chapter 3 of this thesis compares the functional responses and behavioural changes among the two species with respect to chemical cues by displaying the differences in feeding strategies which may indicate the degree of invasiveness and ecological impact of the alien species.

Species interactions are important factors to consider when determining the success of an invader (Bulleri et al. 2008; Raw et al. 2015). However, the interactions that affect functional and behavioural responses are not limited to only physical interactions and can include chemical interactions in the form of disturbance pheromones or chemical cues (Raw et al. 2015; Schulte et al. 2015). Chemical cues are a suitable form of communication within aquatic environments as the chemical substance can be dispersed easily and received by surrounding organisms (Wisenden 2000). Although chemical cues may be used as an attractant, especially in courtship, they can also be used as a deterrent in foraging and predator avoidance (Croll 1983). Heterospecific chemical cues are essential when considering foraging (Croll 1983). One species that attain the food source first releases pheromones to communicate to the individuals of another species that the food resource is no longer available (Kohn 1961; Croll 1983; Fink et al. 2006; Raw et al. 2015). The evolutionary benefits of prolonged pheromone communication can assist in deterring other consumers away from food resources and increase the efficiency of locating food with minimal effort (Croll 1983; Fink et al. 2006). A chemoreceptive study conducted by Raw et al. (2013) showed that *T. granifera* had moved closer to the source of chemical cues released by the natives (*M. tuberculata* and *Assiminea* cf. *capensis*) rather than away. Raw et al. (2013) had observed that *T. granifera* was unaffected by the chemical cues of the native species and was able to move freely. However, the heterospecific natives (*M. tuberculata* and *Assiminea cf. capensis* – not previously introduced to *T. granifera*) had moved away from the source of the *T. granifera* chemical cues (Raw et al. 2013). The introduction of an invasive species into a system can affect the feeding abilities of another species. In some cases, the chemical cues released by individuals of one species may have a neutral effect on another species, therefore, influence on the functional or behavioural responses is negligible (Schulte et al. 2015). In this study, the utilization of the FR approach and behavioural changes due to the presence of chemical cues allowed for an opportunity to further investigate changes in feeding responses to chemical cues.

Tarebia granifera is a high impact invader due to factors such as high feeding rates and the ability to adapt to a wide range of environmental conditions (Appleton et al. 2009; Miranda et al. 2010, 2011a; Raw et al. 2016a). Therefore, studying feeding ecology using tools such as SIA and FR (with respect to chemical cues) can provide a more detailed view of the invasion success of *T. granifera*. Biological invasions are important to monitor as the dynamics between the native and the invasive species can change over time, with changes in environmental conditions (e.g. state shifts). During 2016/2017, the state shift at the St Lucia Estuary was due to the GEF project and increased rainfall (Jones et al. 2020), which created favourable environmental conditions and allowed for the range expansion of *T. granifera* (Perissinotto et al. 2014). The two species now occur sympatrically, providing the opportunity to investigate their diet and to determine whether the presence of one species affects the feeding dynamics of the other.

Aims and objectives

The main aim of this thesis was to investigate dietary preferences, niche overlap and differential feeding dynamics of an invasive (*T. granifera*) and native (*M. tuberculata*) gastropod.

The objectives of this thesis were to:

- Determine the diet of *T. granifera* and *M. tuberculata* by assessing their stable carbon and nitrogen isotopic ratios and that of the available food sources (Chapter 2).
- Determine the dietary niche width and overlap of *T. granifera* and *M. tuberculata* (Chapter 2).
- Determine the functional responses of *T. granifera* and *M. tuberculata* and the potential feeding impact of the invasive species (Chapter 3).
- Determine if the presence of heterospecific chemical cues influences the functional responses of *T. granifera* and *M. tuberculata* (Chapter 3).
- Determine if the physical presence of one species affects the behaviour and feeding activities of the other (Chapter 3).

CHAPTER 2

Stable Isotope Analysis: Dietary preferences and niche widths of invasive (*Tarebia granifera*) and native (*Melanoides tuberculata*) gastropods

A recurring challenge in invasion biology is determining the potential ecological impacts of invaders on native populations within ecosystems. The invasive gastropod *Tarebia granifera* has outnumbered and displaced many native gastropods within South African estuaries. *Tarebia granifera* was first recorded in the St Lucia Estuary in 2005, occurring in densities of over 6000 ind.m^{-2} and outnumbering the native species, *Melanoides tuberculata*. The diets of *T. granifera* and *M. tuberculata* in isolation are known but the recent state shift in the St Lucia Estuary to a freshwater system allowed for the range expansion of *T. granifera*. The two species now co-exist, providing the opportunity to investigate diet preferences, niche widths and overlaps between these consumers, which can aid in understanding how invasive populations interact with native populations within an ecosystem. Stable Isotope Analysis (SIA) is a tool used to provide a temporal and spatial-based view of consumers' diets. This study aimed to explore the feeding dynamics of *T. granifera* and *M. tuberculata* by investigating their dietary preferences and niche overlaps in the St Lucia Estuary at two sites (Mouth and Mpophomeni Stream) using stable isotope analyses (SIAs). The two gastropod species exhibited a high degree of dietary plasticity and consumed similar food sources (such as detritus, *Cladophora* sp. and MPB). There was a similar dietary niche overlap (~36%) between the gastropods at both study sites. Food sources were, however, plentiful in the St Lucia Estuary, allowing for niche partitioning. The results obtained from this study suggested that competitive exclusion could have been averted, and the gastropods can coexist with limited dietary competition. Factors such as high reproductive rates, wide tolerance of physicochemical parameters, limited predation and lack of parasitism may have assisted in the invasive potential and success of *T. granifera*.

Key words: Coexistence, feeding, resource availability, St Lucia Estuary, invertebrates.

2.1) Introduction

Invasive species can have significant ecological impacts on multiple levels of organisation, i.e. from the individual to populations and ecosystems (Parker et al. 1999; Miranda and Perissinotto, 2012). Invasive species can disrupt the functioning of ecosystems by affecting biotic (other organisms) and abiotic (e.g. bioturbation, light intensity and oxygen availability) factors (Lockwood et al. 2007; Miranda et al. 2011a). The introduction of new species into an ecosystem can have many evolutionary impacts whereby the native species are subjected to selective pressure/s that may affect factors such as reproduction, growth, feeding activity, distribution and behaviour (Trussell 1996; van der Velde et al. 2006). Invasive species are introduced into ecosystems via a purposeful introduction (e.g. biological control agents) and accidental introduction (e.g. via aquarium industries or incorrect disposal of species) (Appleton 2003; Miranda et al. 2010). Estuarine and coastal environments are most susceptible to the introduction of non-native species as these systems experience large amounts of activity such as shipping or boating, aquaculture and are a source of food (Ruiz et al. 2000; Padilla and Williams, 2004; Williams and Grosholz, 2008).

The potential impacts that invasive species may have on native species are concerning, as disruption within the ecosystem can lead to displacement or, worse, the extinction of a native species (Karatayev et al 2009; Miranda et al. 2010). *Tarebia granifera* was introduced into South African estuarine ecosystems in the early 1990s via the aquarium trade (Appleton and Nadasan, 2002; Appleton et al. 2009). This invasive gastropod successfully invades South African estuaries due to their high reproductive rates, generalist diet (allows for resource partitioning) and the ability to adapt to a wide range of physicochemical factors (Miranda et al. 2010). *Tarebia granifera* is regarded as an r-selected species (Appleton 2003; De Kock and Wolmarans, 2008) that can disrupt trophic structures and alter the biodiversity of communities (Miranda and Perissinotto, 2012). *Tarebia granifera* was first recorded in the St Lucia Estuary (iSimangaliso Wetland Park) in 2005, occurring in densities of over 6000 ind.m^{-2} and outnumbering the native species, *Melanoides tuberculata* (Miranda et al. 2010; Raw et al. 2016a, 2016b). The St Lucia Estuary is the largest estuary in Africa and forms an essential part of the iSimangaliso Wetland Park, a UNESCO World Heritage Site (Taylor et al. 2006). Both gastropods are considered herbivores/detritivores and deposit feeders with a generalist diet that links primary food sources with higher trophic levels (Miranda et al. 2011a, 2011b; Perissinotto et al. 2014; Raw et al. 2016b). *Tarebia granifera* is known to have a high feeding impact and prefers to feed on resources such as detritus, sedimentary organic matter (SOM), filamentous algae (e.g. *Cladophora* sp.) and microphytobenthos (MPB) (Miranda and Perissinotto, 2012). Miranda and Perissinotto (2012) and Raw et al. (2016b) assessed the diets of *T. granifera and M. tuberculata* when these species did not co-occur. The recent state shift to a freshwater system due to the Global Environmental Facility (GEF) project (Jones et al. 2020) created favourable environmental conditions that allowed for the range expansion of *T. granifera* at the St Lucia Estuary (Perissinotto et al. 2014). The two species now occur within the same region, which provided an opportunity to investigate the diets of the two species in the presence of one another.

Observing diet preference, niche width and overlap between consumers can aid in understanding how invasive populations interact with native populations within an ecosystem (Post 2002; Caut et al. 2006). Understanding ecosystem processes, which include energy flows and exchange of organic matter (Middleburg 2014) from one trophic level to the next, is essential when depicting trophic links and organic matter pathways within estuarine food webs (Krumins et al. 2013). By assessing organisms' feeding interactions and observing changes in ecosystem processes, one can determine the feeding impact that an invasive species has on trophic links and pathways of organic matter (Miranda and Perissinotto, 2012). Stable Isotope Analysis (SIA) has successfully been used in several studies to determine the feeding impacts of invasive species within aquatic ecosystems (Miranda and Perissinotto, 2012; Middleburg 2014; Hill et al. 2015). This analysis is a quantitative tool used to provide a temporal and spatial view of the diet of an organism, thereby, providing a more accurate representation of dietary composition over time (Miranda and Perissinotto, 2012; Hill et al. 2015). Stable Isotope Analysis considers the digested food to depict the assimilated diet as this contributes to a consumer’s overall nutrition (Bearhop et al. 1999; Votier et al. 2003). Stable carbon (C) and nitrogen (N) isotopes are most frequently used in determining trophic structures as well as making energy and organic matter flow inferences within food webs (Middleburg 2014). The stable isotope ratios (such as $\delta^{13}C$ and $\delta^{15}N$) can also be used to quantify isotopic niches and niche widths of consumers, which helps identify overlaps in niches of an invasive and native species (Middleburg 2014; Swanson et al. 2015).

Minor variations in stable isotope ratios due to changes within chemical, physical and biological processes can cause isotope fractionation (Middleburg 2014). Isotope fractionation refers to the changes in stable isotope ratios which are primarily produced by naturally-occurring processes (e.g. physical, chemical and biological process produced during primary production or subsequent heterotrophic processing) (Craig 1953; Middleburg 2014). Stable carbon isotope ratios (δ13C) of the primary producers, found within herbivorous consumer tissues, reflect energy flows and types of food sources due to minor fractionation between energy transfers (DeNiro and Epstein, 1978; Peterson and Fry, 1987; Middleburg 2014). Stable nitrogen isotopes ratios ($\delta^{15}N$) fractionate much more than the stable carbon isotope ratios which allows for the estimation of the position of organisms within food webs (Minagawa and Wada, 1984; Middleburg 2014). All stable isotope mixing models assume that there is stepwise trophic level enrichment that occurs in the carbon and nitrogen isotopes and that this enrichment varies by food sources and the consumer (Mill et al. 2007; Parnell et al. 2013). Factors such as diet quality, feeding rate, body size and nutritional stress can affect the magnitude of this stepwise isotopic fractionation (Δ_C or Δ_N) (Hobson and Welch, 1995; Pinnegar et al. 2001; Mill et al. 2007).

Stable Isotope Analysis (such as Stable Isotope Mixing Models) in combination with various other models (such as Stable Isotope Bayesian Ellipses) can be used to evaluate the contribution of food sources to the diet composition of the gastropods and to determine the dietary niche width and overlap (Parnell et al. 2010; Jackson et al. 2012; Miranda and Perissinotto, 2012; Phillips et al. 2014; Hill et al. 2015). Due to changes in environmental conditions in the St Lucia estuarine lake (e.g. increased freshwater input from 2016), the invasive species increased in abundance and expanded its range into areas where it did not exist before (Miranda and Perissinotto, 2012; Raw et al. 2016a, 2016b). The ranges of *M. tuberculata* and *T. granifera* now overlap, providing the opportunity to assess whether the diets of these species shift, when both species occur within the same region. The “competitive exclusion principle” proposed by Gause (1934) states that “two species with identical niches (and competing for a single resource) cannot coexist together indefinitely”. The coexisting species should have at least one food source that differs when there are overlaps in diet to avoid competitve exclusion (Hutchinson 1957; Wang et al. 2018). Therefore, stable isotope models can also assist in understanding the type of competitive effect that invasive populations can have on the native populations (Schmidt et al. 2007).

This study aimed to investigate dietary preferences and niche overlap of an invasive (*Tarebia granifera*) and native gastropod (*Melanoides tuberculata*) at two independent study sites within the estuarine lake where the species co-exist. The objectives were: 1) to determine the stable isotopic ratios of carbon and nitrogen as well as assess the resource contribution to infer the diet composition of the two gastropods and 2) to determine the niche width and dietary niche overlap of the two gastropod species. Previous studies (Miranda et al. 2011a; Perissinotto et al. 2014; Raw et al. 2016b) showed that both gastropods are considered herbivores/detritivores with generalist diets. Therefore it was hypothesized that the diet preferences of both gastropods would be similar within and between sites in the St Lucia Estuary and that the dietary niches of these species overlap significantly.

2.2) Materials and Methods

2.2.1) Study Site

The native and invasive gastropods and potential food sources were collected from the St Lucia Estuary, specifically, the St Lucia Estuary Mouth (28°23'0" S, 32°25'0" E) and Mpophomeni Stream in False Bay (27°57'33"S, 32°21'15"E) (Figure 2.1) in January 2017. The gastropods and potential food sources were sampled during the wet season (Raw et al. 2016b). Rainfall reached approximately 183 mm in January 2017 (Jones et al. 2020) at the St Lucia, which could have influenced food resource availability (Raw et al. 2016b). These study sites were chosen as both species had co-existed during the time of collection and were used in comparison with a previous study conducted by Raw et al. (2016b) at the same sites, where the gastropods did not co-exist. The gastropods and food sources at St Lucia Estuary Mouth were collected from a freshwater channel that flows through to the northern bank of the estuary which is dominated by a mangrove forest (Raw et al. 2016b). The Mpophomeni Stream is a small, brackish stream that flows through an area that is predominately sand forest vegetation into the False Bay Basin of Northern Lake St Lucia (Raw et al. 2016b). The food sources included microphytobenthos (MPB), sedimentary organic matter (SOM), particulate organic matter (POM), detritus and various vegetative samples (e.g. *Cladophora* sp.). Raw et al. (2016b) found that at the St Lucia Estuary Mouth, the detritus was mostly made up of decomposing mangrove leaves. Other vegetation that was found in the habitat but did not contribute significantly to the gastropods' diets included mangrove tree (*Avicennia marina* and *Bruguiera gymnorrhiza*) leaf litter, the common reed (*Phragmites australis*) and *Ceratophyllum* sp. (submerged macrophyte) (Raw et al. 2016b). *Melanoides tuberculata* and *Tarebia granifera* were collected by hand or with the use of a sweep net. The gastropods were frozen to preserve tissue until processed in the laboratory for SIA in 2018.

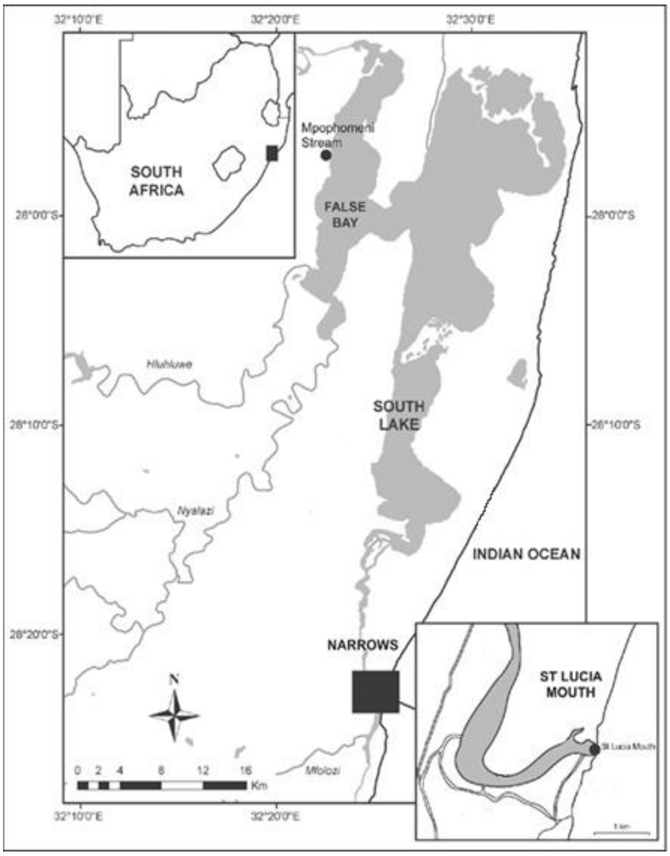


Figure 2.1: Locality map of study sites, St Lucia Estuary Mouth and Mpophomeni Stream in KwaZulu-Natal, South Africa (adapted from Carrasco and Perissinotto, 2012).

2.2.2) Sample Preparation

The gastropod samples were prepared according to protocols outlined in Carrasco and Perissinotto (2011) and Miranda and Perissinotto (2012) and are summarised here. This study included five individuals of each species within each of the three size classes (small, medium and large). Fifteen individuals from each species with shell heights ranging from 9 - 23 mm, were analysed. A Grubbs outlier test was conducted to remove the outliers, therefore, the sample sizes of *T. granifera* and *M. tuberculata* varied at the St Lucia Estuary Mouth (n= 9; n=12, respectively) and Mpophomeni Stream (n=10, n=14, respectively). The food resource samples were collected randomly from each site to capture diversity

The shells of the gastropods were removed. Sterilized (70% ethanol) forceps and a blade were used to carefully separate the muscle tissue of the foot from the gut and operculum. Forceps and blade sterilization were done after each tissue extraction. The tissue was rinsed with distilled water to remove any debris. The foot of each gastropod was then placed in 5 mL vials to dry at 50°C in an air-circulated oven for at least 48 hours. The samples were packaged in Eppendorf microcentrifuge tubes and transported to the Mammal Research Institute at the University of Pretoria for isotopic analysis of $\delta^{13}C$ and $\delta^{15}N$ signatures.

Microphytobenthos was collected by scraping the upper 1 cm layer of dense algal mats and sediments. To resuspend the MPB, filtered (Econofilt RGF) estuarine water was added and the mixture stirred so that the sediments settled at the bottom and the MPB stayed in suspension (Carrasco and Perissinotto, 2011; Carrasco et al. 2012). The suspension was then filtered using a vacuum pump onto pre-combusted (450°C, 6 hours) Econofilt RGF filters. There were three replicates for MPB at each site to account for variability.

Triplicate subsurface water samples were collected for the analysis of Particulate Organic Matter (POM) at each site. Water samples were filtered onto pre-combusted Econofilt RGF filters. Particulate Organic Matter included phytoplankton and particulate suspended detritus which may settle in benthic environments and become a readily available food resource for gastropods. The filters of both MPB and POM were washed with 1% Hydrochloric acid (HCl), rinsed with distilled water and then oven-dried in an air-circulated at 50ºC oven for 48 hours. The samples were placed in aluminium foil envelopes and transported to the University of Pretoria for isotopic analyses.

Sedimentary organic matter (SOM) was sampled from each site by collecting two sediment cores using 20 mm diameter Twin corer, which were placed in crucibles in 100 mL pill vials and the upper 1 cm was removed as this largely contains microalgae. The samples were acid washed with 1% HCl in order to remove any inorganic carbonates ($CaCO_3$). The sediment samples were then washed with distilled water and placed in an air-circulated oven at 50ºC, and dried for at least 48 hours. After drying, the samples were crushed using a pestle and mortar to form a powder and were packaged into Eppendorf microcentrifuge tubes and transferred to University of Pretoria for isotope analysis.

