The invasive potential of the freshwater snail *Radix rubiginosa* recently introduced into South Africa

By

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As the candidate's supervisor I have approved this dissertation for submission.

Professor C.C. Appleton Date

Dedication

This dissertation is dedicated to my parents. I love you Mum and Dad, for helping to make me who I am, for teaching me to be proud of who I am, for showing me how to be strong, for giving me the courage and strength to always strive for better, no matter what. Thank you for giving me the wisdom to know when to turn away and when to charge ahead...you are my rock and foundation.

Abstract

Invasions of ecosystems by exotic species are increasing and they may often act as a significant driver of the homogenization of the Earth's biota, resulting in global biodiversity loss. Moreover, the addition of exotic species may have dramatic effects on ecosystem structure and functioning which may result in the extirpation of indigenous species. In 2004, a large population of an unknown lymnaeid was found in the Amatikulu Hatchery, northern KwaZulu-Natal, South Africa, and was subsequently found in few garden fish ponds in Durban. In 2007, it was identified using molecular techniques as *Radix rubiginosa* (Michelin, 1831) – a species widespread in southeast Asia. An invasion by *R. rubiginosa* is however likely to go unnoticed because its shell morphology resembles some forms of the highly variable and widely distributed indigenous lymnaeid, *Lymnaea natalensis* Krauss, 1848.

Accurate and "easy" species identifications would permit the ready assessment of introduction histories and distributions, but in the present case identification was difficult due to unclear and contradicting accounts of the indigenous *L. natalensis* in the literature. A redescription of *L. natalensis* with emphasis on conchological and anatomical characteristics was therefore presented. This will help to distinguish variation between *R. rubiginosa* and *L. natalensis* and also assist those carrying out rapid bioassessment (SASS) surveys in South African rivers in recognising *R. rubiginosa* should it spread.

For this, shells of *R. rubiginosa* and *L. natalensis* from both the UKZN Pond and the Greyville Pond were selected into either size class 1 (shell length < 10 mm) or size class 2 (shell length ≥ 10 mm). Six shell characters, shell length (height), shell width, aperture length (height), aperture width, length of last body whorl and spire height for each specimen was measured and analysed using principal component analysis (PCA) and

discriminant functions analysis (DFA). The most useful discriminant conchological characters were shell length, length of the last body whorl and aperture width. Use of these shell characters provided simple yet effective criteria for the separation of *R. rubiginosa* and *L. natalensis*. For both size classes *R. rubiginosa* had larger, more broadly ovate shells with longer (higher) body whorls than either of the two populations of *L. natalensis* that exhibited smaller, elongated shells with shorter (lower) body whorls. Also, *R. rubiginosa* had a narrower aperture width compared to the larger, wider aperture of the UKZN Pond *L. natalensis* population. The Greyville *L. natalensis* population was found to have narrower apertures than both *R. rubiginosa* and *L. natalensis* (UKZN Pond).

The morphology of the radula and the reproductive anatomy of *R. rubiginosa* and *L. natalensis* from both the UKZN and Greyville Ponds showed little variation. The species did however vary in the relative numbers of radula teeth in each field and this serves as an additional useful diagnostic character. Both *L. natalensis* populations had similar mantle pigmentation patterns but that of *R. rubiginosa* was different. The mantle surface of *R. rubiginosa* was mottled black with patches of pale white to yellow. There were also large unpigmented fields and stripes that were not observed in *L. natalensis*. Having found characters to conveniently separate the alien *R. rubiginosa* from the indigenous *L. natalensis*, it became increasingly important to assess the potential invasiveness of this introduced species and its likely impact.

The potential invasiveness of *R. rubiginosa* was assessed in relation to the already invasive North American Physidae *Physa acuta* Draparnaud, 1805 and the indigenous *L. natalensis*. This was particularly important in view of the success of *P. acuta* as an invader in South Africa. The hatching success, frequency of egg abnormalities, embryonic development, growth, survivorship, fecundity and life history parameters (GRR, R_o, r_m, T and λ) for the four snail populations were assessed at three experimental temperatures (20^{o}C, 25^{o}C and 30^{o}C).

The results showed that *R. rubiginosa* and *P. acuta* had a higher growth coefficient (K), longer survivorship, higher fecundity (higher hatching success, fewer egg abnormalities, longer duration of oviposition), shorter incubation period, greater life history parameters (GRR, R_o, r_m and λ) and wider temperature tolerances than the two *L. natalensis* populations tested.

The high adaptability of *P. acuta* to changing environmental factors such as temperature, is in agreement with the fact that it is now more widespread in South Africa than the indigenous species *L. natalensis*. This has important implications for *R. rubiginosa*, since this species displayed reproductive attributes and a temperature tolerance that were similar and in certain cases even exceeded the performance of the invasive *P. acuta*. This therefore implies that *R. rubiginosa* has the potential to colonize a wider geographical and altitudinal range than *L. natalensis*, and perhaps even *P. acuta*. Also, the superior reproductive ability of *R. rubiginosa* over *L. natalensis* is likely to present a situation that allows for its rapid spread as well as a possible impact on the indigenous *L. natalensis* that might render it vulnerable.

Preface

The research work described in this dissertation was carried out in the School of Biological and Conservation Sciences, University of KwaZulu-Natal, Westville Campus, Durban under the supervision of Professor C.C. Appleton.

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

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1

General Introduction

1.1 Biological Invasions

Anthropogenic alterations of natural ecosystems and human-assisted dispersal of species outside of their native ranges have caused an unprecedented redistribution and homogenization of the Earth's biota (Olden *et al*., 2004). The expansion of a species range is a natural process, but non-indigenous introductions are growing increasingly frequent as species are moved across geographical barriers, either intentionally or unintentionally (Perrings *et al*., 2000; Ricciardi, 2006).

The process of invasion can be divided into three successive stages namely; introduction, establishment and integration. Introduction involves the dispersal of a non-indigenous species from its native range to that of the recipient range. Through local reproduction and recruitment the new population is established (Vermeij, 1996; Richardson *et al*., 2000). This would eventually augment or replace dispersal from the native range as a means for the sustainability of the invading population. Integration occurs when the invading species develops ecological links with other species in the recipient region (Vermeij, 1996; Richardson *et al*., 2000).

Although research on biological invasions is often weighted toward terrestrial ecosystems, the importance of understanding and preventing non-indigenous species introductions in aquatic systems is highlighted by the increasing number and rate of freshwater invasions, the high endemicity of freshwater ecosystems and the importance of freshwater ecosystems for human health and the economy (Johnson *et al*., 2009). Considering that freshwater covers only about 0.8% of the Earth's surface (Gleick, 1996), this makes freshwater ecosystems extremely species-rich habitats and particularly

vulnerable to non-indigenous species introductions. Since abiotic conditions in freshwater ecosystems are generally more homogenous and less fluctuating than in terrestrial habitats (Cohen and Carlton, 1998; Padilla and Williams, 2004; Gollash, 2006), the initial chances of survival for an aquatic non-indigenous species may be higher (Cook, 1990). Once introduced into an ecosystem, dispersal (either intentional or unintentional) may be easier for freshwater than terrestrial species. This is expected since fewer dispersal barriers exist for freshwater ecosystems.

Pathways of intentional introduction for freshwater species are the trades in live aquatic organisms including aquaculture (Naylor *et al*., 2001), nursery plants (Reichard and White, 2001; Maki and Galatowitsch, 2004), live food (Weigle *et al*., 2005), pet (Padilla and Williams, 2004; Rixon *et al*., 2005) and bait trades (Mills *et al*., 1993). Other pathways arise for the unintentional transfer of freshwater species. These include transport in ballast tanks of intercontinental ships (Mills *et al*., 1993; Ricciardi, 2006), inter-basin and inter-catchment transfer schemes for water supply purposes and introduction as contaminants of aquatic plants (Maki and Galatowitsch, 2004).

1.2 Factors affecting Biological Invasions

In general, the species that become successful invaders are predicted to be species that, in their native ranges, display traits that prompt them to successfully survive conditions encountered during transport, introduction, establishment and integration (Suarez and Tsutsui, 2008). Two main attributes of biological invasions are: invasiveness, i.e. the traits that enable a species to invade a habitat and invasibility, i.e. the characteristics of the new habitat that determine its susceptibility to the establishment and integration of an invasive species (Lonsdale, 1999; Alpert *et al.*, 2000; Marco *et al.*, 2002).

Successful invaders possess characteristics associated with effective dispersal, rapid growth, short generation times, high fecundity, a high degree of phenotypic plasticity, broad physiological tolerance (euryhalinity and eurythermy) and a broad diet (Rejmanek

and Richardson, 1996; Williamson and Fitter, 1996; Reid and Orlova, 2002; Ruesink, 2005; Moyle and Marchetti, 2006; Keller *et al*., 2007; Suarez and Tsutsui, 2008).

Also, the abiotic environment sets clear limits on species invasibility. Invasion site characteristics hypothesized to favour frequent or rapid invasion include (a) similarity to the native ranges of the invasive species, (b) a history of recent natural or anthropogenic disturbance, and (c) a low niche diversity within the habitat (Elton, 1958; Moyle and Light, 1996; Moyle and Marchetti, 2006). It should be noted though that caution must be taken when using such characteristics for predicting invasions as each invader uses the biotic and abiotic environment in a different way.

1.3 Impacts of Biological Invasions

While not all introduced species become invasive, successful colonisers can have major ecological, economic and health implications. Ecological impacts are focused on faunal composition, community structure and ecosystem functioning (Mack *et al*., 2000). All these impacts are mediated by numerous processes that act at the individual, community and ultimately the ecosystem level (Simon and Townsend, 2003). At the level of the individual, invasive species may alter the behaviour of indigenous species, influencing habitat use and foraging. At the population level, the invasive species may influence changes in the abundance or distribution of other species. According to both Gurevitch and Padilla (2004) and Ricciardi (2004), the introduction of non-indigenous species acts as a significant driver of global biodiversity loss, second only to habitat destruction.

These changes in or even loss of biodiversity may occur through predation, competition, hybridization with the indigenous species, extirpation of competitively inferior species and alteration of the abiotic environment (Vitousek *et al*., 1996; Ricciardi *et al*., 1998; Mack *et al*., 2000; Clavero and Garcia-Berthou, 2006). At the community level, invaders may alter both direct and indirect interactions among populations and finally, at the ecosystem level invasive species may change the pathways and magnitude of movements of energy and nutrients (Simon and Townsend, 2003).

Economic impacts result from the effects of introduced species on the indigenous biota, as well as funds expended for costly control or eradication programmes (Perrings *et al*., 2000). Introduced species have also affected industrial constructions such as reservoirs, pumps and water pipes. For an example, in the United States, it is estimated that non-indigenous species cost the national economy $120 billion to $137 billion per year (Pimentel *et al*., 2005), while for the United Kingdom, Australia, South Africa, India and Brazil together, costs exceeded $200 billion (Pimental *et al*., 2001). Finally, invasive species can impact human health either directly when they are infectious or pathogenic to humans, or indirectly by promoting the transfer of disease.

1.4 Invasive freshwater snails in South Africa

In an assessment of the ecological impact and economic consequences of invasive freshwater snails in South Africa, Appleton (2003) listed a prosobranch (*Tarebia granifera* Lamarck, 1822) and three pulmonates (*Lymnaea columella* Say, 1817, *Physa acuta* Draparnaud, 1805 and *Aplexa marmorata* Guilding, 1828) as being of concern. *Lymnaea columella* and *P. acuta* were introduced in the early 1940s (Brown, 1994; Appleton and Brackenbury, 1998; Appleton, 2003), while *A. marmorata* was collected for the first time in this country in 1986 (Appleton *et al*., 1989). The recently introduced species, *T. granifera*, was reported for the first time in Africa by Appleton and Nadasan (2002) after it was discovered in 1999 in a reservoir supplying water to a paper mill in KwaZulu-Natal.

1.5 First Report of *Radix rubiginosa* in South Africa

In 2004, a large population of an unknown lymnaeid was found in a prawn and tropical fish breeding facility in Amatikulu, northern KwaZulu-Natal, South Africa. Since Asia is a frequent source of supply for tropical fish and plants for the South African aquarium trade, and the fact that several other snails in this facility were of Asian origin, it was thought likely that this new lymnaeid was Asian as well. Available keys to Asian freshwater snails (Brandt, 1974; Burch, 1980) suggested that the new snail belonged to

the genus *Radix* but could be any of several species known from the region. In 2007 snails supplied by myself from the Amatikulu Hatchery were identified using molecular techniques as *Radix rubiginosa* (Michelin, 1831) – a species widespread in southeast Asia (J. Lamb and K. Pillay, unpubl. data).

Radix rubiginosa has been listed as a "hothouse" alien species in Great Britain, Ireland and Israel (Mienis 1986; Anderson, 2005), becoming established in greenhouses, aquaria within greenhouses and similar artificially-heated habitats. Dondero and Lim (1976) and Mienis (1986) have commented that it is easy to breed *R. rubiginosa* in aquaria and this was also found to be the case in this study. The indigenous *Lymnaea natalensis* Krauss, 1848 is not as easy to breed and this raises the question, "If *R. rubiginosa* spreads in South Africa, will it do so at the expense of *L. natalensis*?" The natural occurrence of *L. natalensis* in KwaZulu-Natal appears to have decreased already perhaps due to the invasiveness displayed by yet another lymnaeid, *Lymnaea columella*. If *R. rubiginosa* populations become established in the same area then this could increase pressure on the indigenous *L. natalensis* and eventually lead to its extirpation (C.C. Appleton pers. comm.).

The presence of *R. rubiginosa* in a nearby reservoir on the Amatikulu facility, suggests that the spread of this species may already have taken place. Furthermore, accidental escape or deliberate release may well go unnoticed due to its strong resemblance to the indigenous *L. natalensis*. This is because the shell morphology of *L. natalensis* is notoriously variable and some of its variants resemble *R. rubiginosa*. There was thus a clear need to be able to differentiate the two lymnaeid species but identification was difficult due to unclear and contradicting accounts in the literature. This is further complicated by increasing evidence suggesting that the forms of what is widely called *L. natalensis* in Africa may in fact comprise more than one species (Brown, 1994).

Snails introduced into new settings provide opportunities for new host-parasite associations to develop and according to Mas-Coma and Bargues (1997), a broad range of lymnaeid species can serve as hosts of fasciolid parasites. In South Africa, the

intermediate host of the giant liver fluke *Fasciola gigantica* is *Lymnaea natalensis* and this fluke is confined to the subtropical lowland regions. The intermediate host for the common liver fluke *Fasciola hepatica* is *Lymnaea truncatula* Müller, 1774, however this species is confined to the cooler areas of the eastern highlands in South Africa (altitude above approximately 800 m), and also the low-lying parts of the Eastern and Western Cape, South Africa (Brown, 1994). *Radix rubiginosa* serves as the intermediate host for *F. gigantica* over much of south-eastern Asia (Srihakim and Pholpark, 1991; Malone, 1997) and if it were to become invasive in South Africa, it could exacerbate the fascioliasis problem in the country. *Radix rubiginosa* has also been identified as the intermediate host for the avian blood fluke, *Trichobilharzia* sp., a cause of schistosome dermatitis (Nithuithai *et al*., 2004), *Schistosoma incognitum* (Bunnag *et al*., 1983) and various echinostomes (Charoenchai *et al*., 1997).

The overall aim of this study was to assess the invasive capability of *R. rubiginosa* in South Africa. By comparing specific characteristics between *R. rubiginosa* and the already invasive North American snail *Physa acuta* (Physidae), it was possible to assess the potential impact of this introduced lymnaeid on the indigenous *L. natalensis*.

This study is arranged in seven chapters. Chapter 1 provides an overview of the literature on biological invasions giving emphasis on factors affecting invasion, as well as the possible or proven impacts of such invasions.

Chapter 2 provides a review of the family Lymnaeidae. In this Chapter the phylogeny of the Lymnaeidae is addressed based on conchological, anatomical and molecular characteristics.

Invasion by *R. rubiginosa* is likely to go unnoticed because the shell morphology of *L. natalensis* is highly variable and some of its variations resemble those of *R. rubiginosa*. There was thus a clear need to be able to differentiate the two lymnaeid species but identification was difficult due to a lack of clarity in the literature. Chapter 3 presents a

redescription of the indigenous species *L. natalensis* with emphasis on conchological and anatomical characters.

In Chapter 4, the traditional morphometric approach was used to assess the suitability and efficacy of conchological characters to distinguish shell variations and patterns within and between populations of two species of Lymnaeidae, the introduced *R. rubiginosa* and the indigenous *L. natalensis*. This includes an examination of the radula, the reproductive anatomy and a description of the pigmentation patterns on the mantle. These characters were then used as criteria to easily recognize and separate *R. rubiginosa* from *L. natalensis*.

Chapter 5 describes and compares the effects of three experimental temperatures on the hatching rates and embryonic development of four populations of three snail species: *R. rubiginosa* from the Amatikulu Hatchery, *L. natalensis* from both the UKZN and Greyville Ponds and *P. acuta* from the Greyville Pond. A description of the morphology of each developmental stage is provided. In addition, a study of the frequency of various egg abnormalities and their relation to the breeding intensity of these species are assessed.

In Chapter 6 the invasiveness of *R. rubiginosa* is assessed in relation to the indigenous *L. natalensis* and the already established invader, the North American, *Physa acuta*. This investigation determined the growth, survivorship, fecundity and life history parameters of the three species and the role of temperature in causing observed differences. This was seen as particularly important in view of the success of *P. acuta* as an invader in South Africa. The growth, survivorship, fecundity and life history parameters were then comparatively analysed to allow for a more precise focus on the specific attributes that may determine the invasive success of *R. rubiginosa*.

Chapter 7 presents a general discussion of the present study, integrating the key findings and conclusions from the previous chapters.

2

Review of the Family Lymnaeidae

The pulmonate basommatophoran superfamily Lymnaeoidea includes several families of freshwater snails, among which is the Lymnaeidae. The Lymnaeidae inhabit a wide variety of freshwater habitats, and as such display a tremendous morphological diversity, both conchological and anatomical. This high level of morphological diversity makes phylogenetic studies of the Lymnaeidae difficult. Despite this, interest in phylogeny of the lymnaeids is important, because firstly, many lymnaeid species are intermediate hosts for trematode parasites and secondly, lymnaeids are part of a growing number of freshwater taxa that are threatened due to the increasing destruction of freshwater ecosystems.

2.1 The systematic – taxonomic confusion in the family Lymnaeidae

About 1800 species and 34 genera of lymnaeids have been named in the past, with classifications recognising a single genus (Walter, 1968), two genera (Hubendick, 1951; Te, 1976; Jackiewicz, 1998) or more than two genera (Burch, 1965, 1980; Malek, 1985; Jackiewicz, 1993; Glöer and Meier-Brook, 1998), while Kruglov and Starbogatov (1993) recognised up to 26 different genera within the family.

Despite several approaches being used to evaluate the taxonomy and relationships within the family, consensus has not yet been reached because of the poor/inadequate systematic resolution of the information (Hubendick, 1951; Burch, 1965; Inaba, 1969; Walter, 1969; Burch and Lindsay, 1968; Burch and Ayers, 1973; Rudolph and Burch, 1989; Remigio and Blair, 1997). Disagreement between the results of morphological studies on the shell, radula and prostate gland with those from karyological and biochemical methods

(Bargues *et al*., 2001), suggests that morphological and anatomic homoplasy is common among lymnaeids. Hence species systematics and delineation within the Lymnaeidae are obscure due to the great number of described species and the morphological similarities between them. These have often made identification of specimens of the Lymnaeidae difficult (Mas-Coma and Bargues, 1997).

2.2 Phylogeny of the Family Lymnaeidae

The phylogeny and classification of the Lymnaeidae has traditionally been based on the use of shell characters, however, once the variable nature of the shell was demonstrated, workers started to take a more anatomical focus in species determination. In recent years, various cytological, biochemical and molecular studies have proven to be very useful tools in resolving some of the phylogenetic questions.

2.2.1 Shell Characters and their use in Phylogeny

Historically, the shape and sculpture of the shells of different lymnaeid species were considered to be consistent and were generally the primary characteristics used in species identifications (Puslednik, 2006). However, intraspecific variation in shell shape has been demonstrated to be common throughout the Lymnaeidae and is thought to be a response to the relative transience of many freshwater habitats (Russell-Hunter, 1978). Freshwater environments are often dominated by short-term, small scale isolation. In these environments, different populations of a given species may be subjected to different environmental conditions and selection pressures. This results in much inter-population diversity, although very little of this diversity results in speciation (Russell-Hunter, 1978; Britton and McMahon, 2004).

Phenotypic plasticity is therefore an important adaptive trait in the family (Via *et al*., 1995). This is supported by several studies within the Lymnaeidae (Hubendick, 1951; Arthur, 1982; Lam and Calow, 1988; Evans, 1989; Oviedo *et al*., 1995; Ward *et al*., 1997; Wullschleger and Jokela, 2002), where shell variation is seen to be a result of

environmental effects. These environmental effects include factors such as habitat type, water movement and predation (Arthur, 1982; Lam and Calow, 1988; Crowl, 1990; De Witt, 1998).

The exclusive use of shell characters to understand evolutionary relationships can also be problematic when differentiation is limited. An absence of obvious shell diversification can result in the incorrect assumption of a single evolutionary lineage. Cryptic speciation has been demonstrated in a number of freshwater molluscs (Baker *et al*., 2003; Liu *et al*., 2003; Pfenninger *et al*., 2003) and examples from within the Lymnaeidae are the South American taxa *Lymnaea viatrix* Orbigny, 1835 and *Lymnaea cubensis* Pfeiffer, 1839. These species are genetically and anatomically distinguishable, but they have identical shells (Jabbour-Zahab *et al*., 1997; Samadi *et al*., 2000; Durand *et al*., 2002).

2.2.2 Anatomical Characters and their use in Phylogeny

Anatomical studies of the soft parts of snails have proved useful in the past for identifying and separating species. However, at the same time, these characteristics are also thought to be problematic and have obscured systematic relationships (Inaba, 1969). Some authors have proposed that anatomical characters are too variable and should be avoided in phylogenetic studies, since they are prone to selective processes and hence more homoplastic than other characters (Hubendick, 1951; Bargues *et al*., 2001; Remigio, 2002). Differences in anatomy due to variable relaxation/contraction during fixation is also a factor. Other authors advocate that only a small, limited set of anatomical characters is useful in determining species, such as the distal genitalia, prostate and radula teeth (Hubendick, 1951; Walter, 1968; 1969).

Radula morphology is generally considered stereotypic within species and is frequently used as a taxonomic character for studies on molluscan systematics (Fretter and Graham, 1994; Padilla, 1998; deMaintenon, 2004). The shape and form of the radula teeth are typically unique to a species or genus and some features of the radula, such as tooth

numbers have been used to investigate higher level molluscan taxonomic relationships (deMaintenon, 2004).

More recent descriptions of lymnaeids have identified numerous characteristics of the outer body, kidney, nervous system and digestive system that are useful in distinguishing species (Paraense 1976; 1982; 1984; 1994; 1995; Ponder and Waterhouse, 1997; Samadi *et al*., 2000). In the description of the European lymnaeids, Jackiewicz (1959; 1984; 1986; 1988; 1993; 1998) placed a strong emphasis on the male and female reproductive systems as characteristics to identify various species. Russian workers (Kruglov and Starbogatov, 1981; 1989; 1993) based their designations within the Lymnaeidae on very minor differences in the reproductive system, such as the changes in the shape of the oothecal gland. More recent studies have also examined the value of other characters such as the size and shape of the tentacles (Jackiewicz, 1990; Jackiewicz and Buksalewicz, 1998) and the pneumostome (Jackiewicz and Dudzien, 1998) in the identification of lymnaeids.

Despite the variation of anatomical characters within populations, some of the characters referred to above have proven to be more useful in discriminating taxa than shell morphology (Samadi *et al*., 2000). Thus, the value of anatomical characters in understanding the systematics of the Lymnaeidae should not be underestimated.

2.2.3 Biochemical and Molecular Studies and their use in Phylogeny

To overcome problems associated with the phenotypic plasticity of the shell and variation in anatomical characters, cytological, biochemical and molecular studies have been carried out within the Lymnaeidae. These techniques include crossbreeding experiments (Pagulayan and Enriquez, 1983; Kruglov and Starbogatov, 1985), enzyme electrophoresis (Evans, 1989; Monzon *et al*., 1994; Jabbour-Zahab *et al*., 1997; Durand *et al*., 2002), body surface chromatography (Wright, 1964), cytology (Burch and Lindsay, 1969; Garbar and Korniushin, 2002; 2003), immunological studies (Burch, 1973; Burch and Lindsay, 1973; Burch and LoVerde, 1973; Burch and Hadzisce, 1974), allozymes

(Coutellec-Vreto *et al*., 1994), PCR-RFLP's (Carvalho *et al*., 2004), RAPD analysis (Rybska *et al*., 2000) and DNA sequencing (Márquez *et al*., 1995; Bargues and Mas-Coma, 1997; Bargues *et al*., 1997; Remigio and Blair, 1997; Stothard *et al*., 2000; Bargues *et al*., 2001; Remigio, 2002; Bargues *et al*., 2003; Puslednik, 2006).

The majority of studies listed have generally been focused on defining species limits, and understanding the taxonomy and distribution of the lymnaeids. Of these techniques, DNA sequencing has been the most successful tool in understanding speciation. However, major inconsistencies have been identified between relationships predicted from DNA gene sequencing compared to those predicted from the use of traditional shell and anatomical characters.

Molecular phylogenies have shown that taxa with the same number of prostate folds or identical radula dentition (characters considered to be phylogenetically important within the family), are not necessarily closely related (Remigio and Blair, 1997; Remigio, 2002; Puslednik, 2006). For example, morphological phylogenies have indicated that *Stagnicola palustris* Müller, 1774 was distantly related to *Stagnicola corvu*s Gmelin, 1791 and *Lymnaea stagnalis* Linnaeus, 1758, whereas the molecular relationships showed *S. palustris* and *S. corvus* to be sister taxa, with *L. stagnalis* sister to the *S. corvus* and *S. palustris* clade (Remigio, 2002; Puslednik, 2006). Despite these inconsistencies, molecular approaches have over the past decade proven their value not only in resolving phylogenetic issues, but also in providing a sense of the time scale of evolutionary divergence (Thollesson, 1999; Remigio and Herbert, 2003).

2.3 Lymnaeids in parasite transmission

Species of this family are of parasitological importance due to their capacity to act as intermediate hosts for numerous helminth parasites (Brown, 1978; 1994; Bargues and Mas-Coma, 1997). These include several digenean flukes of medical and veterinary importance, such as *Fasciola hepatica* and *Fasciola gigantica* (Malek, 1980; Boray,

1982; Chen and Mott, 1990; Bargues and Mas-Coma, 1997), as well as certain cestode and nematode species.

Lymnaeids worldwide participate in the life cycles of at least 71 trematode species belonging to 13 families whose members use birds and both domestic and sylvatic mammals as definitive hosts (Brown, 1978; Bargues *et al*., 2001). For example, nearly 30 cercarial species have been recorded from *Lymnaea stagnalis* in Europe (Erasmus, 1972); 21 species from the North American *Lymnaea emarginata* Say, 1821 (Cort *et al*., 1937) and in South Africa, Porter (1938) found 43 species in *Lymnaea natalensis* Krauss, 1848. The trematode parasite *Fasciola* is specific to lymnaeids, with at least 12 named species acting as natural intermediate hosts for one or both of the two economically important species of *Fasciola* (Malek, 1980; Boray, 1982; Mas-Coma and Bargues, 1997). Both *Fasciola* species have wide distributions; *F. hepatica* is cosmopolitan while *F. gigantica* is limited to the more tropical regions (Brown, 1978; Torgerson and Claxton, 1999).

Fascioliasis has an important economic impact on livestock because it results in reduced weight gain, progressive decrease in milk yield, lowered fertility and abortion (Dargie, 1987). Other causes of economic loss are the condemnation of infected livers at slaughter and the cost of control strategies. The disease has been reported to cause significant economic losses amounting to between US$20 million to US$107 million in countries such as the Philippines, Cambodia and Indonesia, where the prevalence of infection is high (Spithill *et al*., 1999). The same authors estimated losses due to *Fasciola* infection between 1975 and 1997 to exceed US$3200 million in tropical countries alone.

While fascioliasis has traditionally been a veterinary problem, there have been an increasing number of outbreaks in people (Chen and Mott, 1990; Esteban *et al*., 1998; Mas-Coma *et al*., 1999), resulting in a major public health problem in several areas of the world, including Bolivia, Ecuador, Peru, the Caribbean Islands, the Nile Delta in Egypt and central Vietnam. The largest problem areas are the Caribbean Islands and South

America, with the highest levels of infection rates being reported from the Bolivian Altiplano (highland) region, with up to 66.7% of individuals from this region being infected. As a consequence, this has led to human fascioliasis being listed as an emerging medical disease (Mas-Coma *et al*., 1995; Esteban *et al*., 1997; Mas-Coma *et al*., 1999; Esteban *et al*., 2003; Curtale *et al*., 2005; Keiser and Utzinger, 2005; Mas-Coma *et al*., 2005).

2.4 Conservation status of the Lymnaeidae

The increasing destruction of freshwater ecosystems through a decline in water quality, pollution, increased sediment deposition, diversion and damming has resulted in freshwater molluscs representing one of the most threatened groups of animals in the world (Saunders *et al*., 1999; Lydeard *et al*., 2004; Strong *et al*., 2008). Despite comprising only ~5% of the world's gastropod fauna, freshwater molluscs account for ~20% of recorded mollusc extinctions (Strong *et al*., 2008).

In North America, the increasing number of species being listed as endangered or threatened reflects this growing crisis. For example, according to Riccardi *et al*., (1998), 72% of North America's recognised mussel species are listed as endangered, threatened or of special concern. Puslednik (2006) stated that in the state of Idaho, six lymnaeid species have been listed federally as endangered or threatened. In Europe, the decline of *Myxas glutinosa* Müller, 1774 (considered to be one of the rarest freshwater molluscs) has been attributed to eutrophication, increased turbidity and the regulation of water flow in lakes (Whitfield *et al*., 1998; Puslednik, 2006).

Despite this, only five lymnaeid species have been listed as threatened on the IUCN Red List (IUCN, 2009). These include two North American taxa, *Stagnicola bonnevillensis* Call, 1884 and *Stagnicola utahensis* Call, 1884; *Myxas glutinosa* from Europe; *Erinna newcombi* Adams, 1855 from the Hawaiian islands and the fifth species *Lantzia carinata* Jousseaume, 1872 which is restricted to Reunion Island in the Indian Ocean (IUCN, 2009). This indicates that the threat to lymnaeids is not a localised problem, but a

phenomenon that is occurring across the world. In the draft IUCN Red Data List the two indigenous lymnaeids in southern Africa, *L. natalensis* and *Lymnaea truncatula* Müller, 1774 are listed as having "no threats" and are therefore of "least concern", as far as their conservation status goes (C.C. Appleton pers. comm.), though competition from the invasive *Lymnaea columella* Say, 1817 is mentioned as a possible threat.

Also as discussed previously, many lymnaeids are intermediate hosts for *Fasciola* spp., which have a large economic impact on agriculture. Various control measures such as the draining of wetlands and the use of molluscicides have resulted in over 90% of the snail populations in a targeted area in the United States of America being killed (Graczyk and Fried, 1999). This wide scale destruction of temporary ponds and the snails within them poses an additional threat to indigenous freshwater species (Pusledník, 2006).

3

Redescription of *Lymnaea natalensis* Krauss, 1848 from its type locality

3.1 Introduction

Species delineation within freshwater snails of the genus *Lymnaea* Lamarck, 1799 (Gastropoda: Lymnaeidae) is controversial and characterised by a long and confused systematic history largely due to problems associated with variability in shell shape. The family Lymnaeidae has a world-wide distribution and they have the potential to colonise many fresh water environments (Stothard *et al*., 2000). Several species are of medical and veterinary importance because they serve as intermediate hosts of numerous trematode parasites, including the liver flukes *Fasciola hepatica* and *Fasciola gigantica*, as well as certain cestode and nematode species (Brown, 1978; Malek, 1980; Boray, 1982; Chen and Mott, 1990; Bargues and Mas-Coma, 1997).

As noted in Chapter 1, *Lymnaea natalensis* Krauss, 1848 is the intermediate host of the giant liver fluke *F. gigantica* in South Africa and this fluke is confined to the subtropical lowland regions. *Lymnaea truncatula* Müller, 1774 is the intermediate host for the common liver fluke *F. hepatica*, however this snail species is confined to the cooler areas of the eastern highlands in South Africa (altitude above approximately 800 m), and also the low-lying parts of the Eastern and Western Cape, South Africa (Brown, 1994). Recently, another lymnaeid, *Radix rubiginosa* (Michelin, 1831) was recorded in a tropical fish breeding facility at Amatikulu, northern KwaZulu-Natal, South Africa.

This is important since *R. rubiginosa* serves as the intermediate host for *F. gigantica* over much of south-eastern Asia (Srihakim and Pholpark, 1991; Malone, 1997) as well as the schistosomes *Trichobilharzia* sp. (Nithuithai *et al*., 2004) and *Schistosoma incognitum*

(Bunnag *et al*., 1983) and several echinostomes (Charoenchai *et al*., 1997). Also, the introduction of *R. rubiginosa* is likely to go unnoticed due to its resemblance to the indigenous *L. natalensis*. This is because the shell morphology of *L. natalensis* is notoriously variable and some of its variations resemble *R. rubiginosa*. There was thus a clear need to be able to differentiate the two lymnaeid species but identification was difficult due to unclear and contradicting accounts in the literature. This has important implications for studies of community structure, food web dynamics, biodiversity and biomonitoring that critically depend on the accuracy of species discrimination and identification.

Recent research into the epidemiology of human and bovine fascioliasis has also focused attention on the different "forms" of *L. natalensis* in Africa and this was further complicated by increasing evidence suggesting that what is widely called *L. natalensis* in Africa may in fact comprise more than one species (Brown, 1994; S. Mas-Coma pers. comm. to C.C. Appleton, 2006). This therefore highlights the need for accurate species identification. Because the type locality of *L. natalensis* is 'Port Natal', i.e. Durban, the species is redescribed here based on topotypical specimens from Durban. This redescription of the conchological and anatomical characteristics could then form a basis for new investigations into the true identity of what is currently known as *L. natalensis* across Africa.

3.2 Methodology

3.2.1 The Study Site

In his original description of *L. natalensis*, Krauss (1848) stated, "*In stagnis natalensibus, frequens*" (in still waterbodies in Natal, frequent). However, Herbert and Warén (1999) found probable syntypes of this species in the Stockholm Museum with the locality given as Port Natal. Taking this into consideration and to provide topotypical material, various waterbodies in and around the Durban Metropolitan Area were investigated for the presence of populations that refer morphologically to *L. natalensis*. Of the habitats visited, surprisingly few yielded *L. natalensis* and of these, the UKZN Pond (Cato Manor) was designated as a suitable site (Figure 3.1).

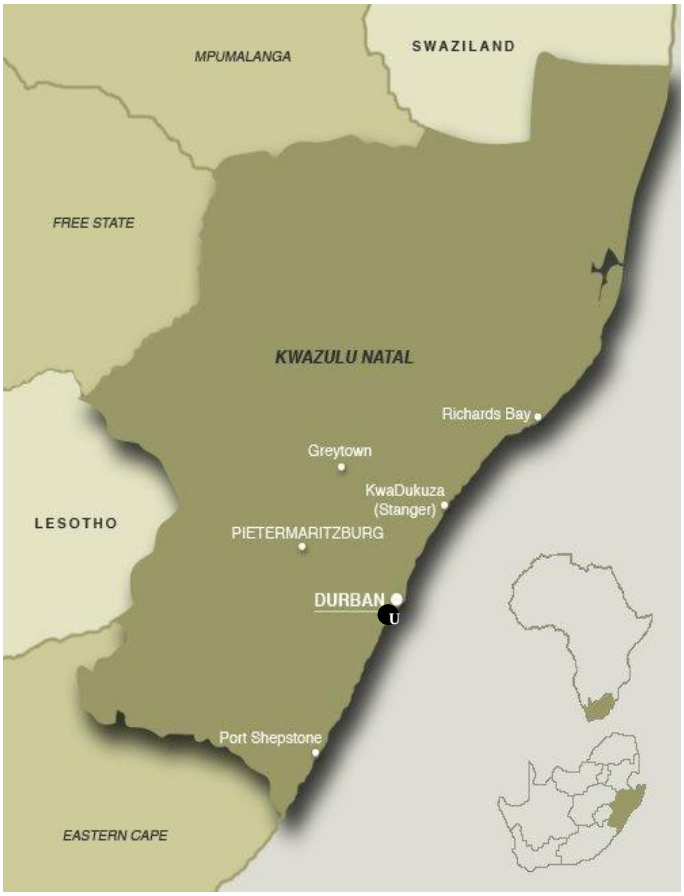


Figure 3.1: Map of KwaZulu-Natal showing the UKZN Pond study site (U), selected for the redescription of *L. natalensis.*

UKZN Pond (Cato Manor, Durban)

This is a permanent but isolated waterbody (S 29^{o} 52' 02.6" E 30^{o} 58' 03.6", the altitude is 24 m), located close to a highway and a sports and recreational stadium (Figure 3.2). The aquatic macro-flora comprised of *Marsilea* sp., *Typha capensis*, *Pistia stratiotes*, *Nymphaea nouchali* and several representatives of the Cyperaceae. About 75% of the surface area was covered by *P. stratiotes*, *Marsilea* sp. and *N. nouchali*, with some areas covered by dense mats of filamentous green algae. *Albizia adiantifolia* trees and dense shrubs along the periphery of the pond limited accessibility and provided partial shade.

The temperature in the Durban Metropolitan Area is mild in winter (June to August) and warm to hot in summer (December to February). Mean monthly figures for the minimum and maximum daily air temperatures were 10.3^{o}C to 21.5^{o}C and 22.8^{o}C to 28.9^{o}C respectively (Source: South African Weather Service, Durban). The total annual rainfall usually exceeds 1000 mm, of which the most falls in spring and summer (September to February). The pond is dependent on rainfall and its depth varied between 2.1 m in winter to 4.5 m in summer. It has not been seen to dry out. The water chemistry parameters for the UKZN Pond were measured by the author at a depth of 30 cm, using a YSI 6920 multi-probe data logger. A summary of selected water chemistry parameters is presented in Table 3.1.

Table 3.1: Selected water chemistry parameters for the UKZN Pond. All values measured are indicated as mean (± standard deviation), n = 35.

Parameter	**Mean (± standard deviation)**
pH	8.13 (± 0.12)
Conductivity (mS/cm)	0.57 (± 0.01)
Dissolved Oxygen (mg/L)	8.08 (± 0.81)

Figure 3.2: The UKZN Pond.

3.2.2 Shell Morphology

The snails were identified based on conchological characteristics according to the original description by Krauss (1848) and subsequent authors (Connolly, 1939; Hubendick, 1951; Mandahl-Barth, 1954; de Azevedo *et al.*, 1961; Brown, 1994; Appleton, 1996; Herbert and Warén, 1999). Due to the variable nature of the shell of *L. natalensis* wherever it was found, a molecular study of snails from this site was conducted in 2007 (J. Lamb and K. Pillay unpubl. data). This was the same molecular study that identified the Amatikulu lymnaeid as *R. rubiginosa*. DNA analyses confirmed that the lymnaeid from the UKZN Pond was *L. natalensis* (see Appendix to Chapter 3).

Fifteen adult *L. natalensis* from the study site were selected to measure the shell thickness. In order to compare the snails without size bias, only adult snails of 15-22 mm

in shell length were used. The shell thickness was measured at three points on the shell and expressed as the mean shell thickness (Figure 3.3).

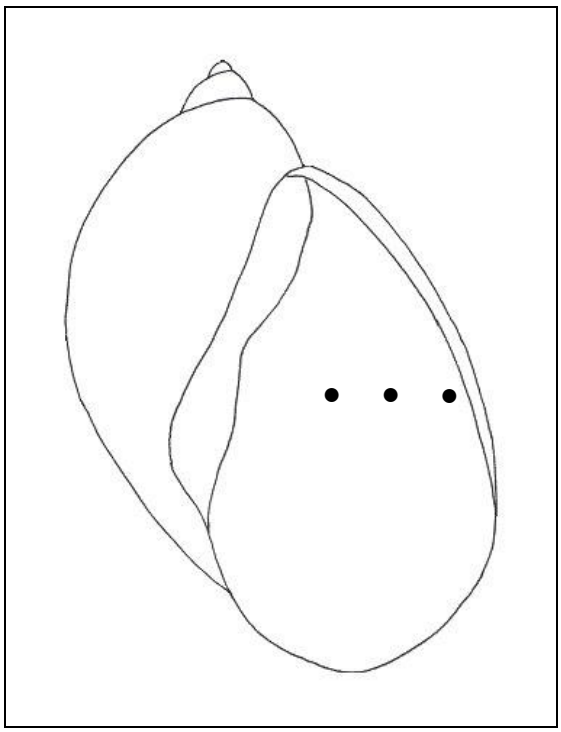


Figure 3.3: Schematic drawing of the shell indicating the points (●) where shell thickness was measured. The mean shell thickness was presented as μm (± standard deviation).

3.2.3 Anatomical Morphology

3.2.3.1 Radula

Before dissection, 15 adult *L. natalensis* from the UKZN Pond were relaxed for 24 hours using menthol crystals. The specimens were fixed in 10% formalin for a further 24 hours and then stored in 75% alcohol. To study the radula, the buccal mass of each relaxed snail was removed and put into a test tube of 5% NaOH solution for 48 hours. This procedure removed the tissues surrounding the radula, leaving only the radular ribbon in the test tube.

The contents of the test tube were emptied into a watch glass and the radula ribbon removed under the dissecting microscope. The extracted radula was then washed in three changes of distilled water, three minutes per rinse and stored in 75% alcohol. The radula was then mounted on a specimen stub using two way laboratory tape and allowed to dry at room temperature. After coating with gold, the radula was viewed using a LEO scanning electron microscope.

The number, shape, size and position of the cusps on the central, lateral and marginal teeth were noted and where appropriate, photographed.

3.2.3.2 Mantle pigmentation patterns

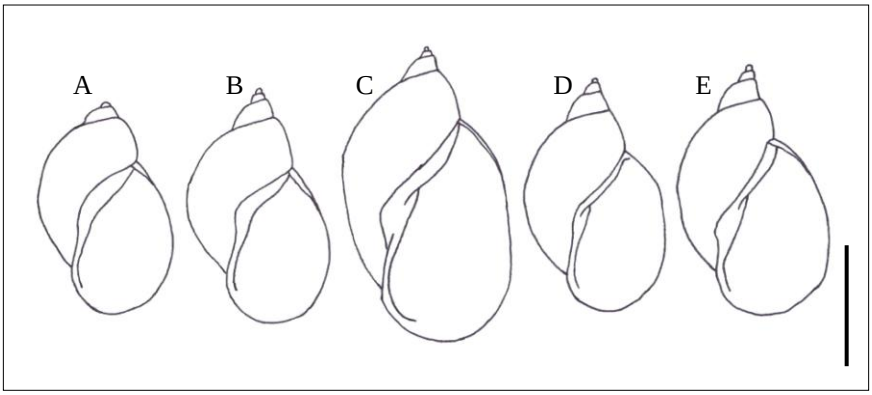
Fifteen adult *L. natalensis* from the study site were relaxed for 24 hours using menthol crystals and then immersed for approximately a minute in hot water (70^{o}C), from which they were transferred to water at room temperature. The soft tissues were separated and dissected following the methodology proposed by Paraense (1976). By holding the cephalopedal mass with a forceps and gently twisting the shell in an anti-clockwise motion, the soft parts were drawn from the shell. Drawings of the mantle pigmentation were made using a camera lucida fitted on a stereomicroscope.

3.2.3.3 Reproductive Anatomy

Fifteen adult *L. natalensis* from the study site were prepared as outlined in section 3.2.3.2. In order to compare the snails without size bias, only adult snails of 15-22 mm in shell length were used. Snails were dissected under a stereoscopic microscope and drawings of the reproductive system were made using a camera lucida.

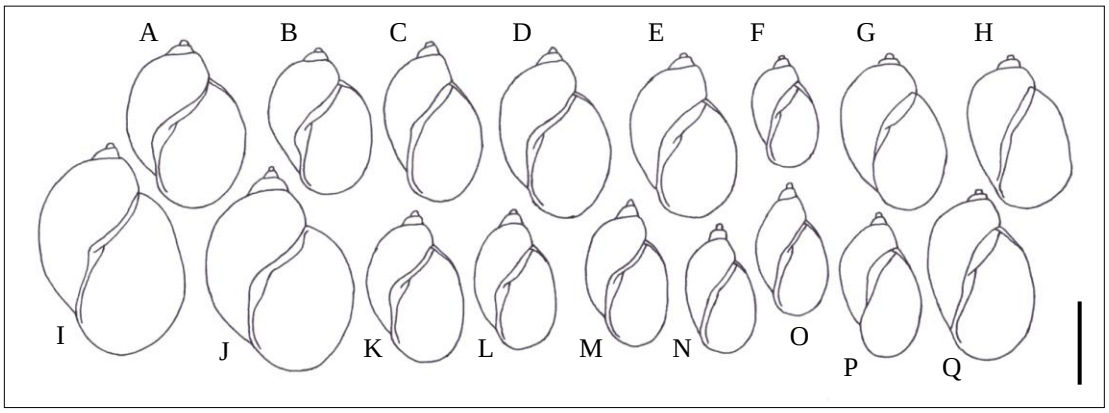
3.3 Classification and Distribution of *Lymnaea* in Africa

A great many forms and species of *Lymnaea* (Figures 3.4 - 3.6) have been described from Africa (Hubendick, 1951). Between the years 1848 and 1939 no less than 11 species were described from or reported to occur in South Africa alone. The first substantial taxonomic revision by Germain (1919) recognised six species of *Lymnaea*: *L. natalensis* Krauss, 1848; *L. africana* Bourguignat, 1883; *L. elmeteitensis* Smith, 1894; *L. tchadiensis* Germain, 1905; *L. vignoni* Germain, 1909 and *L. gravieri* Bourguignat, 1885. Connolly (1939) recognised only two as actually occurring in this country, namely *Lymnaea natalensis* and *Lymnaea caillaudi* Bourguignat, 1883. However, a further reduction by Hubendick (1951) placed all forms and species within the synonymy of *Lymnaea natalensis*. This opinion was followed by Brown (1994) with the caution that there may be more than one taxon involved.



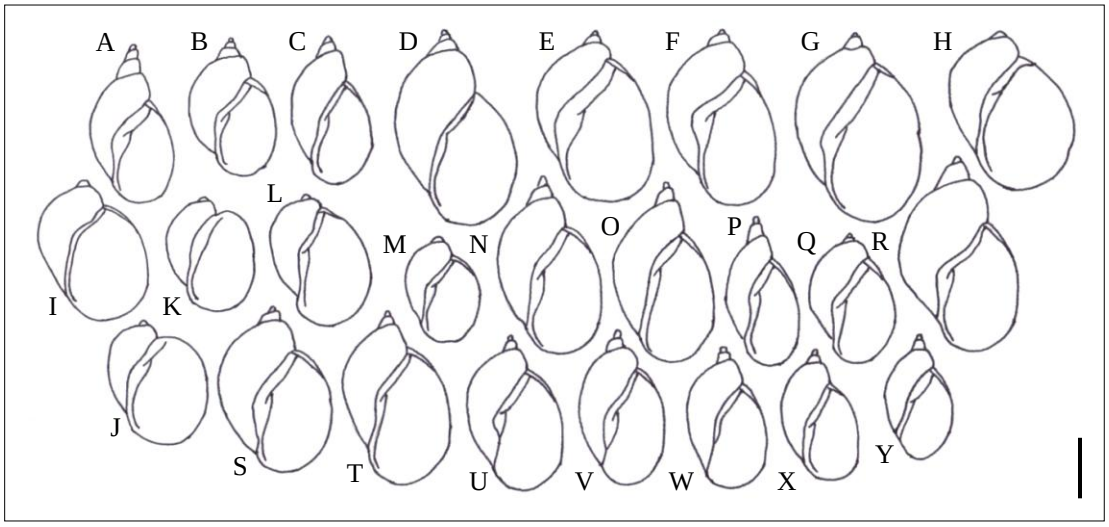
(redrawn from Hubendick, 1951)

Figure 3.4: *Lymnaea natalensis* from Arabia, with the locality given in parentheses, scale bar 10 mm. A-B, *Lymnaea caillaudi* (Mesajia, Yemen); C, *Lymnaea muscatensis* (Muscat); D-E, *Lymnaea caillaudi* (Kalhat, Saudi Arabia).



(redrawn from Hubendick, 1951)

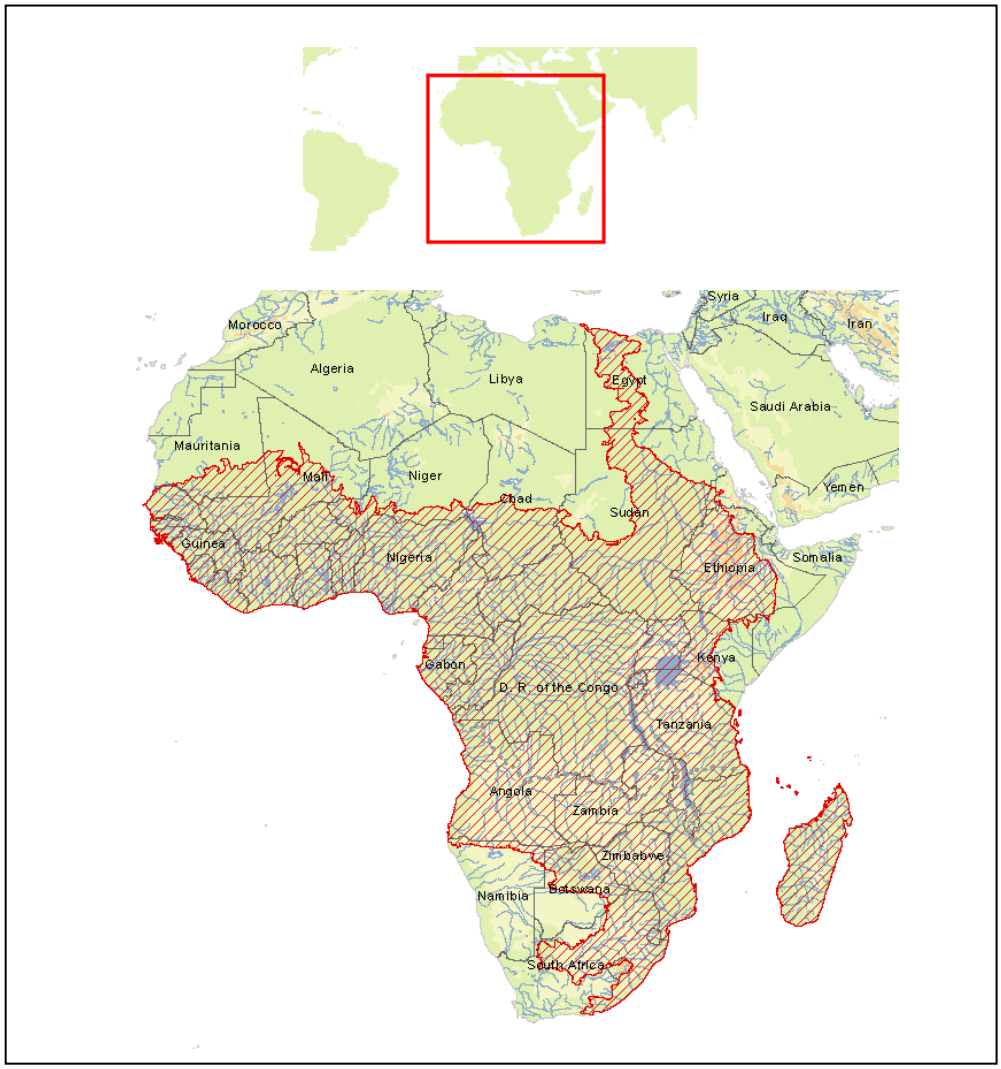
Figure 3.5: *Lymnaea natalensis* from Madagascar, with the locality given in parentheses, scale bar 10 mm.
A-C, *Lymnaea natalensis* (Antisirabe, Madagascar); D-F, *Lymnaea natalensis* (Lake Renobe, Madagascar); G-H, *Lymnaea pacifica* (Ambatondigen, Madagascar); I-J, *Lymnaea specularis* (Ankazoaba, Western Madagascar); K-O, *Lymnaea hovarum* (Antanamena, Madagascar); P, *Lymnaea pacifica* (Ankazoaba, Western Madagascar); Q, *Lymnaea electa* (Ankazoaba, Western Madagascar).



(redrawn from Hubendick, 1951)

Figure 3.6: *Lymnaea natalensis* from Africa, with the locality in which they were described given in parentheses, scale bar 10 mm.
A-B, *Lymnaea exsertus* (Sweet water Canal, Suez); C, *Lymnaea pharaonum* (Egypt); D, *Lymnaea caillaudi* (Alexandria, Egypt), E-G, *Lymnaea caillaudi* (Nuruya, Darfur); H-I, *Lymnaea ribeirensis* (San Antao Island, Cape Verde Island); J-M, *Lymnaea nyansae* (Entebbe, Uganda); N, *Lymnaea elmeteitensis* (Northern Uganda); O-P, *Lymnaea undussumae* (Ndola Swamp, Northern Zimbabwe); Q, *Lymnaea nyansae* (Luanshyla, Northern Zimbabwe); R-S, *Lymnaea caillaudi* (Northern Zimbabwe); T-W, *Lymnaea natalensis* (Port Natal, South Africa); X-Y, *Lymnaea natalensis* (Durban, South Africa).

After acknowledging the intraspecific variation characteristic of *Lymnaea*, Brown (1994) followed Hubendick (1951) in accepting that Africa had a single widespread species, *Lymnaea natalensis* Krauss, 1848. Furthermore *L. natalensis* has been found to occur in Madagascar, the Cape Verde Islands and Tenerife as well as Yemen and Oman in the Arabian Peninsula (Figure 3.7).



(Seddon *et al*., 2011)

Figure 3.7: The distribution of *Lymnaea* spp. in Africa and Madagascar. Occurrences in the Cape Verde Islands are not shown on the map. According to Hubendick (1951) and later Brown (1994) there is little justification for maintaining most of the many named species in Africa; the whole range seems to be inhabited by a single but variable species, *Lymnaea natalensis*. The shaded regions are intended to represent the main areas of occurrence, however, continuity of distribution is not implied and there may be significant discontinuities within the shaded areas.

In common with other species of *Lymnaea*, there is considerable conchological variation within *L. natalensis* that appears continuous across populations whilst local populations may be uniform and distinctive. Haas (1936) discussed the different forms of *L. natalensis* and considered that certain forms were characteristic to certain parts of Africa. This suggestion was not confirmed by Hubendick (1951), stating that the species has a vast range of irregular microgeographical variation. According to Hubendick (1951), *L. natalensis* was treated as the African part of the *Lymnaea auricularia* Linnaeus, 1758 super-species which extended throughout the Palearctic and Oriental regions. However, according to Brown (1994), it appears that certain forms are indeed distinct to certain parts of Africa.

For example, the succineiform forms of *L. natalensis* are most common in, and even possibly restricted to, South Africa. On Madagascar the extremely broad and bulging forms seem to be more common and their shape possibly more pronounced, than in other regions of Africa. Also, *Lymnaea hovarum* Tristram, 1863, from Madagascar has been treated as distinct (Starmühlner, 1969), but seems to be a form of *L. natalensis* according to Brown (1994). Furthermore, the name *Lymnaea caillaudi* Bourguignat, 1883, has often been used to describe snails from eastern Africa, belonging to a form narrower than the typical *L. natalensis* (Brown, 1994). Mandahl-Barth (1954) reduced *L. caillaudi* to a subspecies of *L. natalensis* but later expressed the opinion that even the subspecies could no longer be regarded as being distinct from *L. natalensis* (Pretorius and van Eeden, 1969).

In addition, three widespread European species, *Lymnaea palustris* Müller, 1774, *Lymnaea peregra* Müller, 1774 and *Lymnaea stagnalis* Linnaeus, 1758 also occur in North Africa, while *Lymnaea truncatula* Müller, 1774 has an eastern distribution from Egypt to South Africa. Also, *Lymnaea columella* Say, 1817, an introduction of North American origin, is now well established in southern Africa (van Eeden and Brown, 1966; Brown, 1994; Appleton, 2003). Recent research into the epidemiology of human and bovine fascioliasis has renewed interest in the identification and classification of the different "forms" of *L. natalensis* in Africa. It has called into question the placing of so

many forms across the continent in a single species. As a starting point for unraveling this problem, it seems appropriate to redescribe *L. natalensis* using topotypical material from Port Natal, i.e. Durban, South Africa.

3.4 Results

3.4.1 Original Description

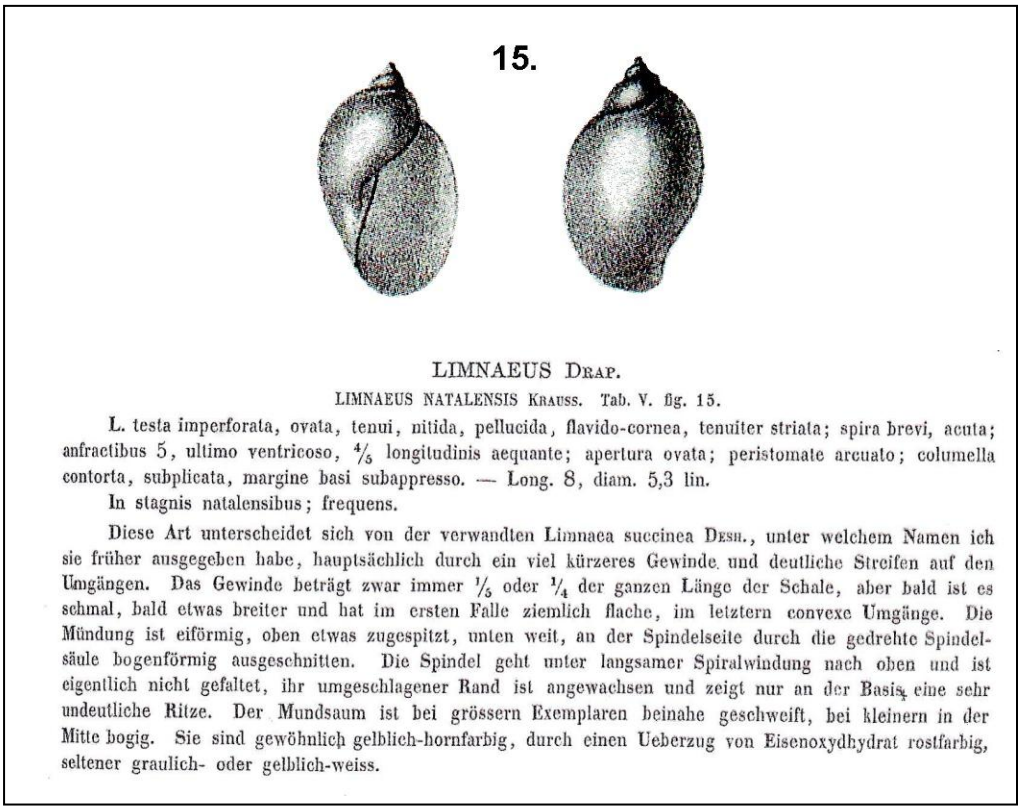
The systematic position of *L. natalensis* is as follows:

Family Lymnaeidae Rafinesque, 1815

Subfamily Lymnaeinae Rafinesque, 1815

Genus *Lymnaea* Lamarck, 1799

Lymnaea natalensis Krauss, 1848



15.

LIMNAEUS DRAP.

LIMNAEUS NATALENSIS KRAUSS. Tab. V. fig. 15.

L. testa imperforata, ovata, tenui, nitida, pellucida, flavido-cornea, tenuiter striata; spira brevi, acuta; anfractibus 5, ultimo ventricoso, 4/5 longitudinis aequante; apertura ovata; peristomate arcuato; columella contorta, subplicata, margine basi subappresso. — Long. 8, diam. 5,3 lin.

In stagnis natalensibus; frequens.

Diese Art unterscheidet sich von der verwandten Limnaea succinea DESH., unter welchem Namen ich sie früher ausgegeben habe, hauptsächlich durch ein viel kürzeres Gewinde und deutliche Streifen auf den Umgängen. Das Gewinde beträgt zwar immer 1/5 oder 1/4 der ganzen Länge der Schale, aber bald ist es schmal, bald etwas breiter und hat im ersten Falle ziemlich flache, im letztern convexe Umgänge. Die Mündung ist eiförmig, oben etwas zugespitzt, unten weit, an der Spindelseite durch die gedrehte Spindelsäule bogenförmig ausgeschnitten. Die Spindel geht unter langsamer Spiralwindung nach oben und ist eigentlich nicht gefaltet, ihr umgeschlagener Rand ist angewachsen und zeigt nur an der Basis eine sehr undeutliche Ritze. Der Mundsaum ist bei grössern Exemplaren beinahe geschweift, bei kleinern in der Mitte bogig. Sie sind gewöhnlich gelblich-hornfarbig, durch einen Ueberzug von Eisenoxydhydrat rostfarbig, seltener graulich- oder gelblich-weiss.

Figure 3.8: The original description, in Latin and German, of *L. natalensis* transcribed from Krauss (1848: 85), supplemented with the original figure of the shell of a specimen from a lentic pool in what is now the city of Durban (see, Figure 15 in text).

3.4.2 Shell Morphology

Although it is subject to considerable variation in detail, the shell of *L. natalensis* is remarkably constant in its general characters (Figure 3.9). The shell is succineiform, dextral and thin (110.60 μm ± 22.14, n = 15 see Figure 3.3). The colour of the shell varies from glossy, pale yellowish, brownish to dark brownish. There is an elongate, tapering spire with an acute apex. These characteristics of the spire however, were variable. There are generally four tightly coiled and convex whorls that are separated by well-impressed and constricted sutures. The body whorl is markedly swollen and forms the greatest portion of the shell.

Figure 3.9: Shells of *L. natalensis* (UKZN Pond) showing the variation found in the population, scale bar 10 mm.

The aperture is large and ovate, with a fold in the middle part of the parietal wall on which a thin white callus can be observed. The base of the aperture joins the columella in a broadly rounded curve. The peristome is thin and sharp. The outer lip of the aperture is generally evenly rounded; inner lip is closely appressed to the parietal wall. The umbilicus is completely closed by the expanded and reflected inner lip. The columella is

short, straight and attenuate at the base; the columellar axis is generally gyrate or twisted. The sculpture consists of growth lines only; these are distinct on the body whorl, but less prominent on the preceding whorls.

3.4.3 Anatomical Morphology

3.4.3.1 Radula

The individuals from the UKZN Pond had between 68 – 80 rows of teeth, with each transverse row of teeth conforming to a radula formula of 12: 8 – 10: 1: 8 – 10: 12. The radula consisted of a single longitudinal row of central teeth found at the middle of the radula, 8 – 10 pairs of lateral teeth (two pairs were identified as being intermediate teeth) and 12 pairs of marginal teeth.

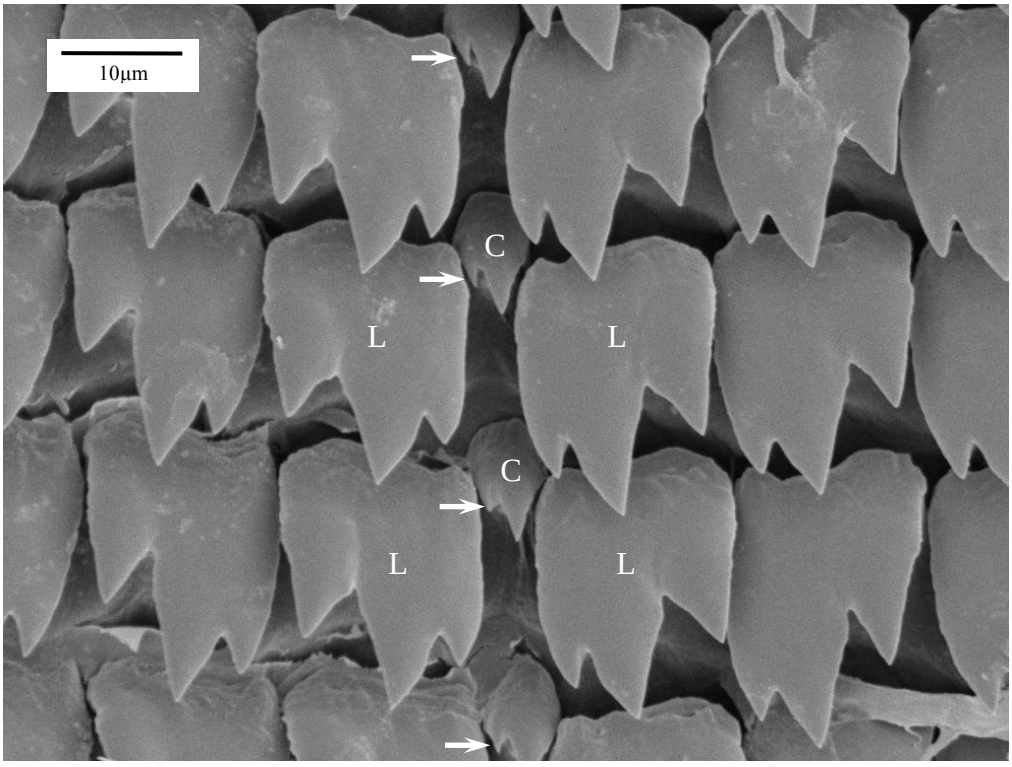


Figure 3.10: Scanning electron micrograph of the central tooth and lateral teeth of *L. natalensis* (UKZN Pond). A smaller accessory cusp is located on the central tooth (indicated by the arrow), scale bar 10 µm. C – central tooth; L – lateral tooth.

The central tooth was asymmetrically bicuspid and very small compared to the other teeth on the radula (Figure 3.10). The main cusp was larger, having a sharp spade-like triangular shape. The cutting point was sharp and acute. A smaller accessory cusp was located on the left hand side towards the base. This accessory cusp was curved and directed towards the larger cusp of the central tooth (see arrows, Figure 3.10). This smaller cusp diminished in size and seemed to disappear after the 10^{th} – 15^{th} transverse row.

From right to left in Figures 3.10 and 3.11, the laterals are rather broad, quadrate and asymmetrically tricuspid. The small, short and spade-shaped endocone was fused to the mesocone. The mesocone was about three times as wide as the endocone. The ectocone was much larger than the endocone and was placed approximately halfway down the mesocone, on its outer margin. For lateral teeth 6-8, the endocone and mesocone appeared very acute in shape and gradually became sub-equal in length (Figure 3.11).

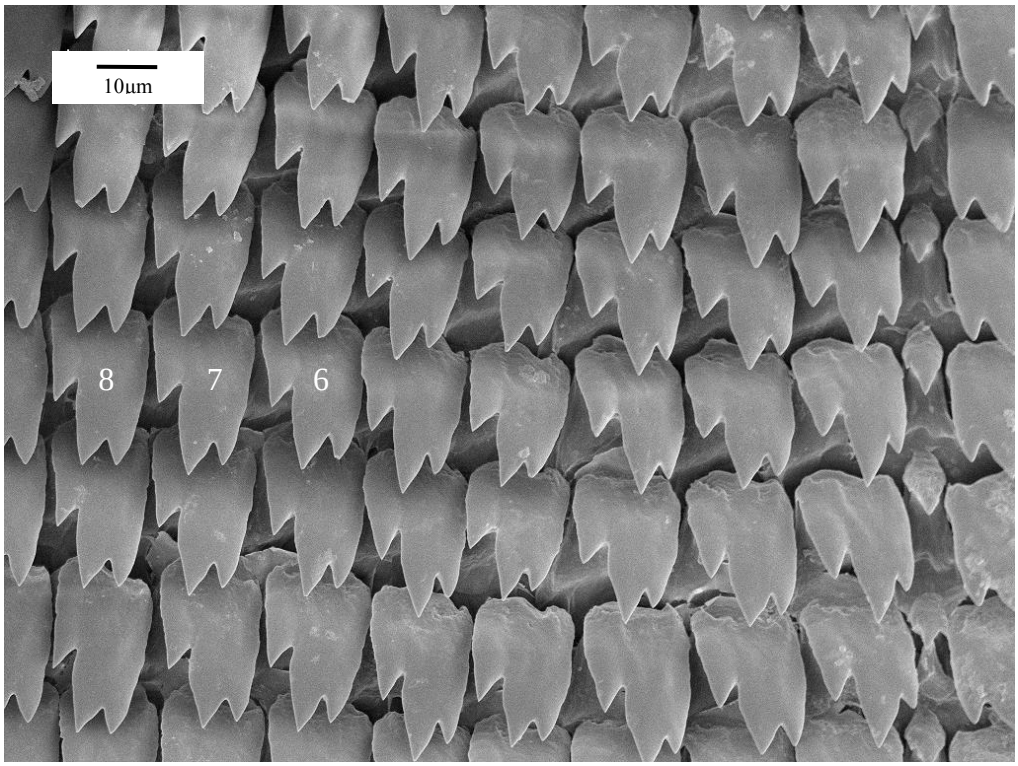


Figure 3.11: Scanning electron micrograph of the lateral teeth from the left side of a transverse row of *L. natalensis* (UKZN Pond). For lateral teeth 6-8, the endocone and mesocone appeared very acute in shape and gradually became sub-equal in length, scale bar 10 µm.

The transition from the lateral to marginal teeth was very abrupt with the 9th and 10th pair of laterals being the transitional or intermediate teeth (Figure 3.12). These intermediate laterals were tricuspid with the endocone and mesocone subequal in length. The ectocone was smaller than the preceding laterals, but still placed approximately midway on the outer margin of the mesocone. In the 9th pair, the ectocone split into two smaller, acute-shaped denticles (see arrow, Figure 3.12). The ectocone of the 10th pair did not split into two smaller denticles and they resembled the tricuspid shape and pattern of the 7th and 8th laterals. This bicuspid pattern of the ectocone was unique for the 9th intermediate laterals only.

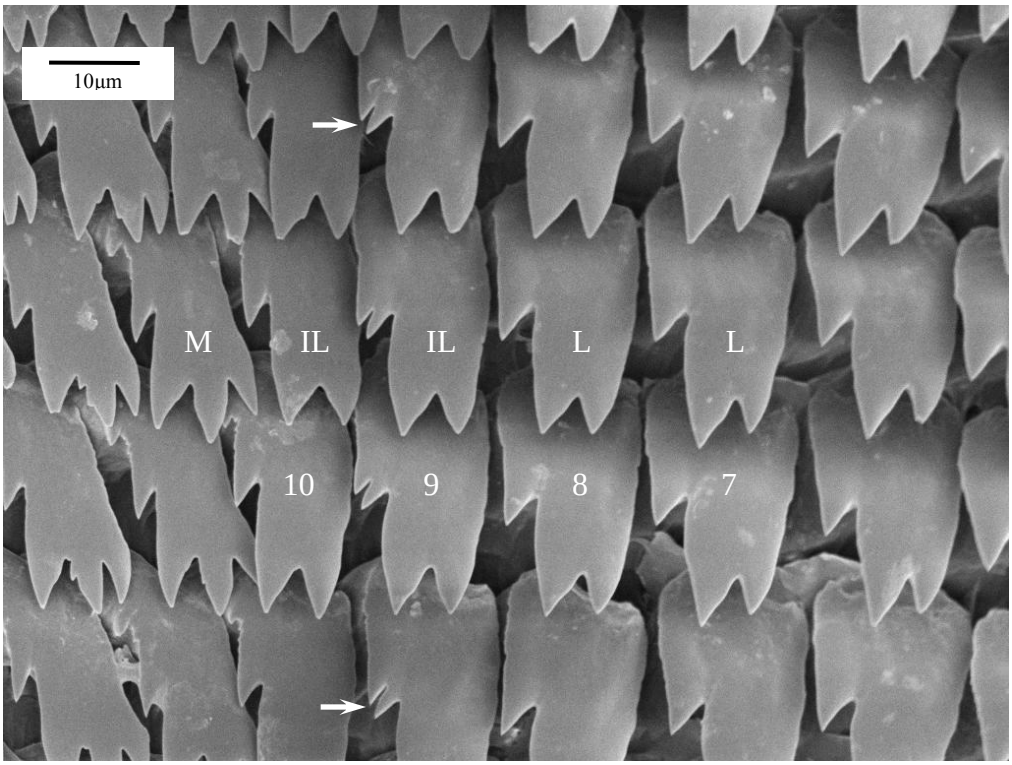


Figure 3.12: Scanning electron micrograph of the intermediate laterals (9th and 10th pair of teeth) of *L. natalensis* (UKZN Pond). In the 9th pair, the ectocone split into two smaller, acute-shaped denticles (indicated by the arrows). The ectocones of the 10th pair did not split into two smaller denticles and they resembled the tricuspid shape and pattern of the 7th and 8th laterals, scale bar 10 µm.
IL – intermediate lateral tooth; L – lateral tooth; M – marginal tooth.

The marginal teeth commenced at the 11th tooth and there were 12 pairs of these multicuspidate teeth on each half of a transverse row (Figure 3.13). The mesocone

persisted throughout the marginal teeth, while the endocone split up into several smaller cusps. The cusps of the endocone and the mesocone were approximately subequal in length and this characteristic was maintained throughout the marginals. In the 1st and 2nd marginals the endocone split into two cusps (Figure 3.13). The ectocone was still placed approximately midway on the outer margin of the mesocone. In addition, smaller denticles appeared below the ectocone, towards the base of these marginals (1st and 2nd marginal teeth). For the marginal teeth 3-12, the mesocone and endocone usually consisted of four bluntly rounded claw-like cusps. In addition, two to three smaller denticles were observed below the ectocone. Proceeding laterally, the teeth became longer and more slender. At the same time, some of the denticles disappeared progressively until only a few were left at the margin of the radula.

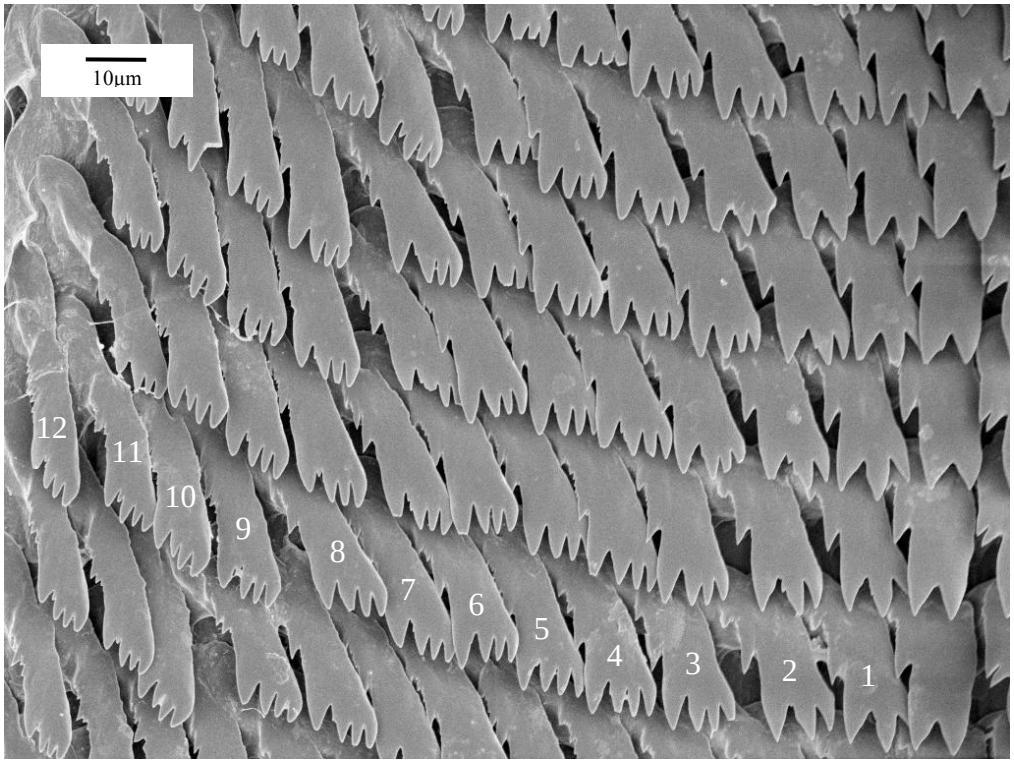


Figure 3.13: Scanning electron micrograph of the marginal teeth (indicated by numbers 3-12) of *L. natalensis* (UKZN Pond). The marginal teeth were multicuspid having four cusps that were short, bluntly rounded and obliquely placed, scale bar 10 µm.

3.4.3.2 Mantle pigmentation patterns

The entire mantle of *L. natalensis* (UKZN Pond) was gray to black in colour but interspersed with unpigmented spots that were usually circular and regular in outline. These spots appeared to be numerous in the region above the kidney and towards the mid region of the mantle. The visceral coil was unpigmented (Figure 3.14). The head was diffusely grayish with scattered pigmentation. Only the outer margin of the foot was pigmented (Figure 3.14).

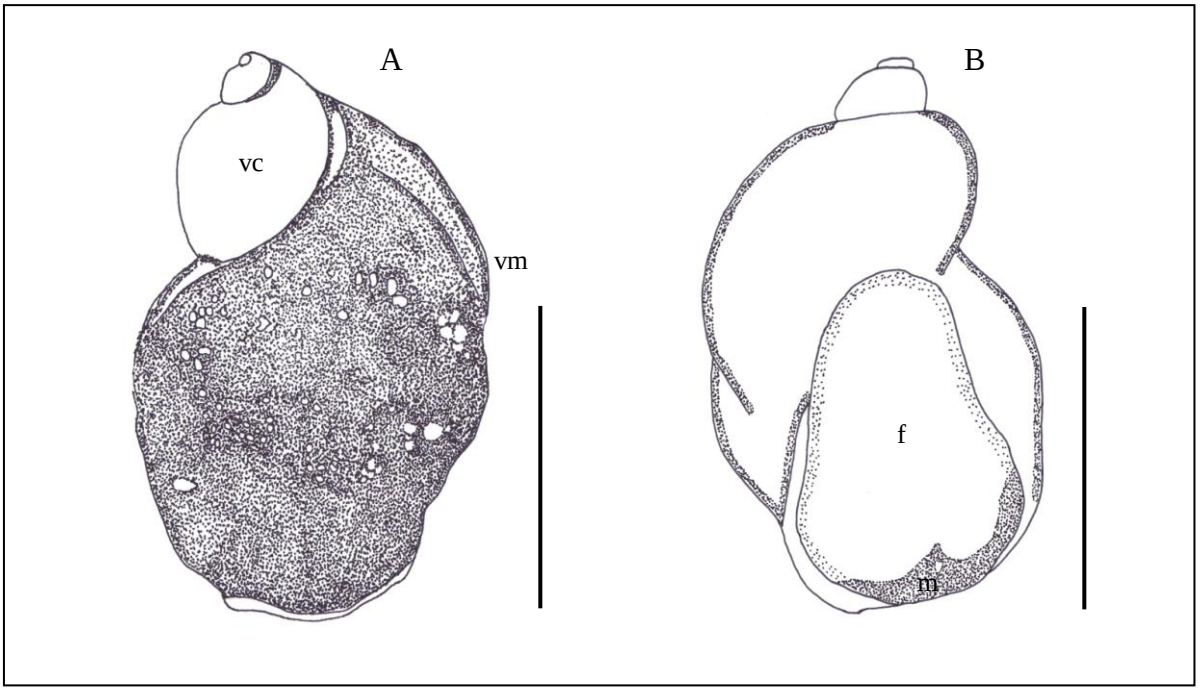


Figure 3.14: *Lymnaea natalensis* (UKZN Pond) – animal with shell removed to show pigmentation patterns.