Detritus in the form of decomposing plant material was sampled near the edge of the water surface from each site. The detritus was washed with 1% HCl to remove any biogenic carbonates, thereafter, thoroughly washed with distilled water and oven-dried in an air-circulated oven at 50ºC for 48 hours. Once oven-dried, the detritus was crushed into a powered form using a mortar and pestle, and the triplicate samples were packed into Eppendorf microcentrifuge tubes and transferred to University of Pretoria for isotope analysis.

Vegetation such as *Cladophora* sp. was collected and identified according to guides by van Oudtshoorn (1999), Wyk et al. (2002), Moll (2009) and Cho (2016). The vegetation was thoroughly washed with distilled water to remove any excess debris and oven-dried (air-circulated oven) at 50ºC for 48 hours and crushed into a powder form as above. Triplicates of each vegetative material (powder) were packed into Eppendorf microcentrifuge tubes and transferred to University of Pretoria for isotope analysis.

2.2.3) Stable Isotope Analyses

The Stable Isotope Laboratory, Mammal Research Institute at the University of Pretoria conducted the Stable Isotopic Analyses. The muscle tissue samples from the gastropods foot were weighed to approximately 0.5 - 0.6 mg and the gastropod samples were placed into tin capsules that were cleaned with toluene. Approximately 1.1 - 1.2 mg of vegetation samples (n = 3 per site) and 2.2 - 2.5 mg of the detrital samples were weighed for analysis. Organic residues (n = 3 per site) were scraped off the Econofilt RGF filter papers and weighed (9.0 - 11.0 mg) into tin capsules that were cleaned in toluene. The sediment samples (n = 3 per site) were also weighed to ~21.0 mg - 22.0 mg.

The Stable Isotope Laboratory conducted the isotopic analysis on a Flash EA 1112 Series elemental analyser which was joined to the Delta V Plus stable light isotope ratio mass spectrometer. The laboratory standards used included Merck Gel and DL-Valine. Values obtained for the Merck Gel during each run were used for the data corrections. The values for the DL-Valine standard informed of the ± error for each run. The isotopic ratios were expressed as the conventional delta values using a per mille (‰) notation, which is relative to the atmospheric N_2 standard for N and Vienna PeeDee Belemnite standard for C (Coplen 2011; Miranda and Perissinotto, 2012; Giménez et al. 2017), using the standard equation:

$$\delta X(‰) = [(\mathrm{Rsample}/\mathrm{Rstandard}) - 1]\mathrm{x}1000$$

where X= ^{13}C or ^{15}N and R = ^{14}N/^{15}N or ^{12}C/^{13}C, respectively.

2.2.4) Data Analyses

A Grubbs Outlier test was conducted to remove outliers in the dataset. Stable Isotope Mixing Models in R v 0.4.1 (SIMMR) were used to create iso-space biplots, which showed the δ^{13}C and δ^{15}N signatures of the gastropods and primary carbon sources (Parnell et al. 2013). Boxplots were used to represent the comparison of dietary proportions/composition of the two gastropod species using the δ^{13}C and δ^{15}N signatures (Parnell et al. 2010; Jackson et al. 2012; Miranda and Perissinotto, 2012; Phillips et al. 2014; Hill et al. 2015). The credibility intervals of the boxplots are reported as the median (50%) and measures of variability (25% and 75%). Before creating the plots, the δ^{13}C and δ^{15}N signatures were corrected using trophic enrichment factors (TEFs), as this accounts for the isotopic fractionation that occurs between each trophic level (DeNiro and Epstein, 1978; Carrasco et al. 2012; Miranda and Perissinotto, 2012; Hill et al. 2015). The TEFs used for this study were 0.4 ± 1.3 SD for δ^{13}C and 2.2 ± 1.6 SD for δ^{15}N (McCutchan et al. 2003; Raw et al. 2016b). The δ^{13}C and δ^{15}N signatures are reported as mean ± SD.

Stable Isotope Bayesian Ellipses in R v 2.1.4 (SIBER; Jackson et al. 2011) were used to calculate the standard ellipse area (SEA). The isotopic standard ellipses for each species were calculated with 95% confidential intervals. Correction for the small sample size to calculate $\mathrm{SEA_C}$, which is the core isotopic niche space, was achieved by inputting the (n-2) denominator in the following equation (Jackson et al. 2012):

$$SEA_C = SEA \times [\frac{n-1}{n-2}]$$

The overlap of $\mathrm{SEA_C}$ represented a comparison of the isotopic niche width and niche overlap (Denton et al. 2018; Hill et al. 2015) of the two gastropods. A function called "bayesianOverlap" represents three values which are calculated areas of the first ellipses, second ellipses and the overlap between the two areas (Pastore 2018). The overlap is expressed as a "proportion of the sum of non-overlapping areas of the ellipses", which provides a range from 0% (absence of ellipses overlapping) to 100% (complete overlap of ellipses) (Pastore 2018). Bayesian ellipses were calculated using a Markov Chain Monte Carlo (MCMC) algorithm (i.e. 20 000 permutations) and described the data in terms of likelihood (Jackson et al., 2011; Raw et al. 2017).

2.2.5) Statistical Analyses

All statistical analyses were conducted using the software R v 3.6.1. Shapiro-Wilk and Levene's tests were conducted to test the data for normal distribution and equal variances, respectively. The carbon and nitrogen values of the gastropods from the St Lucia Estuary Mouth and the Mpophomeni Stream assumed normal distribution and equal variances. However, the carbon values of the gastropods from the Mpophomeni Stream were transformed using the square root transformation to achieve normality and equal variance. The Two Sample T-tests were conducted to compare the mean differences in the isotopic values of the gastropods within each study site. The data were then combined per isotopic value (e.g. all carbon values of the gastropods from the St Lucia Estuary Mouth were combined with the carbon values at the Mpophomeni Stream). The combined carbon and combined nitrogen values of the gastropods did not assume normality and equal variance after transformations. Therefore, the Wilcoxon rank sums tests were conducted to compare the median differences in the isotopic values of the gastropods between study sites. A Bonferroni correction was used to reduce the risk of a Type I error (Bonferroni 1936). A Multivariate Analysis of Variance (MANOVA) was conducted to determine differences in the isotopic signatures of the food sources between study sites.

2.3) Results

2.3.1) Isotopic signatures of food sources and consumers

At the St Lucia Estuary Mouth, there was no significant differences between the $\delta^{13}C$ and $\delta^{15}N$ signatures of *M. tuberculata* and *T. granifera* ($\delta^{13}C$: t = 1.32, df = 18.08, p = 0.20 and $\delta^{15}N$: t = 0.31, df = 11.23, p =0.76). In the St Lucia Estuary Mouth, SOM was the source most enriched in $\delta^{13}C$ (-23.97 ± 0.35 ‰), and POM was the source most depleted in $\delta^{13}C$ (-27.25 ± 0.27 ‰) (Figure 2.2; Table 2.5: Appendix). Microphytobenthos (MPB) had the most enriched $\delta^{15}N$ signature (5.06 ± 1.01 ‰) while *Cladophora* sp. was the source most depleted in $\delta^{15}N$ (1.26 ± 0.04 ‰) (Figure 2.2, Table 2.5 Appendix).

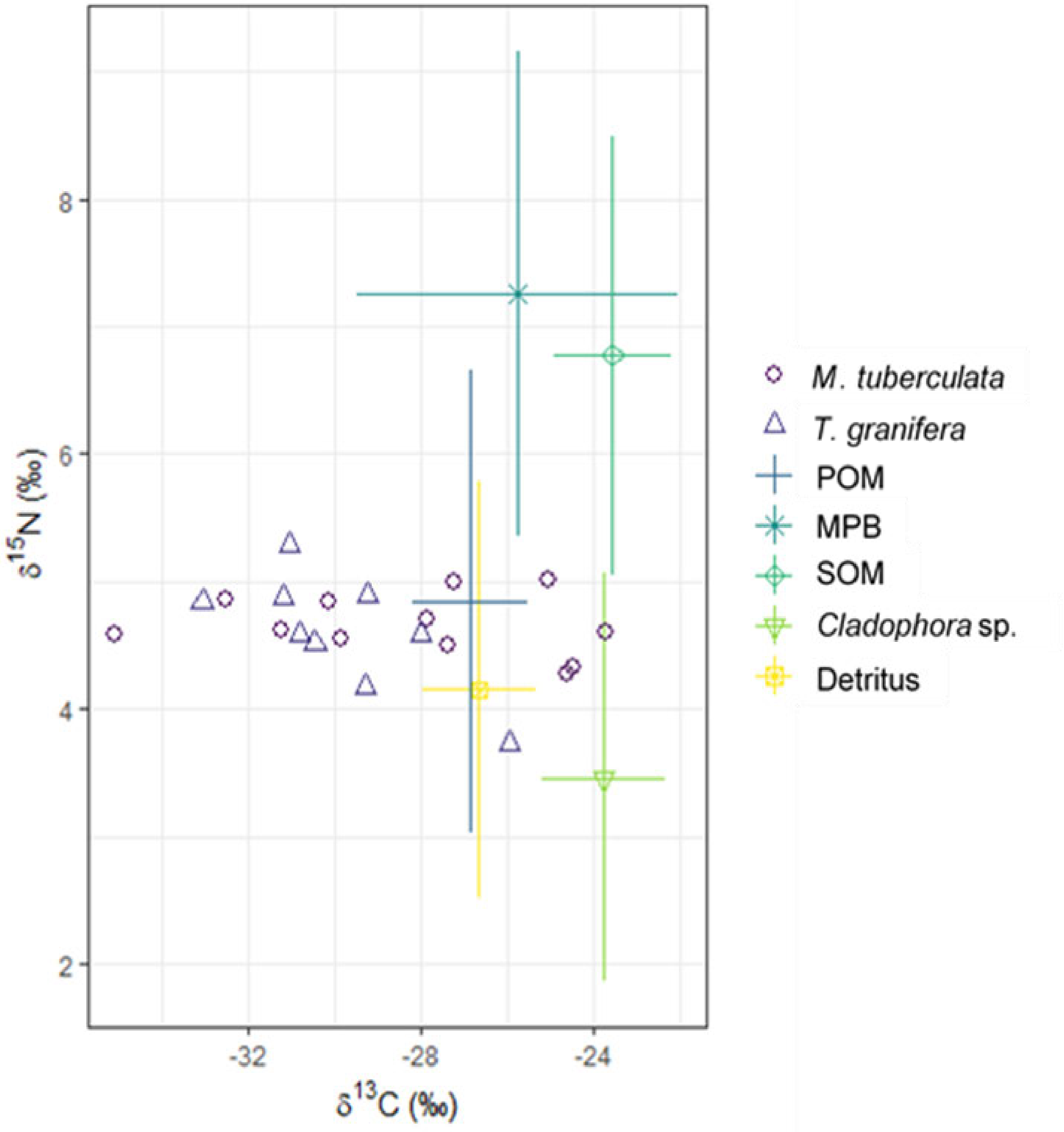


Figure 2.2: Stable Isotope Mixing Model in R (SIMMR) iso-space biplot representing the two gastropods and the source $\delta^{13}C$ and $\delta^{15}N$ (mean ± SD) signatures at the St Lucia Estuary Mouth. The data were corrected for trophic enrichment using the standard fractionation of 0.4 ± 1.3 SD for $\delta^{13}C$ and 2.2 ± 1.6 SD for $\delta^{15}N$.

At the Mpophomeni Stream, *M. tuberculata* was more enriched in δ^{13}C and δ^{15}N signatures (δ^{13}C: -25.31 ± 0.74 ‰ and δ^{15}N: 20.00 ± 0.66 ‰) compared to *T. granifera*, which was more depleted (δ^{13}C: -26.46 ± 1.00 ‰ and δ^{15}N: 19.06 ± 0.67 ‰) (Table 2.2: Appendix). There was a significant difference in the δ^{13}C ($t = -3.14$, $df = 16.14$, $p = 0.006$) and δ^{15}N signatures ($t = 3.44$, $df = 19.28$, $p = 0.003$) of *M. tuberculata* and *T. granifera* at the Mpophomeni Stream (Figure 2.3; Table 2.2: Appendix). At Mpophomeni Stream, δ^{13}C signatures ranged from -21.61 (± 0.17 ‰) for MPB to -29.88 (± 0.43 ‰) for *Cladophora* sp. (Figure 2.3; Table 2.5: Appendix). The δ^{15}N signatures ranged from for 5.22 (± 0.19 ‰) for detritus to 14.68 (± 0.19 ‰) for *Cladophora* sp. (Figure 2.3; Table 2.5: Appendix).

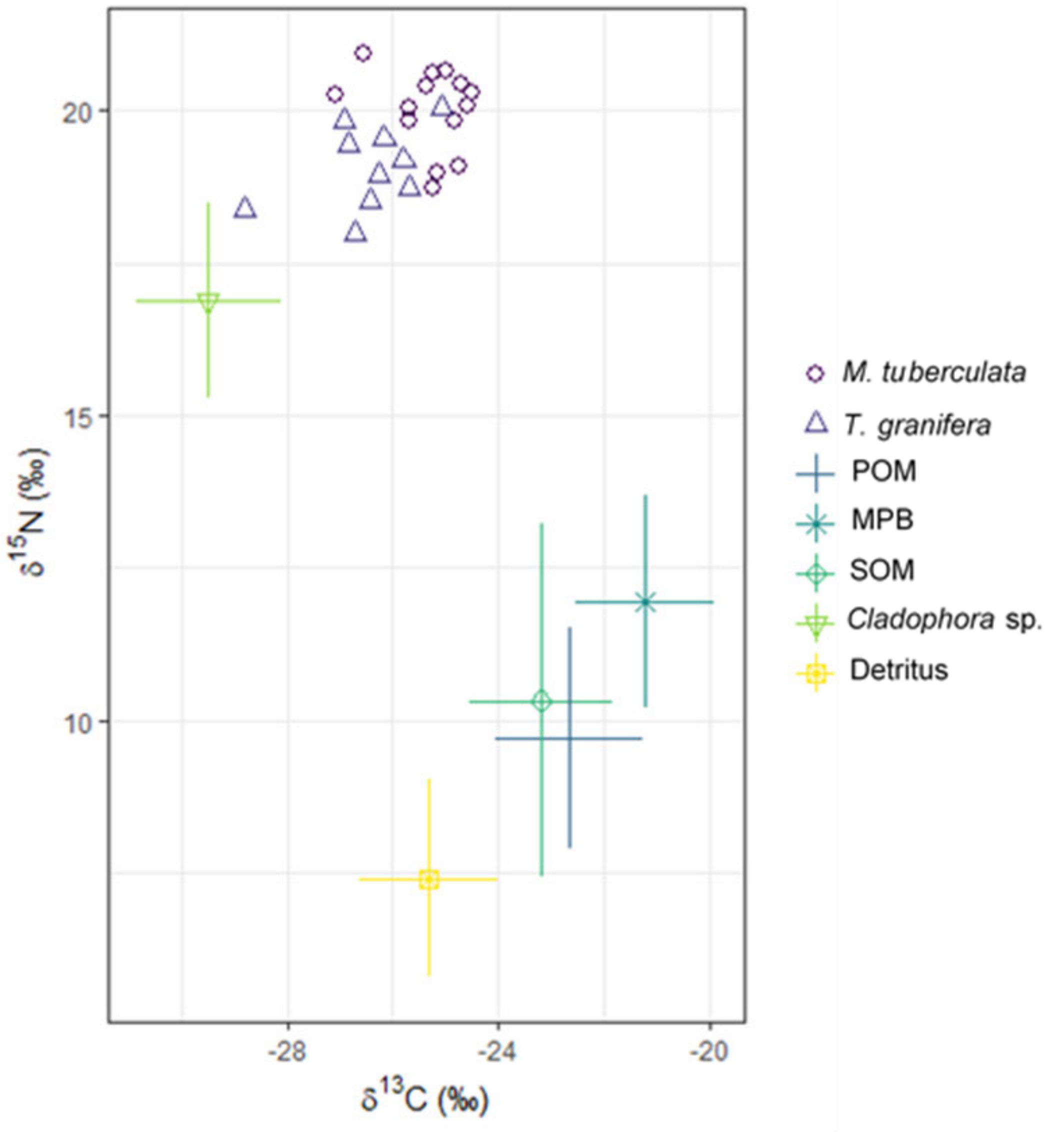


Figure 2.3: Stable Isotope Mixing Model in R (SIMMR) iso-space biplot representing the two gastropod species and the resource δ^{13}C and δ^{15}N (± SD) signatures at the Mpophomeni Stream. The data were corrected for trophic enrichment using the standard fractionation of 0.4 ± 1.3 SD for δ^{13}C and 2.2 ± 1.6 SD for δ^{15}N.

Overall, the $\delta^{13}C$ and $\delta^{15}N$ signatures of food sources differed significantly between the St Lucia Estuary Mouth and Mpophomeni Stream ($\delta^{13}C$: F = 19.43; df = 4; p = 0.0001 and $\delta^{15}N$: F = 25.57; df = 4; p = 0.0001). The $\delta^{13}C$ and $\delta^{15}N$ signatures of the gastropods were also significantly different between the St Lucia Estuary Mouth and Mpophomeni Stream ($\delta^{13}C$: W = 403; p = 0.0004 and $\delta^{15}N$: W = 504; p = 0.0001). The mean $\delta^{13}C$ and $\delta^{15}N$ signatures of both gastropods were more enriched at the Mpophomeni Stream than at the St Lucia Estuary Mouth (Table 2.2: Appendix).

2.3.2) Dietary contribution of sources

At the St Lucia Estuary Mouth, detritus and POM (mean ± SD%) contributed the highest to the diets of *M. tuberculata* (detritus: 31.1 ± 13.9% and POM: 23.0 ± 12.5%) and *T. granifera* (detritus: 32.0 ± 16.7% and POM: 24.9 ± 15.2%) (Figure 2.4; Table 2.3: Appendix). Sedimentary organic matter (SOM) and MPB contributed the least to the diets of *M. tuberculata* (SOM: 11.5 ± 6.7% and MPB: 11.8 ± 6.4%) and *T. granifera* (SOM: 10.7 ± 6.8 % and MPB: 11.7 ± 6.9%) (Figure 2.4; Table 2.3: Appendix).

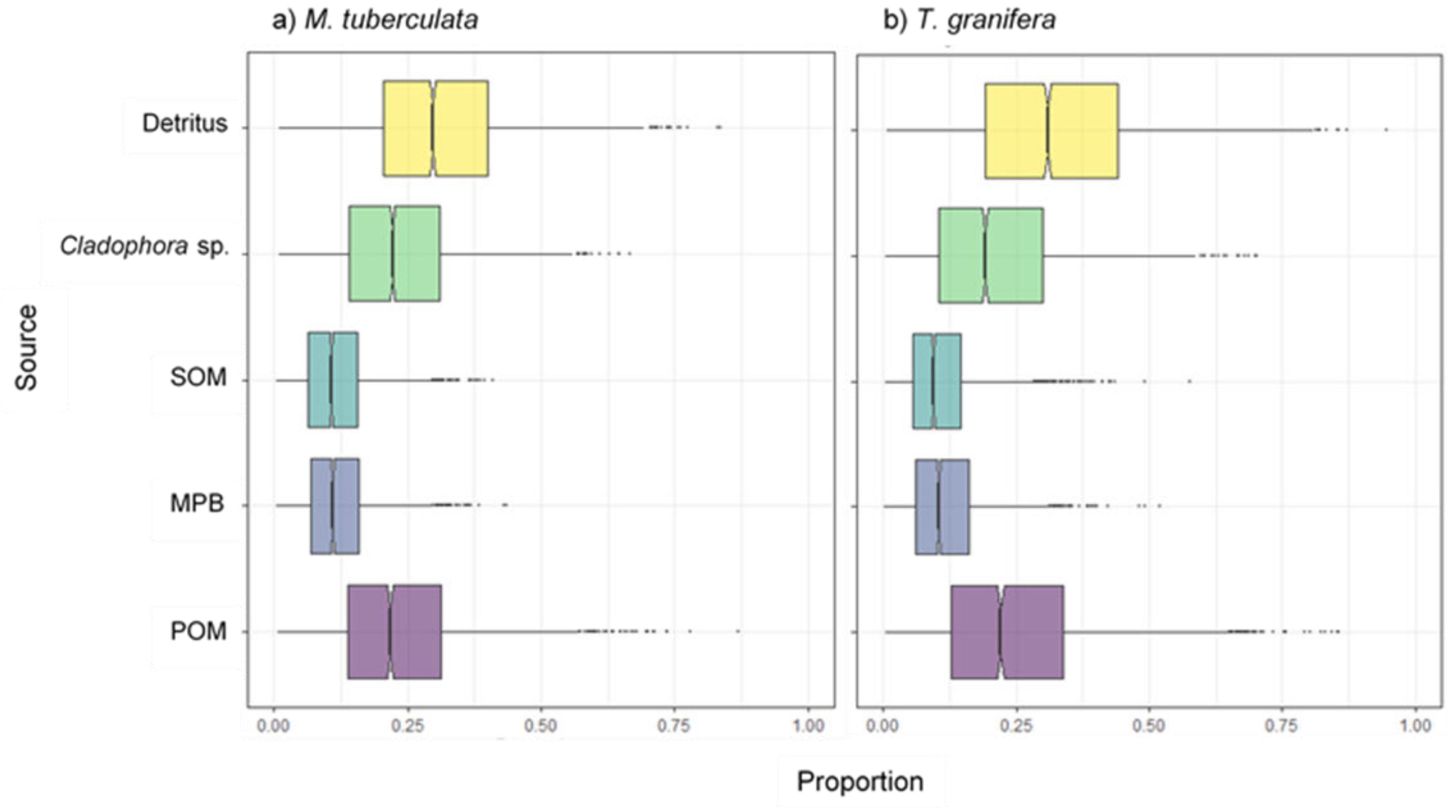


Figure 2.4: Stable Isotope Mixing Model in R (SIMMR) boxplot representing the proportional contribution of each source to the diet of a) *Melanoides tuberculata* and b) *Tarebia granifera* at the St Lucia Estuary Mouth. Percentage proportions with 25%, 50% (median) and 75% credibility interval levels. Dietary sources are coded as follows: Particulate Organic Matter (POM), Microphytobenthos (MPB) and Sediment Organic Matter (SOM).

At Mpophomeni Stream, the sources contributing the highest to both *M. tuberculata* and *T. granifera* diets were *Cladophora* sp. (39.4 ± 5.7% and 56.7 ± 6.6%, respectively) and MPB (22.2 ± 10.8% and 13.9 ± 7.6%, respectively) (Figure 2.5; Table 2.4: Appendix). Detritus contributed the least to the diets of *M. tuberculata* and *T. granifera* (9.7 ± 7.9% and 8.1 ± 6.6%, respectively) (Figure 2.5; Table 2.4: Appendix).

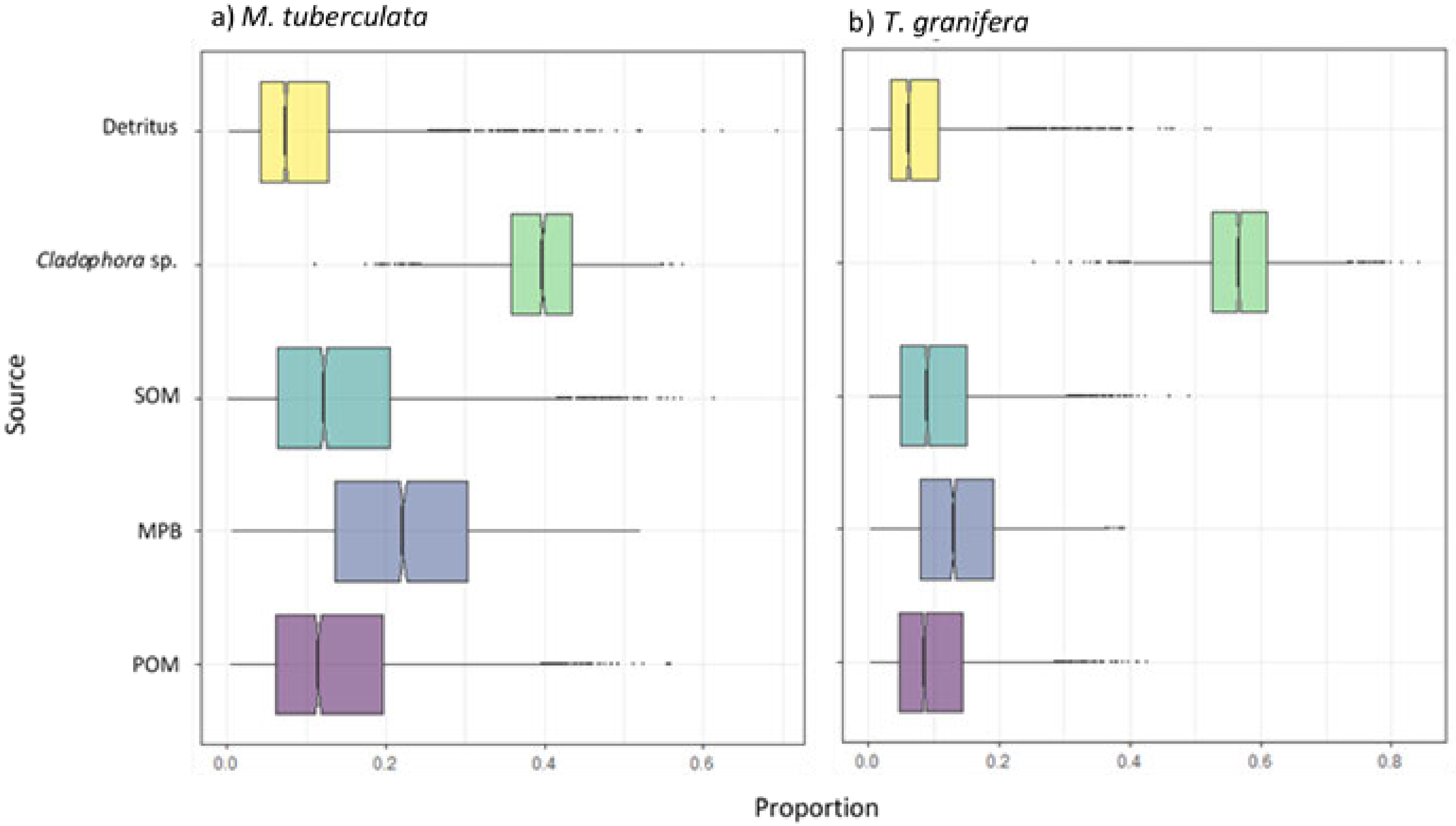


Figure 2.5: Stable Isotope Mixing Model in R (SIMMR) boxplot representing the proportional contribution of each source to the diet of a) *Melanoides tuberculata* and b) *Tarebia granifera* at the Mpophomeni Stream. Percentage proportions with 25%, 50% (median) and 75% credibility interval levels. Dietary sources are coded as follows: Particulate Organic Matter (POM), Microphytobenthos (MPB) and Sediment Organic Matter (SOM).

2.3.3) Dietary niche width and overlap

At the St Lucia Estuary Mouth, *T. granifera* had a smaller niche width compared to *M. tuberculata*, whereas, at the Mpophomeni Stream *T. granifera* had a larger niche width than *M. tuberculata* (Figure 2.6a and b).

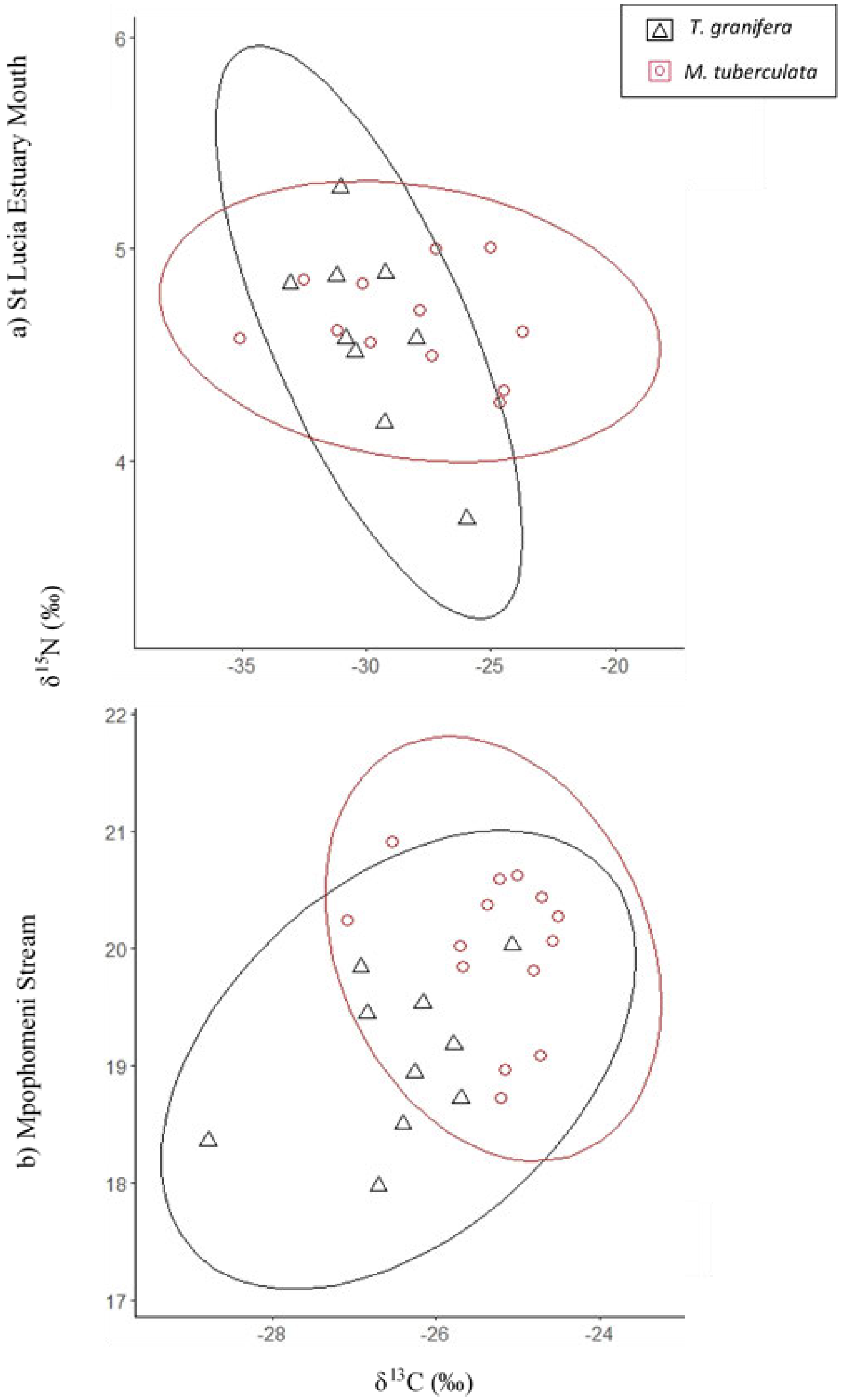


Figure 2.6: Stable Isotope Bayesian Ellipses comparing the core isotopic niche widths and niche overlaps of *Melanoides tuberculata* and *Tarebia granifera* in a) St Lucia Estuary Mouth and b) Mpophomeni Stream. The isotopic standard ellipses of each species were calculated with 95% confidential intervals.

The niche overlaps were similar at the St Lucia Estuary Mouth (36.51%) and the Mpophomeni Stream (36.52%) (Table 2.1)

Table 2.1: Bayesian ellipses (95% confidence interval) showing the area of *Melanoides tuberculata* and *Tarebia granifera* area of overlap and the percentage of overlap between the two gastropods at the two study sites.

Sites	**Area (‰^{2})**			**Overlap (%)**
	M. tuberculata	*T. granifera*	Overlap	
St Lucia Estuary Mouth	17.00	13.96	8.28	36.51
Mpophomeni Stream	9.61	12.70	5.97	36.52

2.4) Discussion

The recent state shift at the St Lucia Estuary was due to the GEF project during 2016/2017 (Jones et al. 2020) which created favourable environmental conditions and allowed for the range expansion of *T. granifera* (Perissinotto et al. 2014). The two species now occur sympatrically, providing the opportunity to investigate their diet and see whether one species feeds any differently in the presence of the other. The highly abundant invader, *T. granifera*, is known to exploit resources efficiently due to their high feeding rates (Miranda et al. 2010), thereby, reducing the availability of food resources for *M. tuberculata* (Hill et al. 2015). Multiple organisms consuming or favouring a single food resource can cause potential trophic shifts in a food web over time (Hill et al. 2015). The δ^{13}C and δ^{15}N signatures of consumers can be used to quantify energy flows, types of food sources and trophic links (DeNiro and Epstein, 1978; Minagawa and Wada, 1984; Peterson and Fry, 1987; Middleburg 2014). The current study aimed to investigate dietary preferences and niche overlap of the sympatric, *M. tuberculata* and *T. granifera*. The results of this study showed that the overall δ^{13}C and δ^{15}N signatures of the gastropods and food sources were significantly different between the St Lucia Estuary Mouth and Mpophomeni Stream. The gastropods at the Mpophomeni Stream had a higher mean δ^{15}N signature compared to those from the St Lucia Estuary Mouth, possibly due to nutrient enrichment (Miranda and Perissinotto, 2012; Peer et al. 2015). Nutrient enrichment occurs when there is an increase in nutrients from naturally occurring sources, agriculture runoff, domestic or industrial effluent (Peer et al. 2015; Nunes et al. 2017). Previous studies have shown that nutrient enrichment of the St Lucia Estuary could be due to factors such as increased rainfall, the joining of the Mfolozi River Mouth to the estuarine system as well as faecal matter of various animals such as hippopotami and waterfowl (Miranda and Perissinotto, 2012; Subalusky et al. 2015; Raw et al. 2016b; Nunes et al. 2017). Increased dissolved nutrients affect vegetation growth, which could have influenced the abundances of varying food sources (Miranda and Perissinotto, 2012; Nunes et al. 2017).

This study exhibited findings similar to previous literature which indicated that *M. tuberculata* and *T. granifera* have generalist diets (Miranda et al. 2011a, 2011b; Miranda and Perissinotto, 2012; Raw et al. 2016a, 2016b; Jones et al. 2017). Sheldon and Walker (1997) and Raw et al. (2016a, 2016b) showed that in mangrove habitats, *M. tuberculata* consumed filamentous algae and organic detritus that boost energy stores. This was also observed at the St Lucia Estuary Mouth. Detritus, POM and *Cladophora* sp. contributed the most to the *M. tuberculata* and *T. granifera* diets, which indicated that both species have very similar diets. A study conducted by Raw et al. (2016b) showed that detritus (decomposing mangrove leaves) and POM contributed greatly to the diet of *M. tuberculata* at the St Lucia Estuary Mouth. In comparison to *Cladophora* sp., detritus is considered a nutritionally-poor food source for gastropods as it lacks the ability to fulfil energy stores as efficiently (Levinton et al. 1984; Raw et al. 2016a). Mangrove leaf fall increases in wet summer months (Steinke 1999) which could explain why detritus contributed the most to both gastropods' diets. Reichard et al. (2002) also noted the significant abundance of detrital food source in mangrove systems. The evidence of POM in the gastropods' diets could be attributed to the consistent availability of POM in this system due to frequent mixing near the sediment-water interface in shallow water regions (Rand 1995; Raw et al. 2016b). At the St Lucia Estuary Mouth, the food sources that contributed the least to both gastropods were SOM and MPB. This may have occurred due to the higher accessibility to other food sources such as detritus, *Cladophora* sp. and POM, therefore, the gastropods were potentially consuming food sources that were readily available (Raw et al. 2016b). Food availability also depends on seasonal fluctuations (Werner and Gilliam, 1984; Juncos et al. 2015; Peer et al. 2015; Raw et al. 2016b) which may result in the two gastropods exhibiting temporal shifts in diet related to food availability (Juncos et al. 2015; Raw et al. 2016b). Microphytobenthos becomes increasingly available during dry seasons, May to September (Raw et al. 2016b), which could have accounted for MPB contributing the least to both gastropods' diets.

At the Mpophomeni Stream, the food sources that contributed the most to *M. tuberculata* and *T. granifera* diets were *Cladophora* sp. and MPB. A study conducted by Miranda and Perissinotto et al. (2012) showed similar results and suggested that the highly abundant *Cladophora* sp., was a food source consumed by both the native and invasive gastropods. Nutrient enrichment via nitrogenous effluent from local farms, domestic run-off and industrial activities could have influenced the increased growth of algal populations such as *Cladophora* sp. (Peer et al. 2015), therefore, making this food source more readily available. In the Mpophomeni Stream, detritus was the least important food source to both gastropods' diets. The Mpophomeni stream flows through an area dominated by sand forest (Kirkwood and Midgley, 1999), where environmental conditions differ from the mangrove dominated St Lucia Estuary Mouth (Whitfield and Taylor, 2009), therefore, this could have affected the types and abundances of foods available for the gastropods to consume.

Miranda et al. (2010) showed that both gastropod species can survive a wide range of environmental conditions. Shifts in the gastropods diets can be caused by seasonal fluctuations, which influence the environmental conditions that subsequently affect the type and abundance of food resources that may be available in the system (Werner and Gilliam, 1984; Juncos et al. 2015; Peer et al. 2015; Raw et al. 2016b). However, the results of the current study showed that both gastropods consume variable proportions of similar foods between the two study sites. This result may indicate a high degree of diet plasticity and potential adaptive traits (Raw et al. 2016b; Schalk et al. 2017). Therefore, the hypothesis that stated diet preferences of both gastropods were similar within and between sites, was supported as similar resources were consumed between sites.

This study showed that at the St Lucia Estuary Mouth, *M. tuberculata* had a slightly larger niche width than *T. granifera*. Contrary to that, at the Mpophomeni Stream, *T. granifera* had the larger niche width compared to *M. tuberculata*. There was a significant difference between the $\delta^{13}C$ and $\delta^{15}N$ signatures of the gastropods at the Mpophomeni Stream compared to the St Lucia Estuary Mouth, which could have explained the changes in niche sizes, as the isotopic signatures influence the niche widths of each species. The use of a wide range of food sources and a larger niche are typical of a generalist diet, whereas a smaller niche implies an organism tends towards a specialist diet (van Valen 1965; Bolnick et al. 2007; Cummings et al. 2012). Specialist species tend to favour a specific food source and prosper when changes to environmental conditions are limited (Wilson and Hayek, 2015). Specialist species are more vulnerable, as factors such as climate change and the introduction of a non-native species can threaten the amount of food available (Wilson and Hayek, 2015). *Tarebia granifera* and *M. tuberculata* were found to be generalist feeders as they both had large niche widths in the respective study sites. However, *T. granifera* consumed an overall larger range of food sources than *M. tuberculata* at the Mpophomeni Stream. *Melanoides tuberculata* has a smaller niche width compared to *T. granifera* at the Mpophomeni Stream which gives this invasive the potential competitive advantage over *M. tuberculata*. Contrary to this, a study conducted by Pettitt-Wade (2016) showed that the success of invasive invertebrates was less likely to depend solely on the large dietary niche widths due to limited mobility and feeding strategies. The success of invasive invertebrates could depend greatly on the ability to tolerate wide ranges of temperatures, salinities, dispersal tactics and reproductive abilities (switching from sexual to asexual reproduction) (Pettitt-Wade 2016). Hill et al. (2015) stated that generalist feeders can survive in a variety of environmental conditions as they are not only limited by what they consume.