A – Dorsal view of animal showing the mantle pigmentation pattern, scale bar 10 mm.

B – Ventral view showing foot and mouth, scale bar 10 mm.

f – foot; m – mouth; vc – visceral coil; vm – visceral mass.

3.4.3.3 Reproductive Anatomy

Figure 3.15 shows the reproductive anatomy of *L. natalensis*. The ovotestis (ot) occupies the upper whorls of the body and is embedded in the digestive gland. Externally, it appears as an elongated sac composed of a cluster of irregular lobules or acini that empty into the ovispermiduct. The ovispermiduct (osd) is a convoluted tube pale white in colour, that passes from the ovotestis to the under side of the albumen gland. This duct can be distinguished into three regions. The first has a short and thin proximal segment connecting the atrium of the ovotestis with the second region, a bosselated swelling of the ovispermiduct. This second region termed the seminal vesicles (sv) is characterised by several blind diverticulae. The third region forms the longest part of the ovispermiduct. It narrows into a distal segment that extends from the seminal vesicles and terminates in the carrefour.

The albumen gland (ag) is a large kidney-shaped organ that is slightly concave on the ventral surface and convex on the dorsal surface. This gland obscures the carrefour and the hindmost portions of the oviduct and ovispermiduct. The albumen gland opens laterally into the carrefour from a broad duct. On the same side as the opening of the albumen gland duct, the ovispermiduct divides into the oviduct and the upper portion of the prostate.

The oviduct is a transverse tubular structure that emerges ventrally from the carrefour. It follows a convoluted course from left to right between the albumen and nidamental glands, and is divided into two distinct regions. The region proximal to the albumen gland (od_1) is smaller than the distal region (od_2), which is formed by larger, dilated tubes. Near its distal end, prior to the junction with the nidamental gland, the oviduct opens into the oviducal caecum. The oviducal caecum (odc) is pouch-like, oval in shape and situated under the oviduct. The oviduct then proceeds towards the head, continuing into the nidamental gland.

The nidamental gland (ng) constitutes the largest part of the female genital system. This voluminous gland is oblong to pyriform in shape, widely convex dorsally and flattened ventrally. The outer surface is characterised by a smooth surface with distinct transverse folds that give it a striated appearance. Its ventral aspect is depressed into a medial groove that is occupied by the spermiduct and the prostate.

The spermatheca (sp) varies from slightly elongated to spherical in shape, is yellowish in colour and joined to the vagina by the spermathecal duct (spd). This duct is uniformly thin, about as long as the body of the spermatheca and gradually widens as it reaches the vaginal wall. The term vagina, as employed by different authors, does not always denote exactly the same structure. According to Baker (1928), it indicates that portion of the female duct which is situated distally to the entrance of the spermathecal duct while de Larambergue (1928) and Hubendick (1951) used the term for the slender portion of the female duct distally to the broader nidamental gland. In this study the vagina (va) is considered that part of the female genital system which extends distally from the base of nidamental gland to the female genital pore (Pretorius and van Eeden, 1969).

The spermiduct (sd) emerges from the carrefour beside the distal portion of the oviduct and continues along a depression in the middle of the ventral region of the nidamental gland. On reaching about half the length of the nidamental gland it narrows and then swells again to form the prostate gland.

Externally, the prostate gland (pr) can be distinguished into two regions, the apical region and the basal region. The apical region, continuous with the spermiduct, is a long flattened ribbon-like structure. It is yellowish in colour and possesses a granulated outer wall. The basal region of the prostate gland is bulbous in shape. At the distal end of the prostate gland, an inwardly directed fissure is formed by a folding of its margin.
The vas deferens (vd) emerges from within the distal portion of the prostate gland as a comparatively wide tube which gradually becomes smaller in diameter. The vas deferens interweaves with the surrounding tissue and continues somewhat thicker to the penis that lies within the penial sheath.

The penial sheath (ps) is wider than the vas deferens and is swollen at the proximal end. It is a regularly cylindrical muscular tube of about the same length as the praeputium. The praeputium (pp) is a hollow cylindrical structure of at least twice the width of the penial sheath and characterised by highly muscular walls. This structure tends to lie at an angle to the longitudinal axis of the body and opens directly to the exterior through the male genital pore near the base of the right tentacle. The pigmentation of the praeputium is evenly distributed over the whole organ and stout muscle bands attach the praeputium to the body wall and the columellar muscle.

The extrinsic muscles of the penial complex are usually two main retractors and several smaller retractors and protractors. A retractor muscle arising from the columellar muscle divides into two branches. One of them, the penial sheath retractor (rps), is inserted into the top of the penial sheath and the other, the praeputial retractor (rpp), is inserted into the juncture of the penial sheath with the praeputium. Besides these two main retractors, smaller extrinsic muscles are inserted into the praeputial wall. The protractors of the praeputial wall (ppp) connect the wall of the praeputium to the wall of the head while the opposing retractors (rpp') originate from a branch of the columellar muscle.

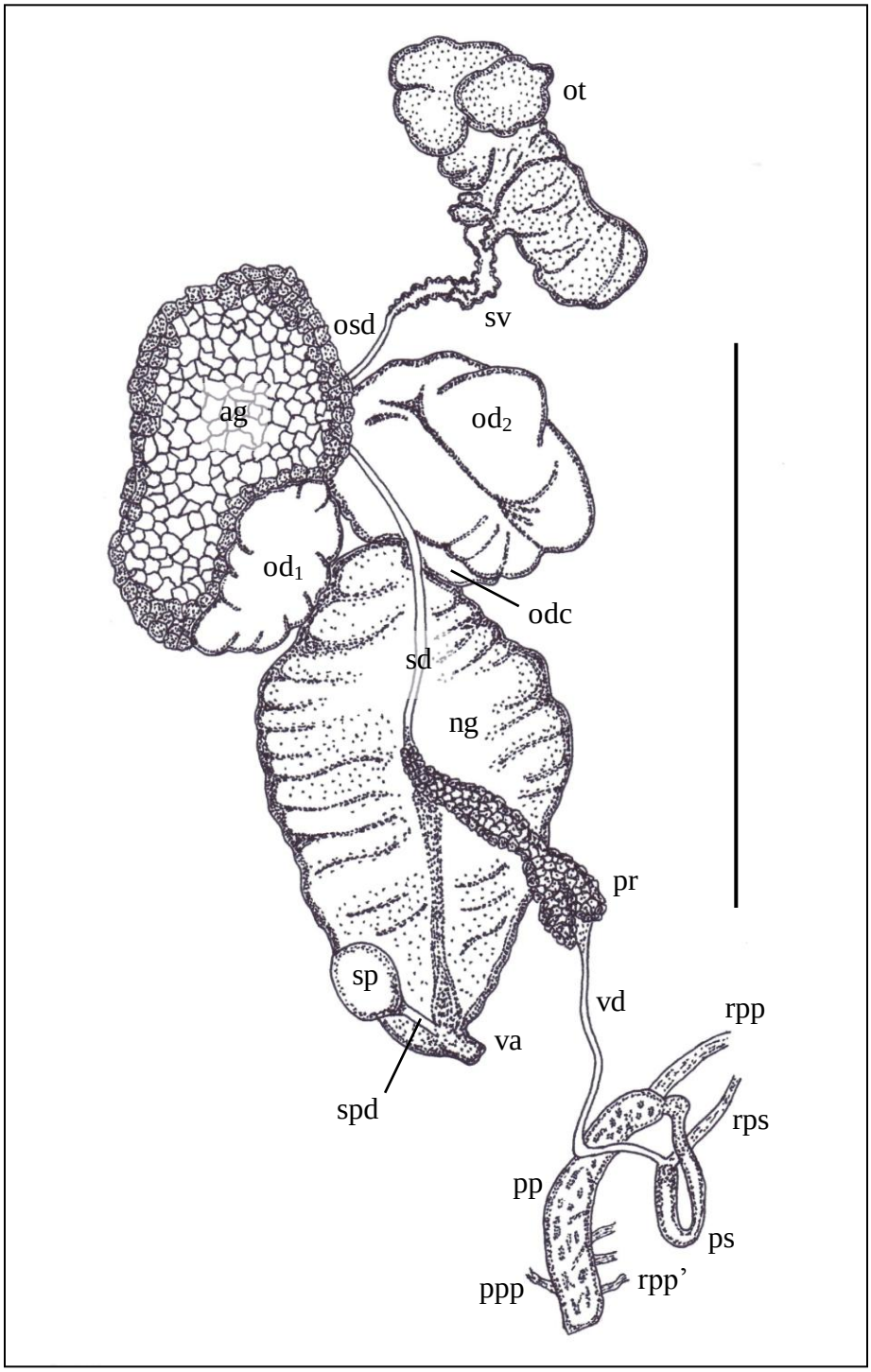


Figure 3.15: Reproductive anatomy of *L. natalensis* (UKZN Pond), scale bar 10 mm.
ag – albumen gland; ng – nidamental gland; od1 – proximal portion of oviduct; od2 – distal portion of oviduct; odc – oviducal caecum; osd – ovispermiduct; ot – ovotestis; pp – praeputium; ppp – protractor muscle of praeputium; pr – prostate; ps – penial sheath; rpp – retractor muscle of praeputium; rpp' – smaller retractor muscle of praeputium; rps – retractor muscle of penial sheath; sd – spermiduct; sp – spermatheca; spd – spermathecal duct; sv – seminal vesicle; va – vagina; vd – vas deferens.

3.5 Discussion

An understanding of the speciation and taxonomy in the Lymnaeidae is often hampered by the considerable morphological plasticity of individuals within the family (Hubendick, 1951; Burch, 1968; Burch and Lindsay, 1973; Arthur, 1982; Lam and Calow, 1988; Evans, 1989; Oviedo *et al*., 1995; Ward *et al*., 1997; Wullscheleger and Jokela, 2002). Despite the shell shape variation observed in *Lymnaea*, the shell characteristics of *L. natalensis* from the UKZN Pond were similar to the descriptions of the species given by other authors (Krauss, 1848; Connolly, 1939; Hubendick, 1951; Mandahl-Barth, 1954; de Azevedo *et al*., 1961; Brown, 1994; Appleton, 1996; Herbert and Warén, 1999).

Anatomical studies of the soft parts of snails have proved useful in the past for identifying and separating species. However, some authors have proposed that anatomical characters are too variable and should be avoided in phylogenetic studies, since they are prone to selective processes and hence are more homoplastic than other characters (Hubendick, 1951; Bargues *et al*., 2001; Remigio, 2002). Other authors advocate that only a small, limited set of anatomical characters are useful in determining species, such as the distal genitalia, prostate and radula teeth (Hubendick, 1951; Walter, 1968).

The dentition of the radula has long been established as providing informative characters for the taxonomy of gastropods (Ponder and Lindberg, 1996). The number, shape, size and cuspidal features of the central, lateral, intermediate and marginal teeth play important roles in classification and have proven to be of value at the generic level (Malek and Cheng, 1974; Kilburn, 1988; Monzon *et al*., 1993).

Lymnaea natalensis sampled from the UKZN Pond had a radula formula of 12: 8-10: 1: 8-10: 12. In his discussion of the dentition of *L. natalensis* (Figure 298: 43), Hubendick (1951), mentioned that the central teeth were asymmetrically unicuspid. This unicuspid (with a single rounded cusp) characteristic of the central tooth was also observed by de Azevedo *et al*. (1961) in material from Mozambique.

In this study, however, *L. natalensis* from the UKZN Pond had a central tooth that was asymmetrically bicuspid, with the smaller cusp located on the left side towards the base of the main cusp. This smaller cusp diminished in size and disappeared after the 10th – 15th transverse row, towards the rear of the radula. This could explain the unicuspid characteristic described by Hubendick (1951) and de Azevedo *et al*. (1961). According to Pretorius and van Eeden (1969), the diminished size and disappearance of the accessory cusp could be attributed to detrition. This was found to be the case in this study, as the accessory cusp was present on teeth that had not yet been used for grazing.

The transition from the tricuspid lateral to multicuspid marginal teeth was abrupt with the 9th and 10th pairs of laterals being the transitional or intermediate teeth (Figure 3.12). In the 9th pair, the ectocone split into two smaller, acute-shaped denticles (see arrow, Figure 3.12). This unique characteristic was noted only by de Azevedo *et al*. (1961): "Intermediate teeth with four cusps, irregular, pointed; the internal two larger and joined halfway, and the external two smaller and isolated."

Mantle pigmentation patterns have been used as a useful diagnostic character in the descriptions of many lymnaeid species. Jackiewicz (1993) reported that these patterns on the mantle showed great diversity, being similar in some species only. From a systematic standpoint it is pertinent to know to what extent these patterns are stable or to what extent they vary within a species. The pigmentation pattern was consistent among all individuals sampled from the UKZN Pond. The intensity of the pigmentation on the mantle varied however among individuals, but the distribution pattern of the spots was distinct and similar to those described by other authors (Hubendick, 1951; de Azevedo *et al*., 1961; Pretorius and van Eeden, 1969).

There were no significant differences between the reproductive anatomy of *L. natalensis* from the UKZN Pond and representative of the species described by other authors (Hubendick, 1951; de Azevedo *et al*., 1961; Pretorius and van Eeden, 1969). Some variations were observed within the present material in the dimensions of the

spermathecal duct, penial sheath and praeputium, but these were attributed to different degrees of contraction and relaxation during preservation.

Hubendick (1951) drew attention to the fact that the different structures of the male reproductive anatomy varied so much in shape and intra-species variation within single species of *Lymnaea*, that this system could not be considered as being of taxonomic importance in the Lymnaeidae. In their study of the male reproductive anatomy of *L. natalensis*, Pretorius and van Eeden (1969) observed that in some specimens the penis was much shorter than its penial sheath (Pretorius and van Eeden, 1969; see Figure 84, page 100), while in other specimens it extended beyond the velum well into the praeputium (Pretorius and van Eeden, 1969; see Figure 85, page 100). These conditions reflected the different degrees of retraction and eversion respectively, of the penis at the time of fixation. Similarly, the praeputium was also subject to varying degrees of contraction and relaxation (Pretorius and van Eeden, 1969).

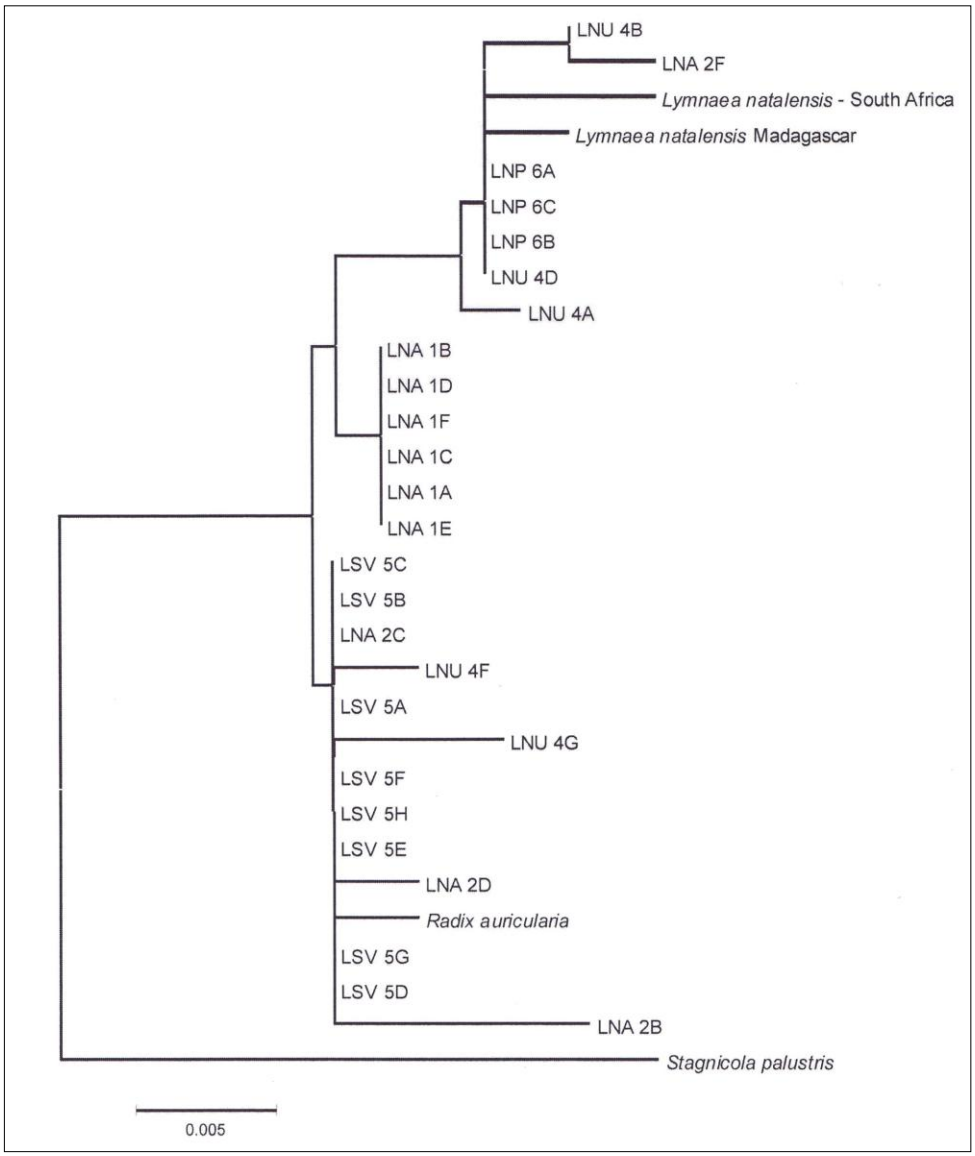
In this study, conchological and anatomical characters were used to identify the snail population from the UKZN Pond as *L. natalensis* Krauss, 1848. Despite some observed variation, the individuals sampled from this study site conformed to accounts made by other authors.

Historically gastropod taxonomy has been based purely on morphological characters, however the large degree of morphological plasticity exhibited by the Lymnaeidae has confused the taxonomy of this group. In the last few decades, the use of molecular techniques in taxonomic studies (Márquez *et al*., 1995; Bargues and Mas-Coma, 1997; Bargues *et al*, 1997; Remigio and Blair, 1997; Stothard *et al*., 2000; Bargues *et al*., 2001; Remigio, 2002; Bargues *et al*., 2003; Puslednik, 2006), often in conjunction with more traditional morphological approaches, has provided increased taxonomic clarity about relationships within molluscan groups. In a study of lymnaeids from the Amatikulu Hatchery and the UKZN Pond, KwaZulu-Natal, South Africa (J. Lamb and K. Pillay, unpubl. data), molecular data were obtained by sequencing three gene regions: two mitochondrial DNA genes (cytochrome oxidase subunit I and 16S rRNA) and one

nuclear DNA gene (18S rRNA). In this molecular study, the cytochrome oxidase subunit I (COI) gene was chosen as it was useful in analysing variation among closely allied taxa (Black *et al*., 1997; Attwood and Johnston, 2001; Attwood *et al*., 2003; Remigio and Herbert, 2003; Staton, 2003; Genner *et al*., 2004). The 16S rRNA region was selected as it had both slow and rapidly evolving regions, allowing for family and genus level delineation (Hillis and Dixon, 1991; Reid *et al*., 1996; Remigio and Blair, 1997; Attwood *et al*., 2003). In addition the slowly evolving 18SrRNA gene was included to resolve any deeper phylogenetic divergences.

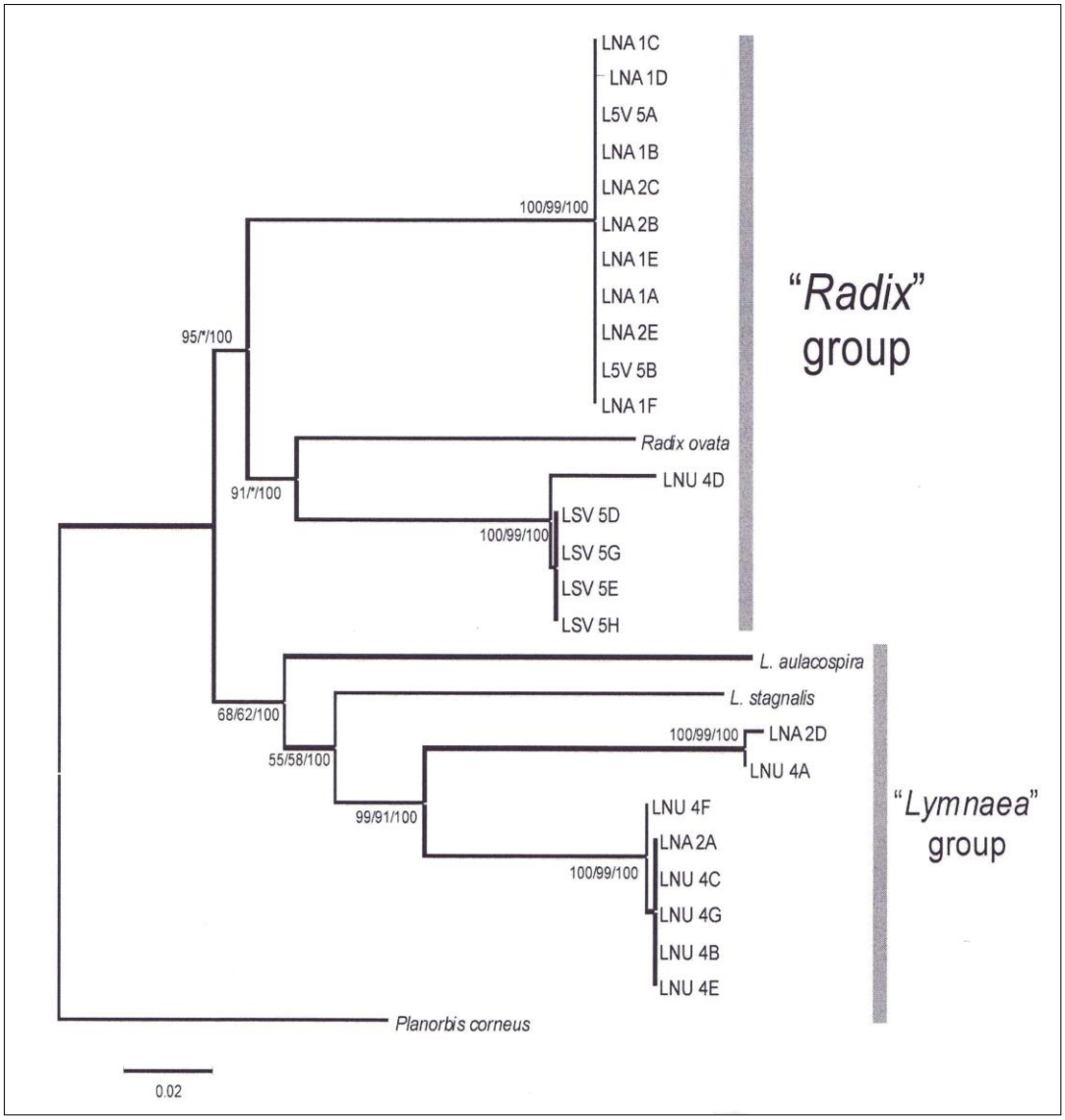
Figures A1.1 - A1.3 in the Appendix to Chapter 3 represent the trees that were constructed using the neighbour-joining method. The results of this comparative molecular study established a clear distinction between the lymnaeids from the UKZN Pond and the Amatikulu Hatchery. The results of the DNA sequencing identified the UKZN Pond population as *Lymnaea natalensis* Krauss, 1848, an indigenous lymnaeid, and the population from the Amatikulu Hatchery as *Radix rubiginosa* (Michelin, 1831), a lymnaeid from southeast Asia.

3.6 Appendix to Chapter 3



(J. Lamb and K. Pillay, unpubl. data)

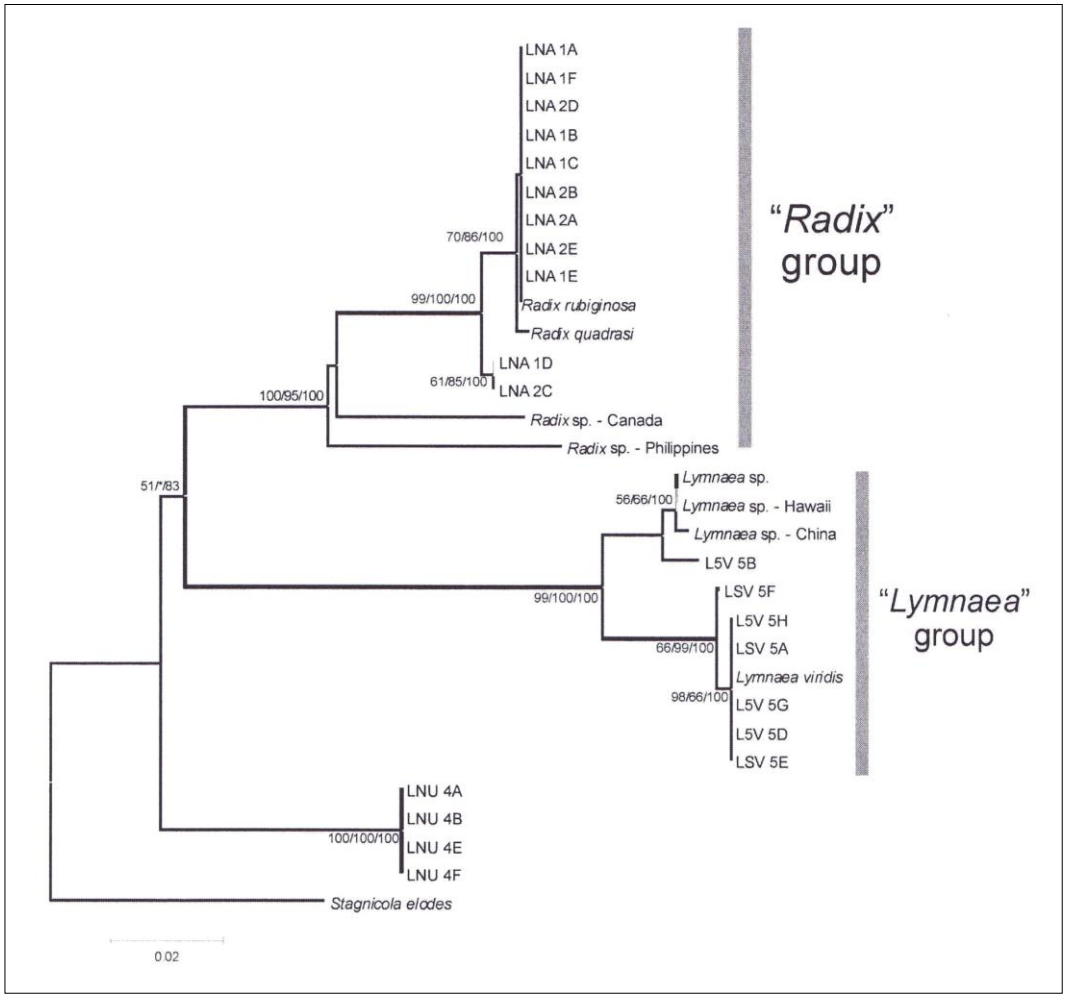
Figure A1.1: Tree based on 389 nucleotides of the 18SrRNA gene from lymnaeid samples, with the outgroup *Stagnicola palustris*. Tree constructed using neighbour-joining method. Bayesian posterior probabilities, neighbour-joining and maximum parsimony bootstrap values were all very weak (<50%) and were thus omitted. The maximum parsimony tree obtained represented a strict consensus of 526 shortest trees. The scale of the branch length of 0.005 represents the number of substitutions per site.
LNA - samples from the Amatikulu Hatchery, Amatikulu, South Africa; LNP - samples from the Nwanetsi River in Mamitwa, Limpopo province, South Africa; LNU - samples from the UKZN Pond, Durban, South Africa; LSV - samples from Hanoi, Vietnam.



(J. Lamb and K. Pillay, unpubl. data)

Figure A1.2: Tree based on 533 nucleotides of the cytochrome oxidase subunit I gene of lymnaeid samples, with the outgroup *Planorbis corneus*. Tree constructed using neighbour-joining method. Numbers on branches represent Bayesian posterior probabilities percentages, neighbour-joining and maximum parsimony bootstrap values, respectively. Only values greater than 50% are shown (* indicates support of < 50% for distance analysis). The maximum parsimony tree obtained represented a strict consensus of 219 shortest trees. The scale of the branch length of 0.002 represents the number of substitutions per site.

LNA - samples from the Amatikulu Hatchery, Amatikulu, South Africa; LNU - samples from the UKZN Pond, Durban, South Africa; LSV - samples from Hanoi, Vietnam.



(J. Lamb and K. Pillay, unpubl. data)

Figure A1.3: Tree based on 360 nucleotides of the 16SrRNA gene of lymnaeid samples, with the outgroup *Stagnicola elodes*. Tree constructed using neighbour-joining methods. Numbers on branches represent Bayesian posterior probabilities percentages, neighbour-joining and maximum parsimony bootstrap values respectively. Only values greater than 50% are shown (* indicates support of <50% for distance analysis). The maximum parsimony tree obtained represented a strict consensus of 210 shortest trees. The scale of the branch length of 0.002 represents the number of substitutions per site.
LNA - samples from the Amatikulu Hatchery, Amatikulu, South Africa; LNU - samples from the UKZN Pond, Durban, South Africa; LSV - samples from Hanoi, Vietnam.

4

Morphological and Anatomical Variation in *Radix rubiginosa* and *Lymnaea natalensis*

4.1 Introduction

The fundamental observation of biology is that morphology and morphological data form the basis of virtually all systematic descriptions (MacLeod, 2002). This is reinforced by the fact that the vast majority of systematic studies begin by grouping organisms on the basis of morphological similarity. Once they are so grouped, relationships amongst the groups are studied, often by careful examination of variation in morphological features, but increasingly often by using these morphologically defined groups as the basis for conducting studies on molecular variation (Jensen, 2003). Hence, morphology remains one of the richest and most reliable sources of information about systematic, evolutionary and ecological relationships (McLellan and Endler, 1998).

Several types of techniques have been devised for the quantitative analysis of shape (Rohlf, 1990; Rohlf and Bookstein, 1990). Most effort has been directed towards those using information about the locations of several points in an image, or landmarks which are measured directly on the specimen (Bookstein, 1989; Marcus, 1990; Rohlf and Bookstein, 1990; Marcus *et al*., 1996; McLellan and Endler, 1998). Various techniques for describing shell morphometrics have been widely used in malacology to show conchological variations. At other times they are used to explore ecological relationships, usually linking morphological variations to a specific set of environmental conditions (Branch and Marsh, 1978; McMahon and Whitehead, 1987; Lam and Calow, 1988; Denny, 2000).

Historically, the shape and sculpture of gastropod shells were considered to be rich sources of taxonomic information in the designation of species and evolutionary relationships (Chiu *et al*., 2002). However, an understanding of the speciation and taxonomy in the pulmonate family Lymnaeidae is hampered by the morphological problems associated with shell shape variation. The problem arises due to the great morphological range of variation within species of the Lymnaeidae (Hubendick, 1951; Burch, 1968; Burch and Lindsay, 1973; Arthur, 1982; Lam and Calow, 1988; Evans, 1989; Oviedo *et al*., 1995; Ward *et al*., 1997; Wullschleger and Jokela, 2002), and this causes real difficulties in studies on species determination.

Anatomical studies on lymnaeids have proved useful in the past for the identification of species, however, similarities among some species and variation within a species have combined to create a number of unusual taxonomic problems (Inaba, 1969). Despite several approaches being used to evaluate the taxonomy and relationships within the Lymnaeidae, consensus has not yet been reached because of the poor systematic resolution of the information and differing interpretations of and emphases on morphological and anatomical features.

The family Lymnaeidae is also of great parasitological importance, due to its capacity to act as intermediate hosts of numerous trematode parasites (Brown, 1978; Bargues and Mas-Coma, 1997). These snail-transmitted helminth diseases, in particular fascioliasis (which is restricted to the family Lymnaeidae), have serious economic and public health impacts (Rim *et al*., 1994; Marquardt *et al*., 2000; Remigio, 2002). Appropriate control strategies for fascioliasis cannot be effective if the specific identity of the snail intermediate host implicated in transmission cannot be readily determined (Pfenniger *et al*., 2006).

Additionally, the growing interest in biodiversity and its evaluation has highlighted the importance of reliable taxonomic identification at the species level (Ronquist and Gärdenfors, 2003; De Meeus *et al*., 2003; Hurtrez-Boussès *et al*., 2005). Also, the presence or absence of freshwater snails (macroinvertebrates) is used in water quality

assessments (Pfenniger *et al*., 2006). Such biological monitoring or biomonitoring is an essential element needed to assess the environmental health of aquatic ecosystem and to protect biological resources (Karr and Chu, 1997). Biomonitoring has gained increasing value since it has been realised that such an assessment can be of critical importance in the understanding of environmental threats and also in the prediction of water quality.

In South Africa this was evident in the formal design and implementation of the River Health Programme (RHP). The South African Scoring System (SASS) is a rapid biomonitoring index using macroinvertebrate families as indicators (Dickens and Graham, 2002) to assess water quality and river health (Dallas, 1997; Vos *et al*., 2002). The prime disadvantage of using invertebrates is the fact that they are difficult to identify to species level, as indicated by the previously used Saprobien Index (Kolkwitz and Marsson, 1909) and the Chutter Biotic Index (Chutter, 1970).

According to Herricks and Schaeffer (1985) organisms should be easily identifiable in order for such a technique to be considered as a valid biomonitoring approach. In addition, the majority of taxonomic identifications in these rapid bioassessments are not made by systematic specialists of the representative taxa, resulting in widespread misidentifications (Gotelli, 2004; Hurtrez-Boussès *et al*., 2005).

This difficulty is complicated by the fact that the aquaculture industry and aquarium trade (which are implicated in the introduction of several exotic species into South Africa) have not been especially concerned with the species-level identification of snails that are accidentally introduced (Rawlings *et al*., 2007). These growing concerns highlight the need for reliable species identifications and a better inspection system at points of entry. While identification for SASS surveys need only be to the family level, the results of such biomonitoring assessments also include important findings, e.g. invasive species.

In this study, the morphometric approach was used to provide an assessment of the suitability and efficacy of conchological characters to help distinguish shell variation patterns within and between populations of two species of the Lymnaeidae, the

introduced *Radix rubiginosa* (Michelin, 1831) and the indigenous *Lymnaea natalensis* Krauss, 1848. This includes an examination of the radula, the reproductive anatomy and the pigmentation patterns on the mantle. These characters were subsequently used as criteria to easily recognise and separate *R. rubiginosa* from *L. natalensis* by non-systematic specialists. It should be noted however that while shell characters and pigmentation patterns on the mantle will be useful to SASS practitioners, characteristics of the radula and reproductive anatomy will not since they both require careful dissection in a laboratory.

4.2 Methodology

4.2.1 The Malacological Study Sites

Three study sites (Figure 4.1) were selected namely; Amatikulu Prawn and Fish Hatchery (Amatikulu), University of KwaZulu-Natal (UKZN) Pond (Cato Manor, Durban) and Greyville Race Course Pond (Greyville, Durban). All these waterbodies were artificial but were chosen because of the depauperate nature of the malacofauna in natural waterbodies within the Durban Metropolitan Area.

Figure 4.1: Map of KwaZulu-Natal showing the study sites selected for sampling: (1) – Amatikulu Prawn and Fish Hatchery (Amatikulu); (2) – UKZN Pond (Cato Manor, Durban); (3) – Greyville Race Course Pond (Greyville, Durban).

As noted in Chapter 3, the lymnaeid population from the Amatikulu site was identified using molecular techniques as *R. rubiginosa* while the UKZN Pond population was identified as *L. natalensis*. In addition, the lymnaeid population from the Greyville Pond was also selected as they conformed morphologically to *L. natalensis* from the UKZN Pond.

4.2.1.1 Amatikulu Prawn and Fish Hatchery (Amatikulu)

The hatchery (S 29^{o} 04' 18.8" E 31^{o} 38' 54.0", altitude 12m) is situated in a low lying coastal area on the northern bank of the Amatikulu River, approximately 140 km north of Durban (Figure 4.2A). It is an aquaculture facility supplying tropical fish and prawns to local and overseas markets. Aquatic plants are also cultivated and supplied to the aquarium trade both in South Africa and abroad.

A total of 52 polytunnels are located on the hatchery (Figure 4.2A), with only 14 utilised constantly. Each of these tunnels housed 28 concrete-lined holding tanks (4.0 x 2.5 m, water depth of approximately 1.1 m), used primarily for the breeding and growing of tropical fish (Figure 4.2B). Many of these tanks had blooms of filamentous algae, with *R. rubiginosa* present in high densities on the inner walls and in drains leading from each tank to an external reservoir.

This site had a warm climate, with high summer and moderate winter temperatures. The summer mean air temperature was 23^{o}C with the winter mean of 18^{o}C. The temperature range was 16-37^{o}C with a mean annual rainfall of 1500 mm. The water chemistry parameters at the Amatikulu Hatchery were measured by the author at a depth of 30 cm, using a YSI 6920 multi-probe data logger. Selected chemical parameters of tank water are summarised in Table 4.1.

Figure 4.2 A, B: The Amatikulu Prawn and Fish Hatchery (Amatikulu).

A - Aerial view of Hatchery (Courtesy of G. Upfold).

B - Inside view of tanks in a typical polytunnel.

Table 4.1: Selected water chemistry parameters for the three study sites. All values measured are indicated as mean (± standard deviation), n = 35.

Study Site	pH	Conductivity (mS/cm)	Dissolved Oxygen (mg/L)
Amatikulu	8.26 (± 0.09)	1.31 (± 0.08)	8.20 (± 0.48)
UKZN Pond	8.13 (± 0.12)	0.57 (± 0.01)	8.08 (± 0.81)
Greyville Pond	7.72 (± 0.17)	0.93 (± 0.01)	5.48 (± 0.61)

4.2.1.2 UKZN Pond (Cato Manor, Durban)

To avoid repetition the reader is referred to Chapter 3, Section 3.2.1.

4.2.1.3 Greyville Race Course (Greyville, Durban)

This ornamental pond (S 29^{o} 50’ 35.4” E 31^{o} 00’ 53.1”, altitude 16 m) is located approximately 5.5 km from the UKZN Pond on a golf course within the grounds of Greyville Race Course (Figure 4.3). It supports floating mats of *Nymphaea nouchali*, *Pistia stratiotes* and filamentous algae so that about 30% of the water surface was generally covered. The pond had gently sloping banks with the approximate water depth recorded in winter and summer of 1.8 m and 3.4 m respectively. The temperature and rainfall data for the Greyville race course Pond were similar to the UKZN Pond. The water chemistry parameters at the Greyville Pond were measured by the author at a depth of 30 cm, using a YSI 6920 multi-probe data logger. A summary of the selected water chemistry parameters is presented in Table 4.1.

Figure 4.3: The Greyville Pond.

4.2.2 Snail species occurring in the study areas

A total of nine freshwater snail species belonging to four families were identified from the three study sites (Table 4.2). Three of the nine were prosobranchs and six were pulmonates. *Physa acuta* Draparnaud, 1805 was the most widespread species occurring in all three study sites while *Lymnaea natalensis* Krauss, 1848, *Helisoma duryi* Wetherby, 1879 and *Melanoides tuberculata* Müller, 1774 occurred in two study sites. The remaining species were each present in only one of the sites.

Table 4.2: Snail species identified from the three study sites. (+) indicates presence; (-) indicates absence.

Species	**Study Site**		
	Amatikulu Hatchery	**UKZN Pond**	**Greyville Pond**
Prosobranchs			
Pomacea diffusa	+	-	-
Melanoides tuberculata	+	-	+
Tarebia granifera	+	-	-
Pulmonata			
Lymnaea natalensis	-	+	+
Radix rubiginosa	+	-	-
Gyraulus chinensis	+	-	-
Helisoma duryi	+	-	+
Physa acuta	+	+	+
Aplexa marmorata	-	-	+
Total species	7	2	5
Indigenous species	1	1	2
Introduced species	6	1	3

Compared to the other sites, the Amatikulu site had a greater number of gastropod species (Table 4.2), but six of these seven were introduced. Also, the Greyville Pond was the only site to record the presence of both the physids, *Aplexa marmorata* Guilding, 1828 and *Physa acuta*. In addition, it was noted that the North American lymnaeid, *Lymnaea columella* Say, 1817 was absent from all three sites.