Consumers that have shared diets often indicate trophic overlaps (Wang et al. 2018). The results of this study showed that these gastropods have shared diets, therefore, indicating shared isotopic niche widths and an overlap in niche space. The degree of overlap is represented as a percentage estimate between 0 and 100%. Overlapping estimates that are greater than 60% infer ecologically significant dietary overlaps and potential direct competition for resources (Hill et al. 2015; Pastore 2018). This study demonstrated that the degree of overlap at St Lucia Estuary Mouth (36.51%) and the Mpophomeni Stream (36.52%) was less than 60%, which suggests there is a lower potential for direct resource competition. Therefore, the hypothesis which stated that the dietary niches of these species overlap significantly, was not supported. A study conducted by Miranda and Perissinotto (2012) showed that *T. granifera* and *M. tuberculata* had a significant isotopic dietary overlap (IDO) of 85% at Lake Nhlange (Kosi Lakes in KwaZulu-Natal) in 2008. A larger niche overlap can occur when there is limited supply of the available food sources which can lead to less discrimination in feeding preferences (Calow and Calow, 1975). The results obtained from this study also showed that at the St Lucia Estuary Mouth, there was a wide $\delta^{13}C$ signature spread, this could be attributed to the high abundance and availability of detritus at the St Lucia Estuary Mouth. Mangrove forests consist mostly of decomposing mangrove leaf litter, which is broken down further to form detritus (Raw et al. 2016b; Mulya and Arlen, 2018). Kristensen et al. (2008) stated that in mangrove forests, the dominant sources of autochthonous carbon included mangrove leaf litter and benthic algae. Mangroves also act as carbon sinks due to their balance in carbon production and source decomposition (Twilley et al. 1992; Gonneea et al. 2004).

Competition occurs when an invasive species is introduced into a system that is already occupied by another species of the same trophic level with similar resource requirements (Schoener 1983; Paustian and Barbosa, 2012). If competition favours the invasive species, the native species may experience a decline in population numbers and/or displacement from habitat (Paustian and Barbosa, 2012). In the current study, the gastropods did not experience significant dietary niche overlap (less than 60%) at either site therefore, competitive exclusion could have been averted. According to the “competitive exclusion principle”, consumers that share identical niches and compete for the same food source cannot stably coexist, thereby introducing niche partitioning (Gause 1934; Hutchinson 1957; Miranda and Perissinotto, 2012). At the Mpophomeni Stream, *T. granifera* consumed very low proportions of MPB compared to *M. tuberculata*. This could suggest that even though the native species do consume *Cladophora* sp., there is a possibility that they will be more inclined to switch to the readily available MPB than the invasive. Previous studies (Miranda et al. 2010; Raw et al. 2016a, 2016b) showed that *T. granifera* was successful in displacing the native, *M. tuberculata* within South African estuaries. Niche partitioning is expected when there is competition among species for similar resources (Novack-Gottshall 2016). Partitioning of resources can take many forms, including changes in foraging strategy, behaviour and habitat specialisation (Hutchinson 1959; Novack-Gottshall 2016). Miranda and Perissinotto (2012) showed that niche partitioning could effectively reduce the niche overlap between the invasive and native species. Noticeable individual-level differences at the Mpophomeni Stream may be due to variability in microhabitat source availability and habitat heterogeneity (Doi et al. 2010; Raw et al. 2016b). Raw et al. (2016b) showed that *M. tuberculata* and

T. granifera both have a generalist diet and that *M. tuberculata* had a larger niche width, this could have occurred due to the gastropod utilizing variable proportions of the same resources and at different rates. The St Lucia Estuary had adequate food resources (Miranda and Perissinotto, 2012) which allowed for the competitive coexistence of the two species with generalist diets.

A study conducted by Pacioglu et al. (2019) showed that the invasive (*Faxonius limosus*) and native species (*Pontastacus leptodactylus*) of crayfish competed for food resources over time, as they shared an almost identical diet which almost drove the native species to extinction. However, due to resource partitioning and new adaptive traits (larger and heavier claw of the male native population to improve foraging) the two species were able to coexist (Pacioglu et al. 2019). Findings in Penk et al. (2018) showed that an invasive mysid shrimp (*Heminysis anomala*) and native species (*Mysis salemaai*) were able to coexist due to spatio-temporal niche partitioning. Zwerschke et al. (2018) also showed that the native oyster (*Ostrea edulis*) was able to alter their niche size and the invasive oyster (*Magallana gigas*) was able to shift their niche centroid in response to one another, which could have allowed these two species to coexist. Despite the gastropods showing evidence of niche partitioning and the ability to avert competitive exclusion, previous studies such as Miranda et al. (2010) and Raw et al. (2016a, 2016b) showed that the invasive gastropod was able to dominate and displace native species numerically. Other factors facilitating coexistence between species include the functional response relationships with the food resources, temporal variations and varying densities in resources (Kneitel 2013). The introduction of a new species into a system can affect the feeding abilities of the native species, however, the curvature of functional responses can determine if the two consumers feeding on a single resource are able to coexist (Koch 1974; Abrams and Holt, 2002; Kneitel 2013; Klauschies and Gaedke, 2020).

This study provided a ‘snapshot’ of dietary preferences and niche overlap between native and invasive co-existing species. Stable Isotopes Analysis can be used in conjunction with many other predictive tools to assess the drivers of invasion success over an extended period. *Tarebia granifera* and *M. tuberculata* were both considered generalist feeders that shared similar diets, however, *M. tuberculata* can tend towards a specialist diet depending on locality. A specialist feeder tends to favour fewer resources, therefore, *M. tuberculata* consumed a larger portion of one particular resource and did not favour other resources in the same area (that had abundant food resources) compared to *T. granifera*. Comparison of prior studies that investigated diet of these species individually (Miranda and Perissinotto, 2012; Raw et al. 2016b), with this study (both species in co-existence) show no evidence of alteration in diet in the presence of the invasive species. It is likely that food availability is structuring the diet of these species. However, *M. tuberculata* and *T. granifera* do show a degree of trophic plasticity by altering diets to the varying conditions of the St Lucia Estuary Mouth and the Mpophomeni Stream. The gastropods in this study did not exhibit significant dietary overlap, therefore, averting competitive exclusion due to potential niche partitioning. Many other factors can facilitate the successful invasion of *T. granifera* in South African estuaries, such as high densities due to their high reproductive rates, high feeding rates, ability to adapt to a wide range of physicochemical parameters and lack of predation (Jones et al. 2009; Jones et al. 2017). The feeding preferences and niche overlap are small entities when considering biological invasions, therefore, more studies need to include more complex interactions between an invader and a native species. Future studies should consider all potential food sources that occur within a system and have a seasonal view over a longer period. It is important to note that physiological variations can help in understanding feeding and living preferences of organisms which can aid in determining the coexistence of species. Other factors that should be considered when comparing feeding dynamics between an invasive species to a native species is the functional response and their behavioural changes due to physical interactions. This study can contribute to invasive biology literature and improve the understanding in how these gastropods coexist. Future studies should include long-term and continuous monitoring of current coexistence as ecosystems may experience shifts due to competitive interactions, in the future.

2.5) Acknowledgements

A sincere thank you to Jamila Janna for providing valuable data towards this study. iSimangaliso Wetland Park Authority and Caroline Fox from Ezemvelo KZN Wildlife are thanked for logistical support in the field. The Gastropoda sampled in this study were collected under an iSimangaliso Wetland Park Authority Research Agreement together with an Environmental and Fisheries Research Permit (RES 2017/71) issued by the South African Department of Agriculture, Forestry and Fisheries). Dr Grant Hall of the Stable Isotope Laboratory at the Mammal Research Institute (University of Pretoria) is acknowledged for processing and analysing of the stable isotope samples. This project was funded by the National Research Foundation (NRF, unique grant number 109523).

2.6) Appendix

Table 2.2: The mean (± SD) $\delta^{13}C$ and $\delta^{15}N$ (‰) of *Melanoides tuberculata* and *Tarebia granifera* at St Lucia Estuary Mouth and Mpophomeni Stream.

Species	**$\delta^{13}C$ (± SD) (‰)**		**$\delta^{15}N$ (± SD) (‰)**	
	Mouth	Mpophomeni Stream	Mouth	Mpophomeni Stream
M. tuberculata	-28.26 (± 3.56)	-25.31 (± 0.74)	4.66 (± 0.24)	20.00 (± 0.66)
T. granifera	-29.89 (± 2.07)	-26.46 (± 1.00)	4.61 (± 0.45)	19.06 (± 0.67)

Table 2.3: Contribution of each food source (%) at varying credibility intervals (2.5; 25; 50; 75; 97.5%) for *Melanoides tuberculata* and *Tarebia granifera* at the St Lucia Estuary Mouth. The median is represented by the 50% credibility interval and the mean (± SD) is representative of the whole dataset.

Species	**Credibility Intervals (%)**	**Contributed Each Food Source (%)**				
		POM	MPB	SOM	*Cladophora* sp.	Detritus
M. tuberculata	2.5	4.1	2.1	1.8	3.4	5.7
	25	13.7	6.9	6.4	14.0	21.1
	50	21.4	10.9	10.4	22.0	30.5
	75	30.3	15.9	15.5	30.3	40.4
	97.5	51.8	26.6	27.1	45.9	60.3
	Mean (± SD)	23.0 (± 12.5)	11.8 (± 6.4)	11.5 (± 6.7)	22.6 (± 11.4)	31.1 (± 13.9)
T. granifera	2.5	3.0	1.9	1.6	2.1	4.3
	25	13.1	6.5	5.5	10.3	19.2
	50	22.5	10.6	9.3	19.1	30.8
	75	34.2	15.8	14.8	29.3	43.6
	97.5	59.2	27.5	27.1	49.3	66.7
	Mean (± SD)	24.9 (± 15.2)	11.7 (± 6.9)	10.7 (± 6.8)	20.7 (± 12.9)	32.0 (± 16.7)

Table 2.4: Contribution of each food source (%) at varying credibility intervals (2.5; 25; 50; 75; 97.5%) of *Melanoides tuberculata* and *Tarebia granifera* at Mpophomeni Stream. The median is represented by the 50% credibility interval and the mean (± SD) is representative of the whole dataset.

Species	**Credibility Intervals (%)**	**Contribution of Each Food Source (%)**				
		POM	MPB	SOM	*Cladophora* sp.	Detritus
M. tuberculata	2.5	1.8	3.4	1.5	27.4	1.2
	25	6.2	13.6	6.4	35.8	4.2
	50	11.4	22.2	12.2	39.8	7.4
	75	19.6	30.4	20.5	43.4	12.7
	97.5	38.9	42.6	41.9	49.3	30.6
	Mean (± SD)	14.0 (± 10.0)	22.2 (± 10.8)	14.8 (± 10.8)	39.4 (± 5.7)	9.7 (± 7.9)
T. granifera	2.5	1.3	1.9	1.4	43.8	1.0
	25	4.8	7.8	4.9	52.6	3.4
	50	8.6	13.0	9.0	56.6	6.2
	75	14.3	19.2	15.1	60.9	10.6
	97.5	28.4	29.7	30.4	69.9	25.7
	Mean (± SD)	10.4 (± 7.3)	13.9 (± 7.6)	10.9 (± 7.8)	56.7 (± 6.6)	8.1 (± 6.6)

Table 2.5: The mean (± SD) $\delta^{15}N$ (‰) and $\delta^{13}C$ (‰) signatures of each food source at the St Lucia Estuary Mouth and Mpophomeni Stream.

Sources	**St Lucia Estuary Mouth**		**Mpophomeni Stream**	
	$\delta^{15}N$ (‰)	**$\delta^{13}C$ (‰)**	**$\delta^{15}N$ (‰)**	**$\delta^{13}C$ (‰)**
	Mean (± SD)	**Mean (± SD)**	**Mean (± SD)**	**Mean (± SD)**
POM	2.64 (± 0.87)	-27.25 (± 0.27)	7.53 (± 0.84)	-23.05 (± 0.49)
MPB	5.06 (± 1.01)	-26.15 (± 3.46)	9.75 (± 0.68)	-21.61 (± 0.17)
SOM	4.58 (± 0.63)	-23.97 (± 0.35)	8.13 (± 2.41)	-23.59 (± 0.37)
Cladophora sp.	1.26 (± 0.04)	-24.18 (± 0.58)	14.68 (± 0.19)	-29.88 (± 0.43)
Detritus	1.95 (± 0.32)	-27.04 (± 0.22)	5.22 (± 0.19)	-25.71 (± 0.03)

CHAPTER 3

Comparative functional responses of invasive (*Tarebia granifera*) and native (*Melanoides tuberculata*) gastropods.

Tarebia granifera is an invasive gastropod that has successfully invaded several tropical and subtropical regions. It was introduced to South Africa in the 1990s, outnumbering and displacing native gastropods, including *Melanoides tuberculata*. Chemical cues (such as secondary metabolites) released by invasive gastropods have shown to aid in this invasion. Although the degree of invasiveness and potential ecological impacts are difficult to assess, feeding dynamics can aid in forecasting the environmental impact. The functional responses (FRs) of each species with and without the presence of heterospecific chemical cues were determined in the laboratory by measuring the relationship between the consumption rate of a food resource and its availability. Functional response parameters (i.e. attack rate, handling time and feeding rate) influence the magnitude of the FR curves. Mixed species experiments were also conducted in order to observe physical interactions (active, inactive and feeding) among individuals and measure resource consumption when both species are placed together under high and low food availability. Both species exhibited Type II FRs and had similar feeding rates. However, feeding rates of *M. tuberculata* and *T. granifera* were significantly affected by chemical cues and resource availability. The mixed species experiment supported the FR results where *T. granifera* spent a shorter time feeding at higher food abundances, therefore, consuming less *Ulva*. The invasive species did not always exhibit high FR parameters indicative of its expected high impact potential. Multi-disciplinary approaches and continuous assessment are recommended when assessing ecological impacts of invasive species.

Key words: Feeding dynamics, behaviour, St Lucia Estuary, invasion biology, chemical cues

3.1) Introduction

Biological invasions can affect the way an ecosystem functions by disrupting ecological processes and interactions between species (Alexander et al. 2012, 2014; Dick et al. 2014; Xu et al. 2016). In most cases, invasive species can utilize resources in a new environment more efficiently than a native species (Vitousek 1990; Morrison and Hay, 2011; Dick et al. 2014). The “invasiveness” of non-native populations can be influenced by various factors such as growth/dispersal rates, resource availability, and habitat heterogeneity (Catford et al. 2009). However, determining the degree of invasiveness and potential ecological impact that non-native species have on ecosystems is a recurring challenge faced in invasive biology (Hill et al. 2015; Xu et al. 2016). The relationship between the food density and food consumption rates can be used to assess the stability of food webs within ecosystems and the overall feeding dynamics of populations (Rogers 1972; Jeschke et al. 2002; Kalinkat et al. 2013). Due to the inability to precisely quantify the feeding impacts of invasive species on ecosystems, functional responses have increasingly been used to compare invasive and native consumers against one another in relation to the food consumed (Alexander et al. 2012, 2014; Dick et al. 2014) and to provide insight into how consumers may influence food densities (Abrams 1990).

Functional responses (FRs) are used to determine the potential feeding impact of invaders by measuring the relationship between the food consumed per capita and the food density (Holling 1959a; Jeschke et al. 2004; Alexander et al. 2012; Rosenbaum and Rall, 2018). Functional responses of consumers are represented as FR curves, where the magnitude of the curve is determined by feeding parameters such as attack rate (a), handling time (h) and maximum feeding rate (F_{max}) (Holling 1959b; Dick et al. 2014; Rosenbaum and Rall, 2018). Attack rate is the capture or searching efficiency of the consumer which controls the invader’s impact at low food resource densities (Rosenbaum and Rall, 2018). Handling time is the time taken to consume, subdue or pursue the food resource and controls feeding at high food resource densities (Dick et al. 2014; Rosenbaum and Rall, 2018; Cuthbert et al. 2019). The F_{max} corresponds to the inverse of handling time (Rosenbaum and Rall, 2018; Cuthbert et al. 2019). These feeding parameters can be used to estimate the type of feeding impact an invader exhibits: 1) low a and high h, indicating a low impact invader; 2) low h and high a, indicating a high impact invader (Cuthbert et al. 2019; South et al. 2019). However, in certain circumstances the consumer may exhibit either a low a and h or a high a and h, this is indicative of an intermediate impact invader (Cuthbert et al. 2019; South et al. 2019).

An intermediate impact invader may be difficult to identify and contradictory impact estimates may emerge, therefore, considering the feeding parameters as separate entities may hinder the prediction of invader feeding impacts (Cuthbert et al. 2019; South et al. 2019). A combination of the feeding parameters using the functional response ratio (FRR) metric may increase the robustness and reliability of predicting the impacts of an invader and the degree of "invasiveness" (Cuthbert et al. 2019). The functional response ratios are calculated using attack rates and handling times which are generated by the FR analyses (FRR = a/h). If the FRR of the invasive species is higher than the native species, the invader is considered a higher predicted impact invader and if the FRR of the invasive species is lower than the native species, the invader is considered a lower predicted impact invader (Cuthbert et al. 2019; South et al. 2019). Functional response ratios have been successful in distinguishing ecologically damaging invaders from various trophic groups, ecological systems, taxa and habitats (Cuthbert et al. 2019; South et al. 2019).

There are three types of functional response curves that describe the relationships between the food consumed and food density. Type I FR curve is a linear, density-dependent curve that indicates as food density increases, the food consumed increases proportionately (Holling 1959a, b; Alexander et al. 2013; Figure 3.1). The Type II FR curve illustrates a hyperbolic curve with a linear rise in food consumed as food density increases, until a threshold is reached (Holling 1959a, b). Thereafter, the food consumed remains constant as food density increases until a plateau is reached (Figure 3.1). The response curve is inversely density-dependent and reaches an asymptotic line, possibly due to handling time constraints (Rosenbaum and Rall, 2018). A Type II FR is typical of a specialist consumer, a consumer feeding on a single food resource (Holling 1959a, b). These consumers are known to destabilize populations by depleting a particular food resource (van Leeuwen et al. 2007; Alexander et al. 2012). The Type III FR curve is sigmoidal and density-dependent. This response occurs when the consumer search time increases as the food density decreases (Holling 1959a, b; Hassell 1978; Alexander et al. 2013). A Type III FR (Figure 3.1) is typical of a generalist consumer (a consumer feeding on multiple food resources), which is known to impart stability to the dynamic between the consumer and food resources (van Leeuwen et al. 2007; Alexander et al. 2012). This response resembles an S-shaped curve which shows an accelerating phase where at low food densities, there is an increase in the food consumed that leads to an exponential consumption (Holling 1959a, b; Hassell 1978; Taylor and Collie, 2003; van Leeuwen et al. 2007; Alexander et al. 2013). Thereafter, there is a 'learning' phase as consumer numbers increase and is then followed by a plateau that is similar to Type II (Holling 1959a, b; Hassell 1978; Taylor and Collie, 2003; Alexander et al. 2013). The functional response curve of each consumer serves as a key indicator to whether the food populations are at risk of extinction or if the food can continue to persist with the existence of that consumer (Alexander et al. 2013). Functional response curves of invasive and native species may identify differences or similarities in feeding dynamics (Alexander et al. 2013; Dick et al. 2014). Observing the feeding regimes and behavioural responses between consumers may, therefore, provide useful information on how invasive populations interact with native populations within an ecosystem (Alexander et al. 2014; Dick et al. 2014; Hoxha et al. 2018).

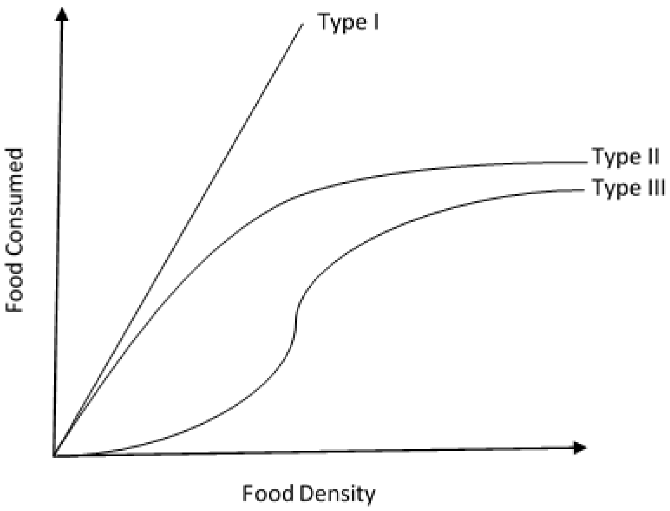


Figure 3.1: Functional response curves (Types I-III) showing the relationships between food density and food consumed (adapted from Staddon 1983). Type I FR indicates that food consumed rises linearly with food density; Type II FR indicates food consumed rises with food density but gradually decreases until a plateau is reached and a Type III FR is characterized by a ‘learning’ phase. It is an S-shape (slow increase, fast increase, slow increase, plateau).

Successful biological invasions are influenced by the interactions between the native and alien species which are related to the dispersal, foraging, courtship, species recognition and predator avoidance of organisms within an ecosystem (Dionne and Dubin, 1994; Lockwood et al. 2007; Hawkes 2009). In turbid benthic environments, visual cues are hampered, which subsequently hinders the abovementioned factors (Dodson et al. 1994; Fink et al. 2006). Therefore, the use of chemical cues, as an attractant or a deterrent, may play a vital role in functional and behavioural responses (Sotka et al. 2009; Raw et al. 2015). Interactions between individuals or species are important factors that affect the success of an invader (Bulleri et al. 2008; Raw et al. 2013, 2015). However, the interactions that affect functional and behavioural responses are not limited to only physical interactions and can include chemical interactions in the form of disturbance pheromones or other chemical cues (Raw et al. 2013; Schulte et al. 2015). Chemical cues are a suitable form of communication within aquatic environments as the chemical substance can be easily distributed to reach a receiver (Wisenden 2000). Food resources such as benthic algae have volatile organic compounds (VOCs) or infochemicals (chemical substances that transport important information) that are released and dispersed over a large distance and they are good indicators of the algal food’s presence (Fink et al. 2006). The VOCs are also released as secondary metabolites by the gastropod or any microbial symbiont that can play a vital role in facilitating interactions between the biota within the ecosystem (Raw et al. 2013). The strong scents of the VOCs are easily picked up by chemoreceptors of the osphradium in the gastropod’s mantle cavity (Wedemeyer and Schild, 1995; Fink et al. 2006). Chemoreceptive studies are approached in three ways: 1) anatomy, histology and morphology of the receptors, 2) behavioural response to the chemical stimuli and 3) direct physiology through measuring impulses/electrical activity generated by sensory nerves (Kohn 1961). Approaches one and two have been used most often in chemoreceptive studies of gastropods (Kohn 1961).

Chemical cues released by organisms can vary depending on the situation that the organism may encounter, therefore, affecting the duration and range of the signals (Wisenden, 2000). Although chemical cues may be used as an attractant, especially in courtship, they can also be used as a deterrent in foraging and predator avoidance (Croll 1983). Heterospecific chemical cues are essential when considering foraging, as one individual of one species attains the food source first, the involuntary release of the pheromones communicates to the individuals of another species that the food resource is “off-limits” (Kohn 1961; Croll 1983; Fink et al. 2006; Raw et al. 2015). The release of the pheromones is executed to potentially allow for recognition of another species or an individual, and this may deter the individual/species away from the food source (Croll 1983; Wisenden 2000). In some cases, the chemical cues released by individuals of one species may have a neutral effect on another species, therefore, there is no influence on the functional or behavioural responses of the receptive species (Schulte et al. 2015).

There is limited information regarding the ecological impacts of invasive freshwater molluscs on South African estuaries (Weyl et al. 2020). This study aimed to address this research gap by investigating differential feeding dynamics between the invasive and native gastropod species. The main objectives were to: 1) determine the FRs of the two gastropod species, 2) estimate the FRR and potential feeding impact of the invasive species, 3) determine if chemical cues can influence the FRs of the gastropods and 4) determine if the physical presence of one species affects the behaviour and feeding activities of the other species. In the FR experiments, the gastropods were subjected to increasing resource densities with and without the presence of heterospecific chemical cues. It was hypothesized that chemical cues of one species would affect the FR parameters of another species. Mixed species experiments were also conducted in order to observe physical interactions among individuals, in the presence of food. It was hypothesized that invasive species would spend more time feeding during the mixed species experiment.

3.2) Materials and Methods

3.2.1) Study Site:

The St Lucia Estuary (28°23'0" S, 32°25'0" E) is located on the East coast of South Africa. The average depth of St Lucia Estuary is ± 0.9 m with a surface area of approximately 325 km^2 (Taylor et al. 2006). The specimens of this study were collected from a freshwater mangrove environment in the mouth area of the St Lucia Estuary (Figure 3.2).

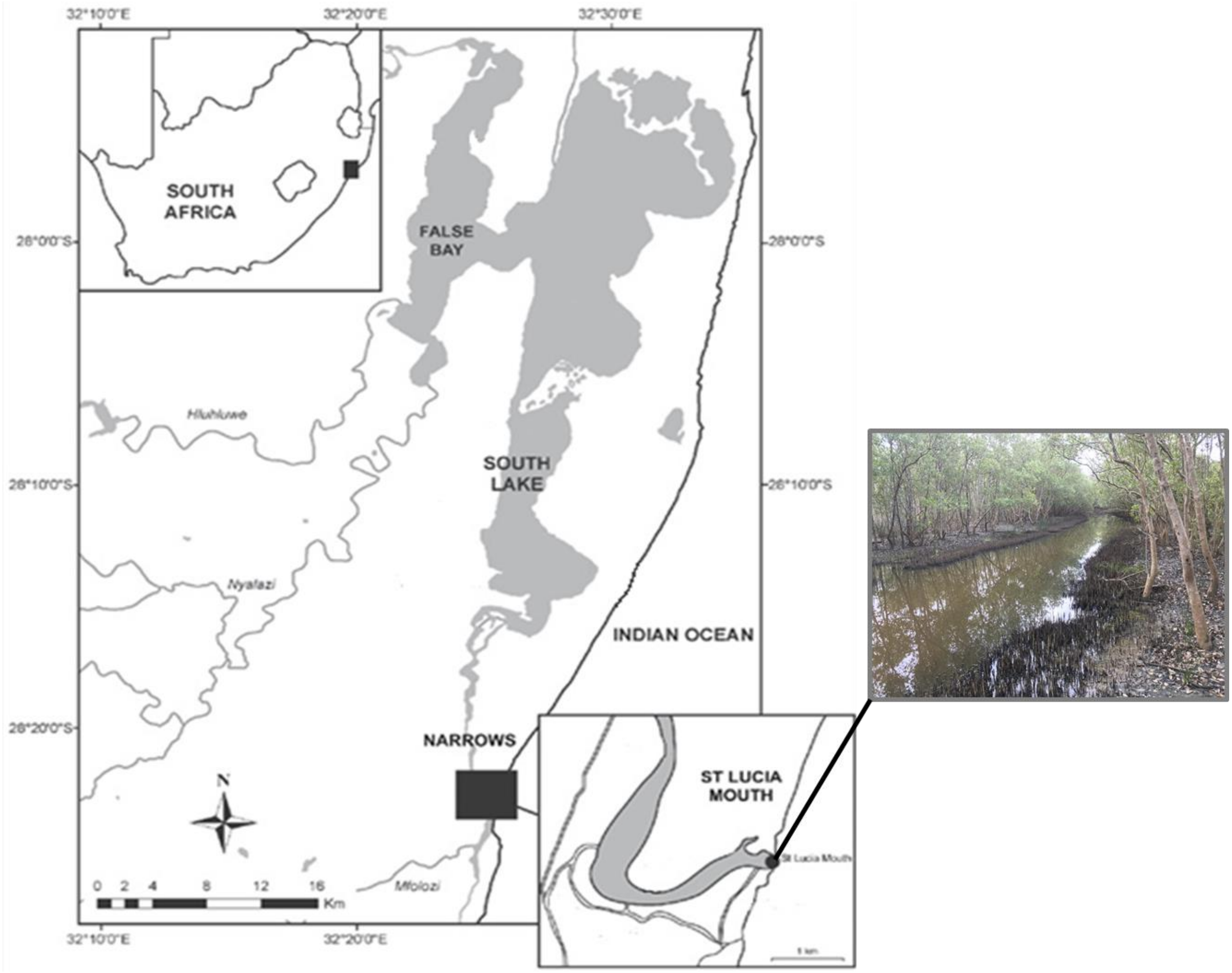


Figure 3.2: Locality map illustrating the mangrove sampling site (•) at the St Lucia Estuary Mouth (28°23'0" S, 32°25'0" E) (adapted from Carrasco and Perissinotto, 2012).

The invasive, *T. granifera*, and the native, *M. tuberculata,* gastropods were collected on multiple occasions (from March until July 2019) and experimentation was consistently conducted within 1 month of collection. Approximately 100 individuals per species were collected by hand on each occasion. The native and invasive gastropods were separated and kept in 20 L plastic buckets that contained water from the mangrove and natural feed (*Cladophora* sp.) before transportation to the laboratory for acclimation to experimental conditions.

3.2.2) Functional Response Curves: Single Species Experiment

All freshwater gastropods were acclimated for 48 hours prior to experimentation, where each species was moved to a new bucket that had distilled, uncontaminated water to reduce salinity stress (Miranda et al. 2010) and they were then fed *Ulva lactuca* (referred to as *Ulva* from hereon). The *Ulva* was sampled at Reunion Park Beach, Isipingo, KwaZulu-Natal. Distilled water produced in an Elix® Essential 10 (Water Purification System) by boiling water and leaving the water to condense, was used during experimentation. Macroalgae such as *Ulva* has been used as a viable feed in aquariums in some research studies (e.g. Oglesby 1977) and was the most durable feed in the preliminary trials. The natural feed (*Cladophora* sp.) is a filamentous alga that tore apart when mildly centrifuged and varied largely when weighed to the required food treatments. Microalgae (such as Spirulina) was used as food during preliminary trials, however, due to inconsistent fluorescent readings, this method was not considered viable for this study. Factors such as the duration of 12:12 hour light:dark cycles, the type of feed and general behaviour of the gastropods were determined during preliminary trials. A programmed timer was used to mimic conditions of the 12:12 hour light:dark conditions over the 48 hour experimental period. The shells of the gastropods were cleaned of any algae using 5% ethanol solution and gentle scrubbing with a toothbrush. The gastropods were left for 30 minutes in 5 L buckets with clean distilled water to recover (all gastropods were out of their shells) from the cleaning. Food was withheld for 24 hours prior to experiments to ensure evacuation of gut contents. The *Ulva* was spun dry by mild centrifugation (10 spins in alternating directions) and then blotted to remove excess water. After the excess water was removed, the *Ulva* was weighed to the feeding treatments: 10, 30, 50, 70 and 90 mg (Grahame 1973; Guidone et al. 2012).

There were four replicates with four different individuals in each replicate, per feeding treatment (i.e. n=16/feeding treatment) as well as two controls (without animals) per feeding treatment to account for any changes in the *Ulva* growth. This procedure was performed simultaneously for *M. tuberculata* and *T. granifera*. For the duration of the experiment, the gastropods were kept in open glass petri dishes (~15 cm x 2 cm) containing 150 mL of distilled water (Figure 3.3). This experiment was repeated three times to increase reliability and accuracy of the results.

To account for the potential growth of the *Ulva* the following equation was used (Xu et al. 2016):

$$\text{Potential Plant Growth (PPG)} = \mathrm{M}_{\text{Control48}} - \mathrm{M}_{\text{Control T0}}$$

Where, $\mathrm{M}_{\text{ControlT0}}$ and $\mathrm{M}_{\text{Control48}}$ represent the mass of *Ulva* at the start and end of the experimental period of 48 hours.

The gastropods were fed for 48 hours and the *Ulva* was mildly centrifuged and weighed again. The amount consumed in each feeding treatment was calculated by subtracting the final *Ulva* mass from the initial mass, considering any PPG from the control. Significant outliers were removed using the

Grubbs outlier test (Adikaram et al. 2015). The gastropod shell heights (15-36 mm) were measured (to the nearest millimetre) after the experiment using a Vernier calliper.

To account for the varying sizes of individuals the data were standardized for shell height as follows:

$$Ulva \text{ consumed} = \text{mean } Ulva\ c\text{onsumed} \left(\frac{\text{mean shell height of all individuals}}{\text{mean shell height of four individuals per replicate}}\right)$$

3.2.3) Functional Response Curves: Chemical Cues Experiment

In order to determine if chemical cues influenced the FRs of the gastropods, the above procedure was replicated and the native gastropod was exposed to distilled water that had been first infused with the chemical cue of the invasive and vice versa. The gastropods that were collected on previous occasions were used to create the chemical cue water. The ‘chemical cue’ water was generated by adding approximately 200 gastropods per species into separate 10 L buckets containing three litres of distilled water in each bucket. The gastropods were kept for 12 hours with *Ulva* to allow for the release of naturally occurring chemical cues. Thereafter, the water was filtered through an Econofilt RGF filter to remove any debris, accumulated microbes and particulate organic wastes (Raw et al. 2013). The “chemical cue” water was then stored separately in glass Schott bottles prior to use in experiments as above. Food was withheld from the gastropods 24 hours prior to experimentation to allow for gut evacuation and the gastropods were kept in 10 L plastic buckets containing distilled water. Before the experiment began, the food was placed into petri-dishes and 150 mL’s of the chemical cue water was then decanted into the petri-dishes. The water level was determined based on the size of the petri-dish, this amount was suitable in order to fully submerge the larger gastropods without causing overflow and this was kept constant throughout the experimental process. *Melanoides tuberculata* was placed in the “chemical cue” water produced by *T. granifera* and *T. granifera* was placed in the “chemical cue” water produced by *M. tuberculata*. The consumption of *Ulva* over a 48 hour period was weighed and calculated as described above (section 3.2.2). There were 4 replicates per feeding treatment (mg) which consisted of 4 individuals of each species per replicate and two controls that did not contain any gastropods. The controls were used to estimate PPG.

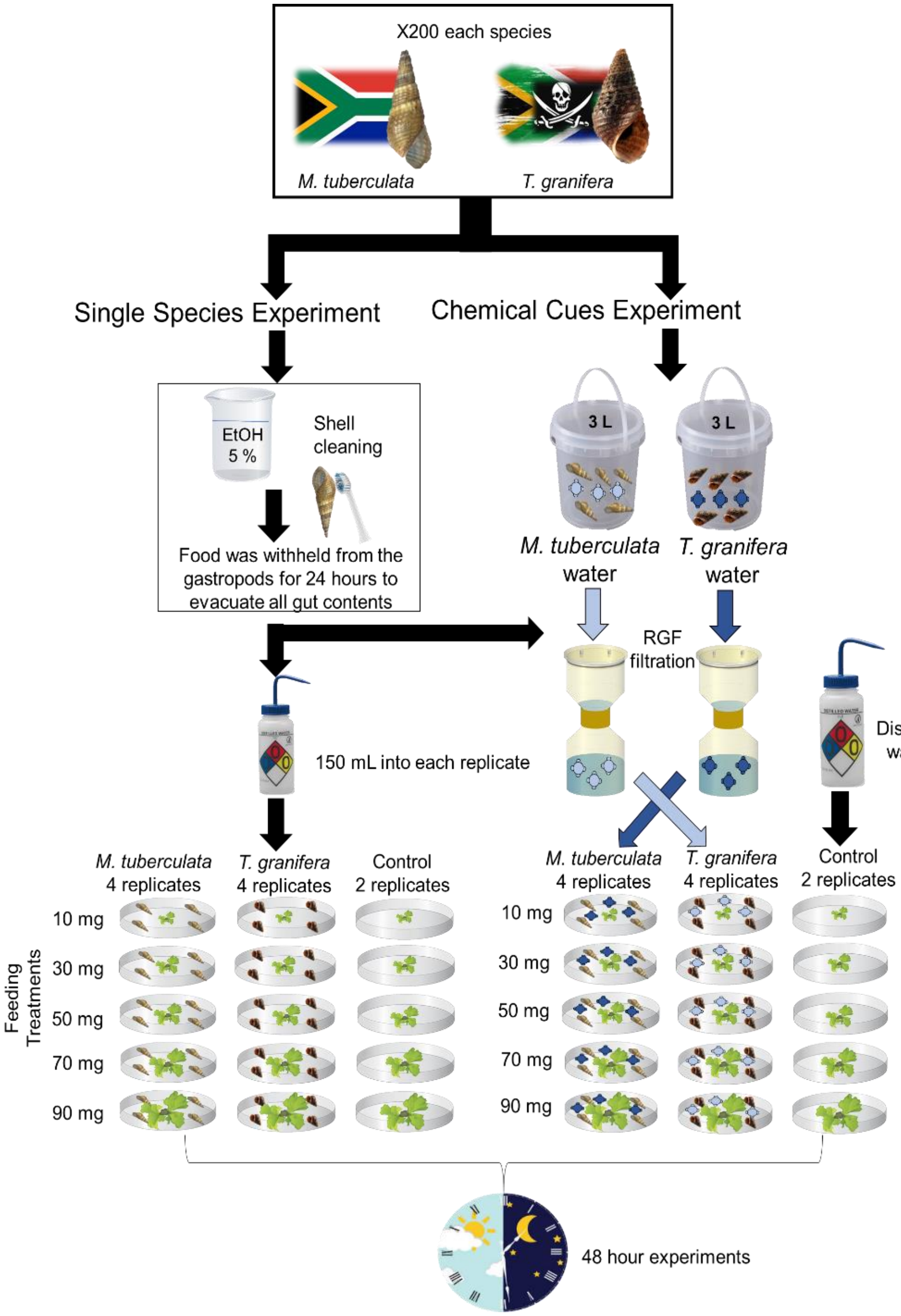


Figure 3.3: Experimental design for the single species experiment and the chemical cues experiment. In the chemical cues' experiments, *Melanoides tuberculata* "chemical cue" water (light blue) was added to the *Tarebia granifera* replicates and *Tarebia granifera* "chemical cue" water (dark blue) was added to the *Melanoides tuberculata* replicates.

Functional Responses were used to estimate the feeding interaction between the consumer and food by creating FR Curves using the "friar" v0.5.100 package in R software v3.6.1. (Pritchard et al. 2017). The "frair" package is an essential tool used for creating and analysing FR curves as well as determining if the experimental organisms follow a Type II or Type III functional response. A typical type II response is a hyperbolic curve where the individual's feeding rate (*F)* is dependent on the 'number' of foods in the environment (*N*).

$$F(N) = \frac{aN}{1 + ahN}$$

Where, *a* is the instantaneous rate of discovery or attack rate and *h* is the handling time.
The "frair_fit" command was used to produce the parameters such as attack rate and handling time. Maximum feeding rate (F_{max}) is the inverse of handling time:

$$F_{max} = \frac{1}{h}$$

The "friar_responses" command was used to test the various functional response models available which was followed by the "frair_test" command and the "Functional Response Coefficient Test", established which of the Type II or Type III response is preferred for that dataset. The equations used in the analyses to create the FR Curves rely on whether there was replacement of feed or not, throughout the experiment.