4.2.3 Vegetation types present in the study areas

Seven macrophyte species were observed (Table 4.3).

Table 4.3: Aquatic plant species present in the three study sites. (+) indicates presence; (-) indicates absence.

Species	Study Site		
	Amatikulu Hatchery	**UKZN Pond**	**Greyville Pond**
Marsilea sp.	-	+	-
Cyperus immensus	-	+	-
Cyperus papyrus	-	+	+
Cyperus textilis	-	+	+
Typha capensis	-	+	+
Nymphaea nouchali	+	+	+
Pistia stratiotes	+	+	+

4.2.4 Shell Morphology and Morphometrics

4.2.4.1 Characters selected for Shell Morphometric Analysis

Morphometric shell characters were chosen based on their representation in previous studies on shell morphometrics and their ability to provide a comprehensive characterisation of shell morphology (Pace, 1973; Brandt, 1974; Liu *et al*., 1979, Burch, 1980; Lam and Calow, 1988; Chiu *et al*., 2002). To assess the morphology of *R. rubiginosa* and *L. natalensis* shells (Figure 4.4) in a repeatable, objective fashion, a traditional morphometric approach was used.

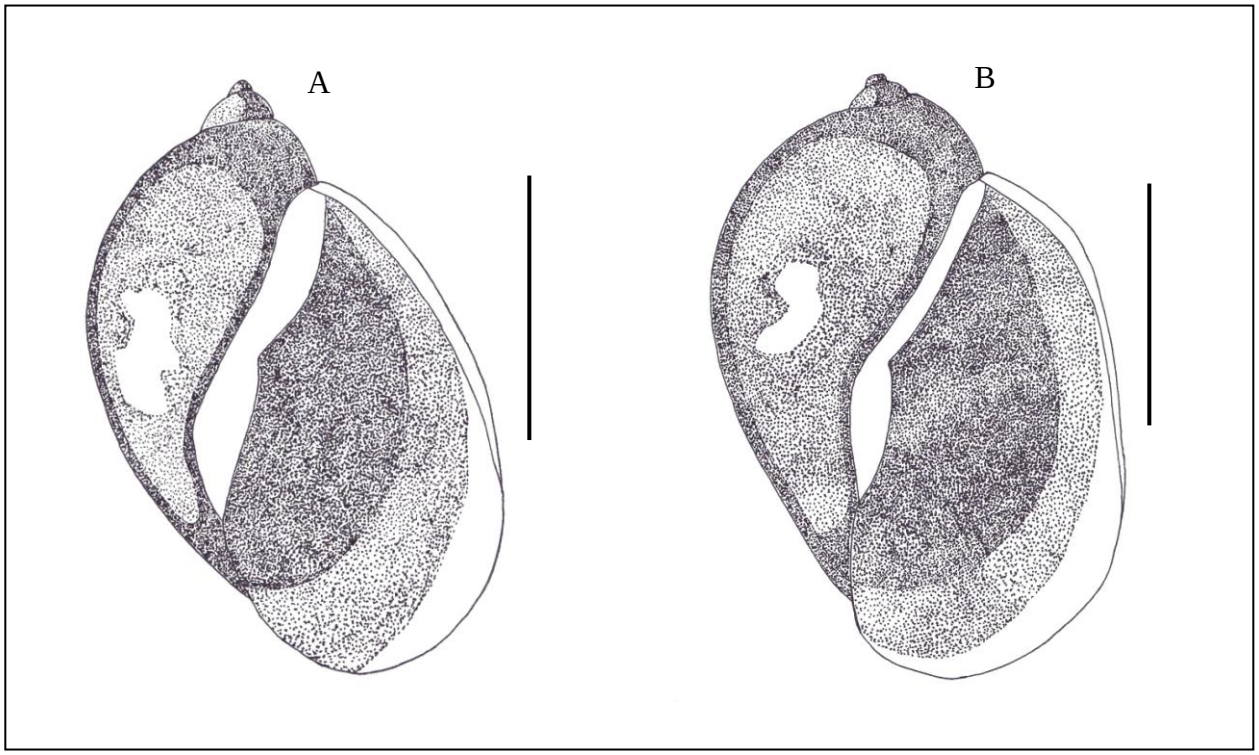


Figure 4.4: Representatives of the family Lymnaeidae, identified from the study areas.
A – *Lymnaea natalensis* Krauss, 1848, scale bar 10 mm
B – *Radix rubiginosa* (Michelin, 1831), scale bar 10 mm

Shells of two size classes were selected (shell length < 10 mm and shell length ≥ 10 mm). An initial suite of six characters for each specimen was measured and used to describe the within-group and between-group variability for the two lymnaeid species. Shell characters were measured to the nearest 0.01 mm using a graticuled eyepiece on a Leica stereomicroscope and a 20 cm vernier caliper. To avoid the effects of bilateral asymmetry, bilateral characters were measured on the right hand side of the shell. Measurements were taken from reference points as denoted in Figure 4.5 for the six characters: shell length (SL), shell width (SW), aperture length (AL), aperture width (AW), length of last body whorl (LBW) and spire height (SPH).

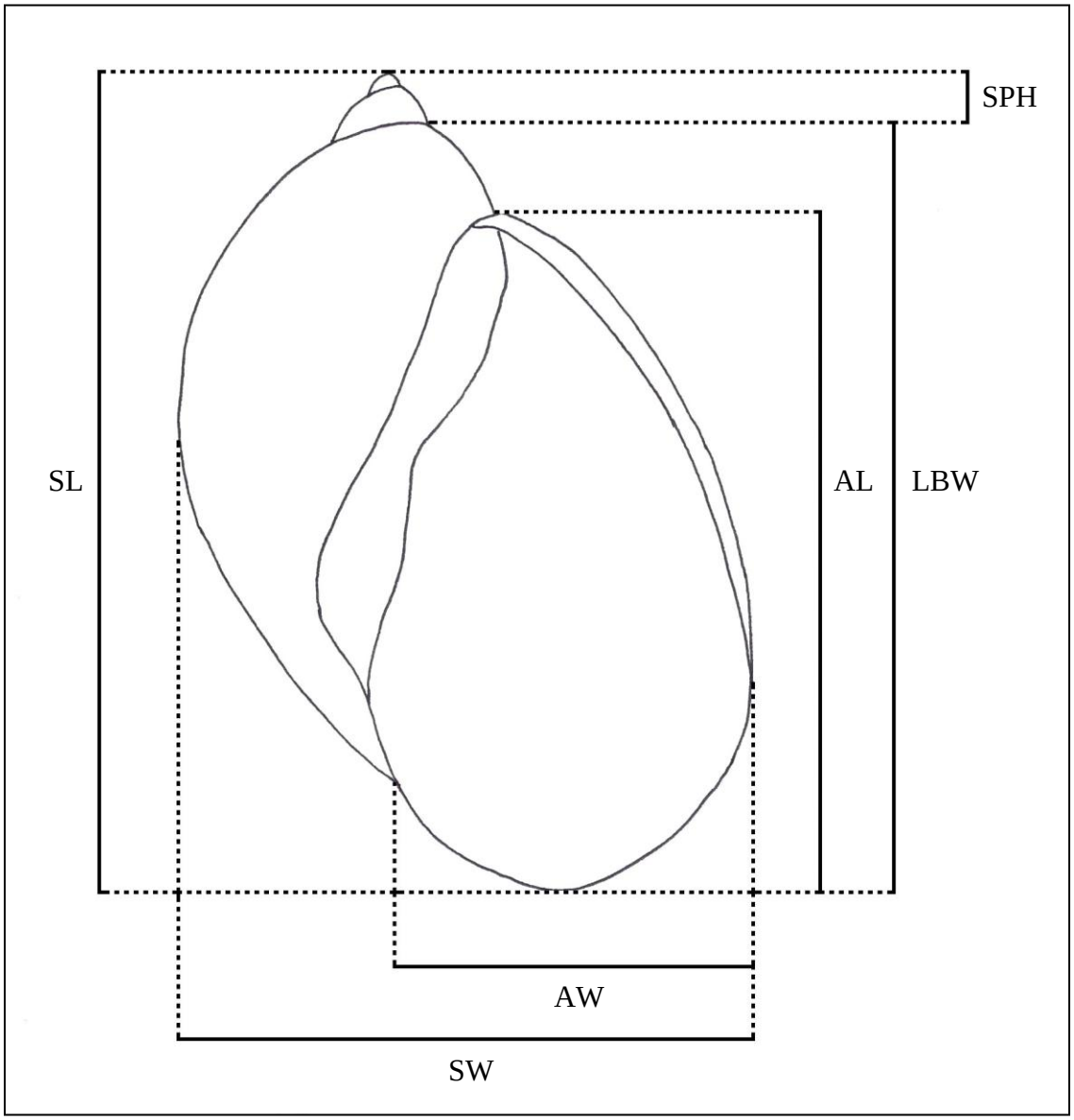


Figure 4.5: Schematic drawing of the six shell characters used for the traditional morphometric approach. AL – aperture length; AW – aperture width; LBW – length of last body whorl; SL – shell length; SPH – spire height; SW – shell width.

4.2.4.2 Statistical Morphometric Analyses

(a) Error Measurements

Within any study utilising morphometric data, variation in characters among populations may arise from heritable components and non-heritable random variation components

that often confound true taxonomic and evolutionary relationships (Pankakoski *et al*., 1987; Richards, 2007). Measurement error describes that component of non-heritable morphological variation arising from variability of repeated measurements of a particular character taken on the same individual, relative to its variability among individuals in a particular group (Bailey and Byrnes, 1990).

According to Bailey and Byrnes (1990), repeatability of measurements of a particular character varies depending on the level of precision relative to the total variability among individuals in a particular group. Precision, in this sense, is described as the 'closeness' of repeated character measurements to each other and is considered the converse of measurement error (Taylor *et al*., 1990; Zar, 1999). Furthermore, a term often incorrectly used in synonymy with precision is accuracy, which describes the 'closeness' of a measurement to the actual value of the character measured (Zar, 1999).

To assess the associated error levels, 30 individuals with a complete suite of shell characters were randomly chosen, five individuals from each of the three study sites and the two size classes. As a test of measurement error, the six shell characters were measured once a day for three days. The series of three measurements were thus independent of each other and allowed the measurement error to be assessed.

Percentage measurement error (%ME) is the within-individual error relative to the between-individual error, and was calculated using methods outlined in Pankakoski *et al*. (1987). Mean within-individual coefficients of variation were calculated for each individual and character, using arithmetic means and standard deviations. According to Pankakoski *et al*. (1987), the effects of differences in character means between the 30 individuals were excluded by calculating separate coefficients of variation for each individual, for each character measurement (CVc). The overall within-individual error (CV_{WI}) corresponded to the means of the 30 individual CVc measurements. The overall between-individual error (CV_{BI}) was calculated from the total variability of each of the three replicates of character measurements for the 30 individuals.

The primary objective, using repeated measures of the shell characters, was to assess the associated error levels. This was done to ensure that only characters with low error levels were included in the subsequent analyses.

(b) Principal Component Analysis (PCA)

Since shell character measurements are highly correlated, the morphometric data were analysed by PCA (Statistica for Windows Release 5.1, StatSoft Inc., 1996). The PCA maintained the morphological distances among species, yet removed the redundancy of highly correlated shell characteristics. Therefore the primary objective of using PCA was to summarise the variation from a correlated multi-attribute to a set of uncorrelated components.

The total suite of six shell character measurements was pre-examined for homogeneity and transformed to natural logarithms to enhance normality and equalize variances. The proportion of the variance subsumed by each component was then expressed as an eigenvalue. This provided a series of loadings showing the correlation of the measured characters with the principal components (Blackith and Reyment, 1971; Kuris and Brody, 1976; Wellington and Kuris, 1983; Rohlf, 1996). The eigenvalues were then used to create a multivariate plot using forward stepwise discriminant function analysis.

(c) Discriminant Functions Analysis (DFA)

A discriminant functions analysis is an inferential analysis that maximises between-group variability and minimises within-group variability, thus allowing for a high percentage of correct groupings for conchologically similar species (Flury and Riedwyl, 1983; Norušis, 1990; Armbruster, 1995). The significance of the overall discriminatory power of the analysis was tested using the Wilks' Lambda standard statistic, while the standardised coefficients were studied to examine each shell character's contribution in the discriminant function.

The analysis was performed using Statistica for Windows Release 5.1, Statsoft Inc., 1996. All assumptions required for the DFA to be performed were met. These were that no two morphometric characters should be highly correlated and that there was no significant deviation from normality.

4.2.5 Anatomical Morphology

To avoid repetition the reader is referred to Chapter 3 as indicated below.

4.2.5.1 Radula

See Section 3.2.3.1 of Chapter 3.

4.2.5.2 Mantle pigmentation patterns

See Section 3.2.3.2 of Chapter 3.

4.2.5.3 Reproductive Anatomy

See Section 3.2.3.3 of Chapter 3.

4.3 Results

4.3.1 Shell Morphology and Morphometrics

4.3.1.1 Shell Description

(a) *Radix rubiginosa* (Amatikulu Prawn and Fish Hatchery)

The shell is broadly ovate and dextral (Figure 4.6). *Radix rubiginosa* has a hard, relatively thick shell (172.60 μm ± 14.10, n = 15 see Figure 3.3 of Chapter 3) with moderate to prominent sutures. The spire is variable and elongate and its contours merge gradually into those of the body whorl. The colour of the shell varies from glossy pale yellow to dark brown. The body whorl is markedly swollen and forms the greatest portion of the shell.

Figure 4.6: Shells of *R. rubiginosa* (Amatikulu) showing the variation found in the population, scale bar 10 mm.

The aperture is relatively large, wide and semi-ovate to ear-like. The peristome is thick with the lower lip of the columella that merges with the peristome curved back or

deflected slightly. The outer lip of the aperture is often expanded; the inner lip is more or less elevated and continuous across the body. The upper region of the peristome is usually relatively straight and directed outwards; below this region the peristome runs almost parallel to the main axis of the shell. The umbilicus is completely closed. The columellar margin is narrowly reflexed and generally twisted or obliquely folded where it joins the parietal wall; the columellar axis is twisted but not gyrate. The sculpture consists of growth lines only; these are distinct on the body whorl, but less distinct on the preceding whorls.

(b) *Lymnaea natalensis* (UKZN Pond)

The shell is succineiform (Figure 4.7), dextral and thin (110.60 μm ± 22.14, n = 15 see Figure 3.3 of Chapter 3). The colour of the shell varies from glossy, pale yellowish, brownish to dark brownish. There is an elongate, tapering spire with an acute apex. These characteristics of the spire however, were variable. There are generally four tightly coiled and convex whorls that are separated by well-impressed and constricted sutures. The body whorl is markedly swollen and forms the greatest portion of the shell.

Figure 4.7: Shells of *L. natalensis* (UKZN Pond) showing the variation found in the population, scale bar 10 mm.

The aperture is large and ovate, with a fold in the middle part of the parietal wall on which a thin white callus can be observed. The base of the aperture joins the columella in a broadly rounded curve. The peristome is thin and sharp. The outer lip of the aperture is generally evenly rounded; inner lip is closely appressed to the parietal wall. The umbilicus is completely closed by the expanded and reflected inner lip. The columella is short, straight and attenuate at the base; the columellar axis is generally gyrate or twisted. The sculpture consists of growth lines only; these are distinct on the body whorl, but less prominent on the preceding whorls.

(c) *Lymnaea natalensis* (Greyville Pond)

The shell morphology *L. natalensis* from the Greyville Pond (Figure 4.8) was similar to that described for *L. natalensis* from the UKZN Pond. The shell was however thinner (102.89 μm ± 6.21, n = 15 see Figure 3.3 of Chapter 3) than the UKZN Pond *L. natalensis* population.

Figure 4.8: Shells of *L. natalensis* (Greyville Pond) showing the variation found in the population, scale bar 10 mm.

4.3.1.2 Error Measurements

Table 4.4 provides some of the descriptive statistics and values for within-individual and between-individual variability for each shell character in 30 randomly selected specimens, i.e. both size classes combined. The percentage measurement error (%ME), arranged in increasing order of magnitude, ranged from 0.29% to 3.03%. Shell length (SL) had the lowest %ME (0.29%), while aperture width (AW) had the highest (3.03%).

Table 4.4: Descriptive statistics for the six shell characters (n = 30), arranged in order of increasing percentage measurement error (%ME). CV_{WI} = overall within-individual error and CV_{BI} = overall between-individual error. Minimum (min), maximum (max) and mean values are provided for each character. To assess the associated error levels, 30 individuals with a complete suite of shell characters were randomly chosen, five individuals from each of the three study sites and the two size classes.

Character	**Min Value (mm)**	**Max Value (mm)**	**Mean (mm)**	**CV_{WI}**	**CV_{BI}**	**%ME**
Shell Length (SL)	4.39	25.30	12.01	0.15	53.50	0.29
Length of Last Body Whorl (LBW)	3.89	22.21	10.69	0.26	53.21	0.48
Shell Width (SW)	2.00	14.20	6.25	0.49	56.30	0.87
Aperture Length (AL)	3.00	18.93	8.72	0.88	54.97	1.61
Spire Height (SPH)	0.35	3.24	1.32	1.07	59.02	1.81
Aperture Width (AW)	1.50	10.00	4.60	1.60	52.90	3.03

Shell characters with high %ME were often associated with high within-individual variability and could not be accurately measured. Aperture length (AL) and aperture width (AW) displayed %ME values of 1.61% and 3.03% respectively. These high %ME values were a result of the difficulty in recording such measurements accurately, the fragility of the aperture and the inability to locate definable and consistent endpoints across the individuals examined.

Spire height (SPH) had a relatively high %ME (1.81%), even though it was defined using two unambiguous points on the shell. This was probably because the three-dimensional

curvature of the shell made it more difficult than expected to get an accurate measurement of this height each time.

Following Taylor *et al*. (1990) characters with %ME greater than 10% were considered unreliable and should be discarded from the data set. No characters displayed %ME greater than 10%, hence the full suite of six characters was used in further analyses of the shells (Table 4.4).

The consequences of high %ME are important. In univariate analyses, statistical tests of differences among groups will be more conservative for those measurement variables with a substantial %ME. Since covariances between measurement variables will also be affected by the high %ME, the correct biological interpretation of discriminant or principal component axes would be difficult (Bailey and Byrnes, 1990).

Between-individual coefficients of variation were uniformly high, reflecting the large size range of snails in the randomly selected sample of 30 individuals (Table 4.4). Within-individual coefficients of variation were low, ranging from 0.15 to 1.60, for shell length and aperture width respectively.

4.3.1.3 Normality, Skewness and Kurtosis

Results of the basic statistics and tests for normality, skewness and kurtosis for the shell characters of size classes 1 and 2 are presented in Tables 4.5 and 4.6 respectively. Shell characters were skewed or kurtotic if data values exceeded twice the value of the standard error of g_1 (skewness) or g_2 (kurtosis).

In size class 1 (shell length < 10 mm), standard error levels for g_1 were 0.264 and 0.523 for g_2. Characters with g_1 values greater than 0.528 were skewed and characters with g_2 values greater than 1.046 were kurtotic. All characters in the data set were therefore normally distributed ($p > 0.05$), non-skewed and non-kurtotic (Zar, 1999).

Table 4.5: Basic statistics (arithmetic mean and standard deviation) for the six shell characters of size class 1 (shell length < 10 mm) from the three study sites (n = 100). The results of the normality (Kolmogorov-Smirnov Test), skewness (g_1) and kurtosis (g_2) tests are also given.

Character	**Mean (mm)**	**SD**	**KS-Test**	**g_1**	**g_2**
Shell Length (SL)	5.77	2.28	0.546	-0.241	-0.602
Shell Width (SW)	2.92	1.16	0.464	0.021	-0.518
Aperture Length (AL)	4.09	1.62	0.576	-0.149	-0.384
Aperture Width (AW)	2.24	0.90	0.814	0.141	-0.256
Length of Last Body Whorl (LBW)	5.16	1.99	0.873	-0.252	-0.549
Spire Height (SPH)	0.61	0.33	0.844	-0.229	-0.566

In size class 2 (shell length ≥ 10 mm), standard error levels for g_1 were 0.204 and 0.406 for g_2. Characters with g_1 values greater than 0.408 were skewed and characters with g_2 values greater than 0.812 were kurtotic. All characters in the data set were normally distributed ($p > 0.05$), non-skewed and non-kurtotic, except spire height. Spire height was not normally distributed and skewed to the right ($g_1 = 0.899$).

Table 4.6: Basic statistics (arithmetic mean and standard deviation) for the six shell characters of size class 2 (shell length ≥ 10 mm) from the three study sites (n = 100). The results of the normality (Kolmogorov-Smirnov Test), skewness (g_1) and kurtosis (g_2) tests are also given.

Character	**Mean (mm)**	**SD**	**KS-Test**	**g_1**	**g_2**
Shell Length (SL)	16.06	3.19	0.731	0.250	0.133
Shell Width (SW)	8.47	1.69	0.822	0.283	0.330
Aperture Length (AL)	11.70	2.26	0.592	0.074	0.409
Aperture Width (AW)	6.28	1.17	0.571	0.304	0.531
Length of Last Body Whorl (LBW)	14.13	2.71	0.913	0.165	0.322
Spire Height (SPH)	1.93	0.62	0.005	0.899	0.386

4.3.1.4 Size Class 1 (shell length < 10 mm)

(a) Principal Component Analysis

Six principal component loading values (correlation coefficients) were derived from the six shell characters for size class 1 (Table 4.7). Principal component 1 described 97.85% of the variance and yielded high loading values for shell length, length of last body whorl, aperture length and shell width (Table 4.7). This suggests that PC1 is a measure of size.

The remaining five principal components (PC2, PC3, PC4, PC5 and PC6) accounted collectively for the remaining 2.15% of the variance. Spire height showed the highest loading value for PC2. Aperture width loaded the highest for PC3, shell width for PC4, aperture length for PC5 and shell length for PC6.

Table 4.7: Component loadings (correlation coefficients) of shell morphological characters for *R. rubiginosa* and *L. natalensis* from size class 1 (shell length < 10 mm). The component loadings were derived from principal component analysis of the six shell characters after natural logarithm transformation. Values with the highest component loadings are in bold.

Variable	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
Log_{10}SL	**-0.998214**	0.015822	-0.050174	0.002547	0.027680	**0.005256**
Log_{10}SW	**-0.992021**	-0.004263	0.090009	**0.088111**	-0.003445	-0.000118
Log_{10}AL	**-0.992994**	0.076177	-0.077374	0.003415	**-0.046502**	0.000234
Log_{10}AW	-0.987409	0.097121	**0.101994**	-0.072020	-0.001307	0.000024
Log_{10}LBW	**-0.996042**	0.057504	-0.060752	0.004381	0.029336	-0.004744
Log_{10}SPH	-0.968282	**-0.248256**	-0.002656	-0.027463	-0.006162	-0.000681
Cumulative %	97.8536	99.1943	99.7061	99.9351	99.9992	100.0000

(b) Discriminant Function Analysis

The DFA extracted two highly significant functions (Wilks' Lambda = 0.14707, F = 19.559, *d.f.* = 12, 146, $p < 0.00001$). Functions 1 and 2 explained 85.15% and 14.85% of the total morphometric variation respectively. Within the first function, principal component 1 was an important parameter of discrimination (Table 4.8). The shell characters contributing the highest loading values for principal component 1 (see Table 4.7) were shell length (0.998214), length of last body whorl (0.996042), aperture length (0.992994) and shell width (0.992994). These shell characters were identified as those having the longest dimensions of the shell. Function 1 was therefore interpreted as representing the overall size component.

Table 4.8: Standardised canonical discriminant function coefficients of principal component loadings for *R. rubiginosa* and *L. natalensis* from size class 1 (shell length < 10 mm). Only the results of those parameters that contributed significantly to the DFA model are shown.

Variable	**Function 1**	**Function 2**	**Wilks' λ**	**F to remove**	***p***
PC 3	-0.827665	**-0.719902**	0.326239	44.46592	<0.00001
PC 1	**0.916043**	-0.605516	0.330573	45.54144	<0.00001
PC 4	-0.826474	0.029758	0.237058	22.33290	<0.00001
PC 2	0.773824	-0.099656	0.224934	19.32408	<0.00001
PC 5	-0.536413	0.079098	0.181850	8.63142	<0.00043
Eigenvalue	3.310254	0.577503			
Cumulative %	85.15	100.00			
Wilks' λ	0.147071	0.633913			
χ^2	144.7214	34.4162			
d.f.	12	5			
p	<0.00001	<0.00001			

The remaining variance must then be attributed to factors other than size, i.e. aspects of shape. Principal component 3 (PC3) contributed the most to Function 2 (see Table 4.8). Aperture width contributed the highest character loadings on PC3 (see Table 4.7). Function 2 therefore represented a part of the overall shape component.

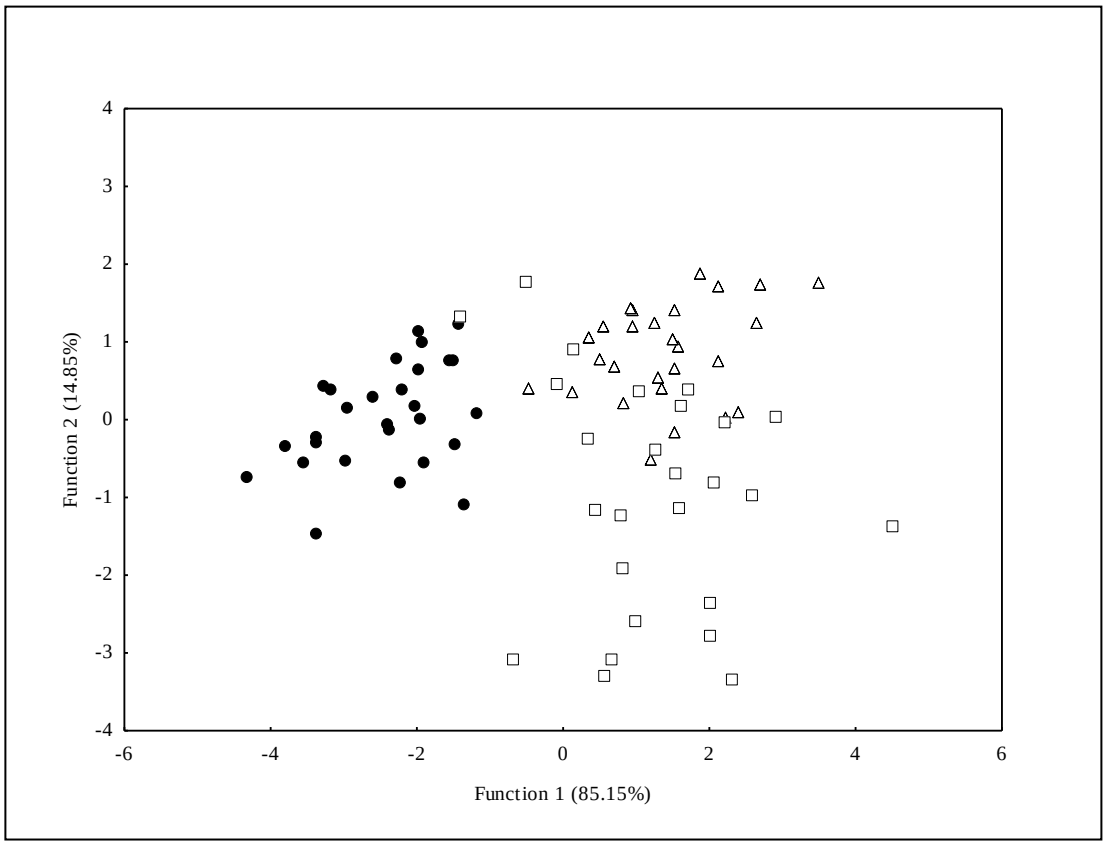


Figure 4.9: Plot of canonical scores defined by functions 1 and 2 from forward stepwise DFA of shell morphological characters for size class 1 (shell length < 10 mm). *Radix rubiginosa* from the Amatikulu site is represented by the closed circles (●). *Lymnaea natalensis* from the UKZN Pond and Greyville Pond are indicated by the open triangles (△) and open squares (□) respectively.

The plot of canonical scores Figure 4.9 separated the lymnaeids into two main distinct clusters. *Lymnaea natalensis* from the UKZN Pond and Greyville Pond were separated from *R. rubiginosa* along function 1. This axis ordinated the shells in a gradient from larger, more broadly ovate and larger body whorl characteristics (negative scores), to smaller, elongated shells with smaller body whorl characteristics (positive scores).

Function 2 ordinated the shells in a gradient from those with a larger, wider aperture (positive scores) to a narrower aperture (negative scores). Aperture width was a highly discriminant parameter for *R. rubiginosa* and *L. natalensis* (UKZN Pond), grouping them closely together. This shell character however, displayed considerable variation for the Greyville Pond *L. natalensis* population, as indicated by the range of both positive and negative scores along function 2 of the canonical plot (Figure 4.9).

4.3.1.5 Size Class 2 (shell length ≥ 10 mm)

(a) Principal Component Analysis

Six principal component loading values (correlation coefficients) were derived from the six shell characters for size class 2 (Table 4.9). Principal component 1 described 90.24% of the variance and yielded high loading values for shell length and length of the last body whorl (Table 4.9). This suggests that PC1 is a measure of size.

Table 4.9: Component loadings (correlation coefficients) of shell morphological characters for *R. rubiginosa* and *L. natalensis* from size class 2 (shell length ≥ 10 mm). The component loadings were derived from principal component analysis of the six shell characters after natural logarithm transformation. Values with the highest component loadings are in bold.

Variable	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
Log_{10}SL	**-0.994800**	-0.000103	-0.068332	0.050334	-0.055741	**0.007940**
Log_{10}SW	-0.977739	0.028319	-0.098430	**-0.182905**	0.009077	0.000035
Log_{10}AL	-0.977583	0.136401	-0.093734	0.074552	**0.106688**	-0.000051
Log_{10}AW	-0.927529	0.197646	**0.317010**	-0.011457	-0.000123	0.000013
Log_{10}LBW	**-0.984631**	0.119344	-0.092902	0.053804	-0.068457	-0.006793
Log_{10}SPH	-0.826713	**-0.558552**	0.064457	0.016365	0.011854	-0.001459
Cumulative %	90.2422	96.6538	98.9275	99.6748	99.9981	100.0000

The remaining 9.76% of the variance was explained collectively by the five principal components (PC2, PC3, PC4, PC5 and PC6). Spire height showed the highest loading

value for PC2, aperture width loaded the highest for PC3, shell width for PC4, aperture length for PC5 and shell length for PC6.

(b) Discriminant Function Analysis

The DFA extracted two highly significant functions (Wilks' Lambda = 0.24276, F = 27.593, *d.f.* = 10, 268, $p < 0.00001$). Function 1 representing 77.97% of the total morphometric variation extracted principal component 1 as an important parameter of discrimination (Table 4.10). The shell characters contributing the highest loading values for principal component 1 (see Table 4.9) were shell length (0.994800) and length of last body whorl (0.984631). Since these characters represented the longest dimensions of the shell, function 1 was interpreted to explain the overall size component.

Table 4.10: Standardised canonical discriminant function coefficients of principal component loadings for *R. rubiginosa* and *L. natalensis* from size class 2 (shell length ≥ 10 mm). Only the results of those parameters that contributed significantly to the DFA model are shown.

Variable	**Function 1**	**Function 2**	**Wilks' λ**	**F to remove**	***p***
PC 2	0.825842	0.509240	0.426735	50.77415	<0.0001
PC 1	**0.886745**	0.044926	0.402903	44.19682	<0.0001
PC 5	-0.647446	0.419957	0.337082	26.03086	<0.0001
PC 3	0.181302	**-0.788722**	0.309722	18.47974	<0.0001
PC 4	0.414621	-0.371306	0.283538	11.25344	<0.0001
Eigenvalue	1.754104	0.495672			
Cumulative %	77.97	100.00			
Wilks' λ	0.242763	0.668596			
χ^2	192.53	54.75			
d.f.	10	4			
p	<0.0001	<0.0001			

Function 2 explained 22.03% of the morphometric variation and was considered an aspect of shape. Along function 2, function principal component 3 (PC3) contributed the

highest coefficient (see Table 4.10). Table 4.9 shows that aperture width contributed the highest character loadings on PC3. Function 2 therefore represented a part of the overall shape component.

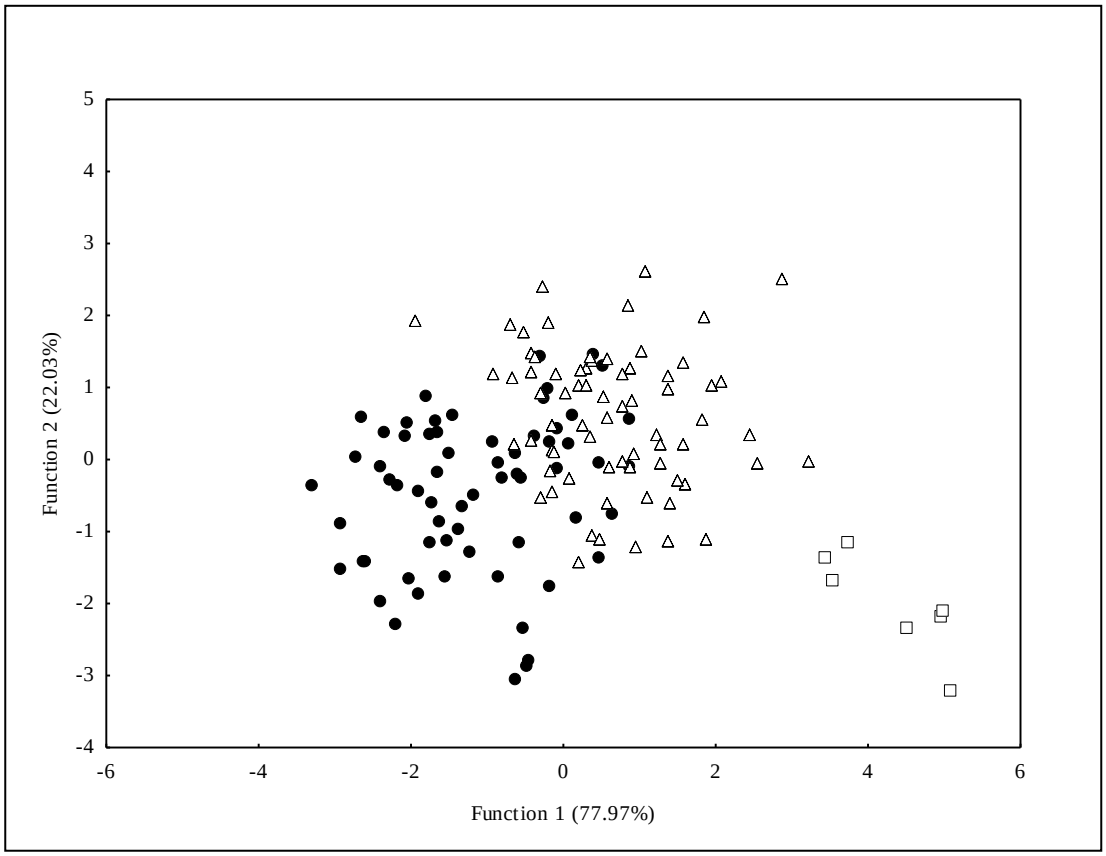


Figure 4.10: Plot of canonical scores defined by function 1 and 2 from forward stepwise DFA of shell morphological characters for size class 2 (shell length ≥ 10 mm). *Radix rubiginosa* from the Amatikulu site is represented by the closed circles (●). *Lymnaea natalensis* from the UKZN Pond and Greyville Pond are indicated by the open triangles (△) and open squares (□) respectively.

The plot of canonical scores (Figure 4.10) separated the lymnaeids into two distinct clusters along function 1. Function 1 ordinated the shells in a gradient from larger, more broadly ovate and larger body whorl (negative scores), to smaller, elongated shells with smaller body whorl (positive scores).

The shell variability of *R. rubiginosa* and *L. natalensis* (UKZN Pond) overlapped in the central morphospace of the canonical plot, forming a cluster that was significantly

different from that of *L. natalensis* (Greyville Pond). The Greyville Pond *L. natalensis* population formed a distinct cluster in the lower right quadrant of the DFA plot, displaying characteristics of smaller shell lengths and a much reduced last body whorl in comparisons to those of the '*Radix rubiginosa – Lymnaea natalensis* (UKZN Pond)' cluster.

Function 2 had principal component 3 (aperture width) contributing the highest loading value (see Table 4.10). Shells were ordinated on this axis from those with larger, wider apertures (positive scores) to narrower apertures (negative scores).

Function 2 separated both the populations of *L. natalensis*. The *L. natalensis* population from the UKZN Pond overlapped with *R. rubiginosa* forming a cluster in the central morphospace of the canonical plot, indicating a large overlap of aperture width measurements. *Lymnaea natalensis* from the Greyville Pond however, formed a distinct cluster in the lower right quadrant of the DFA plot. Hence, aperture width proved to be a highly discriminant parameter, between both *L. natalensis* populations.

4.3.2 Anatomical Morphology

4.3.2.1 Radix rubiginosa

(a) Radula

The teeth were divided into three distinct series, the central, laterals and marginals. An additional series of intermediate teeth was transitional between the laterals and marginals, combining the characteristics of these two series of teeth. Each radula generally consisted of 80 - >100 overlapping rows of these teeth. In studying the radula morphology it is important to consider only the newer perfect teeth, as older teeth near the mouth are usually worn and hence give a false idea of the true form of the cusps.

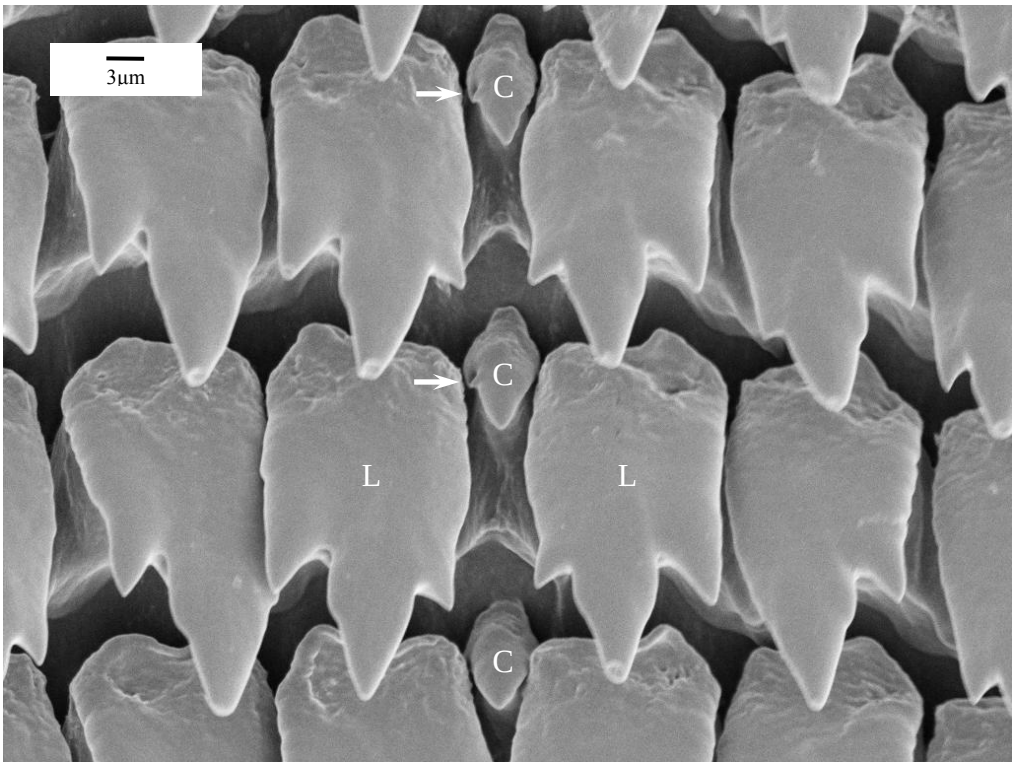


Figure 4.11: Scanning electron micrograph of the central tooth and lateral teeth of *R. rubiginosa*. A smaller accessory cusp is located on the left side towards the base of the central tooth (indicated by the arrows), scale bar 3 µm.
C – central tooth; L – lateral tooth.