This study was conducted without replacing the food throughout the experiment which indicates that the Rogers' 'random predator' type II functional response equation is suitable (Roger 1972; Juliano 2001; Alexander et al. 2012):

$$N_e = N_0\left(1 - e^{\left(a(N_e h - T)\right)}\right)$$

Where N_e is the amount of food eaten, N_0 is the initial amount of food and *T* is the total time available. The command "fr_rogersII" represents the Rogers "random predator" type II functional response which is a decreasing food function (Rogers 1972). The FR curves were created to graphically observe the difference in magnitudes between the native and invasive species. The frair package calculates the statistical difference between feeding parameters within treatments (e.g. the *a* of *M. tuberculata* compared to the *a* of *T. granifera*, within single species experiment) and calculates a single *a* and *h* value for the respective experiments. The F_{max} is a derivative of handling time (1/*h).*

3.2.3) Behavioural Response: Mixed Species Experiment

To determine if there was any influence of one species on another species, two individuals from each species were observed together in a "mixed species experiment". The individuals were selected randomly and the shell heights (17-26 mm) were standardized as above. To identify and record gastropod behaviour, one individual of each species (generally the larger individual) was marked with a dot on the top of the shell with a correction pen as it was easy to apply and non-toxic. Based on the results of the FR curves, the feeding treatments of interest were narrowed down to the lowest (10 mg) and highest (90 mg) *Ulva* masses. The consumption of *Ulva* over a 48 hour period was weighed and calculated as described above (section 3.2.2). There were four replicates per feeding treatment and two controls that had *Ulva* only, this was done to account for PPG. The experiment was run on a 12:12-hour light:dark cycle over 48 hours, as described above (sections 3.2.2 and 3.2.3). The experiment did not include "chemical cue" water, instead, 150 mL's of distilled water was decanted into each petri-dish. The behaviour traits of each gastropod in each replicate were recorded according to three different types of activity, adapted from Miranda et al. (2010): 1) inactive - quiescent with operculum closed or head out of shell (foot not anchored); 2) active - foot anchored or moving (not feeding); 3) feeding (stationary feeding or actively moving and feeding). Over 48 hours the behaviour of the gastropods was monitored daily, in the morning, afternoon and evening (7-9 am, 2-4 pm and 6-7 pm, respectively) from initial time (0 minutes) followed by 30 minute intervals for the first hour, thereafter at 60 minute intervals (excluding evening) for the duration of the time period. The average counts of each behaviour trait were represented graphically as percentages. After the activity in each petri-dish was recorded, there were photographs taken to visually document behaviour at that time interval. This experiment was only repeated once over 48 hours.

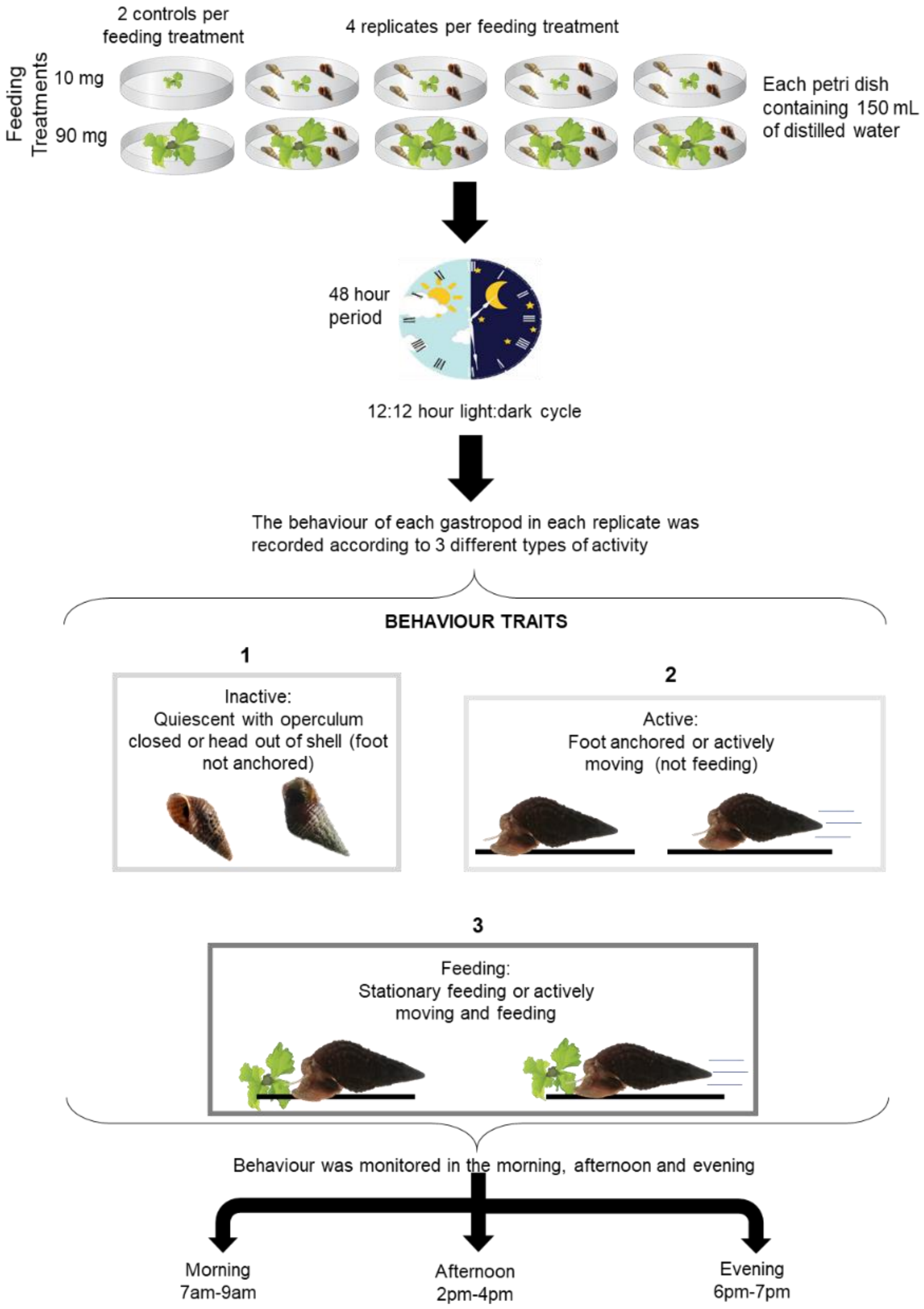


Figure 3.4: Experimental design for the mixed species experiment, to observe three behavioural responses (inactive, active and feeding) of *Tarebia granifera* and *Melanoides tuberculata* at two feeding treatments (10 and 90 mg) over 48 hours.

3.2.4) Statistical Analyses:

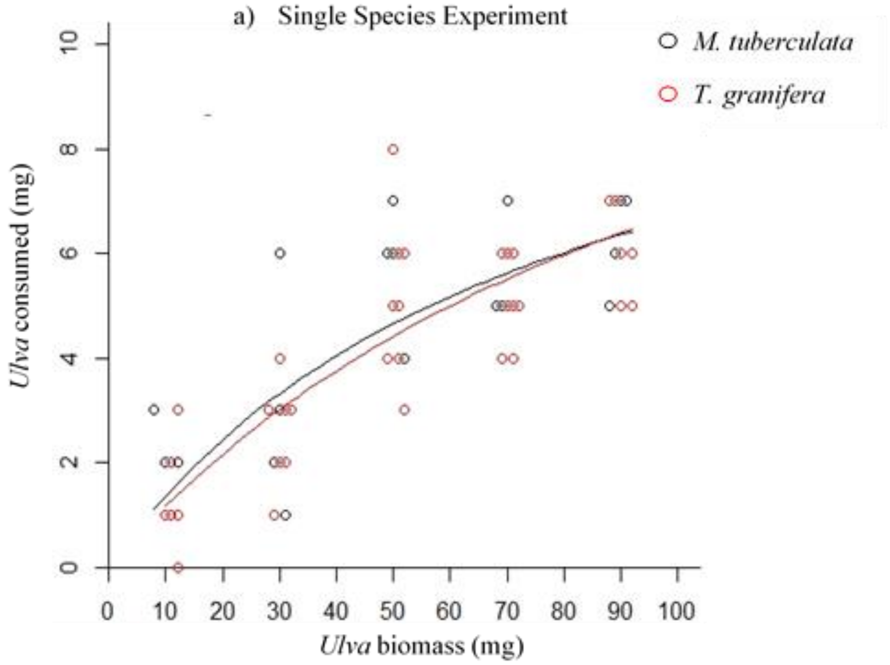
All statistical analyses were conducted using the software R v 3.6.1. For the FR experiments, depending on the outcome of the Shapiro-Wilk and Levene's test either the nonparametric Generalised Least Squares (GLS) model or the parametric Analysis of Variances (ANOVA) was conducted to determine if the amounts of *Ulva* consumed differed between species (invasive and native) and between the various *Ulva* biomasses (10, 30, 50, 70 and 90 wet weight in mg) ($p = .05$). Lastly, a post hoc Dunns (non-parametric)/TukeyHSD (parametric) test was conducted to see which variables (initial *Ulva* biomass, species or the interaction between *Ulva* biomass and species) in the dataset were significantly different from one another. All values are reported as mean (± SD). For the behaviour aspect of this study (mixed species experiments), the data did not assume normal distribution and the data could not be transformed, therefore, nonparametric tests: Permutational Multivariate Analysis of Variances (PERMANOVA) and a PERMANOVA pair-wise post hoc (pairwise.adonis, package: vegan), were conducted. The mixed species experiments were conducted over a 48 hour period, where behaviour traits were observed in feeding treatments and monitoring periods. Therefore, the average counts of each behavioural trait were calculated over two 24 hour periods. The PERMANOVA was conducted to test the average 24 hour counts of the behaviour traits within three factors: 1) feeding treatments (10 and 90 mg), 2) the three behaviour traits (inactive, active and feeding) and 3) monitoring periods (morning, afternoon and evening) as well as the interaction between these factors. Lastly, a PERMNAOVA pair-wise post hoc was conducted with a Bonferroni correction (Bonferroni 1936) to assess differences between factors.

3.3) Results

3.3.1) Functional Response Curves

The FR curves indicated that the invasive and native gastropods both exhibited Type II FRs during the single species experiment and the chemical cues experiment (using heterospecific chemical cue water) (Figure 3.5a and b, respectively). There was no significant difference in the amount of food consumed between the two gastropod species in the single species experiment (GLS: $t = -0.548$; $p = 0.585$; Figure 3.5a) and chemical cues experiment (ANOVA; $F = 0.684$; $p = 0.415$; Figure 3.5b). However, there was a significant difference in *Ulva* consumed across the varying *Ulva* biomass in the single species experiment (GLS: $t = 15.296$; $p < 0.001$; Figure 3.5a; Table 3.4: Appendix) and chemical cue experiment (ANOVA; $F = 14.561$; $p <0.001$; Figure 3.5b; Table 3.4: Appendix). There was no significant interaction in the amount of food consumed between species and *Ulva* biomasses in the chemical cues experiment (ANOVA; $F = 0.646$; $p = 0.634$; Figure 3.5b). Overall, in the single species experiment, both gastropods exhibited similar feeding rates, however, in the presence of chemical cues, *T. granifera* had a higher feeding rate at lower *Ulva* biomasses (Figure 3.5a and b).

a) Single Species Experiment



b) Chemical Cues Experiment

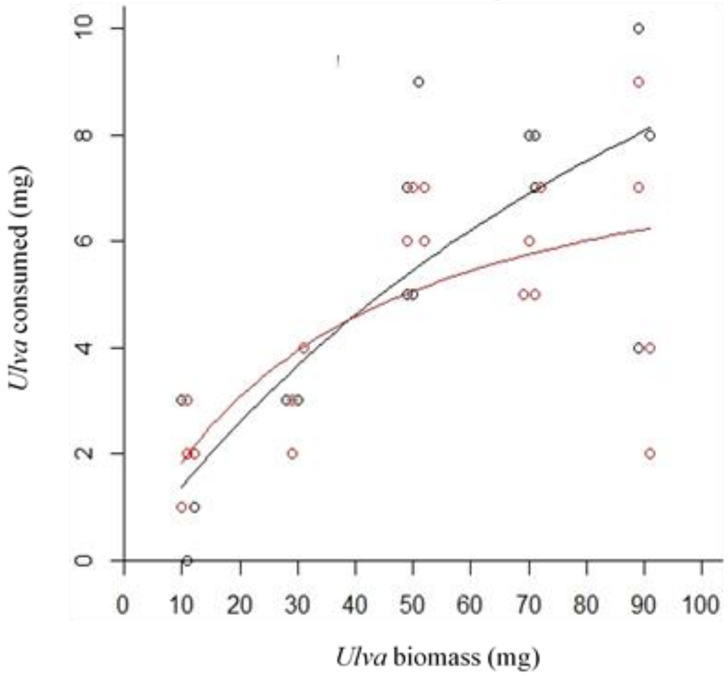


Figure 3.5: Type II FRs representing the amount of *Ulva* consumed (mg) by *Melanoides tuberculata* (black open circle) and *Tarebia granifera* (red open circle) at varying feeding treatments (10, 30, 50, 70 and 90 mg) during the a) single species experiment and the b) chemical cues experiment. Type II FR curves were modelled using the Rogers' random predator equation.

Melanoides tuberculata had a higher attack rate and handling time (*a* = 0.083; *h* = 0.175; Figure 3.6a) than *T. granifera* (*a* = 0.067; *h* = 0.142; Figure 3.6a) in the single species experiment. However, in the presence of chemical cues, *Tarebia granifera* had a higher attack rate and handling time (*a* = 0.13; *h* = 0.233; Figure 3.6b) than *M. tuberculata* (*a* = 0.0802; *h* = 0.103; Figure 3.6b). In the single species experiment, the maximum feeding rate of *T. granifera* was higher (F_{max} = 7.065; Figure 3.6c) than *M. tuberculata* (F_{max} = 5.707; Figure 3.6c) and in the presence of chemical cues the maximum feeding rate of *M. tuberculata* was higher (F_{max} = 9.728; Figure 3.6c) than *T. granifera* (F_{max} = 4.300; Figure 3.6c). There was no significant difference between the feeding parameters of the two gastropod species in the single species experiment (attack rate, p = 0.537 and handling time, p = 0.671; Figure 3.6a-c) and chemical cues experiment (attack rate, p = 0.414 and handling time, p = 0.196; Figure 3.6a-c).

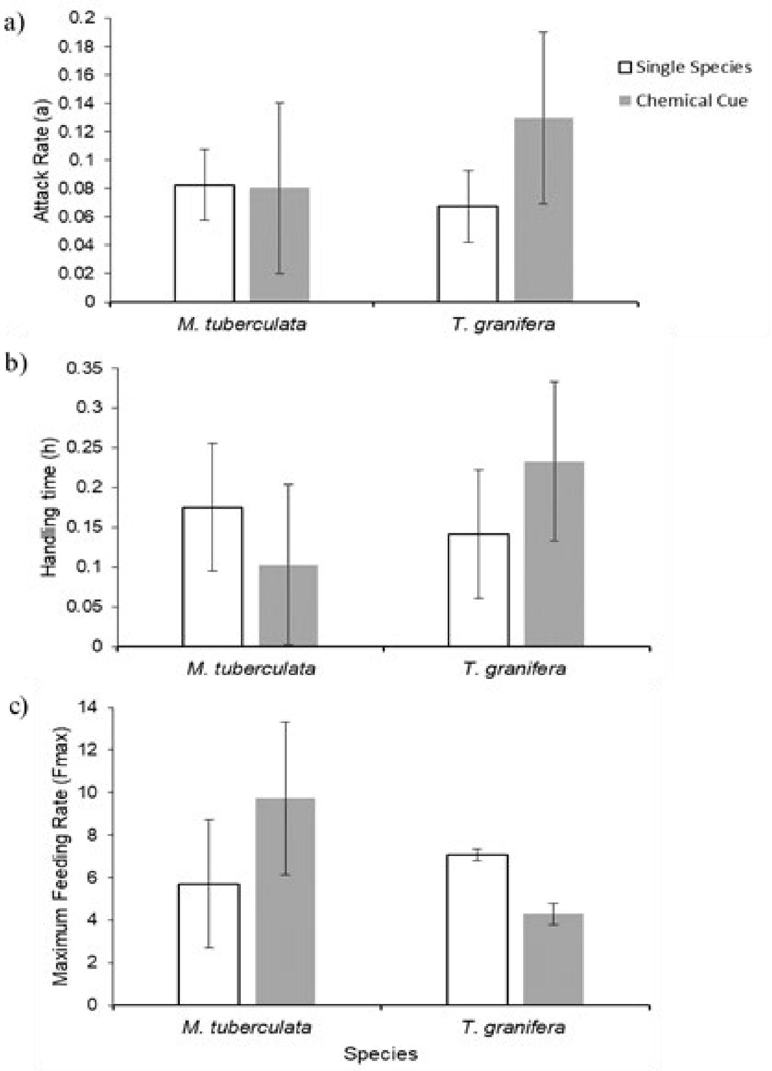


Figure 3.6: Comparison of the FR feeding parameters: a) attack rate; b) handling time and c) maximum feeding rate, that were generated for the *Melanoides tuberculata* and *Tarebia granifera*. Error bars represent standard errors.

In the single species experiment, *T. granifera* and *M. tuberculata* had similar FRRs, however, in the presence of chemical cues, *T. granifera* exhibited a lower FRR than *M. tuberculata* (Table 3.1).

Table 3.1: Functional response ratios based on the feeding parameters (*a* and *h*) of Melanoides tuberculata and Tarebia granifera during the single species experiment and chemical cues experiment.

	M. tuberculata	*T. granifera*
Single Species Experiment	0.471	0.475
Chemical Cues Experiment	0.780	0.557

3.3.2) Behavioural Responses: Mixed Species Experiment

There was no significant difference in the factors (monitoring periods and feeding treatments) and no significant interactions between the factors (Table 3.2). Results from this study showed that there was a significant difference (PERMANOVA: pseudo-F = 7.35, df =1, p = 0.002) between feeding, inactive and active behaviour traits (Table 3.2). There was a significant difference between inactive and active behaviour traits (Pairwise PERMANOVA post-hoc: F = 8.44, df =1, p = 0.009), inactive and feeding (F = 13.76, df =1, p = 0.009) as well as active and feeding (F = 21.74, df =1, p = 0.006) (Table 3.2). *Tarebia granifera* fed for a higher percentage of time (actively moving and stationary, 2%) compared to *M. tuberculata* (1%) at 10 mg over 48 hours (Table 3.3: Appendix). Whereas *M. tuberculata* (actively moving and stationary feeding, 2%) fed for a highest percentage of time compared to *T. granifera* (1%) over the 48 hours at 90 mg (Table 3.3: Appendix).

Table 3.2: Permutational Multivariate Analysis of Variances conducted to test differences in the average counts of behaviour among three factors: monitoring periods (morning, afternoon and evening), behaviour traits (inactive, active and feeding) and feeding treatments (10 and 90 mg).

Factors	**df**	**Pseudo-F**	**p-value**
Behaviour	1	7.351	0.002 **
Monitoring periods	2	0.461	0.803
Feeding treatments	1	0.304	0.882
Behaviour * Monitoring periods	2	0.480	0.808
Behaviour * Feeding treatments	1	0.562	0.628
Monitoring periods * Feeding treatments	2	0.285	0.967
Behaviour * Monitoring periods * Feeding treatment	2	0.432	0.840

* Interaction between factors

** Significant difference: $p < .05$

In the mixed species experiment, there was a higher mean *Ulva* consumed at 90 mg (7.85 ± 2.31 mg) than 10 mg (2.60 ± 1.39 mg) (Figure 3.7). Overall, the mixed species experiment had the highest consumed *Ulva*, compared to the single species and chemical cues experiments at both feeding treatments (10 and 90 mg) (Figure 3.7), however, this difference was not significant (F = 2.838, df = 2, p = 0.0755).

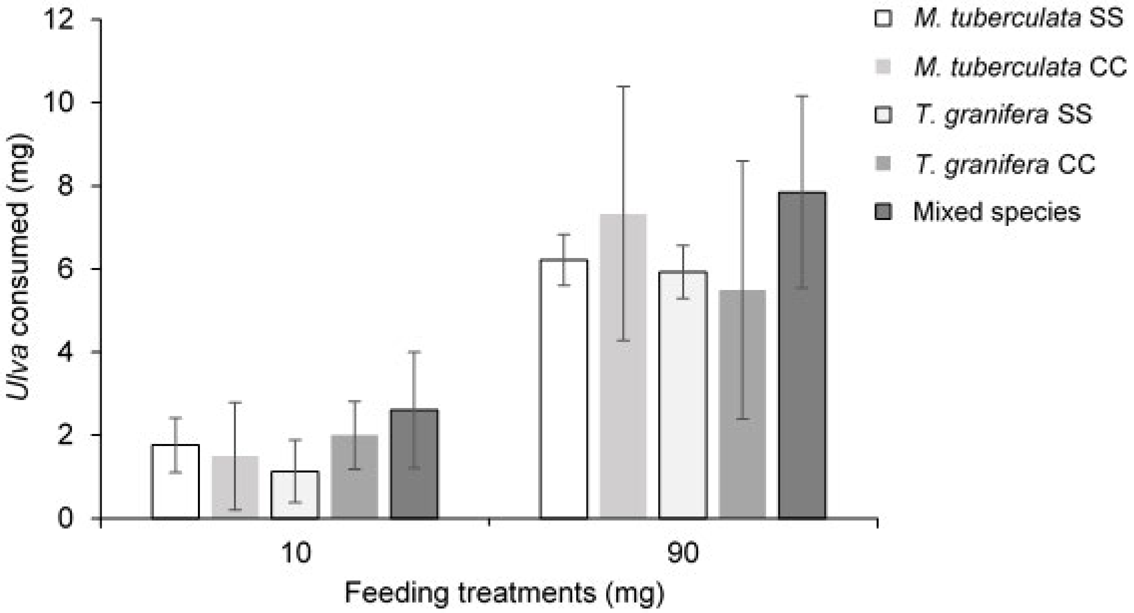


Figure 3.7: Mean *Ulva* consumed by *Melanoides tuberculata* and *Tarebia granifera* at two feeding treatments (10 and 90 mg) during the single species (SS), chemical cues (CC) and mixed species experiments. Chemical cues and single species experiments were conducted with and without the presence of heterospecific chemical cues, respectively. The mixed species experiment included two individuals per species (*M. tuberculata* and *T. granifera*) in each replicate, to observe behaviour.

3.4) Discussion

Chemical cues have been shown to influence the invasion success and behavioural responses of *T. granifera* (e.g. Raw et al. 2013, 2015). However, these studies were conducted before the St Lucia Estuary experienced a state shift to a fresh/oligohaline system (Jones et al. 2020), which created favourable environmental conditions and allowed for the range expansion of *T. granifera* (Perissinotto et al. 2014). The current study utilized the FR approach to compare the feeding impacts of the native (*M. tuberculata*) and invasive (*T. granifera*) gastropods, with and without the presence of chemical cues. Although, previous research observed physical interactions of gastropods (e.g. Raw et al. 2013, 2015), this study also examined the feeding and physical impacts that the now sympatric gastropods may have on one another. Therefore, the current study aimed to investigate differential feeding dynamics between the invasive and native gastropod species by using the FR approach and behavioural responses with respect to chemical cues. The results of this study demonstrated that *M. tuberculata* and *T. granifera* both exhibited Type II FR curves which suggested that at higher food densities, consumption rates decreased, presumably due to the consumers being limited by their capacity to pursue, subdue and consume food (Dick et al. 2014; Rosenbaum and Rall, 2018; Cuthbert et al. 2019). Although both gastropods are considered to have generalist diets (Miranda et al. 2011a; Raw et al. 2016b; Chapter 2), these consumers were limited to a single food source in this study. Limiting a consumer to a single food source could prevent the generation of a Type III curve (van Leeuwen et al. 2007; Alexander et al. 2012). It is possible for consumers to switch between Type II and III as they should modify attack rates according to food type and abundance (van Leeuwen et al. 2007). A typical biological invasion would be associated with both Type II and Type III FR curves, but most prominently shown as a Type II FR curves which is largely dependent on the feeding parameters that determine the shape and magnitude of the FR curves (Dick et al. 2014).

Previous studies (Giboda et al. 1997; De Kock and Wolmarans, 2008; Karatayev et al. 2009; Jones et al. 2017; Kesner and Kumschick, 2018) showed *T. granifera* to be a high impact invader due to high feeding rate. However, the findings from this study show that *T. granifera* did not always have a higher feeding rate. This result was shown in the single species experiment where *T. granifera* consumed less *Ulva* at low food abundances and the species took a shorter time to consume the food. In the chemical cues experiment, *T. granifera* consumed more *Ulva* at lower biomasses, however, *M. tuberculata* had an overall higher feeding rate than *T. granifera*. This could have accounted for the larger mass of food consumed at the higher *Ulva* biomasses. The hypothesis which stated that chemical cues released by one species would affect the FR parameters of the other species was supported.

An ecologically damaging, high impact invader would typically have a high attack rate and a shorter handling time as they would establish themselves rapidly and more efficiently within a new system (Alexander et al. 2012; Xu et al. 2016). A high impact invader could potentially cause local extinctions of trophically analogous species and food resources, therefore, potentially disrupting ecological functioning of an ecosystem (van Leeuwen et al. 2007; Alexander et al. 2012; Dick et al. 2014; Xu et al. 2016). The results of the current study showed contradictory invader impact predictions of *T. granifera* when the feeding parameters were examined separately. *Tarebia granifera* demonstrated characteristics typical of both low impact and high impact invaders (Cuthbert et al. 2019). However, in the presence of chemical cues, the attack rate of *T. granifera* showed that the invasive gastropod was a more impactful invader, but the handling time showed that *T. granifera* was a less impactful invader (Cuthbert et al. 2019). The standard feeding parameters from this study suggested that during the single species and chemical cues experiment, *T. granifera* was an intermediate impact invader. Previous studies conducted by Ricciardi et al. (2012) and Barrios-O'Niell et al. (2014) had shown contradictory prediction results when the feeding parameters were considered separately, however, Cuthbert et al. (2019) provided a clearer prediction of the impact that the invader, bloody red shrimp, *Hemimysis anamala,* had on native mysids and other zooplankton populations. Therefore, due to the contradictory results of invader impact prediction in the current study, functional response ratios (FRRs) were conducted to improve the robustness and reliability of the invader impact (Cuthbert et al. 2019).

Cuthbert et al. (2019) had stated that if the FRR of the invasive species is higher than the native species, the invader is considered a higher predicted impact invader and if the FRR of the invasive species is lower than the native species, the invader is considered a lower predicted impact invader. The FRRs from this study indicated that *T. granifera* was a more impactful invader (FRR = 0.475) during the single species experiment as the invader had a higher FRR than the native (FRR = 0.472). Although, the single species experiment showed that *T. granifera* was a more impactful invader, it was important to have observed the influence the invader would have had in the presence of heterospecific chemical cues (Raw et al. 2013). Previous studies (Giboda et al. 1997; De Kock and Wolmarans, 2008; Karatayev et al. 2009; Jones et al. 2017; Kesner and Kumschick, 2018) suggested that *T. granifera* was a high impact, ecologically damaging invader in the presence of a native species. Contrary to that, the results from the current study showed that *T. granifera* was a lower impact invader in the presence of the heterospecific chemical cues. The FR approach is used as a predictive tool that measures the interactions between the consumer and a food resource within a particular area (Vonesh et al. 2017), this was reflected in the methodology of the current study, where the gastropods were limited to a single food resource as well as a designated experimental area. However, in their natural habitat these gastropods are not limited/restricted by space and resource availability (Chapter 2). Other factors such as resource abundances (McCoy et al. 2011), consumer density (Rall et al. 2008) and morphology (Altwegg et al. 2006) can influence the magnitude of an FR response and its ability to predict invader impact.

A native species subjected to new selective pressures could obtain adaptations and potential biotic tolerance to an invasive species (Stotz et al. 2016). Changes in the FRs of *M. tuberculata* in the presence of the heterospecific chemical cues, could have been an indication of stress or presented a potential competitive opportunity (suggesting that the native gastropod was capable of species recognition which triggered a response to feed at a higher rate) (Kohn 1961; Croll 1983; Wisenden 2000; Fink et al. 2006; Raw et al. 2015; Stotz et al. 2016). New selective pressures exerted onto the native species could result in changes in life processes such as movement, reproduction, sensitivity, growth, respiration, excretion and nutrition (Moran and Alexander, 2014). The feeding parameters (*a* and *h*) of *T. granifera* increased from the single species experiment to the chemical cues experiment which suggested that the presence of heterospecific chemical cues influenced their feeding rates at lower *Ulva* biomasses. However, *M. tuberculata* showed a different FR in the presence of *T. granifera* chemical cues, where they increased the amount of food consumed when food abundance was higher, which contributed to the increase in FRR from the single species to the chemical cue experiments. *Tarebia granifera* has successfully invaded South African estuaries due to their high fecundity (high reproductive output) leading to high densities (Appleton et al. 2009; Miranda et al. 2011b). Research conducted by Abbott (1952), Chaniotis et al. (1980) and WHO (1981) stated that *T. granifera* can give birth to one juvenile every 12 hours, whereas *M. tuberculata* was estimated to give birth to one juvenile every day (Keller et al. 2007). The preliminary results observed in the current study (Table 3.5: Appendix) showed that *T. granifera* birthed at rates 8 times higher than the native, *M. tuberculata* within a 24 hour period. However, the number of offspring can be influenced by environmental conditions and morphological type (Pointier 1989). Therefore, this data represents a snapshot comparison of birth rates between the two gastropods. An invader may also have a greater advantage of invading new niches due to its ability to adapt to a wide range of physicochemical factors (Miranda et al. 2010), remain unaffected by the presence of heterospecific chemical cues and exhibit high reproductive rates.

The adaptation of different evolutionary responses normally occurs when a species undergoes a selective pressure such as the introduction of a new species (Maron and Alexander, 2014; Stotz et al. 2016). Selective pressures can cause phenotypical changes (e.g. shell colour and pattern) in organisms which can take years to become apparent, however, behavioural (e.g. feeding strategies, locomotion) changes can occur more rapidly (Maron and Alexander, 2014). Behavioural responses can be considered to have high plasticity when changes take <60 years to occur and some gastropods (e.g. *Littorina obusata* in Edgell et al. 2009) have been known to alter behavioural responses as a result of new selective pressures (e.g. introduction of alien species, state shifts and resource depletion) (Maron and Alexander, 2014; Stotz et al. 2016). Edgell et al. (2009) found that when invasive crabs were introduced to an environmental niche the native gastropods exhibited high plasticity behavioural responses, where they adjusted behaviour in order to adapt to the introduction of the crabs. The mixed species experiment in this study was conducted to observe behaviour and consumption of the gastropods. Results from this study in the mixed species experiment showed that *T. granifera* exhibited a higher percentage of time feeding compared to *M. tuberculata* at 10 mg over 48 hours, supporting the findings of the chemical cues experiment that *T. granifera* had a higher feeding rate at lower food abundances. Contrary to this, *M. tuberculata* exhibited a longer average time feeding at 90 mg compared to *T. granifera* over the 48 hours. Therefore, this showed that *M. tuberculata* consumed more at higher food abundances. The hypothesis which stated that invasive species would spend more time feeding was not supported as time spent feeding was dependent on food density.

Behavioural response is one of the approaches used in chemoreceptive studies to identify any reaction to chemical stimuli, which is used to either encourage or discourage the habitat selection, dispersal, courtship, foraging and predator avoidance of organisms (Lockwood et al. 2007; Hawkes 2009; Raw et al. 2013). Therefore, the reaction of one individual can influence the activity of the others (Lockwood et al. 2007; Hawkes 2009; Turley and Findlay, 2009). Behavioural responses of the freshwater gastropods in this study can also be a result of evolutionary history, where the native species may display avoidance or competitive behaviour based on previous encounters with other consumers that coexist within communities (Raw et al. 2013, 2015). Native species that are unfamiliar with other consumers could exhibit avoidance behaviour (Turner et al. 2006). Raw et al. (2013) showed that *T. granifera* had moved closer to the source of chemical cues released by the natives (*M. tuberculata* and *Assiminea* cf. *capensis*) rather than away. However, the natives (*M. tuberculata* and *Assiminea* cf. *capensis* – not previously introduced to *T. granifera*) had moved away from the source of the *T. granifera* chemical cues (Raw et al. 2013). This behavioural response can also contribute to the dispersal of *T. granifera* to new habitats that are already inhabited by other native species, causing range expansions (Raw et al. 2013). Raw et al. (2013) had also observed that the avoidance behaviour is largely instinctive due to evolutionary histories, where prior introduction or lack thereof, to another species did not influence the behaviour of the native consumer. However, if the native species frequently encounters other consumers, a competitive behavioural response can be displayed due to the selective pressure (Raw et al. 2015). These competitive behavioural responses caused by selective pressure can make chemical communication negligible (Allan et al. 2009).

Another commonly studied aspect of behavioural responses is the bold-shy continuum, where bold indicates a higher chance of risky consumer behaviour and shy refers to reserved consumer behaviour (Hedgespeth et al. 2018). The boldness of a predator/consumer is an important behavioural strategy as it could become a heritable trait (Wilson et al. 1994). The degree of boldness is affected by factors such as predation risk, metabolism and feeding rates (Godin and Smith, 1988; Krause et al. 1998; Ioannou et al. 2008). Bolder predators/consumers spend more time searching for food (low a) and approaching the food sooner after detection (low *h*) (Ioannou et al. 2008). The single species experiment of the current study, implied that *T. granifera* showed to be a bolder predator/consumer which supported its suitability to invade new areas and *M. tuberculata* was considered to be a shy consumer. However, in the presence of the heterospecific chemical cues, *T. granifera* appeared to become a shy consumer in comparison to *M. tuberculata*, whilst *M. tuberculata* switched to become a bolder consumer. Recent studies have showed that changes in behaviour of consumers can have significant effects on the food webs over time (Werner and Peacor, 2003; Hedgespeth et al. 2018). Therefore, in this study, changes in feeding were not only noticed in the FR curves but in the behavioural responses of the gastropods as well.

In conclusion the results of this study showed that both native and invasive gastropods exhibited Type II FRs, however, feeding rates of *M. tuberculata* and *T. granifera* had differed from the single species experiment to the chemical cues experiment. Overall, *T. granifera* was considered a lower impact invader in the presence of *M. tuberculata* chemical cues. In the presence of *T. granifera* chemical cues, *M. tuberculata* had increased feeding rates when food abundances increased. Changes in the observed bold-shy consumer behaviour of both species were consistent with the results of the FR experiments. Previous studies that conducted research on these species generally focused on one approach, whereas this study used two approaches (e.g. FR and chemical cues) which gave a more reliable snapshot of invasion. This snapshot of invasion and co-existence may change over a longer period. Evolutionary history and co-habitation can influence the response of native species to the effect of chemical cues released by antagonistic species. Responses of the native species to avoid potential competition with invasive species may include temporal variation or dispersal. Future studies should consist of physical interactions of the gastropods as these could show a clearer picture of how the different species behave in the presence of conspecific and heterospecific chemical cues. Furthermore, studies could focus on the active chemical compounds and their effects on different lineages of the gastropods. The response by the native species may be related to potential biotic resistance in adaptation to the invasive species (David et al. 2017). Therefore, continuous monitoring using long-term studies could aid in showing changes in feeding dynamics between native and invasive species due to the varying chemical cue interactions. Future studies should implement more sensitive measurements in their methodology to account for minute differences in consumption of individuals, as well as different food sources, which will allow for more accurate behavioural responses. Alternative methodologies (e.g. gut pigment content analysis) can be used to incorporate viable food sources such as Cladophora sp. and MPB more accurately. *Tarebia granifera* has the potential to be a high impact invader in systems that are resource limited (Alexander et al. 2012; Cuthbert et al. 2019), however, the St Lucia Estuary and many other estuarine systems in KwaZulu-Natal have high abundances in varying food resources. *Tarebia granifera* has many other adaptations, such as adapting to a wide range of physicochemical variables, higher reproductive rates, numerical dominance, and niche partitioning, which allows *T. granifera* to successfully invade South African estuaries (Miranda et al. 2010). The use of the new metric developed by Cuthbert et al. (2019) assisted in the clarification of invader impact. Future research should divulge in more studies that include FRRs in more complex species interactions. Resource availability, life-history strategies (e.g. bold-shy continuum) and the overall biotic tolerance of the native species within that system are all aspects that should be considered when determining the degree of impact/invasiveness of a non-native species.

3.5) Acknowledgements

A sincere thank you to Caroline Fox from Ezemvelo KZN Wildlife for access to sampling site and the National Research Fund (NRF, unique grant number 109523) for funding this project. The Gastropoda sampled in this study were collected under an iSimangaliso Wetland Park Authority Research Agreement together with an Environmental and Fisheries Research Permit (RES 2019/93) issued by the South African Department of Agriculture, Forestry and Fisheries). Ruven Pillay, Merusha Govender, Njabulo Mdluli, Natleen Govender and Mandy Jones, are thanked for their assistance and continuous support in the field/laboratory.

3.7) Appendix

Table 3.3: Percentage of time spent (%) on the three behavioural traits of Melanoides tuberculata and Tarebia granifera at a) 10 mg and b) 90 mg, over 48 hours. Behaviour traits: Inactive - quiescent with operculum closed or head out of shell (foot not anchored), Active - foot anchored or moving (not feeding), Feeding (stationary feeding or actively moving and feeding).

		Behaviour Traits (%)		
Species	**Feeding treatments (mg)**	Inactive	Active	Feeding
M. tuberculata	10	40	59	1
	90	34	64	2
T. granifera	10	32	65	2
	90	22	77	1

Table 3.4: Statistical differences in *Ulva* consumed across varying feeding treatments (10, 30, 50, 70, 90 mg) in the single species and chemical cues experiments. In the single species and chemical cues experiments, Dunns post hoc test and Tukey/HSD tests were conducted, respectively.

***Ulva* Biomass (mg)**	**Single Species Experiment**	**Chemical Cue Experiment**
	p-value	p-value
10-30	0.999	0.362
10-50	0.0001*	0.0001*
10-70	0.0001*	0.0001*
10-90	0.0001*	0.0001*
30-50	0.0001*	0.003*
30-70	0.999	0.005*
30-90	0.0001*	0.008*
50-70	0.0001*	0.999
50-90	0.999	0.999
70-90	0.999	0.999

* Significant difference: $p < .05$

The gastropods were monitored regularly during the feeding period to remove any offspring that may have affected the *Ulva* consumed. The number of offspring were counted when removed, during three 48 hour experiments, which contributed towards preliminary reproduction observations (Table 3.5).

Table 3.5: Average number of births (± SD) produced by *Melanoides tuberculata* (*M. tuberculata*) and *Tarebia granifera* (*T. granifera*) over 48 hours. The data were averaged over three 48 hour experiments, where each experiment contained 80 individuals per species. The number of births was then calculated over 24 hours per individual.

Time	***M. tuberculata***	***T. granifera***
48 hours	54.67 (± 46.46)	435.33 (± 281.74)
24 hours	0.34	2.72

CHAPTER 4

General Discussion and Conclusion

In the past two decades, human-mediated invasions have become prevalent in scientific research (Blackburn et al. 2011) where determining the effects of invasive species has become a vital element in invasion biology, ecosystem ecology and management (Hill et al. 2015). Aquatic ecosystems are challenging environments to investigate due to the complexity of ecological interactions (Alexander et al. 2012; Miranda and Perissinotto, 2012; Heinrichs et al. 2020). Estuaries experience fluctuating environmental conditions (e.g. state shifts), creating unstable systems that become vulnerable to invasion (Diez et al. 2012; Miranda and Adams, 2013; Wilson et al. 2020). State shifts influence the biotic resistance of the native species in these systems, allowing invasive species (that have efficient adaptive skills) to thrive (Diez et al. 2012). A high impact invader could cause local extinctions of trophically analogous species and food resources, therefore, potentially disrupting the ecological functioning of an ecosystem (van Leeuwen et al. 2007; Alexander et al. 2012; Dick et al. 2014; Xu et al. 2016). There has been an increased focus on aquatic invasive species in South Africa in order to address the knowledge gaps regarding ecological consequences (e.g. competitive exclusion of native species, biotic homogeneity and trophic structure) of invasive species (Miranda et al. 2010, Weyl et al. 2020). *Tarebia granifera* was introduced to South African estuaries in the 1990s via the aquarium trade industries (Cowie 1998; Appleton and Nadasan, 2002; Miranda et al. 2010) and was first recorded in the St Lucia Estuary in 2005 (Miranda et al. 2010). The state shift in the St Lucia Estuary (Perissinotto et al. 2014) allowed for the range expansion of the invasive species, *T. granifera*, which has in the past outnumbered and displaced many native species such as *M. tuberculata* and *Assiminea* cf. *capensis* (Miranda and Perissinotto, 2012; Raw et al. 2013). Feeding ecology is utilized in research to improve the knowledge on aspects such as diet composition, habitat preferences, resource partitioning, competition and trophic structures within ecosystems (Braga et al. 2012).