Examination of representative radula showed 95 – 116 rows of teeth, each row consisting of a central tooth, 11 pairs of lateral teeth (two pairs were identified as intermediate teeth) and 21 pairs of marginal teeth. Each transverse row had a radula formula of 21: 11: 1: 11: 21. A single longitudinal row of median or central teeth was found at the middle of the radula (Figure 4.11). The central tooth was asymmetrically bicuspid, having a sharp spade-like triangular cusp. A smaller accessory cusp was located on the left side towards the base (see arrows, Figure 4.11).

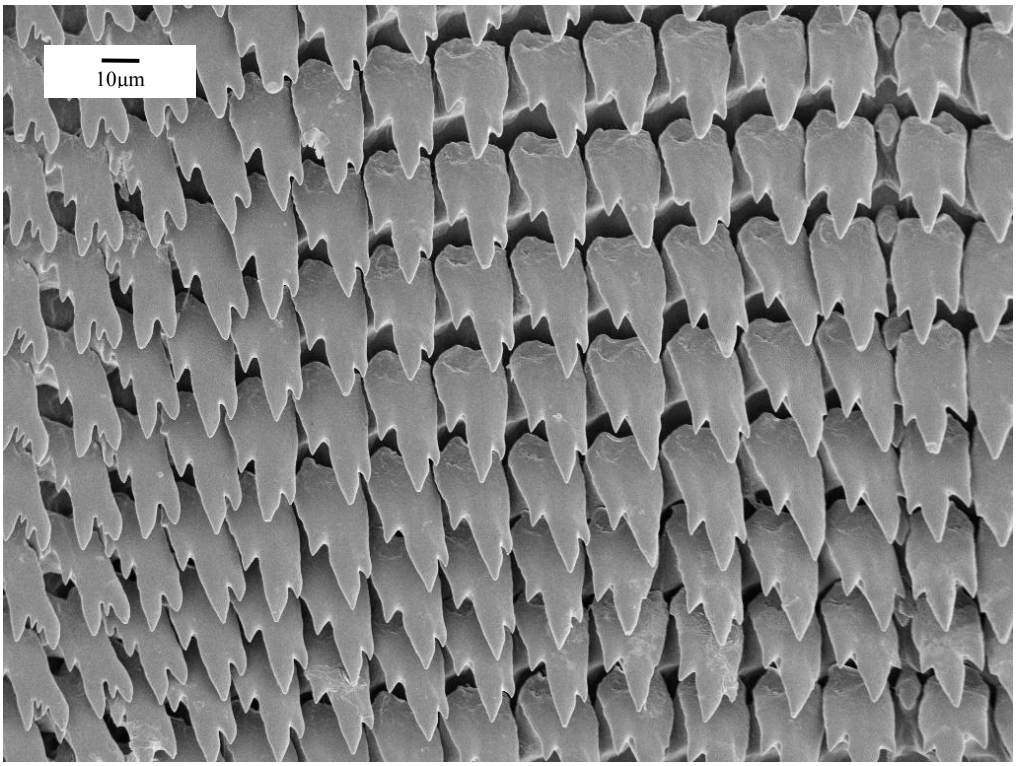


Figure 4.12: Scanning electron micrograph of the lateral teeth from the left side of a transverse row of *R. rubiginosa*, scale bar 10 µm.

The laterals were a little longer than wide, usually asymmetrically tricuspid and were the largest teeth in the radula (Figures 4.11 and 4.12). These teeth had a small, short, spade-shaped endocone (inner cusp) situated close to and adjacent to a much larger and longer mesocone (medial cusp). These cusps were fused. The mesocone was about three times as wide as the endocone and bluntly rounded. The ectocone (outer cusp) was much larger than the endocone and seemed to overlie the fused endocone and mesocone.

In *R. rubiginosa*, the 1st and 2nd pair of laterals displayed a sub-equal endocone and ectocone. The distance down the mesocone, leading to both the endocone and ectocone was similar (Figures 4.11 and 4.12). For lateral pairs 3 – 9, the ectocone began to migrate lower down towards the base of the mesocone and appeared to be prominently overlying (Figure 4.12). The number of lateral teeth on each side of the central tooth was consistent for *R. rubiginosa*.

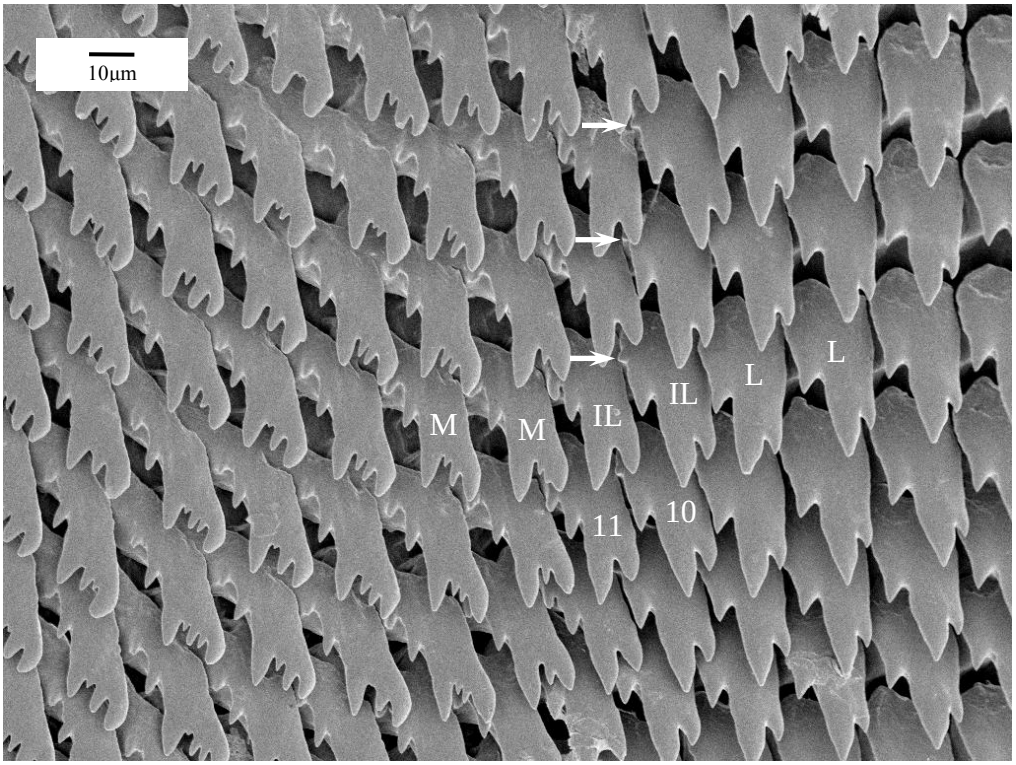


Figure 4.13: Scanning electron micrograph of the intermediate laterals (10th and 11th pairs of teeth) of *R. rubiginosa*. The 10th pair was tricuspid but developed a small enlargement towards the base of the ectocone (indicated by the arrow). In the 11th pair the ectocone, located towards the base of the tooth, split into two cusps, scale bar 10 µm.
IL – intermediate lateral tooth; L – lateral tooth; M – marginal tooth.

The intermediate laterals (10th and 11th pairs of teeth), situated after the nine pairs of lateral teeth, were "transitional" forms, i.e. they began to change from the tricuspid pattern of the laterals into the multicuspid pattern of the marginals (Figure 4.13).

The 10th intermediate pair displayed the tricuspid pattern with the development of a small enlargement towards the base of the ectocone (see arrow, Figure 4.13). In the 11th pair the mesocone was smaller, more spade-shaped and sub-equal with the endocone. The ectocone, located towards the base of the tooth, split into two cusps. This bicuspid pattern of the ectocone was distinct in the marginals, but became progressively degenerate and even absent in the marginals towards the lateral margin of the radula.

The marginal teeth, the last of the morphological tooth types, comprised the outermost group of teeth on each side of a transverse row (Figure 4.14). The endocone and mesocone were subequal and split into tiny denticles giving rise to the multicuspid condition common to most marginals. The marginal teeth of *R. rubiginosa* possessed four to five cusps that were short, bluntly rounded and obliquely placed. A few of the smaller cusps were acute and triangular shaped.

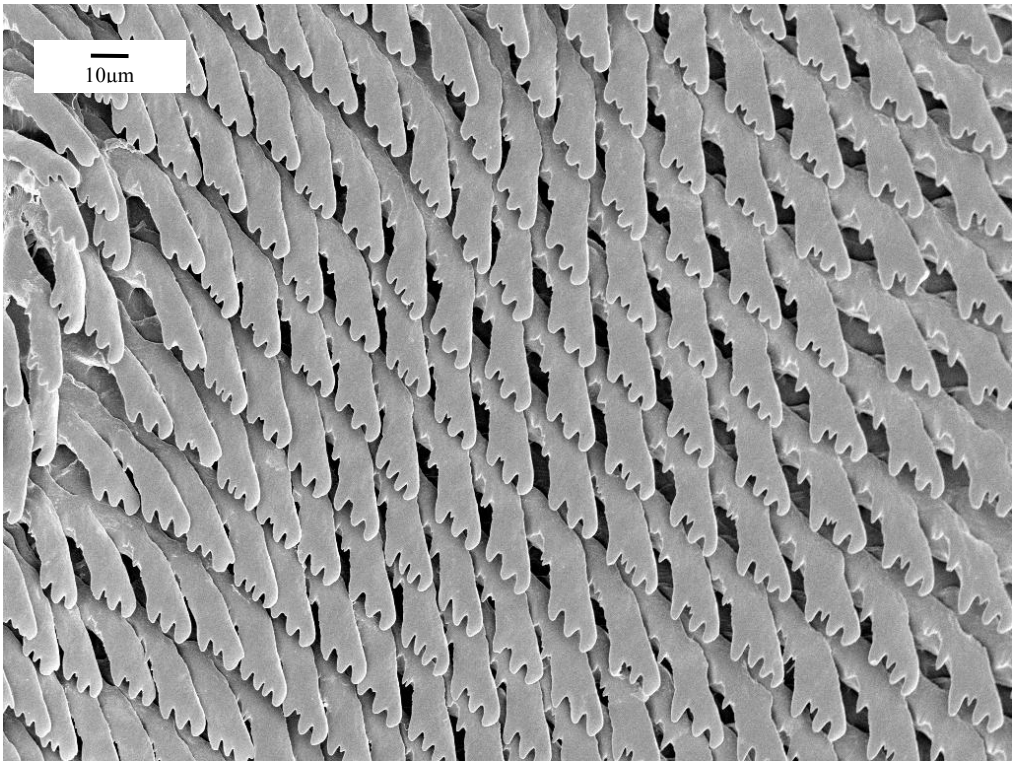


Figure 4.14: Scanning electron micrograph of the marginal teeth of *R. rubiginosa*, scale bar 10 µm.

There were a further two smaller cusps at the outer margin, towards the base of each tooth. These cusps, representing the reduced ectocone, may occur on up to the 4th or 5th last marginal tooth, beyond which they progressively disappeared. Proceeding laterally, there was a reduction of the number of cusps as the size of the tooth itself reduced (Figure 4.14). Marginal teeth occupying the extreme lateral borders of the radula had smaller and poorly defined blunt-shaped cusps. These extreme marginals were claw-like with three small cusps.

(b) Mantle pigmentation

The mantle surface of *R. rubiginosa* was mottled black with patches of pale white to yellow, interspersed with numerous unpigmented spots (Figure 4.15).

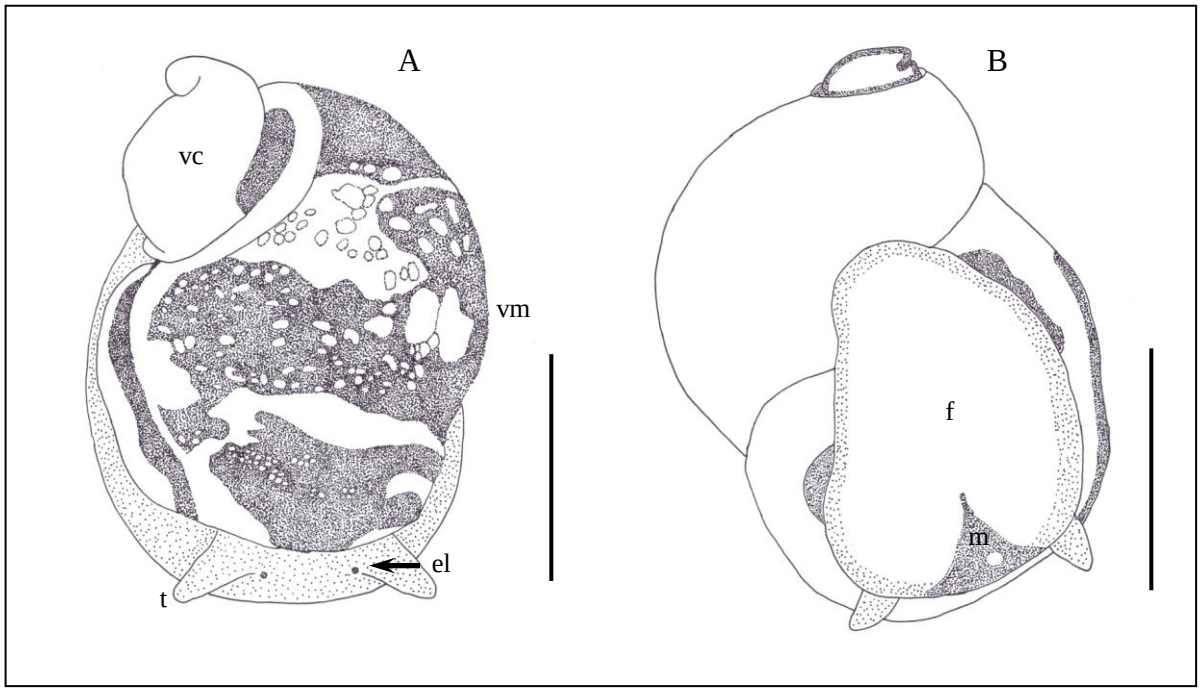


Figure 4.15: External features and pigmentation patterns of *R. rubiginosa* from the Amatikulu hatchery.
A – Dorsal view of animal with shell removed to show the mantle pigmentation pattern, scale bar 10 mm.
B – Ventral view showing foot and mouth, scale bar 10 mm.
el – eye lobe; f – foot; t – tentacle; m – mouth; vc – visceral coil; vm – visceral mass.

These circular spots were numerous in the region above the kidney and towards the mid-region of the mantle surface. There were also large unpigmented fields and stripes.

A pale white collar area bordered the mantle edge (Figure 4.15). Above this collar area the mantle surface was heavily pigmented with a strongly developed, narrow, irregular black stripe. This pigmentation pattern was observed for *R. rubiginosa* but not for *L. natalensis*. The head was diffusely grayish with scattered darker pigmentation. Only the outer margin of the foot was pigmented. Visceral coil pigmentation was absent.

(c) Reproductive Anatomy

The reproductive anatomy of *R. rubiginosa* was similar to that of *L. natalensis* from the UKZN Pond (see Section 3.4.3, Figure 3.15 of Chapter 3).

The penial sheath is wider than the vas deferens and is swollen at its proximal end. It is about twice the length of the praeputium. The praeputium of *R. rubiginosa* displayed a more intense pigmentation pattern than that of *L. natalensis*. The pigmentation of the praeputium of *R. rubiginosa* is also distributed over the whole organ, much like that of *L. natalensis*. The spermatheca is rounded and connected to the vagina by a long, slender spermathecal duct. The number of lobules / diverticula in the prostate is an important character in the planorbids, especially *Biomphalaria*. There was however, no difference in this character between *R. rubiginosa* and *L. natalensis*.

4.3.2.2 Lymnaea natalensis (UKZN Pond)

To avoid repetition the reader is referred to Chapter 3 as indicated below.

(a) Radula

See Section 3.4.3, Figure 3.10-3.13 of Chapter 3.

(b) Mantle Pigmentation

See Section 3.4.3, Figure 3.14 of Chapter 3.

(c) Reproductive Anatomy

See Section 3.4.3, Figure 3.15 of Chapter 3.

4.3.2.3 Lymnaea natalensis (Greyville Pond)

(a) Radula

Examination of representative radulae showed the presence of 108 – 120 rows of teeth, with each half row consisting of a central tooth, eight pairs of lateral teeth (two pairs were identified as intermediate teeth) and usually 28-30 pairs of marginal teeth. Each transverse row therefore had a radula formula of 28-30: 8: 1: 8: 28-30.

The single longitudinal row of central teeth was asymmetrically bicuspid. The main cusp was sharply pointed and triangular in shape (Figure 4.16) with a smaller, bluntly rounded accessory cusp on the left side towards the base. This accessory cusp (see arrow, Figure 4.16), was not as prominent as that observed in *L. natalensis* from the UKZN Pond (see arrow, Figure 3.10).

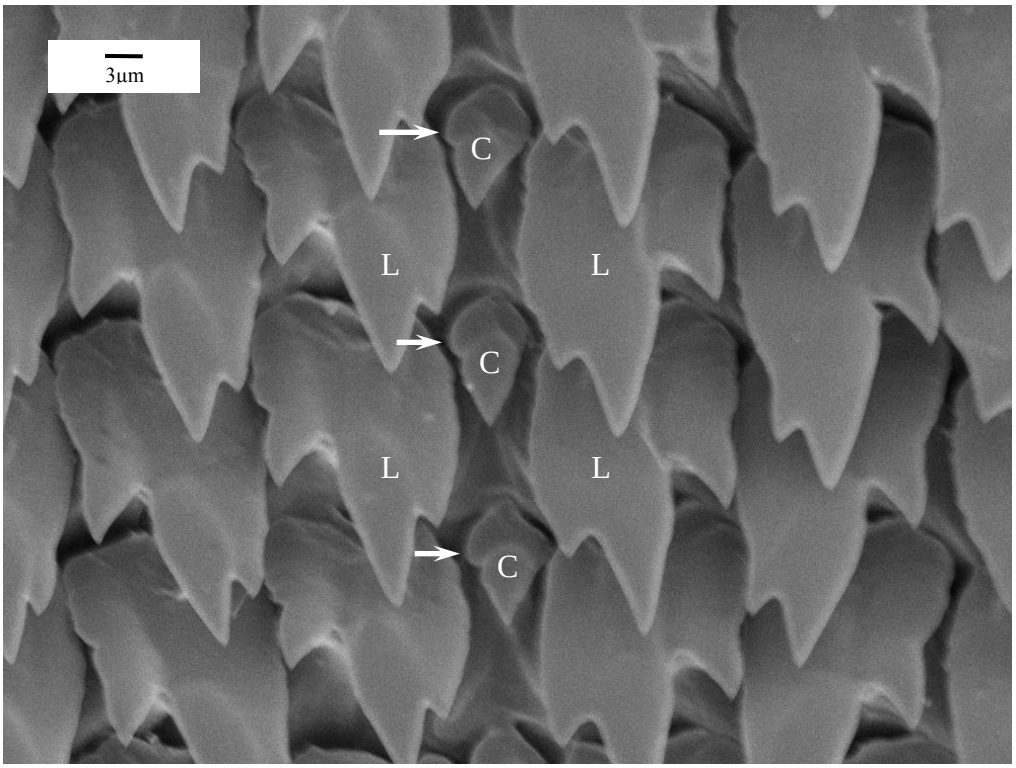


Figure 4.16: Scanning electron micrograph of the central tooth and lateral teeth of *L. natalensis* (Greyville), scale bar 3 µm.
C – central tooth; L – lateral tooth.

The laterals were a little longer than wide and asymmetrically tricuspid (Figure 4.17). These teeth consisted of a small, short and spade-shaped endocone situated adjacent to a much larger and longer mesocone. The endocone was also slightly directed towards the mesocone. Proceeding laterally, the mesocone became more pronounced with the endocone being situated higher up the inner margin of the mesocone (Figure 4.17). The ectocone was much larger than the endocone and seemed to overlie the fused endocone and mesocone more prominently than was observed for the lymnaeids from the UKZN Pond *L. natalensis* population (see, Figure 3.11).

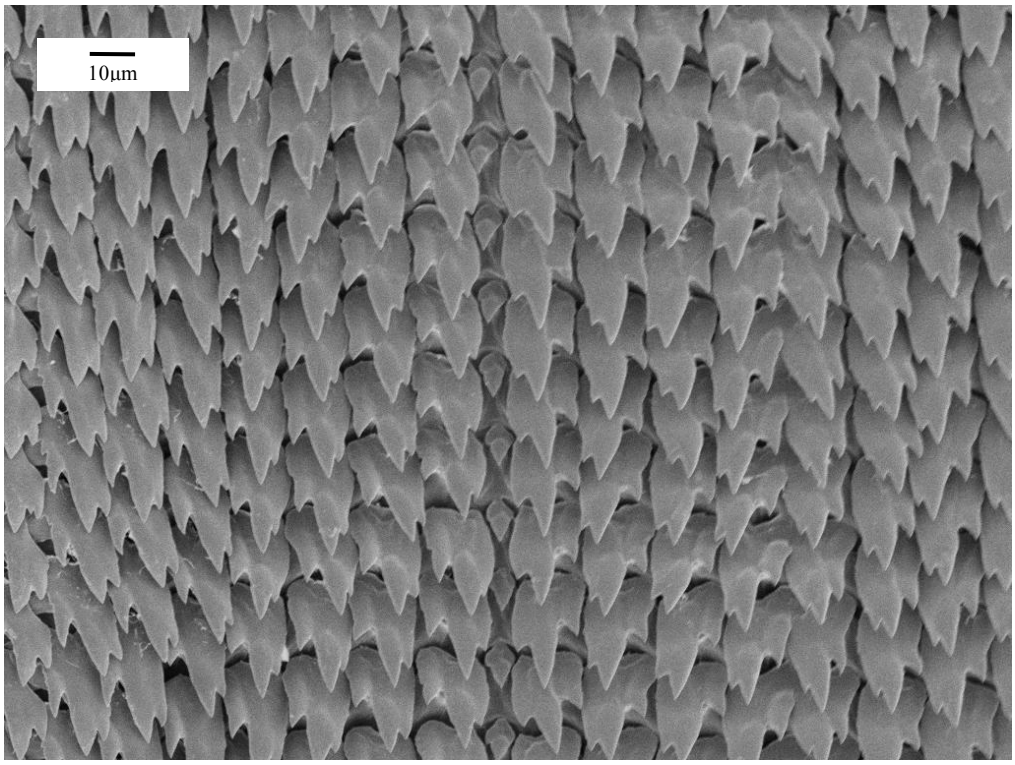


Figure 4.17: Scanning electron micrograph of the lateral teeth of *L. natalensis* (Greyville), scale bar 10 µm.

The intermediate laterals (7th and 8th pair of teeth) were obliquely placed with a reduced and smaller mesocone (Figure 4.18). On the 8th pair, the endocone sometimes split and displayed the formation of a small denticle (see arrow, Figure 4.18).

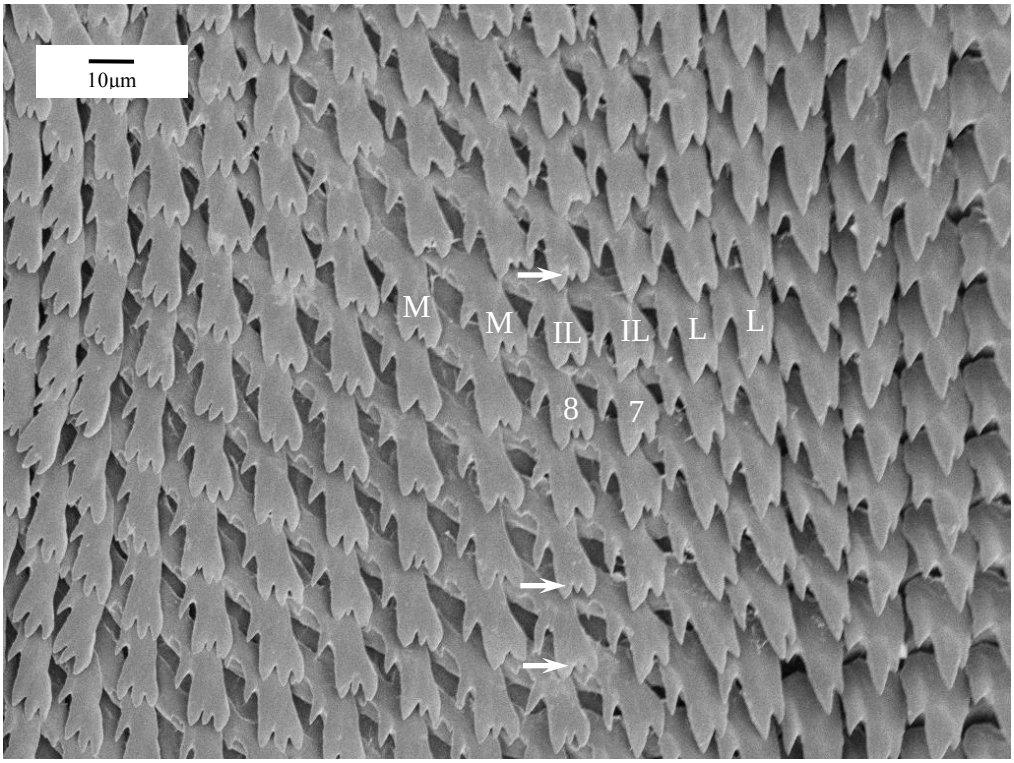


Figure 4.18: Scanning electron micrograph of the intermediate laterals (7th and 8th pair of teeth) of *L. natalensis* (Greyville), scale bar 10 µm.
IL – intermediate lateral tooth; L – lateral tooth; M – marginal tooth.

The mesocone and endocone of the marginal teeth were subequal with usually three to four bluntly rounded cusps (Figure 4.19). The ectocone became much reduced and sometimes absent in the marginals towards the lateral margin of the radula. Proceeding laterally, there was a reduction of the number of cusps as the size of the tooth itself was reduced. These marginal teeth were smaller, narrower and had poorly defined blunt cusps.

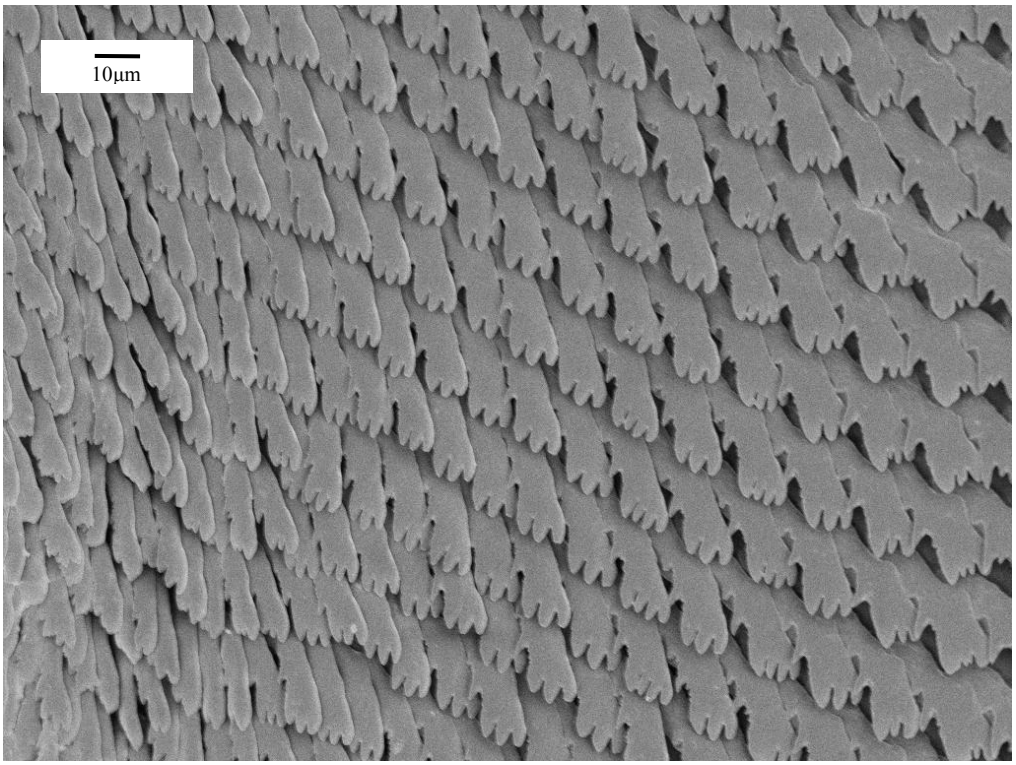


Figure 4.19: Scanning electron micrograph of the marginal teeth of *L. natalensis* (Greyville), scale bar 10 µm.

(b) Mantle Pigmentation

The entire mantle surface was covered by a grayish to black pigmentation, interspersed with clusters of unpigmented spots that were usually circular and regular in outline (Figure 4.20). These spots were numerous in the region above the kidney and towards the mid-region of the mantle.

The mantle boundary was heavily pigmented and described by a strongly developed, fairly broad, irregular black stripe (Figure 4.20). The visceral coil was unpigmented. The head and foot displayed a diffusely gray pigmentation.

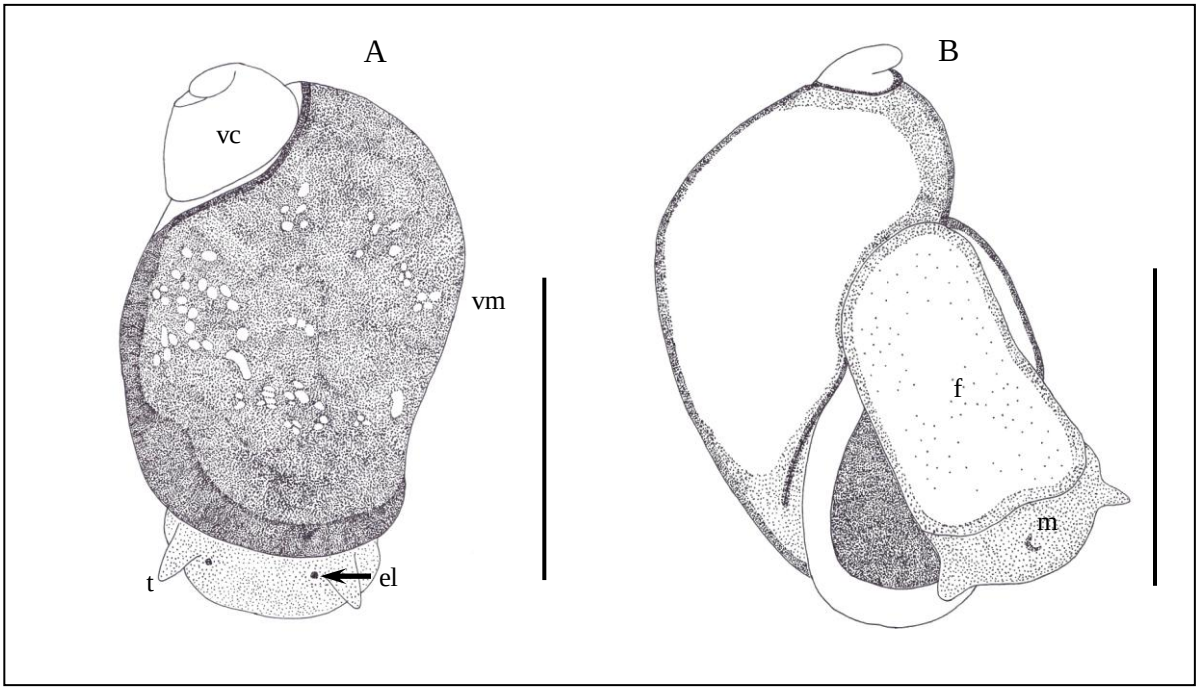


Figure 4.20: External features and pigmentation patterns of *L. natalensis* (Greyville).
A – Dorsal view of animal with shell removed to show the mantle pigmentation pattern, scale bar 10 mm.
B – Ventral view showing foot and mouth, scale bar 10 mm.
el – eye lobe; f – foot; t – tentacle; m – mouth; vc – visceral coil; vm – visceral mass.

(c) Reproductive Anatomy

There were no differences observed between *L. natalensis* from the UKZN Pond and the Greyville Pond. For a description of the reproductive anatomy see Section 3.4.3, Figure 3.15 of Chapter 3.

4.4 Discussion

During the last century, humans have caused an unprecedented redistribution of many organisms, including plants and animals. As introductions of non-indigenous species continue in biological communities the accurate identification of species is fundamental to both basic and applied aquatic research. Studies of community structure, food web dynamics, biodiversity and biomonitoring depend critically on the accuracy of species discrimination and identification. It is therefore increasingly important for ecologists to be able to identify invasives and to understand the spread of the invasive species as well as to predict the impact that a given invasive will have on indigenous species in the invaded habitat (Parker *et al*., 1999; Byers and Goldwasser, 2001).

The genus *Radix* Montfort, 1810 (Gastropoda: Basommatophora: Lymnaeidae), formerly included in *Lymnaea*, has a European and Asian distribution (Pfenninger *et al*., 2006). *Radix rubiginosa* identified from the Amatikulu Prawn and Fish Hatchery, is indigenous to southeast Asia (Monzon *et al*., 1993; Remigio and Blair, 1997). This implies that the snails were introduced to KwaZulu-Natal as a result of trade in the pet and aquarium industry.

The presence of these potentially invasive snails has important implications for freshwater invertebrate diversity and to humans and domestic stock due to their role as intermediate hosts of *F. gigantica* (Srihakim and Pholpark, 1991; Malone, 1997) and the avian blood fluke, *Trichobilharzia* sp., a cause of schistosome dermatitis (Nithuithai *et al*., 2004). *Radix rubiginosa* has also been identified as the intermediate host for *Schistosoma incognitum* (Bunnag *et al*., 1983) and various echinostomes (Charoenchai *et al*., 1997). The natural habitat for *R. rubiginosa* includes the low-lying areas of tropical/subtropical southeast Asia which suggests that if it becomes invasive it could colonize the lowlands KwaZulu-Natal, Mpumalanga, Limpopo and perhaps further north and eastwards. This presents a problem as it could then exacerbate the fascioliasis problem in the country. However efforts aimed at controlling fascioliasis cannot be effective if the specific identity of the snail hosts implicated in transmission has not been

determined (Pfenninger *et al*., 2006). This is particularly true for lymnaeids, given their long history of taxonomic confusion resulting from a purely phenotypic approach.

4.4.1 Shell Morphology and Morphometrics

Molluscan taxonomy has historically been based largely on morphological characters, with shell form, radular structure and anatomy being the most commonly used characters. However, the large degree of morphological plasticity exhibited by lymnaeids has plagued the taxonomy of this family and the value of these morphological characters is therefore under scrutiny. In this study, the morphometric approach was used to provide an assessment of the suitability of conchological characters to be used as identification criteria for separating *R. rubiginosa* and *L. natalensis*.

Size is assumed to correspond to general factors, i.e. linear combinations of appropriate suites of characters (Mosimann and Malley, 1979; Bookstein *et al*., 1985; Sundberg, 1989). There are many ways in which such a general-size factor could be defined. It is most commonly taken as the first principal component obtained from a principal component analysis (PCA) correlation matrix of log-transformed morphometric characters (Jolicoeur, 1963; Reyment *et al*., 1984; Sundberg, 1989). Jolicoeur and Mosimann (1960) argued that the first principal component can be viewed as size if all coefficients of this component display similar loading values (Tables 4.7 and 4.9).

From the analyses for size class 1 (shell length < 10 mm), PC1 was an important parameter of discrimination along function 1 (Table 4.8). Shell length and the length of the last body whorl were the characters with the highest loading values on this component (Table 4.7). Interestingly, these characters were identified as having the longest dimensions of the shell and were therefore interpreted as representing the overall size component. From the canonical plot (Figure 4.9), *R. rubiginosa* was found to have larger, more broadly ovate shells with longer (higher) body whorls than either of the two populations of *L. natalensis* which had smaller, elongated shells with shorter (lower)

body whorls. Importantly, these *L. natalensis* populations could not be separated based on size dimensions of the shell (function 1) for size class 1 (Figure 4.9).

The orthogonality imposed by principal component analysis makes all components independent and if the first component is an indication of size, the remaining components are then indications of shape (Jolicoeur, 1963; Reyment *et al*., 1984; Bookstein *et al*., 1985; Sundberg, 1989; Atchley and Hall, 1991; Costa *et al*., 2004). For size class 1 (shell length < 10 mm), PC3 was the most important parameter of discrimination along function 2 (Table 4.8). Since aperture width contributed the highest loading value to this component, it was interpreted as representing the overall shape component (Table 4.7). *Radix rubiginosa* and *L. natalensis* (UKZN Pond) displayed a similar variation in the width of the aperture (Figure 4.9). This can be explained due to the high morphological variation evident in the family Lymnaeidae.

According to Wullschleger and Ward (1998), characters associated with the aperture are extremely plastic and allow for adaptation to ecological conditions, such as the increase in aperture size due to enlargement of the foot, in order to adequately attach to substrates. Despite the overlap shown by the canonical plot (Figure 4.9), *R. rubiginosa* had narrower apertures in comparison to the UKZN Pond *L. natalensis* population which displayed a larger, wider aperture. The use of aperture width as a character displayed considerable variation in *L. natalensis* from the Greyville Pond. It was apparent that some specimens of this population exhibited the larger, wider aperture and others the narrower aperture characteristics (Figure 4.9).

For size class 2 (shell length ≥ 10 mm), PC1 contributed the highest loading values on function 1 (Table 4.10). Shell length and the length of the last body whorl were the shell characters with the highest loading values for this component (Table 4.9). The above characters for *R. rubiginosa* and *L. natalensis* (UKZN Pond) overlapped in the central morphospace of the canonical plot (Figure 4.10), indicating a degree of similarity in shell variation patterns. Despite this morphological plasticity for size class 2, the shells of *R. rubiginosa* were larger, more broadly ovate with longer (higher) body whorls in

comparison to the smaller, elongated shells with shorter (lower) body whorls of *L. natalensis* (UKZN Pond). For this size class, the Greyville Pond *L. natalensis* population exhibited the smallest shell dimensions (Figure 4.10).

From Table 4.10 it was shown that PC3 contributed the highest loading values for the shape component (function 2) in size class 2 (shell length ≥ 10 mm). Aperture width contributed the highest loading value to this component and was interpreted as representing a part of the overall shape component (Table 4.9). The overlap of aperture width for *R. rubiginosa* and *L. natalensis* (UKZN Pond) was evident from the canonical plot (Figure 4.10). This again confirmed the problem of phenotypic plasticity evident in the family Lymnaeidae. *Radix rubiginosa* did however, have a narrower aperture width in comparison to the UKZN Pond *L. natalensis* population. Furthermore, aperture width was a highly discriminant shell characteristic used in separating both the *L. natalensis* populations. From Figure 4.10 it was shown that *L. natalensis* from the UKZN Pond displayed larger and wider apertures than the Greyville Pond *L. natalensis* population.

4.4.2 Anatomical Morphology

The morphology of the radula for *R. rubiginosa* and both the *L. natalensis* populations showed very little variation in tooth shape and were observed to be homoplastic (the characters of the central, lateral and marginal teeth were similar for all populations). The species did however vary in the relative numbers of teeth in each field and this serves as an additional useful diagnostic character. In this study, each transverse row of the radula of *R. rubiginosa* had a radula formula of 21: 11: 1: 11: 21. The UKZN Pond and the Greyville Pond *L. natalensis* populations had radula formulae of 12: 8-10: 1: 8-10: 12 and 28-30: 8: 1: 8: 28-30 respectively. These differences in the morphology in the two *L. natalensis* populations only approximately 5.5 km apart focuses attention on the phenotypic plasticity that is an important adaptive trait in the family Lymnaeidae.