Stable isotope analysis and functional responses are methods that are frequently used to assess feeding ecology as they may provide a more detailed view (spatially and temporally) of diet composition (Miranda and Perissinotto, 2012; Hill et al. 2015) and assess the feeding strategies of consumers (Dick et al. 2014; Rosenbaum and Rall, 2018). In the past, Miranda et al. (2011a), Miranda and Perissinotto (2012), Raw et al. (2016a, 2016b) focussed on the diet composition and feeding strategies of *T. granifera* and *M. tuberculata* before *T. granifera* expanded its spatial range to overlap with that of *M. tuberculata* in St Lucia. Studies such as Miranda et al. (2011a), Miranda and Perissinotto (2012) and Raw et al. (2016a, 2016b) used various methods including gut content analysis and stable isotope analysis to assess the dietary composition of *M. tuberculata* and *T. granifera*. These studies showed that *M. tuberculata* and *T. granifera* are detritivores/grazers with a generalist diet, therefore, consuming opportunistically on food resources that are readily available. Functional responses and behavioural responses (with respect to chemical cues) were used in previous studies such as Raw et al. (2013, 2015), Xu et al. (2016) and South et al. (2019) to assess the feeding strategies of various native and invasive species (*M. tuberculata*, *T. granifera*, *Pomacea canaliculata* and *Bellamya aeruginosa*). These studies showed how invasive and native species could interact by exposing them to the same factors such as varying food abundances and the presence of heterospecific chemical cues. The results obtained by these studies showed that invasive species had a higher impact as they consumed a greater amount than the native species (Xu et al. 2016; South et al. 2019) and were not influenced by the presence of heterospecific chemical cues (Raw et al. 2013, 2015). The main aim of this thesis was to investigate dietary preferences, niche overlap and differential feeding dynamics of an invasive (*T. granifera*) and native (*M. tuberculata*) gastropod, during coexistence. Therefore, this study observed dietary composition and feeding strategies between these gastropod species which aided in understanding how invasive populations interact with native populations within an ecosystem.

Chapter 2 focused on the dietary preferences, niche width and overlap of *T. granifera* and *M. tuberculata*. The results showed that both species are generalist feeders, which agreed with findings in the literature (Miranda and Perissinotto 2012; Raw et al. 2016b). Both gastropod species consumed similar food resources within sites (Chapter 2), although at varying rates depending on availability (Raw et al. 2016a, 2016b). At the St Lucia Estuary Mouth both gastropods consumed a higher amount of detritus, whereas *Cladophora* sp. was consumed more by both gastropods at the Mpophomeni Stream (Chapter 2). Environmental conditions and seasonal variability affected the abundances and types of food resources that were available (Raw et al. 2016a, 2016b). The high abundance and availability of detritus at the St Lucia Estuary Mouth was due to decomposing of mangrove leaf litter and benthic algae (Kristensen et al. 2008; Raw et al. 2016b; Mulya and Arlen, 2018) Mangroves also act as carbon sinks due to their balance in carbon production and source decomposition (Twilley et al. 1992; Gonneea et al. 2004), this could have also caused the wide δ^{13}C signature spread at the St Lucia Estuary Mouth (Chapter 2). Contrary to the St Lucia Estuary Mouth, the Mpophomeni Stream had a higher mean δ^{15}N signature (Chapter 2), this could be due to nutrient enrichment (Miranda and Perissinotto, 2012; Peer et al. 2015). The current study showed that there was a significant difference between the δ^{13}C and δ^{15}N signatures of the gastropods at the Mpophomeni Stream compared to the St Lucia Estuary Mouth, which could explain the changes in niche sizes, as the isotopic signatures influence the niche widths of each species.

Miranda and Perissinotto (2012) showed an 85% overlap in the dietary niches between *T. granifera* and *M. tuberculata*, however, this study showed that the dietary niche overlap between both gastropods (~36%) was not significant (<60%). This result was unexpected due to these gastropods consuming very similar food resources. Larger overlap of niches occurs when food resources are limited, however, in the St Lucia Estuary, there is an abundance of varying food resources which could have reduced the degree of overlap between *T. granifera* and *M. tuberculata* (Miranda and Perissinotto, 2012). Lower dietary niche overlap suggests that there is reduced potential for direct resource competition (Hill et al. 2015; Pastore 2018). Niche partitioning aided in averting competitive exclusion as these gastropods could have utilized the same food resources in varying ways (Gause 1934; Hutchinson 1957; Miranda and Perissinotto, 2012; Raw et al. 2016b).

Functional response relationships with the food resources, temporal variations and varying densities in resources also aids in facilitating coexistence between trophically analogous species (Kneitel 2013). Chapter 3 focused on the comparative functional responses of *T. granifera* and *M. tuberculata* to determine the relationship between food density and rate of food consumed which aids in identifying the feeding impact these consumers have on an ecosystem (Rogers 1972; Jeschke et al. 2002; Kalinkat et al. 2013). The results from this study showed that both gastropods exhibited Type II functional response curves (Chapter 3), which is typical of invaders (Dick et al. 2014). Type II FR curves largely depend on the feeding parameters that determine the shape and magnitude of the FR curves (Dick et al. 2014). In the single species experiment, *T. granifera* exhibited a lower attack rate (low *a*) and a shorter handling time (low *h*) than *M. tuberculata*. This suggested that *T. granifera* consumed less *Ulva* at low food abundances and the species took a shorter time to consume the food. A chemical cues experiment was conducted to observe the differences in feeding rates of the gastropods. When the experiment was repeated in the presence of heterospecific chemical cue water, *T. granifera* exhibited a high attack rate (high *a*) at lower *Ulva* biomasses, allowing this species to have a higher feeding rate at lower food abundances. However, *M. tuberculata* exhibited a lower attack rate (low *a*) and took a shorter time to consume food (lower *h*). This could have accounted for the larger mass of food consumed at the higher *Ulva* biomasses. The feeding parameters of both gastropods were not significantly different within the single species experiment and the chemical cues experiment (Chapter 3).

These feeding parameters can be used to estimate the type of feeding impact an invader may exhibit: 1) low *a* and high *h*, indicating a low impact invader; 2) low *h* and high *a*, indicating a high impact invader (Cuthbert et al. 2019; South et al. 2019). However, in certain circumstances, the consumer may exhibit either a low *a* and *h* or a high *a* and *h*, this is indicative of an intermediate impact invader (Cuthbert et al. 2019; South et al. 2019). The feeding parameters of both species were examined separately, but this provided conflicting results when trying to determine the invader impacts of *T. granifera*. *Tarebia granifera* was considered an intermediate impact invader. However, to provide more robust and reliable predictions of invader impacts, functional response ratios (FRRs) were utilized. Previous literature (Giboda et al. 1997; De Kock and Wolmarans, 2008; Karatayev et al. 2009; Jones et al. 2017; Kesner and Kumschick, 2018) suggested that *T. granifera* was a high impact, ecologically damaging invader in the presence of a native species.

FRR results from the current study showed that *T. granifera* was predicted to be a more impactful invader in the single species experiment. However, in the presence of heterospecific chemical cues, *T. granifera* was predicted to be a lower impact invader (Chapter 3). *Melanoides tuberculata* showed a different FR in the presence of *T. granifera* chemical cues, where they increased the amount of food consumed when food abundance was higher, which contributed to the increase in FRR from the single species to the chemical cue experiments (Chapter 3). This response by the native species may be related to potential biotic tolerance in adaptation to the invasive species (David et al. 2017). Both gastropod species behaved according to the results observed in the FR experiments, where *T. granifera* consumed more at lower *Ulva* biomasses and *M. tuberculata* consumed more at higher *Ulva* biomasses (Chapter 3). Previous studies such as Raw et al. (2013) showed that *T. granifera* had moved closer to the source of heterospecific chemical cues released by the natives (*M. tuberculata* and *Assiminea* cf. *capensis*) rather than away, whereas *M. tuberculata* would move away from the source of heterospecific chemical cues. This type of behaviour exhibited by the native species can be associated with avoidance behaviour due to unfamiliar interactions with the invasive species (Raw et al. 2013). Behavioural changes could have occurred in *M. tuberculata* as a result of new selective pressures caused by the *T. granifera*, therefore, reduction in competitive exclusion should allow for recent coexistence with *T. granifera* (Stotz et al. 2016). *Tarebia granifera* has a greater ability to disperse, causing range expansions, due to their efficient behavioural responses to heterospecific chemical cues (Raw et al. 2013).

The feeding preferences and niche overlap are small entities when considering biological invasions, therefore, more studies need to include more complex interactions (e.g. include the influence of abiotic factors as well as the long term effects of chemical cues on behaviour, using relative distance) between an invader and a native species. Future studies should consider all potential food sources that occur within a system and include a seasonal view over a longer period. It is important to note that physiological variations (chemical and physical variations that can occur in living systems e.g. chemical cues) can help in understanding the feeding and living preferences of organisms which can aid in determining the coexistence of species. Other factors such as functional and behavioural responses were considered when comparing feeding dynamics between an invasive species to a native species. Both gastropods exhibited Type II responses and generalist diets (Miranda et al. 2011b; Raw et al. 2016b; Chapter 2), however, these consumers were limited to a single food source which could have prevented the generation of a Type III curve (van Leeuwen et al. 2007; Alexander et al. 2012). Chemical cues secreted by one consumer may affect the feeding activities of another, therefore, further studies should focus on the active chemical compounds and their effects on different lineages of the gastropods. Due to the minor changes in amounts of food consumed, future studies should implement more sensitive measurements in their methodology (e.g. gut content analysis) to account for minute differences in consumption of individuals, which will allow for accurate behavioural responses. Due to the contradictory results of invader impact prediction in the current study, future studies should implement the new functional response ratio (FRR) metric developed by Cuthbert et al. (2019) to develop more robust and reliable clarifications of invader impact. Future research should divulge in more studies that include FRRs in more complex species interactions. Other factors must be considered when determining the degree of impact/invasiveness of a non-native species, e.g. reproductive success, environmental tolerances, release from parasites/predators, resource availability, life-history strategies (e.g. bold-shy continuum) and the overall biotic tolerance of the species within a system. Therefore, including a multiple method approach in studies is recommended when assessing ecological impacts of invasive species.

In conclusion, invaders can coexist with other trophically analogous species in the St Lucia Estuary and many other estuarine systems in KwaZulu-Natal, due to high abundances in varying food resources, abiotic tolerances and fecundity. Both chapters of this thesis provided evidence that *T. granifera* and *M. tuberculata* can coexist due to diet plasticity and their ability to adopt varying feeding strategies. This study can contribute to invasive biology literature and improve the understanding on how these gastropods coexist. *Tarebia granifera* and *M. tuberculata* both adopted generalists feeding strategies, where both species feed opportunistically on food resources that were seasonally available. This allows for the gastropods to alter their diets according to readily available food sources and environmental conditions. Niche partitioning and high abundances of food resources reduced direct competition for these food resources and allowed for these gastropods to coexist naturally. *Tarebia granifera* showed efficient feeding dynamics under limited food resources. Therefore, in systems that are resource limited, *T. granifera* becomes the more dominant species as they consume more at lower food availabilities despite the presence of heterospecific chemical cues. There are many other factors that can facilitate the successful invasion of *T. granifera* in South African estuaries, such as high densities due to their high reproductive rates, efficient dispersal strategies, the ability to adapt to a wide range of physicochemical factors as well as lack of predation and parasitism (Jones et al. 2009; Jones et al. 2017). The results obtained in this thesis provided predictions of feeding impacts that the invader, *T. granifera*, may have on the St Lucia ecosystem as well as their effect on a native species within this system. This thesis can contribute to invasive biology literature and improve the understanding in how these gastropods coexist.

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END OF THESIS

Comparative functional responses of an invasive and native gastropod

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INTRODUCTION

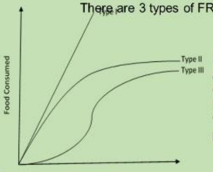
Biological Invasions

Biological invasions disrupt ecosystem processes and species interactions[1,11]. However, estimating the impact of the non-native population is a challenge[5,11]. Invasive species, *Tarebia granifera*, occurs within South African estuaries and has outnumbered the native species, *Melanoides tuberculata*[7].

Figure 1: *Tarebia granifera* at Amanzimtoti Estuary.

Functional Responses

Functional Responses (FR) can be used to determine the potential impact of invaders by measuring the relationship between food consumed and the food density[1,6,10]. There are 3 types of FR curves[6,10]:



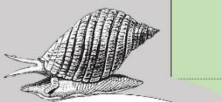
Feeding parameters[10]:
Attack rate (a) – capture efficiency
Handling time (h) – time taken to consume and pursue food.

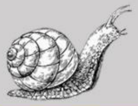
Functional Response Ratios (FRRs) (a/h) - predict potential invader impact[2].

Chemical Cues

Chemical cues are a well-suited form of communication within aquatic environments as the chemical substance can easily distribute sufficiently to gain a response[11].

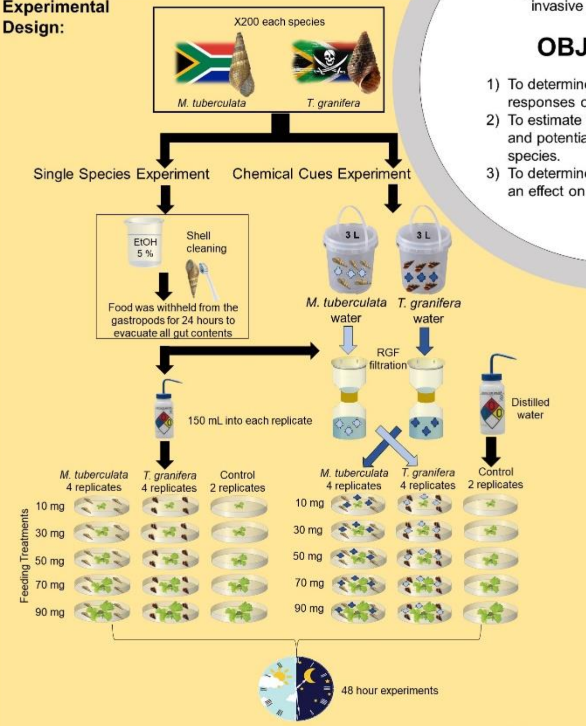
Chemical cues released by *T. granifera* cause behavioral responses which could affect the feeding activities of *M. tuberculata*[4,9].





METHODS

Experimental Design:



X200 each species
M. tuberculata *T. granifera*
Single Species Experiment
Chemical Cues Experiment
EtOH 5 %
Shell cleaning
Food was withheld from the gastropods for 24 hours to evacuate all gut contents
3 L
3 L
M. tuberculata water
T. granifera water
RGF filtration
150 mL into each replicate
Distilled water
M. tuberculata 4 replicates
T. granifera 4 replicates
Control 2 replicates
Feeding Treatments
10 mg
30 mg
50 mg
70 mg
90 mg
48 hour experiments

Functional Response curves were created using the “friar” package in R software v3.6.0[6].

Statistical Analyses:

Kruskal Wallis test or Analysis of Variances (ANOVA) was conducted to determine if the amounts of *Ulva* consumed differed between species (invasive and native) and between the various amounts of *Ulva* biomass (α=0.05).
Dunns (Kruskal Wallis) or TukeyHSD (ANOVA) post hoc tests were conducted to see which variables (initial *Ulva* biomass, species or the interaction between *Ulva* biomass and species) in the dataset were significantly different from one another.

AIM:

To investigate differential feeding dynamics between the native and invasive gastropod species.

OBJECTIVES:

1) To determine the functional responses of two gastropod species.
2) To estimate functional response ratios and potential impact of the invasive species.
3) To determine if chemical cues have an effect on the functional responses.

RESULTS

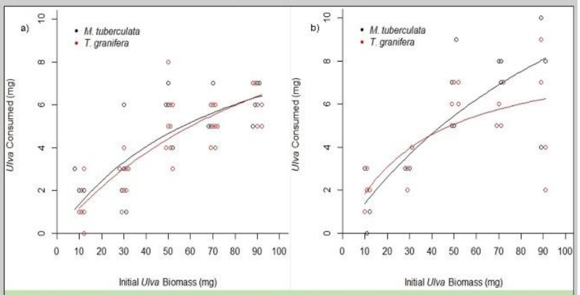


Figure 2: Functional Response curves showing the initial *Ulva* biomass and the amount of *Ulva* consumed by the two gastropod species during a single species experiment (a) and a chemical cues experiment (b).

Table 1: Feeding parameters: attack rate (a), handling time (h) and maximum feeding rate (1/h) created by functional response of the two gastropod species during a single species and a chemical cues experiment.

Experiment	**Species**	**Feeding parameters**		
		a	**h**	**1/h**
Single Species	*M. tuberculata*	0.0826	0.175	5.707
	T. granifera	0.0672	0.142	7.065
Chemical Cues	*M. tuberculata*	0.0802	0.103	9.728
	T. granifera	0.1300	0.233	4.300

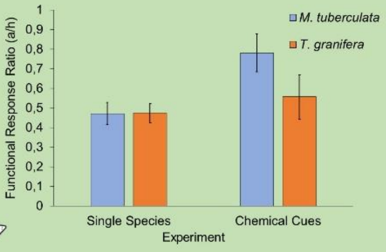
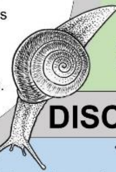
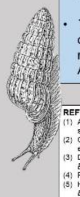


Figure 3: Functional Response Ratios (a/h) of the two gastropod species during a single species and a chemical cues experiment.



DISCUSSION & CONCLUSION

- Both gastropod species exhibited Type II FRs (Figure 2).
- In the single species experiments, *M. tuberculata* and *T. granifera* showed similar feeding rates. However, in the presence of chemical cues, *T. granifera* had a higher feeding rate than *M. tuberculata* at lower *Ulva* biomasses (Figure 2).
- In the chemical cues experiment, *T. granifera* had a higher attack rate and longer handling time, therefore, more *Ulva* was consumed at lower biomasses and they took a longer time to consume the food. However, *M. tuberculata* exhibited a lower attack rate and they took a shorter time to consume food, this could account for the larger amount of food consumed at the higher *Ulva* biomasses (Table 1 and Figure 2).
- The FRRs displayed that *T. granifera* was a higher impact invader based on the combination of feeding parameters which is an expected result of an invasive species[1,2,11]. Contrary to that, *T. granifera* was predicted to be a lower/intermediate impact invader[2] with respect to chemical cues (Figure 3)
- *Melanoides tuberculata* showed a different FR to *T. granifera* chemical cues, where they increased the amount of food that they consumed which contributed to the increase in FRR from the single species to chemical cues experiment[3]. This response by the native species may be related to biotic resistance and tolerance in adaptation to the invasive species in the food web[3].
- *Tarebia granifera* has the potential to be a high impact invader if food resources are limited[1,2], other factors such as the ability to adapt to a wide range of physicochemical variables, higher reproductive rates and resource partitioning, allows *T. granifera* to successfully invade South African estuaries[7].



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