Jackiewicz (1993) suggested that the distribution and intensity of pigmentation patterns on the mantle are a useful diagnostic character in the descriptions of many lymnaeid

species. It was further reported that these patterns showed great diversity, being similar in some species only. In this study, both *L. natalensis* populations had similar mantle pigmentation patterns. The entire mantle was gray to black in colour but interspersed with unpigmented spots that were numerous in the region above the kidney and towards the mid-region of the mantle. *Radix rubiginosa* however, displayed a distinctly different mantle pigmentation pattern. The mantle surface of *R. rubiginosa* was mottled black with patches of pale white to yellow. There were also large unpigmented fields and stripes that were not observed in *L. natalensis*. In addition, the mantle was interspersed with numerous unpigmented spots that were most frequent in the region above the kidney and towards the mid-region of the mantle.

Since the primary objective was to comparatively discern patterns of variation among *R. rubiginosa* and *L. natalensis*, characters limited to those of the shell and mantle pigmentation patterns are summarised below.

Radix rubiginosa was identified based on the following characteristics:

> Larger, more broadly ovate shells with longer (higher) body whorls; shell is hard and thick; narrower apertures; moderate to prominent sutures; spire is variable and elongate; the upper region of the peristome is usually straight and directed outwards, below this region the peristome runs almost parallel to the main axis of the shell; the mantle surface is mottled black with patches of pale white to yellow and large unpigmented fields and stripes; the mantle is also interspersed with numerous unpigmented spots that are most frequent in the region above the kidney and towards the mid-region of the mantle.

Lymnaea natalensis was identified based on the following characteristics:

> Shell is succineiform, smaller, elongate with shorter (lower) body whorls; shell is thin; larger, wider apertures; the base of the aperture joins the columella in a broadly rounded curve; the sutures are well-impressed and constricted; variable, elongate, tapering spire with an acute apex; the entire mantle is gray to black in colour but interspersed with unpigmented spots that are numerous in the region above the kidney and towards the mid-region of the mantle.

These characteristics would assist non-systematic specialists to easily recognise and separate *Radix rubiginosa* from *Lymnaea natalensis*.

5

Embryonic Development of *Radix rubiginosa*, *Lymnaea natalensis* and *Physa acuta*

5.1 Introduction

In general, the species that become invasive are not a random sampling of biodiversity. Instead, successful invaders are predicted to be species that, in their native ranges, display traits that enable them to successfully survive conditions encountered during transport, introduction, establishment and integration (Suarez and Tsutsui, 2008). Successful invaders possess characteristics associated with effective dispersal, rapid growth, short generation times, high fecundity, high degree of phenotypic plasticity, broad physiological tolerance (euryhalinity and eurythermy) and a broad diet (Rejmanek and Richardson, 1996; Williamson and Fitter, 1996; Reid and Orlova, 2002; Ruesink, 2005; Moyle and Marchetti, 2006; Keller *et al*., 2007; Suarez and Tsutsui, 2008).

Because patterns of invasions by exotic species can be hard to predict, research into the reproductive biology of a species is essential for an understanding of its ecology and therefore, of its ability to spread (Sastry, 1979; Borcherding, 1995). Indeed, the success and extent to which a species can spread in a given environment is related mainly to those factors which can limit the species reproduction (Sastry, 1979). In respect of freshwater snails, Russell-Hunter (1978) reported that temperature was probably the most important factor determining development, growth and reproduction. It may also act as an important selection pressure (Hardy, 1979; Lam and Calow, 1990) that can determine the geographical distribution, relative abundance, physiological responses (particularly growth and reproduction) and behaviour of freshwater snails. It was noted by Joubert and Pretorius (1985) that species could become adapted to different thermal regimes over

their geographic distribution. It is therefore essential to investigate the effects of temperatures not only on the adult snails, but on their eggs and embryonic development as well.

Developmental studies are thus crucial in determining the distribution of a species both in terms of its response to environmental parameters and its relation to, and impact on, closely related species (Elliott, 1988; Lillehammer *et al*., 1989; Brittain and Campbell, 1991). Knowledge of the timing of the embryonic stages and the effect of temperature on the duration of embryonic development is important in assessing this.

The introduction of *Radix rubiginosa* (Michelin, 1831) to northern KwaZulu-Natal is important, especially in view of the success of the North American physid, *Physa acuta* Draparnaud, 1805, as an invader in South Africa (Hamilton-Attwell *et al*., 1970; De Kock *et al*., 1989; Brackenbury and Appleton, 1993; Appleton and Brackenbury, 1998; Appleton, 2003; De Kock and Wolmarans, 2007). In their review of introduced freshwater snails worldwide, Madsen and Frandsen (1989) concluded that the aquarium trade was a source of multiple introductions and responsible for the distribution of several of the common species, including *P. acuta*, a view supported by Appleton (2003).

Its superior reproductive capacity (Appleton and Brackenbury, 1998), ability to migrate upstream and to quickly recolonise a water-body (Brakenbury and Appleton, 1993), have resulted in *P. acuta* being one of the most widespread and dominant freshwater gastropod species in South Africa. Given the abundance and easy accessibility of *P. acuta*, this species was selected as a comparison to assess the invasive potential of *R. rubiginosa*.

The aim of this experimental study was to describe and compare the effect of three experimental temperatures (20^{o}C, 25^{o}C and 30^{o}C) on the hatching success and embryonic development of four snail populations of three species: *R. rubiginosa* from the Amatikulu Hatchery, *Lymnaea natalensis* Krauss, 1848 from both the UKZN and Greyville Ponds and *P. acuta* from the Greyville Pond. A description of the morphology

of each developmental stage is also provided. In addition, the frequency of various egg abnormalities and their relation to the breeding intensity of these species was assessed.

5.2 Methodology

The snails used in this study were from breeding colonies maintained in the laboratory. These colonies were originally collected from the three study sites described in Chapter 4, while *P. acuta* was collected from the Greyville Pond. A total of 50 individuals with shells 10 – 15 mm in height were randomly selected from each population and placed into five aerated aquaria (45 x 29 x 12 cm), each containing six litres of dechlorinated tap water (water depth approximately 6 cm). The aquaria were maintained at three constant temperatures (20°C, 25°C and 30°C) and a 12:12 (L:D) photoperiod. To prevent the snails from escaping, the aquaria were covered with a netted mesh.

The snails were fed lettuce daily with the amount adjusted to the maximum daily consumption. In addition the diet was supplemented *ad libitium* with Tetramin® (a commercially available brand of fish food) and Marcus Rohrer® Spirulina (two tablets crushed into a fine powder and added to the water). Leaves of *Nymphaea nouchali* and *Marsilea* sp. were placed in the aquaria to provide resting and egg laying surfaces for the snails (since the snails were observed to lay egg capsules on the under-surfaces of the leaves rather than on the sides of the aquaria). The aquaria water was changed weekly and the faeces were removed daily.

5.2.1 Egg Abnormalities, Viable Eggs and Hatching Success

5.2.1.1 Egg Abnormalities

Egg capsules were isolated and carefully removed with a scalpel (care being taken not to rupture the capsular membrane). The capsules were then transferred to separate containers (10 x 10 x 2 cm) maintained at the three constant temperatures. The number of eggs present in each capsule was counted and recorded.

To determine the effects of species and temperature on the proportion of egg abnormalities, 600 eggs were viewed under a stereomicroscope for each of the four snail

populations. Egg capsules containing 18 – 40 eggs were used in order to provide a satisfactory statistical base for computing the proportion of egg abnormalities. The experiment was replicated three times. Dwarf eggs, non-nucleated eggs, eggs without development and polyvitelline eggs were used to assess the frequency of abnormalities.

5.2.1.2 Viable Eggs and Egg Hatching Success

Immediately prior to hatching the numbers of viable eggs (embryos showing normal development) were counted to calculate hatching success. Egg capsules were then returned to the container and allowed to hatch. For the purposes of these experiments, hatching was defined as the escape of the young snail from the egg, rather than from the egg capsule, since a delay may occur before the young snail finds its way out of the latter. This gave less variable results and so allowed a more accurate definition of the effect of species and temperature on the hatching success (Harris and Charleston, 1977).

Data were not normally distributed even after transformation and were analysed using a non-parametric Kruskal-Wallis test (Zar, 1999). Statistical analyses were performed using SPSS 11.0.1 (SPSS Inc.). Probability values are two-tailed and significance was determined at $p < 0.05$.

5.2.2 Embryonic Development

Leaves of *N. nouchali* and *Marsilea* sp. were carefully examined for the presence of newly laid egg capsules. Examining the leaves and removal of egg capsules were carried out over an eight hour period daily for one week. This procedure ensured an adequate sample of egg capsules to assess the effects of species and temperature on embryonic development.

The time of oviposition was noted and egg capsules were transferred to separate containers (10 x 10 x 2 cm) maintained at the three constant temperatures. The egg capsules were fixed using 0.5% formalin at hourly intervals for the first 24 hours after

oviposition. After this period, samples were fixed and viewed at daily intervals, until hatching was observed. The morphology and developmental stages of the embryo were viewed using a stereomicroscope. The duration of embryonic development (incubation period) was noted and the size of the embryos at different stages was measured.

The average geometric growth rate for the developing embryos was calculated using the formulation of Simpson and Roe (1939).

$$G = \frac{2.303 \log Y_t - \log Y_0}{t}$$

where,

Y_0 = initial size

Y_t = size at time t

t = time measured at intervals of 1 day

G = geometric growth rate

All measurements were recorded using NIS Elements D image analysis software.

5.3 Results

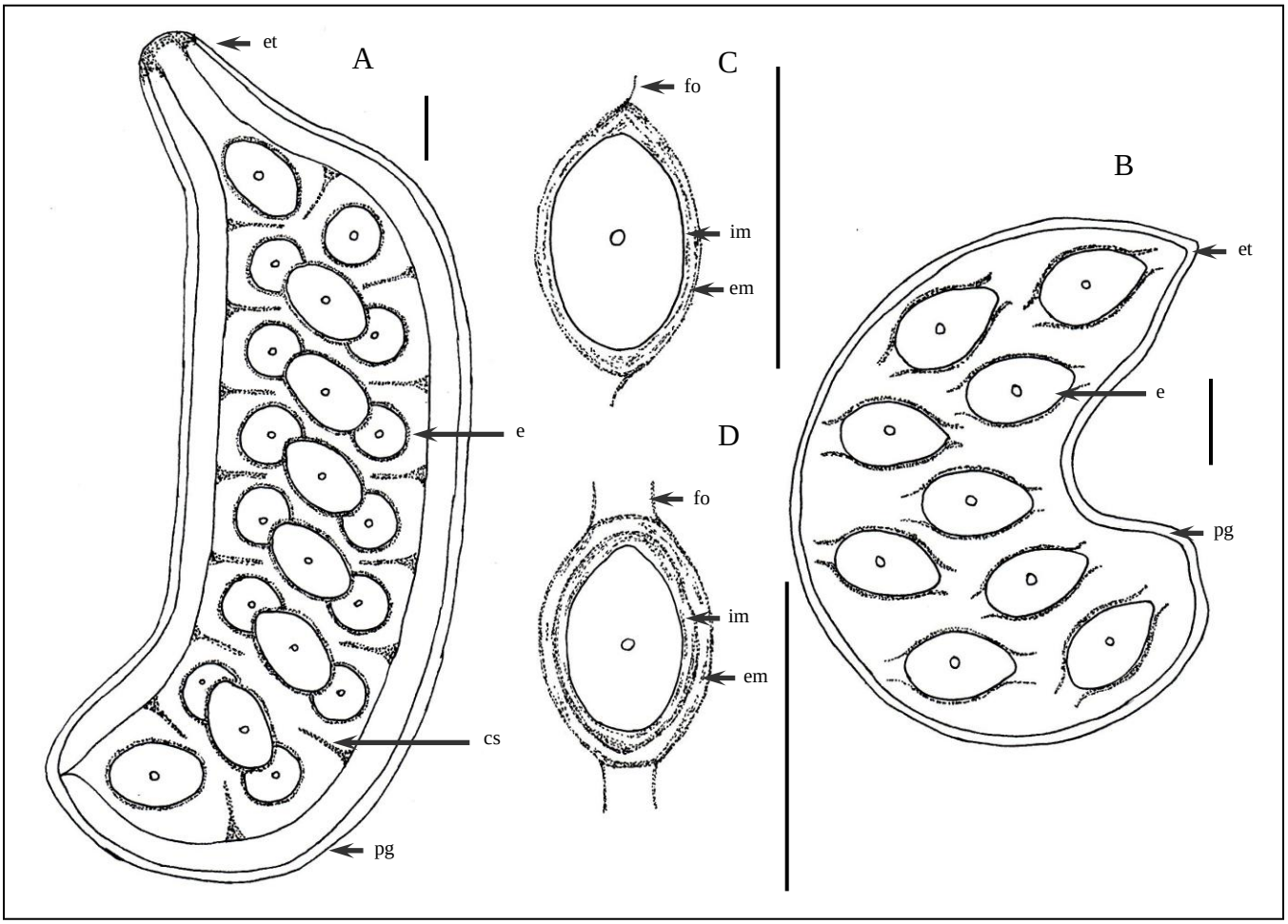
5.3.1 Description of Egg Capsules

During oviposition, the egg masses of freshwater snails are firmly attached to a substratum, such as leaves, stones and submerged pieces of wood (Berrie, 1965). These non-moving objects must be located in habitats with favourable conditions for the development of embryos and hatchlings (Geraerts and Joosse, 1984). The egg masses contain encapsulated embryos that are surrounded by a nutrient (capsular fluid). Therefore, the egg and egg capsule serve the dual purpose of providing a source of nutrition and protection for the developing embryos.

The shape and structure of the eggs and egg masses, as well as the number of eggs per mass and their arrangement within the mass, are characters that should be considered in the identification of snails (Nekrassow, 1929; De Witt, 1954). According to Bondesen (1950), these characters could also be used to determine relationships between families and even higher taxa of gastropods (De Witt, 1954). To obtain clarity and system in the structural features of the egg capsules, the descriptions of the egg capsules for the four snail populations follow Bondesen (1950) and are briefly explained below.

In the Lymnaeidae and Physidae (Figure 5.1), there is a gelatinous slimy outer envelope (*pallium gelatinosum*) and a more distinct inner capsular wall (Bondesen, 1950; De Witt, 1954). The egg consisting of the egg cell (ovum) with its albumen and the internal membrane around it, varied from spherical to ovoid in shape. The albumen was the sole source of food available to the developing embryo until hatching. The internal membrane of the egg was surrounded by a series of fine lamellar structures forming the external membrane. Bondesen (1950) and De Witt (1954) noted that the external membrane continued to form egg strings (*fila ovi*) that connected the individual eggs with each other throughout the capsule. This was also seen in the present study. The terminal point of a capsule (*existus terminalis*) was elongated into a terminal tail that was prolonged into a

spout, a tube or a tapering end. This characteristic terminal part of the capsule was especially important for the orientation of the capsules.



(redrawn from Bondesen, 1950)

Figure 5.1: Characteristic egg capsule morphology for the Lymnaeidae (A) and Physidae (B) showing curvature of the capsule after oviposition. Dextral and sinistral families have the egg capsules curved in opposite directions, *R. rubiginosa* and *L. natalensis* are dextral snails while *P. acuta* is a sinistral snail. Lymnaeid capsules display anticlockwise torsion while physid capsules show clockwise torsion. In lymnaeid capsules (C), distinct capsular strings and egg strings were observed, resulting in the characteristic corkscrew arrangement of the eggs within the capsule. In the Physidae (D), the egg strings were not as well developed as in the Lymnaeidae, scale bar 1 mm.
cs – capsular strings; e – egg; em – external membrane; et – *existus terminalis* (terminal point of capsule); fo – *fila ovi* (egg strings); im – internal membrane; pg – *pallium gelatinosum* (gelatinous slimy outer envelope).

From Table 5.1 it was evident that the egg capsules from the four snail populations varied considerably in dimensions and in the number of eggs per capsule (clutch size).

Table 5.1: Comparison of egg capsule dimensions and clutch sizes for each of the four snail populations (n = 100). Dimensions are presented as mean millimeters (± standard error).

Snail Populations	Egg Capsule Length	Egg Capsule Width	Clutch Size	Egg Length	Egg Width
R. rubiginosa	15.66 (±0.51)	5.00 (±0.08)	52.81 (±2.40)	0.90 (±0.008)	0.73 (±0.005)
L. natalensis (UKZN Pond)	12.85 (±0.33)	3.17 (±0.03)	27.95 (±1.20)	0.83 (±0.008)	0.62 (±0.003)
L. natalensis (Greyville Pond)	13.02 (±0.30)	3.80 (±0.05)	24.38 (±0.88)	0.88 (±0.006)	0.65 (±0.003)
P. acuta	9.92 (±0.37)	4.87 (±0.09)	39.80 (±1.81)	0.75 (±0.004)	0.62 (±0.004)

(a) Radix rubiginosa

The egg capsule was elongated and sometimes a curvature to the left was observed. The distinct terminal tail was a continuation of the capsule. *Radix rubiginosa* had the largest egg capsule dimensions of the three snail species investigated (Table 5.1). The smallest capsule recorded was 5.00 x 2.92 mm, with four eggs enclosed. The largest capsule measured 38.17 x 4.67 mm, with 134 eggs enclosed.

The egg cell of *R. rubiginosa* was darker yellow in comparison to *L. natalensis* and *P. acuta*. The eggs were regularly oval. The mean dimensions of *R. rubiginosa* eggs were 0.90 x 0.73 mm, larger than the eggs of either *P. acuta* or *L. natalensis* from both the UKZN and Greyville Ponds (Table 5.1). *Radix rubiginosa* also produced a higher mean number of eggs per capsule (clutch size).

The corkscrew arrangement of the eggs was difficult to see in egg capsules of *R. rubiginosa*. This was because the capsular strings were not as prominent as in *L. natalensis* and also since *R. rubiginosa* produced more eggs per capsule, the eggs were often orientated very close to each other.

(b) Lymnaea natalensis

The egg capsules for *L. natalensis* from both the UKZN and Greyville Ponds were similar to those of *R. rubiginosa*, except that *R. rubiginosa* eggs and capsules were larger and also had the largest mean clutch sizes (Table 5.1). The egg capsules of *L. natalensis* from both study ponds also displayed a corkscrew spiral arrangement of eggs. This could be attributed to the distinct capsular strings, the loose orientation of the eggs in the capsule and the fact that the eggs were laid almost in a single layer.

For *L. natalensis* from the UKZN Pond, the number of eggs per capsule ranged from 6 – 80. The smallest capsule of this snail population was 6.58 x 2.83 mm and the largest was 24.92 x 2.92 mm. For the Greyville Pond population, the smallest egg capsule recorded was 4.17 x 3.00 mm, with 5 eggs enclosed. The largest capsule recorded was 23.17 x 4.50 mm, with 59 eggs enclosed.

It is evident that while the Greyville lymnaeids displayed larger mean egg capsule dimensions, they produced smaller clutch sizes in comparison to the UKZN pond population (Table 5.1). This could be attributed to the marginally smaller mean egg dimensions (0.83 x 0.62 mm) of the UKZN Pond population, resulting in more eggs being packaged per capsule.

(c) Physa acuta

The egg capsules of *P. acuta* were kidney-shaped but the curvature depended upon the number of eggs in the capsule. Smaller capsules with few eggs were not curved and were round or oval but larger capsules were curved clockwise. The terminal part of the capsule was shortened, or sometimes entirely lacking, resulting in the capsule tapering. The egg cells were oval to pear-shaped and pale yellow in colour.

The number of eggs per capsule ranged from 6 – 99. The smallest capsule recorded for *P. acuta* was 4.75 x 4.00 mm and the largest was 22.83 x 7.17 mm. *Physa acuta* had the

smallest mean egg and egg capsule length dimensions of the three species examined, however, it produced a higher mean number of eggs per capsule when compared to *L. natalensis* from both the UKZN and Greyville Ponds. Of importance to this study is the fact that *R. rubiginosa* produced, on average, more eggs per capsule than the invasive *P. acuta*.

5.3.2 Viability of Eggs and Egg Abnormalities

During embryogenesis, the growth and development of the embryo is subjected to various factors that influence development. These factors can be either intrinsic (lack of fertilisation, quantity of albumen) or extrinsic (crushing of the egg cell during the formation of the capsule, crowding as a result of abnormal development of a neighbouring embryo, changes in the environment).

An assessment of both the hatching success (proportion of viable eggs per capsule) and the frequency of egg abnormalities is presented in Table 5.2. Viable eggs were recorded as those that eventually hatched, while dwarf eggs, eggs without egg cells, eggs without development and polyvitelline eggs were characteristics used to assess egg abnormality.

(a) Hatching Success

At each of the three temperatures, *P. acuta* had the highest mean hatching success followed closely by *R. rubignosa*. *Lymnaea natalensis* from both the UKZN and Greyville ponds had the lowest hatching success (Table 5.2).

All four snail populations displayed a common trend inasmuch as the highest hatching success was at 25^{o}C. *Radix rubiginosa* and both the *L. natalensis* populations had their lowest hatching success at 20^{o}C, while *P. acuta* had its lowest success at 30^{o}C. For all populations, the mean hatching success increased from 20^{o}C to 25^{o}C, but decreased at 30^{o}C.

Table 5.2: Hatching success and egg abnormalities (%) for the four snail populations at the three temperatures (n = 25). The values are presented as percentage means (± standard deviation).

Temperature	Snail Populations	Hatching Success	Dwarf Eggs	Eggs without Egg Cells	Eggs without Development	Polyvitelline Eggs
20°C	*R. rubiginosa*	94.72 (±0.23)	1.96 (±0.04)	1.12 (±0.08)	2.11 (±0.14)	0.09 (±0.16)
	L. natalensis (UKZN Pond)	87.62 (±0.74)	4.42 (±0.29)	3.33 (±0.40)	4.24 (±0.15)	0.39 (±0.09)
	L. natalensis (Greyville Pond)	87.10 (±0.52)	4.68 (±0.20)	3.80 (±0.38)	3.97 (±0.22)	0.45 (±0.11)
	P. acuta	96.88 (±0.21)	1.39 (±0.20)	0.63 (±0.22)	1.01 (±0.11)	0.10 (±0.09)
25°C	*R. rubiginosa*	96.90 (±0.37)	1.24 (±0.40)	0.78 (±0.16)	0.92 (±0.21)	0.15 (±0.14)
	L. natalensis (UKZN Pond)	92.60 (±0.51)	2.91 (±0.12)	1.63 (±0.17)	2.63 (±0.53)	0.23 (±0.09)
	L. natalensis (Greyville Pond)	92.71 (±0.41)	2.89 (±0.16)	1.91 (±0.35)	2.20 (±0.18)	0.29 (±0.13)
	P. acuta	97.86 (±0.18)	0.79 (±0.06)	0.47 (±0.07)	0.73 (±0.14)	0.16 (±0.05)
30°C	*R. rubiginosa*	95.59 (±0.42)	1.85 (±0.19)	0.73 (±0.35)	1.68 (±0.03)	0.15 (±0.16)
	L. natalensis (UKZN Pond)	90.89 (±0.28)	3.02 (±0.30)	2.76 (±0.46)	3.19 (±0.34)	0.15 (±0.14)
	L. natalensis (Greyville Pond)	90.52 (±1.03)	2.71 (±0.61)	3.23 (±0.23)	3.30 (±0.31)	0.25 (±0.09)
	P. acuta	96.17 (±0.31)	1.38 (±0.16)	0.78 (±0.10)	1.54 (±0.18)	0.13 (±0.13)

(b) Dwarf Eggs

Dwarf eggs were smaller in size than the normally proportioned viable eggs and were the most frequently occurring egg abnormality. Bondesen (1950) noted that they were generally found placed first or last in the row of normally proportioned eggs within the capsule, rarely occurring in the middle of the egg capsule and this was also seen in the present study.

For the dwarf egg abnormality (Table 5.2), all four snail populations had the highest percentage at 20°C. For the Greyville Pond population, the lowest occurrence was at 30°C, while the remaining snail populations had the lowest values for this abnormality at 25°C. In addition, at each of the three temperature treatments, *P. acuta* had the lowest mean percentage of dwarf eggs followed by *R. rubignosa* with marginally higher

percentages. *Lymnaea natalensis* from both the UKZN and Greyville Ponds displayed considerably higher rates of occurrence for this abnormality.

(c) Eggs without Egg Cells

In this abnormality, the eggs were of normal proportion but they lacked an egg cell. This could be possibly due to a cessation in the supply of egg cells to the albumen gland (Bondesen, 1950). From Table 5.2, a trend was observed similar to that for the dwarf eggs however at 30^{o}C *R. rubiginosa* and *P. acuta* had the lowest (0.73%) and highest (0.78%) percentages for this abnormality respectively. Again, both *R. rubiginosa* and *P. acuta* had lower percentages than the lymnaeids.

(d) Eggs without Development

Eggs showing this type of abnormality had no characteristic place in the egg-row but were often inserted between the normally developed eggs. For these eggs that did not develop, a trend was observed similar to the previously described abnormalities (Table 5.2). Snails maintained at 25^{o}C had the lowest proportion of undeveloped eggs. *Radix rubiginosa* and the lymnaeids had the highest rate of eggs without development at 20^{o}C, while *P. acuta* appeared to be influenced by the higher temperature of 30^{o}C, resulting in its highest recorded proportion of undeveloped eggs at this temperature.

(e) Polyvitelline Eggs

Usually there is one egg cell per egg, but occasionally more may be present. In most cases these were normally proportioned eggs that contained two egg cells (twins) and were most frequently found first or last in the egg row. This was the least common abnormality of all three temperature treatments (Table 5.2). For each of the three temperature treatments *L. natalensis* from both the UKZN and Greyville Ponds had a higher percentage occurrence for polyvitelline eggs than either *R. rubiginosa* or *P. acuta*.

Table 5.2 also shows that those populations producing the largest numbers of polyvitelline eggs also laid the largest number of eggs lacking egg cells.

The influence of temperature on the viable eggs (hatching success) and egg abnormalities is presented in Table 5.3.

Table 5.3: Kruskal-Wallis analysis of the influence of temperature on viable eggs (hatching success) and egg abnormalities for the four snail populations (n = 25). Probability values are two-tailed and significance was determined at $p < 0.05$.

Snail Populations	Viable Eggs	Dwarf Eggs	Eggs without Egg Cells	Eggs without Development	Polyvitelline Eggs
R. rubiginosa	0.119	0.259	0.484	0.029	0.907
L. natalensis (UKZN Pond)	0.004	0.010	0.012	0.029	0.597
L. natalensis (Greyville Pond)	0.004	0.001	0.009	0.038	0.545
P. acuta	0.389	0.209	0.552	0.298	0.848

The effect of temperature was significant for both *L. natalensis* populations (Table 5.3). The invasive snail, *P. acuta* showed no significant difference in the hatching success or egg abnormalities at the three temperatures (Table 5.3). This has importance since it indicates that the physid could be adapted to reproduce efficiently over a wide temperature range. *Radix rubiginosa* displayed a similar pattern to *P. acuta* but only eggs without development were influenced by temperature ($p = 0.029$). None of the other characters showed a significant temperature effect for *R. rubiginosa*. Polyvitelline eggs had non significant *p*-values ($p > 0.05$) for all snail populations (Table 5.3).

The effects of each of the test temperatures on egg viability and abnormality are presented in Tables 5.4 – 5.6. Due to the rare occurrence of polyvitelline eggs, there was no significant difference between populations for this abnormality (see Tables 5.4 – 5.6).

Table 5.4: Kruskal-Wallis analysis for viable eggs (hatching success) and egg abnormalities between snail populations maintained at 20°C (n = 25). Probability values are two-tailed and significance was determined at $p < 0.05$.

Snail Populations		Viable Eggs	Dwarf Eggs	Eggs without Egg Cells	Eggs without Development	Poly-vitelline Eggs
R. rubiginosa	*L. natalensis* (UKZN Pond)	<0.001	<0.001	<0.001	0.001	0.165
	L. natalensis (Greyville Pond)	<0.001	<0.001	<0.001	0.006	0.075
	P. acuta	0.029	0.101	0.262	0.017	0.921
L. natalensis (UKZN Pond)	*R. rubiginosa*	<0.001	<0.001	<0.001	0.001	0.165
	L. natalensis (Greyville Pond)	0.725	0.584	0.535	0.627	0.659
	P. acuta	<0.001	<0.001	<0.001	<0.001	0.130
L. natalensis (Greyville Pond)	*R. rubiginosa*	<0.001	<0.001	<0.001	0.006	0.075
	L. natalensis (UKZN Pond)	0.725	0.584	0.535	0.627	0.659
	P. acuta	<0.001	<0.001	<0.001	<0.001	0.053
P. acuta	*R. rubiginosa*	0.029	0.101	0.262	0.017	0.921
	L. natalensis (UKZN Pond)	<0.001	<0.001	<0.001	<0.001	0.130
	L. natalensis (Greyville Pond)	<0.001	<0.001	<0.001	<0.001	0.053

At 20°C there was no significant difference in the hatching success or egg abnormalities for either of the *L. natalensis* populations (Table 5.4). These lymnaeids were however, significantly different to both *R. rubiginosa* and *P. acuta*. Despite hatching success being significantly different, *R. rubiginosa* was similar to *P. acuta* for the percentage of dwarf eggs ($p > 0.101$) and eggs without egg cells ($p > 0.262$).

Snail populations maintained at 25°C (Table 5.5) and 30°C (Table 5.6) displayed similar trends. There was no significant difference between *R. rubiginosa* and *P. acuta* for any of the abnormalities (Tables 5.4 - 5.6). The two *L. natalensis* populations were similar to each other but both differed significantly from *R. rubiginosa* and *P. acuta* (Table 5.5).

Table 5.5: Kruskal-Wallis analysis for viable eggs (hatching success) and egg abnormalities between snail populations maintained at 25°C (n = 25). Probability values are two-tailed and significance was determined at $p < 0.05$.

Snail Population		Viable Eggs	Dwarf Eggs	Eggs without Egg Cells	Eggs without Development	Poly-vitelline Eggs
R. rubiginosa	*L. natalensis* (UKZN Pond)	<0.001	0.002	0.014	<0.001	0.509
	L. natalensis (Greyville Pond)	<0.001	<0.001	0.002	0.002	0.461
	P. acuta	0.091	0.153	0.347	0.545	1.000
L. natalensis (UKZN Pond)	*R. rubiginosa*	<0.001	0.002	0.014	0.001	0.509
	L. natalensis (greyville Pond)	0.740	0.660	0.584	0.682	0.923
	P. acuta	<0.001	<0.001	0.001	<0.001	0.509
L. natalensis (Greyville Pond)	*R. rubiginosa*	<0.001	<0.001	0.002	0.002	0.461
	L. natalensis (UKZN Pond)	0.740	0.660	0.584	0.682	0.923
	P. acuta	<0.001	<0.001	<0.001	<0.001	0.474
P. acuta	*R. rubiginosa*	0.091	0.153	0.347	0.545	1.000
	L. natalensis (UKZN Pond)	<0.001	<0.001	0.001	<0.001	0.509
	L. natalensis (Greyville Pond)	<0.001	<0.001	<0.001	<0.001	0.474

At 30°C, however, the UKZN Pond *L. natalensis* population was similar to *R. rubiginosa* ($p > 0.138$) and *P. acuta* ($p > 0.206$) for eggs without development (Table 5.6).

Table 5.6: Kruskal-Wallis analysis for viable eggs (hatching success) and egg abnormalities observed between snail populations maintained at 30°C (n = 25). Probability values are two-tailed and significance was determined at $p < 0.05$.

Snail Populations		Viable Eggs	Dwarf Eggs	Eggs without Egg Cells	Eggs without Development	Poly-vitelline Eggs
R. rubiginosa	*L. natalensis* (UKZN Pond)	<0.001	0.004	<0.001	0.138	0.944
	L. natalensis (Greyville Pond)	<0.001	0.017	<0.001	0.005	0.645
	P. acuta	0.417	0.707	0.648	0.533	1.000
L. natalensis (UKZN Pond)	*R. rubiginosa*	<0.001	0.004	<0.001	0.138	0.944
	L. natalensis (Greyville Pond)	0.356	0.559	0.134	0.303	0.708
	P. acuta	<0.001	0.001	0.000	0.206	0.927
L. natalensis (Greyville Pond)	*R. rubiginosa*	<0.001	0.017	<0.001	0.005	0.645
	L. natalensis (UKZN Pond)	0.356	0.559	0.134	0.303	0.708
	P. acuta	<0.001	0.003	<0.001	0.001	0.624
P. acuta	*R. rubiginosa*	0.417	0.707	0.648	0.533	1.000
	L. natalensis (UKZN Pond)	<0.001	0.001	<0.001	0.206	0.927
	L. natalensis (Greyville Pond)	<0.001	0.003	<0.001	0.001	0.624

5.3.3 Embryonic Development

Embryonic development takes place entirely within the eggs from which the young hatched as crawling juvenile snails. Development was direct and passed through cleavage, blastula, gastrula, trochophore and veliger stages. The egg capsules were transparent, allowing all phases in the embryonic development to be easily monitored. Development in *R. rubiginosa* was used to illustrate and review the sequence and morphology of the embryonic stages that typically occur in pulmonate snails (Figures 5.2 - 5.14).

(a) Egg Cell before Cleavage

Figure 5.2A is of a fertilised egg cell (zygote) before the first cleavage. The egg was isolecithal (nearly uniform distribution of the yolk through the cytoplasm of the egg). An accumulation of yolk material at the vegetative pole of the egg cell resulted in a yellowish colour. With the appearance of the two polar bodies (see arrow, Figure 5.2B), the uncleaved egg cell was observed to contain a relatively yolk free zone at the animal pole, thus giving this region a clear appearance.

(b) First Cleavage (2-cell stage)

During the cleavage period there was a rapid succession of cell division. During this period the size of the embryo does not change but the cleavage cells or blastomeres become smaller with each division (Balinsky, 1970). Initiation of the first cleavage was observed by an indentation (cleavage furrow) that appeared at the animal pole (see arrow, Figure 5.2C). Cleavage of the animal pole region was more rapid than that of the vegetative pole, resulting in the cleavage furrow gradually becoming predominant.

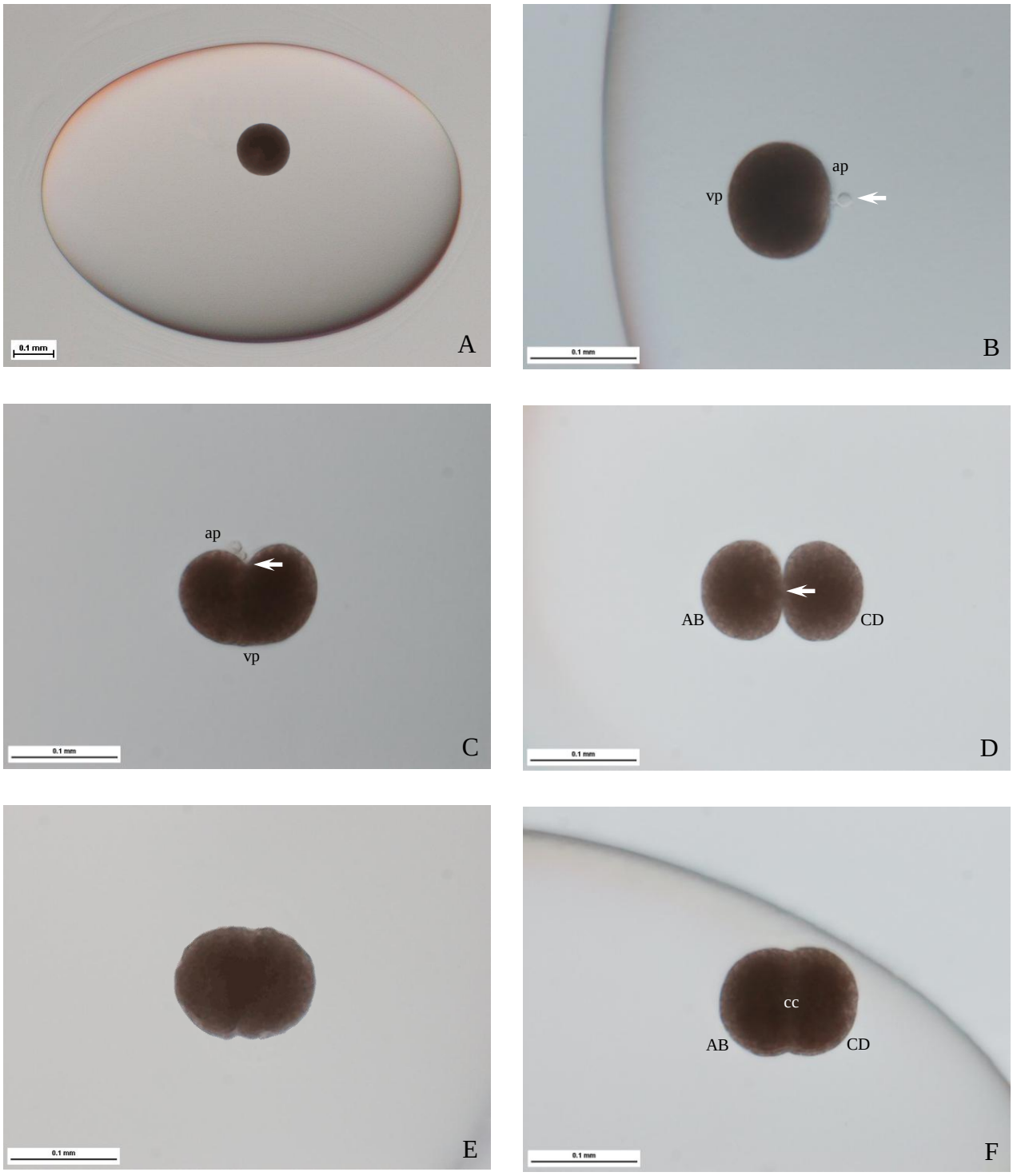


Figure 5.2: Sequence of the morphological characteristics occurring during the first cleavage (2-cell stage).

A – Fertilised egg cell before cleavage.

B – Uncleaved egg cell showing the animal and vegetative poles, with the polar body (see arrow).

C – Cleavage was initiated at the animal pole by the appearance of a cleavage furrow (see arrow).

D – First cleavage divided the egg cell into blastomeres AB and CD. The blastomeres were linked to each other by the cytoplasmic bridge (see arrow).
E – The blastomeres approached each other, increasing their surface contact.
F – The cleavage cavity was observed between the two blastomeres.
ap – animal pole; cc – cleavage cavity; vp – vegetative pole.

This plane of this first division passed through the main axis of the egg dividing it meridionally into two blastomeres AB and CD (Figure 5.2D). Both these blastomeres were rounded and linked to each other by only a small cytoplasmic bridge (see arrow, Figure 5.2D).

After this division the two blastomeres approached each other, increasing their surface contact. The spherical blastomeres were then closely applied to each other over a flat partition (Figure 5.2E), with the formation of a separating blastomeric membrane. This was also noted by Raven (1966) and Kawano *et al*. (1992). A cleavage cavity was observed between the two blstomeres and increased in size pushing the blastomeres apart (Figure 5.2F). According to Raven (1966) and Kawano *et al*. (1992) this cavity has as an osmo-regulatory function.

(c) Second Cleavage (4-cell stage)

The plane of the second division was also meridional and passed through the main axis, but it was at right angles to the first plane of cleavage. The blastomere AB divided into A and B, while CD divided into C and D, resulting in a four cell stage embryo (Figure 5.3). In this four cell stage the blastomeres A and C were situated slightly higher than blastomeres B and D. Blastomeres A and C met each other at the animal pole in the animal cross furrow while blastomeres B and D met each other at the vegetative pole in the vegetative cross furrow (Figure 5.3).

Blastomeres A, B, C and D, also called the quadrants of the embryo, follow an arrangement that may be dextral, i.e. dexiotropic (clockwise) or sinistral, i.e. laeotropic (anticlockwise).

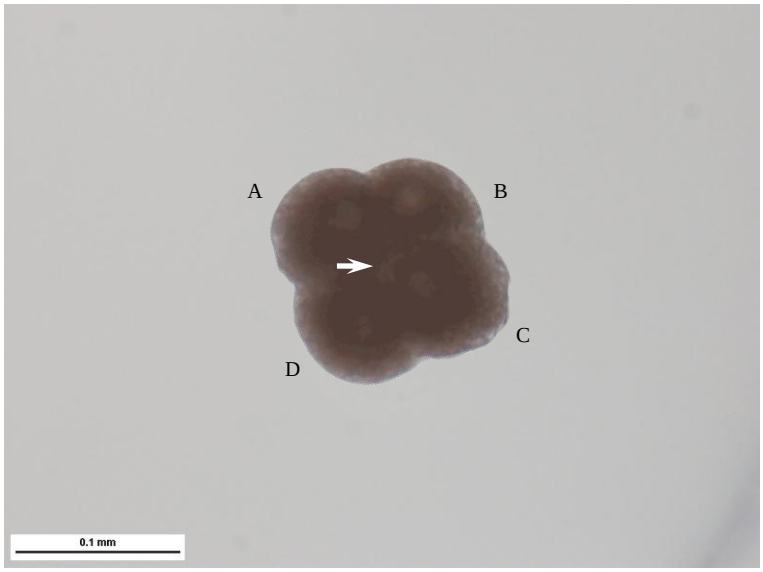


Figure 5.3: During the cleavage period of rapid cell division, the size of the embryo does not change, rather the cleavage cells or blastomeres become smaller with each division. In second cleavage (4-cell stage), the four large blastomeres A, B, C and D were of the same size and orientated side by side. The cleavage furrows linking the alternate blastomeres in the animal and vegetative poles of the embryo were observed. In addition the cleavage cavity (see arrow) reappeared in the central space formed by the furrows of the blastomeres. This regular succession of formation and extrusion of the cleavage cavity continues until the gastrula stage.

(d) Third Cleavage (8-cell stage)

The egg cells of both lymnaeids and physids displayed spiral holoblastic cleavage, despite the presence of a dense isolecithal yolk. The third cleavage occurred at right angles to the first two planes and to the main axis of the egg (Figure 5.4). This cleavage was therefore horizontal or parallel to the equator of the egg. It resulted in the eight cell stage that consisted of four smaller micromeres (1a – 1d) at the animal pole and four larger macromeres (1A – 1D) at the vegetative pole (Figure 5.4).

These two tiers were not orientated precisely one above the other and the upper tier of micromeres was shifted slightly in the same direction in relation to the lower tier of macromeres. This resulted in an orientation where the upper tier was over the junction between each of the vegetal macromeres (Figure 5.4). This unequal division coincided

with the end of division synchrony within the embryo. In further divisions both the micromeres and macromeres divided asynchronously. The cleavage cavity was no longer situated in the centre of the embryo but in the animal half between the micromeres and macromeres (Figure 5.4B).

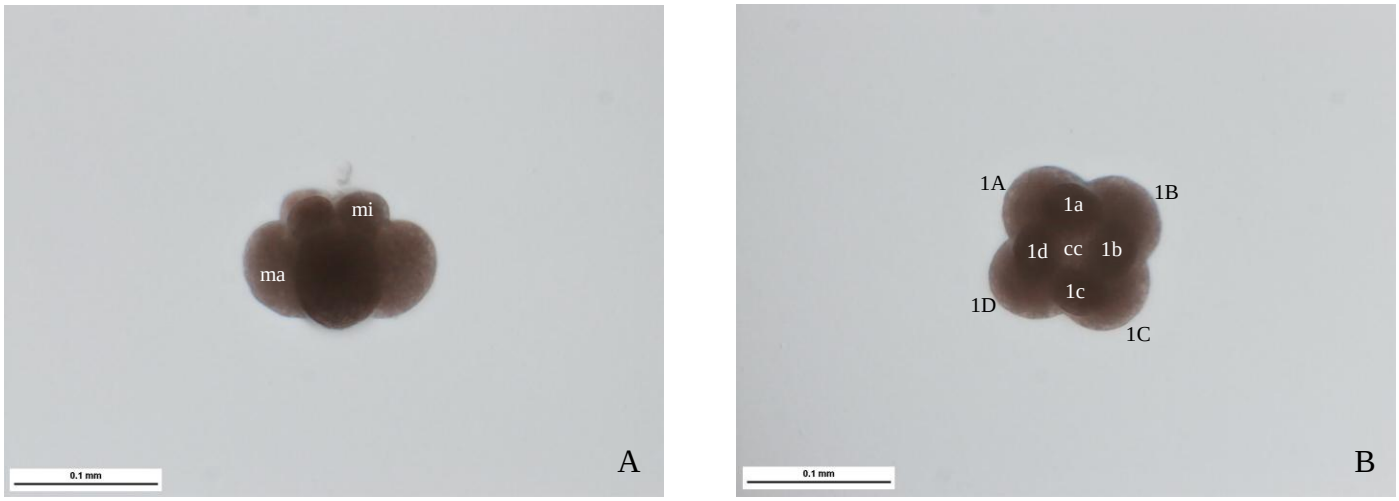


Figure 5.4: Third cleavage (8-cell stage) showing an upper tier of micromeres (1a – 1d) and the lower tier of macromeres (1A – 1D). The micromeres were orientated over the junction between each of the macromeres.
A – Lateral view of the 8 cell stage, showing the upper tier of micromeres and a lower tier of macromeres.
B – Third cleavage when viewed from the egg axis or from the animal pole. The cleavage cavity was observed in the animal half of the embryo.
cc – cleavage cavity; ma – macromeres; mi – micromeres.

A relationship exists between the types of cleavage of the egg in relation to the type of coiling of the adult shell. If, at third cleavage, the micromeres at the animal pole were displaced in a clockwise direction relative to the corresponding vegetal macromeres, the cleavage was dextral or dexiotropic and the adult snail had a dextrally coiled shell, e.g. Lymnaeidae. If the micromeres were displaced anticlockwise, the cleavage was laeotropic and the coiling of the shell was sinistral, e.g. Physidae (Raven, 1966; Balinsky, 1970; Verdonk and Biggelaar, 1983).

(e) Fourth Cleavage (16-cell stage)

The embryo continued to develop according to the general plan of molluscan spiral cleavage and divided faster at the animal pole than at the vegetative pole. According to the law of alternating cleavage, first formulated by Kofoid (1894), a regular succession of dexiotropic and laeotropic divisions followed each other (Verdonk and Biggelaar, 1983). At fourth cleavage, the dexiotropically-formed micromeres 1a – 1d and the macromeres 1A – 1D divided laeotropically (Figure 5.5).

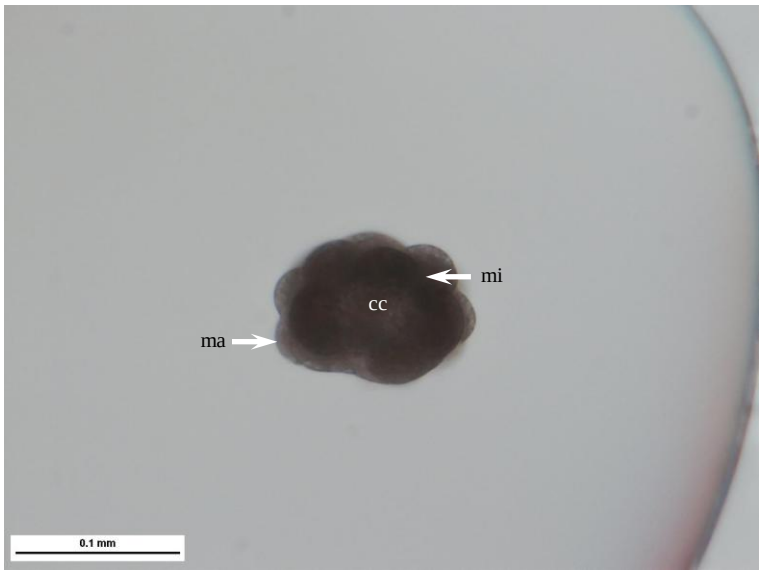


Figure 5.5: The fourth cleavage (16-cell stage). During this stage, the dexiotropically-formed micromeres and marocmeres divided laeotropically.
cc – cleavage cavity; ma – macromeres; mi – micromeres.

The micromeres at the animal pole divided into an upper and lower tier (Figure 5.5). The macromeres present at the vegetative pole divided into the second quartet of micromeres and macromeres. During each cycle the cleavage cavity swelled and thereafter collapsed. A gradual increase in the size of the cleavage cavity was also observed. Up to the 8 – 16 cell cleavage stage, collapse of the cleavage cavity always immediately preceded cell division and no cleavage cavity was present while the cells were dividing.

(f) Fifth Cleavage (24-cell stage)

At the fifth cleavage, dexiotropic division of the macromeres occurred (Figure 5.6). The micromeres of the first quartet did not divide and the embryo passed from a 16 into a 24 cell embryo.

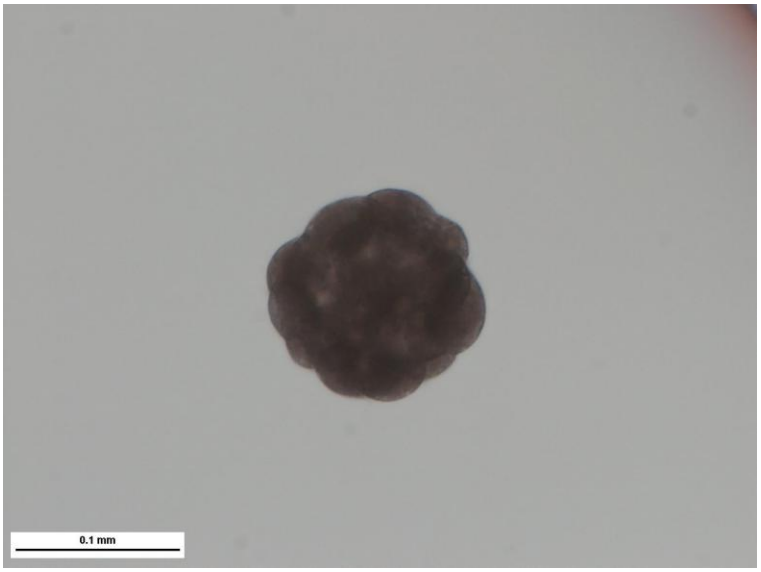


Figure 5.6: At the fifth cleavage (24-cell stage), dexiotropic division of the embryo occurred.

(g) Sixth Cleavage (64-cell stage)

The interval between the fifth and sixth cleavage is generally longer than the preceding intermitotic stages, and is therefore often called a resting stage (Verdonk and Biggelaar, 1983). This was also observed in the present study. The sixth cleavage was laeotropic and lead to the formation of a fourth quartet of micromeres and macromeres (Figure 5.7). At this 64 cell stage the regular character of spiral cleavage was lost and bilaterally symmetrical division took place.

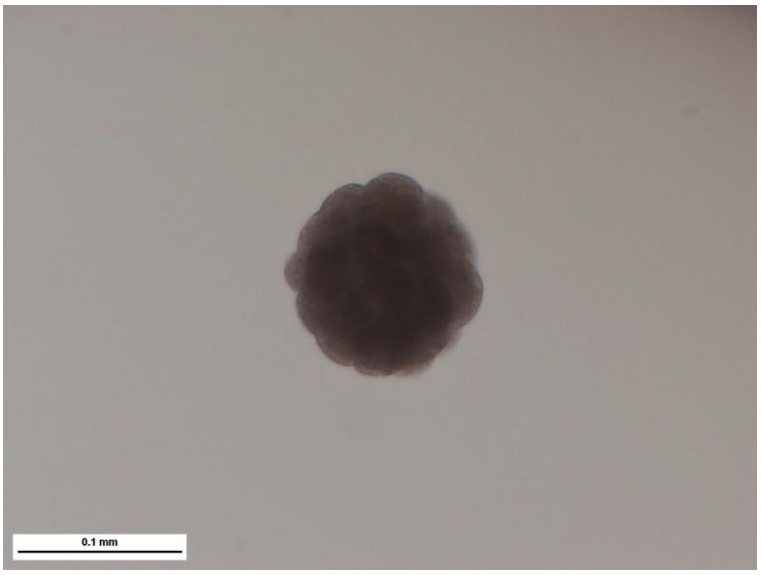


Figure 5.7: During the sixth cleavage (64-cell stage), the micromeres and macromeres divided laeotropically. Also, division synchrony was lost at this stage and bilaterally symmetrical division took place.

(h) Blastula Stage

This stage was characterised by the formation of a space between the animal and vegetative poles (Figure 5.8A). As the vegetal micromeres were larger than the animal micromeres, a well defined cavity (blastocoel) was situated in the animal half of the embryo. Later, a ball shaped layer of cells surrounded the blastocoel (see arrow, Figure 5.8B).

(i) Gastrula Stage

A flattening of the vegetative pole towards the animal pole marked the beginning of gastrulation (Figure 5.9A). This was followed by the invagination of the vegetative region towards the interior of the embryo and resulted in the formation of a spherical opening, the blastopore. As gastrulation proceeded the blastopore gradually became narrower (Figure 5.9B) and was later positioned ventrally. When gastrulation was complete, the embryo developed into a trochophore larva.

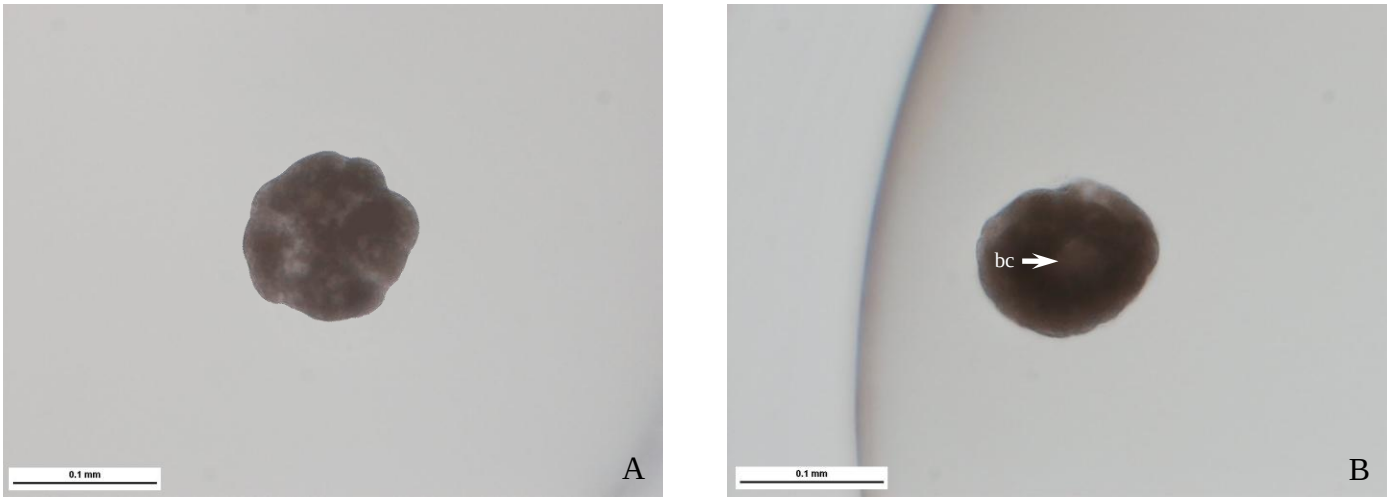


Figure 5.8: The blastula stage.

A – Embryo with space between the animal and vegetative poles.

B – Blastocoel surrounded by cells (see arrow).

bc – blastocoel.

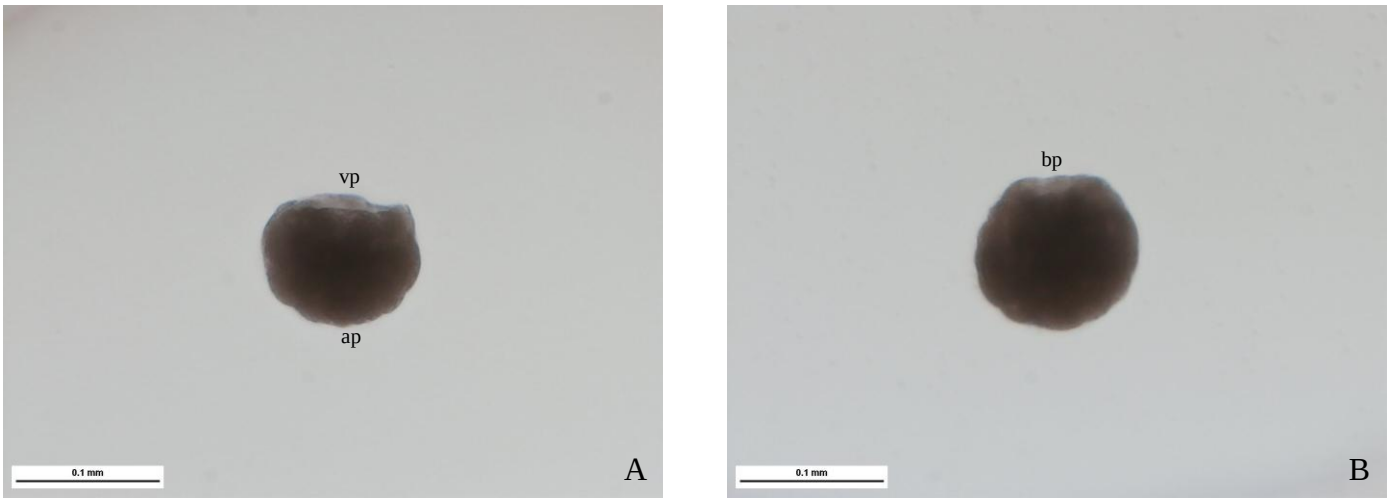


Figure 5.9: During the gastrula stage, the macromeres situated in the centre of the vegetal region changed in shape. They reduced their external surface area whereas the inner part widened forming a pit at the vegetal pole of the embryo. At the beginning of the gastrula stage, this pit (blastopore) was very wide. As gastrulation proceeded, the blastopore narrowed and closed from back to front until only a small opening remained.

A – Young gastrula with a wide pit (blastopore) forming at the vegetative pole.

B – Older gastrula with a reduced blastopore.

ap – animal pole; bp – blastopore; vp – vegetative pole.

(j) Early Trochophore Stage

The first larval stage developing from the gastrula stage was the trochophore and the embryo began to increase considerably in size. The trochophore was characterised by a distinct prototroch around the equator of the embryo, consisting of cells bearing cilia (Figure 5.10). The early trochophore stage was ovoid and revolved slowly and irregularly within the egg. These larval movements later became smoother and more rapid.

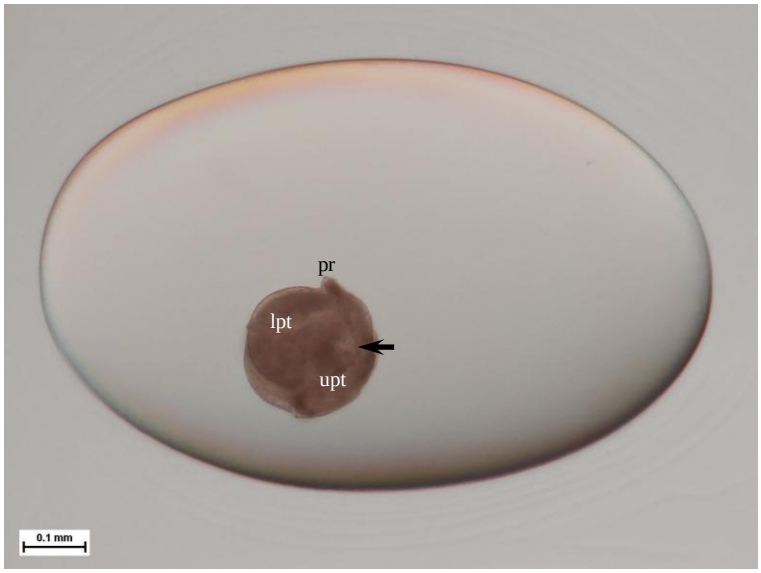


Figure 5.10: Trochophore embryo developed after gastrulation showing the prototroch, a band of ciliated cells, the prototroch, around the equator. The prototroch thus divided the trochophore into the upper pretrochal region and the lower posttrochal region. Smaller cilia also occurred over the rest of the larva. The blastopore moved towards the apical plate and developed into the mouth (see arrow).
lpt – lower posttrochal region; pr – prototroch; upt – upper pretrochal region.

These movements resulted in the formation of an anterior and posterior axis. The prototroch divided the larva into the upper or pretrochal region and the lower or posttrochal region (Figure 5.10). The pretrochal region was formed by a set of cells that gave rise to such larval structures as the apical plate, cephalic plate and the head vesicle. The blastopore moved towards the apical plate and developed into the mouth (see arrow, Figure 5.10). The apical plate, cephalic plate and head vesicle formed the head and

anterior region of the embryo (Raven, 1966; Kawano *et al.*, 1992; Creton *et al.*, 1993). The posttrochal region comprised of the stomodaeum, which formed the mouth and cells that formed the foot and shell gland (Raven, 1966; Kawano *et al.*, 1992; Creton *et al.*, 1993).

(k) Late Trochophore Stage

The late trochophore stage was characterised with the larva beginning to flatten and display a slightly elongated, kidney-like shape. In this stage, the cells responsible for the formation of the head and foot were seen at the anterior region (Figure 5.11). On the dorsal side opposite the mouth, the shell gland was represented by a thickening of the ectoderm (see arrow, Figure 5.11). A single lobe that represents the future foot is observed.

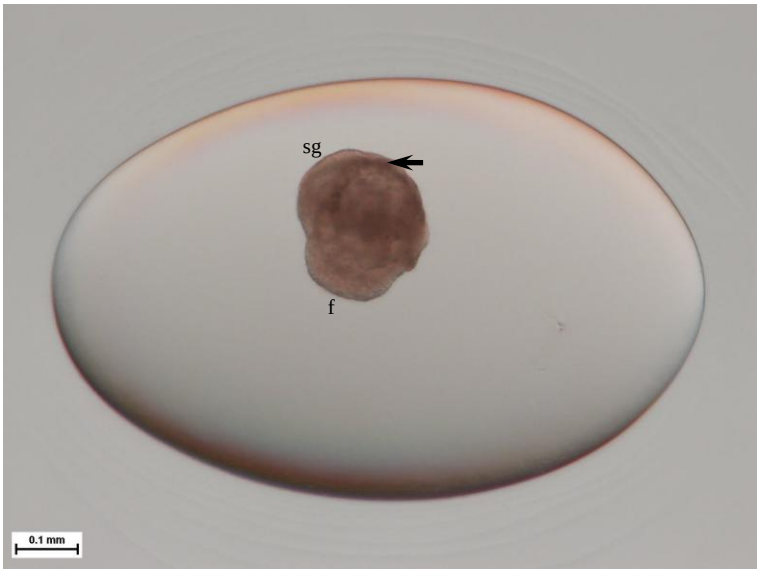


Figure 5.11: Late trochophore showing development of the distinct anterior region and visceral mass, indicated by the accumulation of large vacuolated cells. The formation of the shell gland, represented by a thickening of the ectoderm (see arrow) occurred at the posterior region where the shell spire later develops. No evidence of the shell was seen at this stage. During the late trochophore stage, the larva was still observed to move within the egg.
f – foot; sg – shell gland.

(l) Early Veliger Stage

The characteristic veliger was the larval stage following the earlier trochophore stage. Unlike the trochophore, the veliger had many of the characteristic features of the adult (Figures 5.12A and B). It possessed a muscular foot, eyes, tentacles, a fully developed mouth and a shell. The shell was observed at the edge of the mantle fold and partially covered the visceral mass (Figure 5.12). Structures such as the apical plate, head vesicle and prototroch formed a large part of the veliger's body.

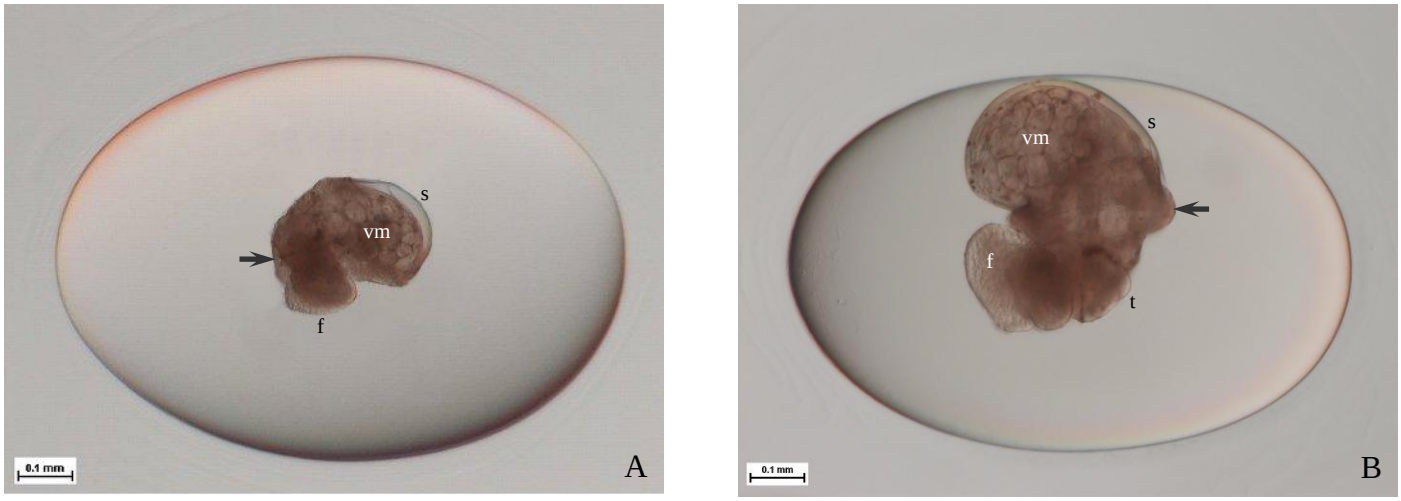


Figure 5.12: The early veliger stage showing the development of a distinct head, shell and foot.
A – The head region was distinguished with aggregations of ganglia forming the eyes (see arrow). The posterior region of the visceral mass was covered by an embryonic shell.
B – The embryo exhibited considerable coordination of movement by use of the muscular foot. Elevations of the tentacle regions were observed as well as a raised ridge marking the margin of the mantle (see arrow). This ridge encircled the lower part of the visceral mass.
f – foot; s – shell; t – tentacles; vm – visceral mass.

(m) Late Veliger Stage

At the late veliger stage, the embryonic shell had greatly increased in size to cover the entire visceral mass. It also started to coil. This unpigmented larval shell began to take on a light brown colour that was more evident just prior to hatching. It was separated from the head region by the mantle ridge that was depressed to form the mantle cavity (see arrow, Figure 5.13). The eyes and tentacles in the pretrochal region were well developed (Figure 5.13). The mouth was situated at the boundary between the head and foot. In the posttrochal region the foot had grown in size and was much more differentiated. A distinct regular heart beat was noted and the embryo still moved actively within the egg.

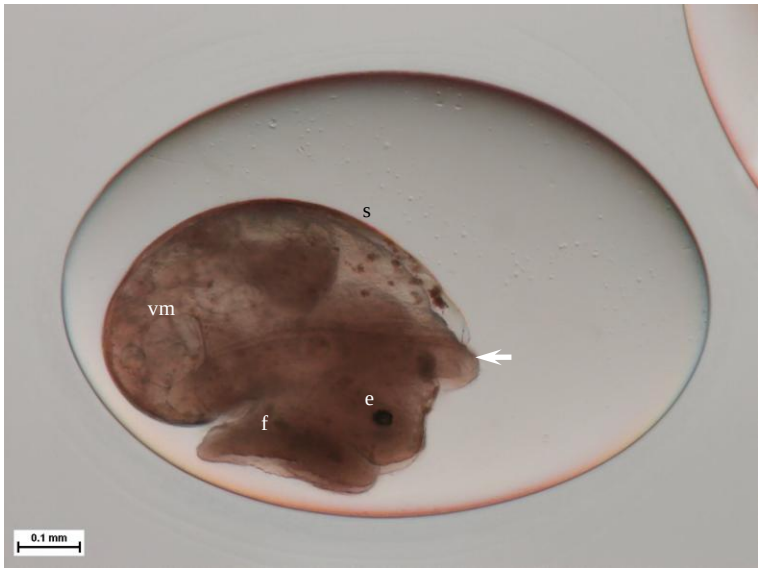


Figure 5.13: The late veliger embryo. The ridge (see arrow) marking the edge of the mantle clearly differentiated the visceral mass from the muscular foot region. The shell was now larger and covered the entire visceral mass. At the anterior head region, the eyes were more prominent. The heart and other organs of the visceral mass were also visible.
e – eyes; f – foot; s – shell; vm – visceral mass.

(n) Hatching Stage

During the stage immediately prior to hatching, the embryo increased in size to fill most of the egg (Figure 5.14). During this period the embryo was observed creeping over and rasping off the inner surface of the egg. Thus, by the time the animal filled the egg, the inner membrane was reduced to a very thin envelope through which the young snail could be seen scraping into the cavity of the egg capsule. Once hatching had occurred, the snail moved freely in the gelatinous matrix of the egg capsule and escaped through an opening made in its outer wall or through an opening made by another snail.

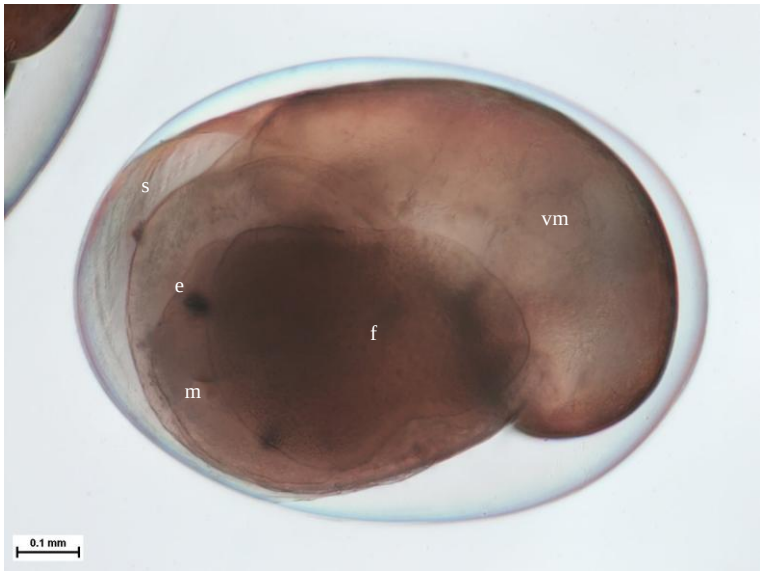


Figure 5.14: Young snail shortly before hatching. The snail occupied the entire interior of the egg. Continued thinning of the internal egg membrane by the movements of the shell and foot resulted in its rupture.
e – eyes; f – foot; m – mouth; s – shell; vm – visceral mass.

5.3.3.1 Analysis of the incubation period, mean size and mean geometric growth rate

Assessments of the incubation period, mean size and mean geometric growth rate of the different embryonic stages of development are presented in Tables 5.7 – 5.10 for the four snail populations.

The time needed for development prior to hatching decreases with increasing temperature (Tables 5.7 – 5.10). *Physa acuta* had the shortest incubation period at all three temperatures (Table 5.10). For this species the longest embryonic development period was 9 days at 20^{o}C, while at 30^{o}C development was completed in 5 days. The incubation period for *R. rubiginosa* (Table 5.7) was marginally longer than *P. acuta*, but followed a similar trend (incubation period increasing with decreasing temperature). In addition, it was comparatively shorter than *L. natalensis* from the UKZN and Greyville Ponds (Tables 5.8 and 5.9 respectively).

Table 5.1 showed that *R. rubiginosa* had the largest mean egg dimensions (0.90 x 0.73 mm), followed by the Greyville and UKZN Pond *L. natalensis*. The eggs of *P. acuta* were the smallest (0.75 x 0.62 mm). An assessment of the mean size at hatching (Tables 5.7 - 5.10) indicated that *R. rubiginosa* had the largest values for all three temperatures, followed by the Greyville and UKZN pond lymnaeids. Despite *P. acuta* having the shortest incubation period, this snail had the smallest mean size at hatching for all three temperatures, a characteristic attributed to its smaller egg size (Table 5.1). The mean size at hatching was therefore limited by the size of the egg and the quantity of albumen.

Despite higher temperatures resulting in the acceleration of development and a shorter incubation period, the final size of the hatched embryo was smallest at 30^{o}C for all four snail populations. At 20^{o}C however, all four snail populations had the largest mean sizes for hatched embryos (Tables 5.7 – 5.10).

Embryonic growth followed the same general pattern observed for most other metazoans (De Witt, 1954). As previously stated, the size of the embryo does not change during cleavage (Tables 5.7 – 5.10) because as the total number of cells increased, the cleavage cells or blastomeres became smaller with each division (Balinsky, 1970). A rapid increase in embryo size followed the gastrula stage of development however and the fastest rate of growth occurred during the trochophore and veliger stages.

Growth rate was influenced by temperature and the length of the incubation period. At each of the three temperatures, the growth rates of the four snail populations were highest 30^{o}C, leading to a shorter incubation period. *Lymnaea natalensis* from both the UKZN and Greyville Ponds (Tables 5.8 and 5.9 respectively) had growth rates that were similar, except that the UKZN population had a higher growth rate for most developmental stages at 30^{o}C. Also, the two *L. natalensis* populations had lower growth rates for most developmental stages when compared to *R. rubiginosa* (Table 5.7) and *P. acuta* (Table 5.10). A comparison of the growth rates from Tables 5.7 and 5.10 indicated that *P. acuta* had higher growth rates than *R. rubiginosa* except for the early veliger stage where *R. rubiginosa* had higher growth rates.

The influence of temperature on the size of the embryonic stages of development is presented in Table 5.11.

Table 5.11: Kruskal-Wallis analysis for the size of the embryonic stages of development for the four snail populations as a function of temperature (n = 15). Probability values are two-tailed and significance was determined at $p < 0.05$.

Snail Populations	Cleavage	Blastula	Gastrula	Early Trochophore	Late Trochophore	Early Veliger	Late Veliger	Hatching
R. rubiginosa	1.000	0.915	0.092	0.000	0.012	0.001	0.000	0.021
L. natalensis (UKZN Pond)	1.000	0.114	0.200	0.034	0.566	0.022	0.017	0.001
L. natalensis (Greyville Pond)	1.000	0.258	0.275	0.007	0.003	0.002	0.003	0.000
P. acuta	1.000	0.890	0.607	0.001	0.003	0.000	0.019	0.098

For each of the four snail populations, the size of the early stages of embryonic development (cleavage, blastula and gastrula) was not significantly influenced by temperature, as indicated by the *p*-values ($p > 0.05$). However, the size of the embryo for the remaining developmental stages (early trochophore, late trochophore, early veliger, late veliger and hatching) was significantly temperature dependent (Table 5.11). For the lymnaeids from the UKZN pond, the size of the late trochophore embryo was not influenced by temperature ($p = 0.566$). Also, *P. acuta* displayed no significant difference ($p = 0.098$) in the size of the hatched embryos, a characteristic that indicates a wide tolerance in temperature (Table 5.11).

5.4 Discussion

5.4.1 Egg Capsule Descriptions

In the freshwater basommatophorans encapsulation of eggs has evolved with internal fertilisation as a means of enhancing reproductive success. Egg capsules provide a source of nutrition and protection for embryos from such environmental stresses as predation (Pechenik, 1979; Perron, 1981), bacterial attack (Lord, 1986), osmotic changes (Pechenik, 1982; Hawkins and Hutchinson, 1988), desiccation (Spight, 1977; Pechenik, 1978), temperature shock (Spight, 1977; Pechenik, 1986) and current action (Perron, 1981).

Further, the stressful nature of the freshwater habitat has necessitated the suppression of the planktonic stages. Because the entire development process is direct rather than planktonic and takes place within the egg, more perivitelline fluid for feeding and more space for development are needed. Hence there must be a greater emphasis on egg size than on numbers of eggs (Fioroni and Schmekel, 1976; Calow, 1978; Geraerts and Joosse, 1984).

From the morphological comparisons of egg capsules for each of the four snail populations (Table 5.1), *P. acuta* produced the smallest eggs and egg capsules. Despite

this, *P. acuta* produced the second highest mean number of eggs per capsule (clutch size), a trait that must enhance its invasiveness. *Radix rubiginosa* had the largest egg capsules and produced the most eggs per capsule as well as the largest eggs. This presents an advantage in that a larger amount of albumen is at the disposal of the embryo, allowing for more rapid development before hatching. *Physa acuta* had the fastest development time (5 - 9 days).

5.4.2 Viability of Eggs and Egg Abnormalities

Even when still *in ovo*, the embryo may be subjected to various influences that interfere with its normal development. Following Bondesen (1950), these included either intrinsic factors (lack of fertilisation, quantity of albumen) or extrinsic factors (crushing of the egg cell during the formation of the capsule, crowding as a result of abnormal development of a neighbouring embryo, changes in the physical and / or chemical environment).

(a) Hatching Success

The mean hatching success (mean percentage viable eggs) increased from 20^{o}C to 25^{o}C, with a decrease recorded for all populations at 30^{o}C (Table 5.2). *Physa acuta* had the highest hatching success at all three temperatures (96.17 - 97.86%). This was due to its high reproductive capacity which Appleton and Brackenbury (1998) believed to contribute to the invasive success of *P. acuta*. Of importance was the observation that *R. rubiginosa* had the second highest mean hatching success (94.72 - 96.90%), only marginally lower than *P. acuta*.

(b) Dwarf Eggs

Dwarf eggs were smaller than normally proportioned eggs and associated with the starting and stopping of a bout of oviposition. If the snail was disturbed during oviposition, it was probable that the albumen production was reduced and could cease (Bondesen, 1950). Dwarf eggs may therefore contain an insufficient quantity of albumen

for nourishment of the embryo. Thus, as the embryo developed, it used a larger proportion of the available albumen causing a decrease in egg size (Bondesen, 1950). If growth of the embryo was restricted by the egg being too small, the embryo would invariably grow deformed and might cease development early.

In Table 5.2 it was noted that *L. natalensis* from both the study ponds had the highest percentage of dwarf eggs (2.71 - 4.68%). These snails also displayed the highest percentage of eggs without development (2.20 - 4.24%). *Radix rubiginosa* had a considerably lower percentage of dwarf eggs than either of the *L. natalensis* populations. This is important since the embryos of *R. rubiginosa* would not be influenced by the smaller size of the egg restricting growth and also by the lesser quantity of albumen present. The embryos of *R. rubiginosa* would therefore be better equipped for survival in comparison to the two populations of *L. natalensis*. The invasive, *P. acuta* had the lowest occurrence of dwarf eggs (0.79 - 1.39%) followed by *R. rubiginosa* (1.24 - 1.96%).

(c) Eggs without Egg Cells

This abnormality affected the growth and development of neighbouring embryos in the egg capsule. As previously stated, due to the growth requirements of the embryo in normally proportioned eggs, large quantities of albumen were used causing a decrease in egg size. As the eggs decreased in size, those possessing no egg cells were rounded off and pressed against the normally proportioned eggs causing it to become considerably narrowed. This resulted in the embryo attaining an abnormal, elongated shape. According to Bondesen (1950), a few of the empty eggs can also be used as extra nourishment for the embryos. This was also seen in the present study where empty egg envelopes were seen. From Table 5.2, *R. rubiginosa* had a considerably lower percentage of eggs without development than both the *L. natalensis* populations. *Physa acuta* had the lowest occurrence of this abnormality at 20^{o}C (0.63%) and 25^{o}C (0.47%). At 30^{o}C, however, *R. rubiginosa* (0.73%) had a percentage lower than *P. acuta* (0.78%).

Eggs with this type of abnormality would restrict the growth and development of neighbouring embryos. This would have an unfavourable effect on the viability of the hatched young when they are exposed to the environmental conditions after emerging. It is therefore suggested that the adjacent eggs would not be adapted for survival and might perish soon after emergence from the egg capsule.

(d) Eggs Without Development

It is possible that in a given egg mass some eggs did not develop because they had not been fertilised previously. However, it was also observed that normal development until the trochophore stage was not always followed by the hatching of juveniles. This condition of embryos not completing development could be attributed to a lack of energy required for radular movements after the rapid utilisation of energy stores during embryonic development (Vaughn, 1953; Costil, 1997). Imai (1937) observed that hatching was influenced by the mechanical action of the radula and that the longer hatching was delayed (due to lower temperatures), the weaker the action of the radula became resulting in the cessation of development (Vaughn, 1953). From Table 5.2, it was noted that snail populations maintained at 20^{o}C had a higher occurrence of eggs without development. *Physa acuta* (0.73 - 1.54%) and *R. rubiginosa* (0.92 - 2.11%) had considerably fewer eggs that did not develop than both *L. natalensis* populations (2.20 - 4.24%).

(e) Polyvitelline Eggs

Polyvitelline eggs were among the rarer abnormalities (< 1%) and the least frequently recorded at the three temperatures (Table 5.2). These included eggs with two or more egg cells and were most frequently observed first or last in the egg row. Of these the majority were situated at the terminal end of the egg capsule and consequently deposited last on the substratum. It was therefore hypothesised that polyvitelline eggs could be attributed to a disruption in the oviposition process (Bondesen, 1950; De Witt, 1954).

According to Crabb (1927) and De Witt (1954), it was probable that active contraction of the gizzard during feeding inhibited the passage of ova down the hermaphrodite duct to such an extent that several ova accumulated in the duct's enlarged part. During a period of reduced activity of the gizzard all the ova could pass into the uterus at the same time. In this way, a number of egg cells were enveloped by the albumen and the egg membrane which would normally cover a single egg cell (De Witt, 1954).

For each of the three experimental temperatures *P. acuta* (0.10 - 0.16%) and *R. rubiginosa* (0.09 - 0.15%) had a lower occurrence of polyvitelline eggs than either of the *L. natalensis* populations (0.15 - 0.45%). Also from Table 5.2, it was noted that there was a tendency for those snails producing the most polyvitelline eggs to also lay the largest numbers of eggs lacking egg cells. The overall effect of both polyvitelline eggs and eggs lacking egg cells at the three temperatures accounted for 0.63 - 0.91% in *P. acuta* and 0.88 - 1.21% in *R. rubiginosa*. The *L. natalensis* population from both the UKZN Pond and the Greyville Pond had an occurrence of 1.86 - 3.72% and 2.20 - 4.25% respectively for both these abnormalities.

It is therefore evident that the individual egg abnormalities comprise a small proportion of the total egg output. However, collectively these abnormalities comprise a higher proportion and would therefore reduce the hatching success of the four snail populations. At each of the three temperatures *R. rubiginosa* and *P. acuta* had lower frequencies in comparison to the two *L. natalensis* populations. This therefore implies that a greater proportion of the eggs produced by *R. rubiginosa* and *P. acuta* are likely to hatch. From Table 5.3 it was evident that temperature had a significant effect on the hatching success and egg abnormalities (dwarf eggs, eggs without egg cells and eggs without development) for both the *L. natalensis* populations. *Radix rubiginosa* and *P. acuta* showed no such difference in hatching success or egg abnormalities (Table 5.3). This has special importance since it indicates that both *R. rubiginosa* and *P. acuta* are adapted to reproduce maximally over a wider range in temperature.

5.4.3 Embryological Development

Temperature induces developmental responses in body size, with larger individuals developing at lower temperatures (Atkinson, 1994; Partridge and French, 1996; Chown and Gaston, 1999; Fischer *et al*., 2003). In a study of the growth of the larval shell of *Lymnaea japonica* under controlled temperature conditions, Imai (1937) noted that higher temperatures resulted in the acceleration of development but the hatching size of this snail species was smaller (Noland and Carriker, 1946). According to Imai (1937), embryos developing at lower temperatures had larger sizes at hatching. It was therefore suggested that the animals grew and developed more rapidly at higher temperatures, using larger amounts of energy and were thus of a smaller size (Imai, 1937; Vaughn, 1953). In this study all four snail populations had larger mean hatching sizes at 20^{o}C (Tables 5.7 - 5.10) than at 25^{o}C or 30^{o}C.

For freshwater snails, a sufficient quantity of nourishment is of importance for the growth and survival of the embryos and that the size of the snail at hatching depends largely upon this. It was noted that *R. rubiginosa* had both the largest mean egg size (Table 5.1) and the largest embryo size at hatching (Table 5.7). These were comparatively higher than both *L. natalensis* populations and *P. acuta*.

The embryonic development period decreased with increasing temperature (Tables 5.7 – 5.10). *Physa acuta* had the shortest incubation period at all three temperatures followed marginally by *R. rubiginosa*. *Lymnaea natalensis* had the longest incubation period at all temperatures.

Growth rates (Tables 5.7 - 5.10) were not constant for successive periods of observation and were dependent on both the temperature and incubation time. At higher temperatures, faster growth rates occurred due to an acceleration of development leading to shorter incubation periods. A rapid increase in embryo size followed gastrulation with the fastest growth rates recorded for the trochophore and veliger stages (Tables 5.7 – 5.10). These faster growth rates were attributed to the development of specialised

structures and organs at these stages. Following this period, the rate decreased as the young snail neared the maximum prenatal size.

In summary, the temperatures at which snails lay eggs and the relationship between egg development and temperature have important implications for the distribution and potential spread of an introduced species (Harris and Charleston, 1977). The ascendancy in abundance and distribution of introduced species generally results in a decline of indigenous species (Zukowski and Walker, 2008).

Unsurprisingly, *P. acuta* proved to have a higher fecundity, a shorter incubation period and wider temperature tolerances than the other species tested. These are characteristics that enhance its success as an invasive. On the basis of this argument, its high adaptability to changing environmental factors such as temperature, is in agreement with the fact that *P. acuta* is more widespread in South Africa than *L. natalensis* (Hamilton-Attwell *et al*., 1970; De Kock *et al*., 1989; Brackenbury and Appleton, 1993; Appleton and Brackenbury, 1998; Appleton, 2003; De Kock and Wolmarans, 2007).

This has important implications for *R. rubiginosa*, since this snail displayed similar characteristics to *P. acuta*, and exhibited greater adaptability and survival over a wider temperature range than *L. natalensis*. Several authors (Dondero and Lim, 1976; Mienis 1986) have also commented that it is easy to breed *R. rubiginosa* in aquaria and this was found to be the case in the present study as well. The indigenous *L. natalensis* is not as easy to breed and this raises the question, "If *R. rubiginosa* spreads in South Africa, will it become invasive, probably at the expense of *L. natalensis*?"

Radix rubiginosa appears to have a number of reproductive advantages over *L. natalensis*. It produces larger egg capsules with more eggs per capsule. The eggs of *R. rubiginosa* are larger, allowing the developing embryo more space and nourishment, increasing its adaptability and tolerance of environmental conditions after emerging. The hatching success of *R. rubiginosa* are also high and only marginally lower than the already invasive *P. acuta*. Also, *R. rubiginosa* has a much higher hatching success than

L. natalensis at all temperatures tested. The lower frequency of egg abnormalities in both *R. rubiginosa* and *P. acuta* further implies that a greater proportion of the eggs produced by these introduced species are likely to hatch.

In addition a shorter incubation period, larger size at hatching, faster rates of growth and development as well as the potential for rapid development over an extended range of temperatures are all advantageous to *R. rubiginosa*. Therefore the competitive superiority of *R. rubiginosa* over the indigenous *L. natalensis* with respect to their reproductive potential presents a situation that allows for rapid spread of the former and possibly the extirpation of the latter.

Table 5.7: Incubation period, mean size and mean geometric growth rate (GGR) of the different embryonic stages of development for *R. rubiginosa* at the three temperature treatments (n = 15). Mean sizes of embryo are given in millimetres (± standard deviation). Gaps in the incubation periods between embryonic stages are due to an absence of synchronous development.

Embryonic Stage	**20°C**			**25°C**			**30°C**		
	Cumulative Incubation Period	**Mean Size (mm)**	**GGR (mm/day)**	**Cumulative Incubation Period**	**Mean Size (mm)**	**GGR (mm/day)**	**Cumulative Incubation Period**	**Mean Size (mm)**	**GGR (mm/day)**
First Cleavage	4 – 6 hours	0.122 (±0.016)		3 – 4 hours	0.122 (±0.016)		2 – 3 hours	0.122 (±0.016)	
Second Cleavage	6 – 7 hours	0.122 (±0.016)		4 – 5 hours	0.122 (±0.016)		3 – 4 hours	0.122 (±0.016)	
Third Cleavage	8 -10 hours	0.122 (±0.016)		5 – 6 hours	0.122 (±0.016)		4 – 5 hours	0.122 (±0.016)	
Fourth Cleavage	10 – 11 hours	0.122 (±0.016)		7 – 8 hours	0.122 (±0.016)		6 – 7 hours	0.122 (±0.016)	
Fifth Cleavage	12 – 14 hours	0.122 (±0.016)		9 – 12 hours	0.122 (±0.016)		7 – 8 hours	0.122 (±0.016)	
Sixth Cleavage	18 – 22 hours	0.122 (±0.016)		16 – 20 hours	0.122 (±0.016)		11 – 14 hours	0.122 (±0.016)	
Blastula	23 – 30 hours	0.127 (±0.006)	0.039	21 – 24 hours	0.126 (±0.007)	0.037	16 – 19 hours	0.125 (±0.008)	0.040
Gastrula	31 – 48 hours	0.139 (±0.006)	0.274	24 – 48 hours	0.135 (±0.015)	0.570	20 – 36 hours	0.131 (±0.010)	0.264
Early Trochophore	3 – 4.5 days	0.221 (±0.014)	0.273	2 – 3 days	0.169 (±0.009)	0.224	1.5 – 2 days	0.165 (±0.008)	0.345
Late Trochophore	4.5 – 6 days	0.347 (±0.031)	0.299	3 – 4 days	0.325 (±0.018)	0.653	2 – 3 days	0.316 (±0.020)	1.304
Early Veliger	6 – 8 days	0.593 (±0.031)	0.358	4 – 5 days	0.559 (±0.038)	0.541	3 – 4 days	0.540 (±0.032)	0.536
Late Veliger	8 – 11 days	0.735 (±0.033)	0.107	6 – 7 days	0.716 (±0.050)	0.124	4 – 5 days	0.659 (±0.025)	0.200
Hatching	12 days	0.875 (±0.041)	0.044	8 days	0.845 (±0.026)	0.083	6 days	0.838 (±0.014)	0.120

Table 5.8: Incubation period, mean size and mean geometric growth rate (GGR) of the different embryonic stages of development for *L. natalensis* (UKZN pond) at the three temperature treatments (n = 15). Mean sizes of embryo are given in millimetres (± standard deviation). Gaps in the incubation periods between embryonic stages are due to an absence of synchronous development.

Embryonic Stage	**20°C**			**25°C**			**30°C**		
	Cumulative Incubation Period	**Mean Size (mm)**	**GGR (mm/day)**	**Cumulative Incubation Period**	**Mean Size (mm)**	**GGR (mm/day)**	**Cumulative Incubation Period**	**Mean Size (mm)**	**GGR (mm/day)**
First Cleavage	4 – 6 hours	0.116 (±0.007)		3 – 4 hours	0.116 (±0.007)		2 – 3 hours	0.116 (±0.007)	
Second Cleavage	7 – 8 hours	0.116 (±0.007)		5 – 6 hours	0.116 (±0.007)		4 – 5 hours	0.116 (±0.007)	
Third Cleavage	9 – 10 hours	0.116 (±0.007)		6 – 7 hours	0.116 (±0.007)		5 – 6 hours	0.116 (±0.007)	
Fourth Cleavage	10 – 13 hours	0.116 (±0.007)		8 – 10 hours	0.116 (±0.007)		6 – 8 hours	0.116 (±0.007)	
Fifth Cleavage	14 – 18 hours	0.116 (±0.007)		12 – 14 hours	0.116 (±0.007)		10 – 12 hours	0.116 (±0.007)	
Sixth Cleavage	26 – 34 hours	0.116 (±0.007)		20 – 28 hours	0.116 (±0.007)		16 – 19 hours	0.116 (±0.007)	
Blastula	38 – 60 hours	0.125 (±0.008)	0.049	30 – 48 hours	0.123 (±0.006)	0.049	21 – 36 hours	0.120 (±0.007)	0.039
Gastrula	2.5 – 3.5 days	0.134 (±0.009)	0.073	2 – 3 days	0.131 (±0.009)	0.084	1.5 – 2 days	0.128 (±0.009)	0.103
Early Trochophore	3.5 – 5 days	0.175 (±0.014)	0.269	3 – 4 days	0.167 (±0.017)	0.242	2 – 3 days	0.162 (±0.009)	0.471
Late Trochophore	5 – 7 days	0.324 (±0.029)	0.410	4 – 6 days	0.315 (±0.022)	0.632	3 – 5 days	0.313 (±0.027)	0.660
Early Veliger	7 – 10 days	0.495 (±0.030)	0.212	6 – 8 days	0.480 (±0.021)	0.211	5 – 6 days	0.455 (±0.049)	0.187
Late Veliger	10 – 13 days	0.671 (±0.022)	0.101	8 – 11 days	0.641 (±0.055)	0.144	6 – 8 days	0.617 (±0.054)	0.304
Hatching	14 days	0.816 (±0.036)	0.049	12 days	0.783 (±0.024)	0.050	9 days	0.744 (±0.070)	0.063

Table 5.9: Incubation period, mean size and mean geometric growth rate (GGR) of the different embryonic stages of development for *L. natalensis* (Greyville pond) at the three temperature treatments (n = 15). Mean sizes of embryo are given in millimetres (± standard deviation). Gaps in the incubation periods between embryonic stages are due to an absence of synchronous development.

Embryonic Stage	**20°C**			**25°C**			**30°C**		
	Cumulative Incubation Period	**Mean Size (mm)**	**GGR (mm/day)**	**Cumulative Incubation Period**	**Mean Size (mm)**	**GGR (mm/day)**	**Cumulative Incubation Period**	**Mean Size (mm)**	**GGR (mm/day)**
First Cleavage	4 – 6 hours	0.119 (±0.006)		3 – 4 hours	0.119 (±0.006)		2 – 3 hours	0.119 (±0.006)	
Second Cleavage	7 – 8 hours	0.119 (±0.006)		5 – 6 hours	0.119 (±0.006)		3 – 4 hours	0.119 (±0.006)	
Third Cleavage	10 – 11 hours	0.119 (±0.006)		6 – 7 hours	0.119 (±0.006)		4 – 6 hours	0.119 (±0.006)	
Fourth Cleavage	12 – 14 hours	0.119 (±0.006)		8 – 10 hours	0.119 (±0.006)		6 – 8 hours	0.119 (±0.006)	
Fifth Cleavage	14 – 20 hours	0.119 (±0.006)		12 – 14 hours	0.119 (±0.006)		10 – 12 hours	0.119 (±0.006)	
Sixth Cleavage	28 – 48 hours	0.119 (±0.006)		20 – 28 hours	0.119 (±0.006)		15 – 20 hours	0.119 (±0.006)	
Blastula	2 – 3 days	0.126 (±0.009)	0.030	1.25 – 2 days	0.125 (±0.009)	0.040	1 – 1.75 days	0.121 (±0.007)	0.017
Gastrula	3 – 4 days	0.135 (±0.015)	0.071	2 – 3 days	0.134 (±0.011)	0.096	2 – 2.5 days	0.129 (±0.009)	0.069
Early Trochophore	4 – 6 days	0.178 (±0.006)	0.274	3 – 4 days	0.175 (±0.005)	0.269	2.5 – 3.5 days	0.169 (±0.008)	0.539
Late Trochophore	6 – 8 days	0.336 (±0.023)	0.318	4 – 6 days	0.319 (±0.018)	0.600	3.5 – 5.5 days	0.309 (±0.014)	0.601
Early Veliger	8 – 11 days	0.512 (±0.025)	0.211	6 – 8 days	0.484 (±0.039)	0.208	5.5 – 7 days	0.463 (±0.037)	0.202
Late Veliger	11 – 14 days	0.705 (±0.030)	0.107	8 – 11 days	0.677 (±0.050)	0.168	7 – 9 days	0.649 (±0.039)	0.225
Hatching	15 days	0.845 (±0.023)	0.045	12 days	0.821 (±0.027)	0.048	10 days	0.793 (±0.029)	0.067

Table 5.10: Incubation period, mean size and mean geometric growth rate (GGR) of the different embryonic stages of development for *P. acuta* at the three temperature treatments (n = 15). Mean sizes of embryo are given in millimetres (± standard deviation). Gaps in the incubation periods between embryonic stages are due to an absence of synchronous development.

Embryonic Stage	**20°C**			**25°C**			**30°C**		
	Cumulative Incubation Period	**Mean Size (mm)**	**GGR (mm/day)**	**Cumulative Incubation Period**	**Mean Size (mm)**	**GGR (mm/day)**	**Cumulative Incubation Period**	**Mean Size (mm)**	**GGR (mm/day)**
First Cleavage	3 – 4 hours	0.112 (±0.008)		2 – 3 hours	0.112 (±0.008)		2 – 3 hours	0.112 (±0.008)	
Second Cleavage	5 – 6 hours	0.112 (±0.008)		4 – 5 hours	0.112 (±0.008)		3 – 4 hours	0.112 (±0.008)	
Third Cleavage	6 – 8 hours	0.112 (±0.008)		5 – 6 hours	0.112 (±0.008)		4 – 5 hours	0.112 (±0.008)	
Fourth Cleavage	9 – 11 hours	0.112 (±0.008)		7 – 9 hours	0.112 (±0.008)		5 – 6 hours	0.112 (±0.008)	
Fifth Cleavage	16 – 18 hours	0.112 (±0.008)		11 – 14 hours	0.112 (±0.008)		7 – 8 hours	0.112 (±0.008)	
Sixth Cleavage	21 – 26 hours	0.112 (±0.008)		18 – 23 hours	0.112 (±0.008)		12 – 14 hours	0.112 (±0.008)	
Blastula	28 – 35 hours	0.118 (±0.012)	0.045	24 – 28 hours	0.117 (±0.010)	0.053	15 – 19 hours	0.116 (±0.007)	0.061
Gastrula	36 – 48 hours	0.129 (±0.011)	0.277	29 – 36 hours	0.125 (±0.012)	0.317	20 – 24 hours	0.125 (±0.100)	0.353
Early Trochophore	2 – 3 days	0.174 (±0.009)	0.594	1.5 – 2 days	0.163 (±0.012)	0.895	1 – 1.5 days	0.154 (±0.014)	1.241
Late Trochophore	3 – 5 days	0.307 (±0.017)	0.567	2 – 3 days	0.291 (±0.012)	1.161	1.5 – 2.5 days	0.283 (±0.015)	1.219
Early Veliger	5 – 6 days	0.455 (±0.027)	0.197	3 – 4 days	0.417 (±0.009)	0.362	2.5 – 3.5 days	0.403 (±0.013)	0.353
Late Veliger	6 – 8 days	0.581 (±0.035)	0.246	4 – 5 days	0.556 (±0.039)	0.287	3.5 – 4.5 days	0.537 (±0.038)	0.287
Hatching	9 days	0.726 (±0.022)	0.074	6 days	0.719 (±0.016)	0.128	5 days	0.710 (±0.017)	0.186

6

Growth and Life History Parameters of *Radix rubiginosa*, *Lymnaea natalensis* and *Physa acuta*

6.1 Introduction

Characteristics of an introduced species are critical to both the success and impact of the invader (Lodge, 1993) and the identification of these characteristics allows recommendations to be made on how to evaluate the invasive potential of these new introductions (Kolar and Lodge, 2001). Factors that have been suggested as predictors of invasive success include abundance and wide range in the native habitat, a broad physiological tolerance (euryhalinity and eurythermy), rapid growth and life history parameters such as short generation times, high fecundity and high intrinsic rate of natural increase (Rejmanek and Richardson, 1996; Williamson and Fitter, 1996; Barrat-Segretain *et al*., 2002; Moyle and Marchetti, 2006; Keller *et al*., 2007; Suarez and Tsutsui, 2008). Therefore the extent to which a species can spread, as well as its success in a given environment, are thought to be mainly due to those factors which may limit growth, reproduction and survival (Sastry, 1979; Borcherding, 1995).

Among ecological factors, temperature is frequently limiting for the growth, distribution and population dynamics of organisms (Vaidya and Nagabhushanam, 1978; Raut *et al*., 1992; Abdul Aziz and Raut, 1996). The influence of such factors is more obvious in poikilothermic organisms like molluscs where the body temperature approximates the temperature of the environment. In general, research on the effects of different temperatures on snails has dealt with geographic distribution, relative abundance and physiological responses, particularly growth and reproduction (McDonald, 1973). In these animals, temperature has an influence on the metabolism and life history

characteristics, and may act as an important selection pressure (Hardy, 1979; Lam and Calow, 1990).

Furthermore, knowledge of the effect of temperature on life history traits is important in assessing interactions between various species and also in determining the potential invasiveness of introduced species (Brittain and Campbell, 1991). A study on interactions between species also requires knowledge of the parameters that may influence the competitive process. Among these growth, survival and fecundity are the most relevant and are necessary components of life tables (Barbosa *et al*., 1992).

The construction of life tables is an important analytical tool for understanding the characteristics and dynamics of a snail population. Parameters such as age specific survival and fecundity are of particular interest because of their close relationship to fitness itself (Charlesworth, 1980; Stearns, 1992; Lessells, 1991; Partridge *et al*., 1995). Therefore, life history analysis has a range of applications that include measuring the growth capacity of a population (Southwood and Henderson, 2000), examining the dynamics of colonising or invading species, predicting life history evolution and estimating extinction probabilities (Granett *et al*., 1983; Trichilo and Leigh, 1985; Carey *et al*., 1988; Omer *et al*., 1992; McPeek and Kalisz, 1993; Vargas *et al*., 1997).

A key parameter of life tables is the intrinsic rate of natural increase (r_m). This biometric parameter is a comprehensive numeric evaluation of a specific environmental factor in terms of the survival rate and fecundity of cohorts of a species (De Kock, 1973; De Kock and van Eeden, 1976; Prinsloo and van Eeden, 1976). The intrinsic rate of natural increase further indicates whether a population will increase, decrease or remain static in numbers. It has been used as a measure of population growth (Lotka, 1943) and as a quantitative expression of the relative favorability of experimental conditions in studies on population dynamics and limiting factors (Birch, 1948; Leslie and Park, 1949; Root, 1960; De Kock, 1973; Prinsloo and van Eeden, 1976).

Since r_m summarises the reproductive capacity in terms of the speed of development, survival rate and fecundity, comparison of r_m values enables an assessment of the potential rate of population increase of a species under specified conditions (Force and Messenger, 1964; De Kock, 1973; Prinsloo and van Eeden, 1976).

The aim of this study was to assess the invasiveness of *Radix rubiginosa* (Michelin, 1831) in relation to the already established invader, the North American Physidae, *Physa acuta* Draparnaud, 1805 and the indigenous *Lymnaea natalensis* Krauss, 1848. This investigation assessed the growth and various life history parameters of the three species and the role of temperature in causing observed differences. This was particularly important in view of the success of *P. acuta* as an invader over a wide geographical and altitudinal range in South Africa (Hamilton-Attwell *et al*., 1970; De Kock *et al*., 1989; Brackenbury and Appleton, 1993; Appleton and Brackenbury, 1998; Appleton, 2003; De Kock and Wolmarans, 2007). The survival rate, fecundity and intrinsic rate of natural increase were then comparatively analysed to allow for a more precise focus on the specific attributes likely to enhance the ability of *R. rubiginosa* to spread.

6.2 Methodology

The methodology for the culture and maintenance of the four snail populations was described in Chapter 5.2. Individual F_1 snails (progeny raised in the laboratory from field collected snails) were pre-acclimated to the experimental temperatures. The F_1 snails were then utilised for the cohorts to study the effects of temperature on the growth and life history parameters for each of the four populations.

At the start of the experiment, a total of 30 individuals with shell lengths measuring approximately 1 mm were randomly selected from each population and placed into aerated aquaria (45 x 29 x 12 cm), containing six litres of dechlorinated tap water (water depth approximately 6 cm). The aquaria were maintained at three constant temperatures (20^{o}C, 25^{o}C and 30^{o}C) and a 12:12 (L:D) photoperiod. To prevent the snails from escaping, the aquaria were covered with a net. Each trial was done in triplicate.

The snails were fed lettuce daily and the amount was adjusted to the maximum daily consumption. In addition the diet was supplemented *ad libitium* with Tetramin® (a commercially available brand of fish food) and Marcus Rohrer® Spirulina (2 tablets crushed into a fine powder and added to the water). Leaves of *Nymphaea nouchali* and *Marsilea* sp. were placed into the aquaria to provide resting and egg laying sites for the snails. The aquaria water was changed weekly and the faeces were removed daily.

6.2.1 Growth

Shell length was measured every week to the nearest 0.01 mm, using either a graticuled eyepiece on a stereomicroscope or a Vernier caliper. Growth was expressed as an increase in the mean shell length. The relationship between size and age for consecutive intervals of growth was used to estimate the rate of growth and maximal size of a given population (Walford, 1946; Ricker, 1975).

An estimate of theoretical growth for the four snail populations was obtained by fitting the observed data to the von Bertalanffy growth equation:

$$L_t = L_\infty (1 - e^{-K(t - t0)})$$

where,
L_t = shell length at age t
L_∞ = asymptotic length
K = growth coefficient (the exponential rate at which length approaches the asymptotic length)
t_0 = time at which length is theoretically zero on the modeled growth trajectory

The von Bertalanffy growth parameters K and L_∞ were estimated by the Ford-Walford method, where L_t at time t along the abscissa is plotted against L_{t+1} at time t + 1 along the ordinate, using as unit time the interval between two successive observations. The coordinate at which the regression line intersects the line y = x is L_∞. The slope of the regression line is e^{-K} (i.e. the growth coefficient is equal to the negative natural logarithm of the slope of the Ford-Walford plot). Using the L_∞ value obtained, a resulting plot of ln (L_∞ - L_t) against t was constructed. The intercept of that regression with the ordinate (i.e. where t = 0) was used to calculate t_0:

$$t_0 = (\text{intercept} - \ln L_\infty) / K$$

The slope of the regression line and therefore K, depend upon the interval between successive measurements. This is allowed for in the von Bertalanffy growth equation.

6.2.2 Survival, Fecundity and Life History Parameters

To evaluate the response of the four snail populations to the three temperatures, the standard statistics for life tables were calculated. Methods of calculations discussed by Birch (1948); Andrewartha and Birch (1954); De Kock (1973); Southwood (1978) and

Gutierrez *et al*. (2000) were followed. A week was taken as the unit time for the life tables. This was because it was envisaged that this time period might reflect subtle differences in the survival, fecundity and growth of the four snail populations, which might otherwise be diluted and unnoticed if recorded over a forthnight, as was done by other workers (Shiff, 1964; De Kock, 1973). As *R. rubiginosa*, *L. natalensis* and *P. acuta* are hermaphroditic, each individual was a potential female and fecundity was calculated from the total number of viable eggs per individual. Therefore data analysis was carried out following the single sex method and the life tables were constructed using the following parameters.

(a) Age (x)

The life table was divided into uniform durations of one week. All data collected in the period of one age group were taken as if they occurred at the mid-point of that age group.

(b) Survival rate (l_x)

This parameter monitored the survival of a cohort through time, i.e. it is the proportion of individuals surviving at age x in relation to the initial size of the cohort. The lack of response to mechanical stimuli was considered a criterion of death. Snails judged as dead were kept under surveillance for 24 hours before the final mortality figure was noted. All dead snails were removed from the aquaria after calculations were completed. Survivorship was recorded weekly until the last snail died. The duration of mortality was also assessed over the period between the first and last mortality.

(c) Fecundity (m_x)

Fecundity was presented as the average number of eggs laid per snail over one week for each of the three temperature treatments. Eggs were removed from the aquaria every day to control for any effects associated with a reduced surface area available for ovipositon.

(d) Gross reproductive rate (GRR)

This parameter represents the sum of eggs produced per snail over the entire duration of the study: $GRR = \Sigma m_x$

(e) The net reproductive rate (R_o)

The net reproductive rate (R_o) represents the actual mean replacement per generation which included the effect of the cohort's survival rate during reproduction. It is the sum of the product of l_x and m_x in each group: $Ro = \Sigma l_x m_x$

(f) Intrinsic rate of natural increase (r_m)

This biometric parameter is a comprehensive numeric evaluation of a specific environmental factor in terms of the survival rate and fecundity of cohorts of a species and indicated whether a population will increase, decrease or remain static in numbers. The intrinsic rate of natural increase (r_m) represents the maximum population growth under the particular conditions of the experiment. The r_m is calculated by iteration of the formula (Lotka, 1925): $\Sigma e^{-rm\,x} l_x m_x = 1$

Since the intrinsic rate of natural increase summarises the reproductive capacity of the four snail populations in terms of the speed of development, survival rate and fecundity, comparison of the respective r_m values enables an accurate assessment of the influence of temperature on the potential rate of population increase of these species (Force and Messenger, 1964).

(g) Mean generation time (T)

The mean duration of a generation can be considered as the time between one birth and the next one. This parameter was calculated using the formula: $T = \log_e R_o / r_m$

where, R_o is the net reproductive rate and r_m is the intrinsic rate of natural increase.

(h) Finite rate of increase (λ)

This parameter indicates the number of individuals of the future cohort that will replace one individual of the existing cohort (number of times the population will multiply itself per unit time). Therefore the finite rate of increase was the natural antilogarithm of the innate capacity of increase: $\lambda = e^{rm}$

To compare the effects of temperature on the survival and fecundity within and between the four snail populations, a non-parametric Mann-Whitney-U test was performed. This test was selected because the data were not normally distributed even after transformation. The life history parameters were subjected to an analysis of variance (ANOVA) and the means were separated using the Tukey HSD. Statistical analyses were performed using SPSS 11.0.1 (SPSS Inc.). Probability values are two-tailed and significance was determined at $p < 0.05$.

6.3 Results

6.3.1 Growth

The growth curves of the four snail populations maintained at the three temperatures are presented in Figures 6.1 - 6.3. At 20°C, *Radix rubiginosa* reached the largest observed shell length (18.20 mm) at 29 weeks (Figure 6.1). *Lymnaea natalensis* from the UKZN Pond attained a maximum shell length of 16.25 mm at 22 weeks while the Greyville Pond population had its largest shell length of 15.65 mm at 19 weeks. The smaller-sized *Physa acuta* attained a maximum shell length of 11.80 mm but this population exhibited the greatest longevity of 35 weeks (Figure 6.1).

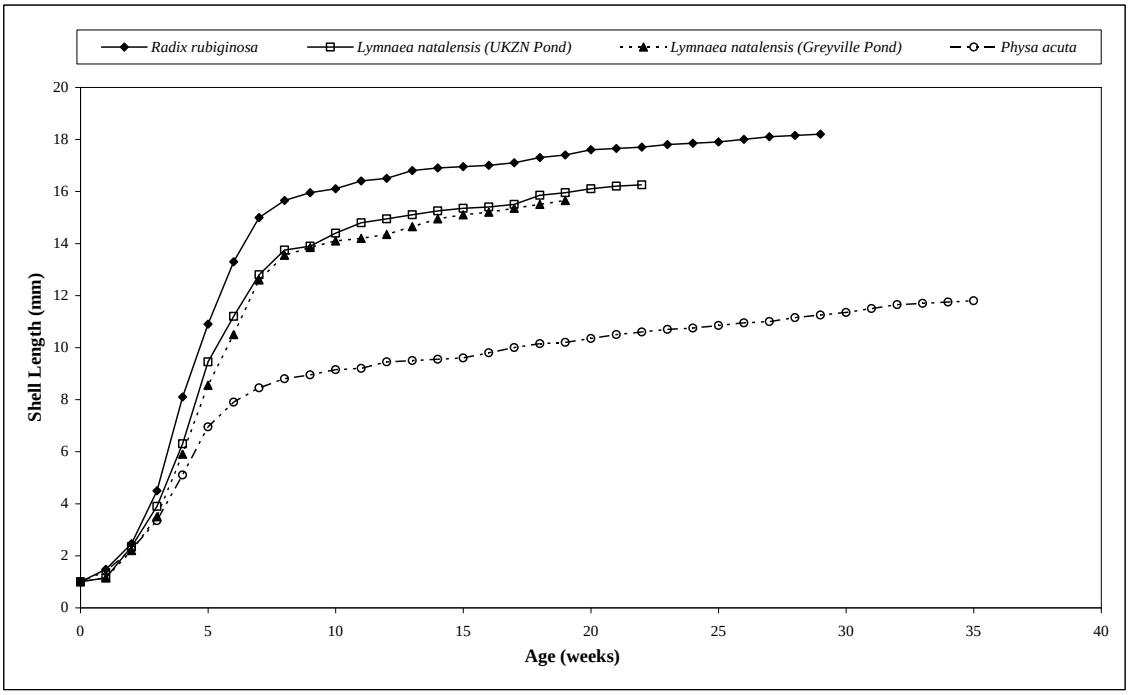


Figure 6.1: Growth expressed as an increase in the mean shell length for *R. rubiginosa*, *L. natalensis* (UKZN and Greyville Ponds) and *P. acuta* at 20°C (n = 90).

At 25°C (Figure 6.2), *R. rubiginosa* attained a maximum shell length of 17.40 mm, while *L. natalensis* from both the UKZN and Greyville Ponds had maximum shell lengths of 13.95 mm and 13.35 mm respectively. The maximum shell length recorded by *P. acuta* at this temperature was 10.20 mm.

Figure 6.2 also shows that *P. acuta* had the greatest longevity of 28 weeks followed by *R. rubiginosa* (25 weeks) and finally *L. natalensis* (18 weeks for the UKZN Pond population and 16 weeks for the Greyville Pond population).

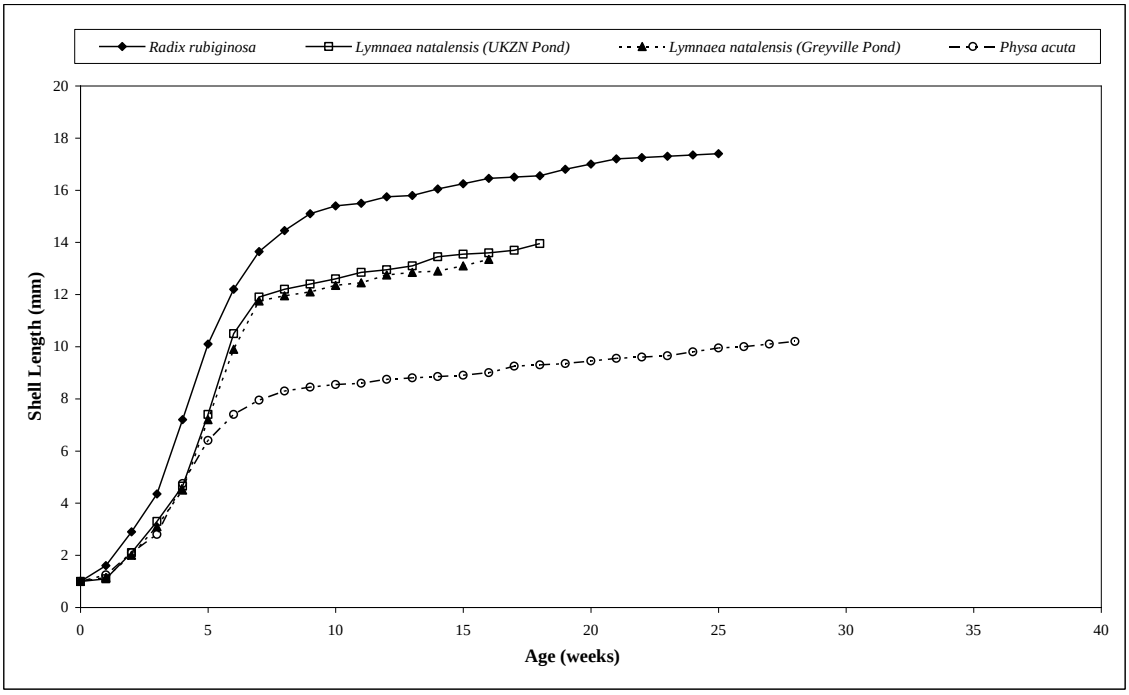


Figure 6.2: Growth expressed as an increase in the mean shell length for *R. rubiginosa*, *L. natalensis* (UKZN and Greyville Ponds) and *P. acuta* at 25°C (n = 90).

At 30°C, the pattern for shell growth and longevity was similar to that observed at 20°C and 25°C. Figure 6.3 shows that the maximum shell length attained by *R. rubiginosa*, *L. natalensis* (UKZN Pond), *L. natalensis* (Greyville Pond) and *P. acuta* were 16.10 mm, 11.25 mm, 11.65 mm and 9.20 mm respectively.

At 30°C (Figure 6.3) *P. acuta* attained the greatest longevity (22 weeks) followed by *R. rubiginosa* (21 weeks). The longevity of *L. natalensis* at 30°C was 12 weeks (UKZN Pond) and 14 weeks (Greyville Pond).

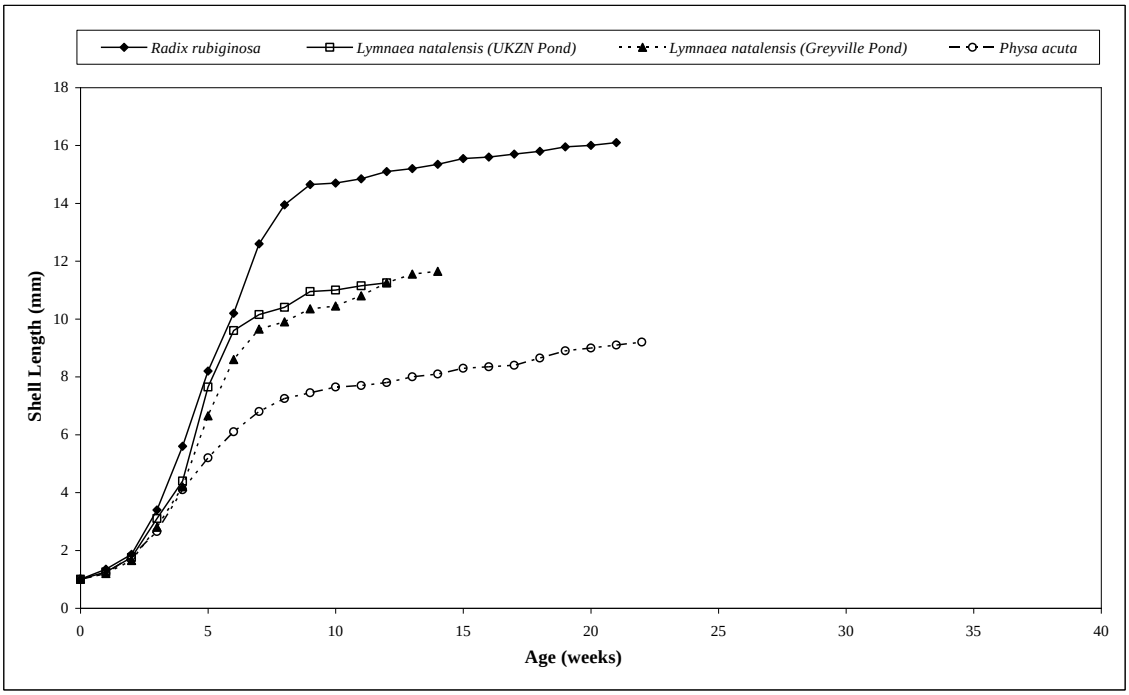


Figure 6.3: Growth expressed as an increase in the mean shell length for *R. rubiginosa*, *L. natalensis* (UKZN and Greyville Ponds) and *P. acuta* at 30°C (n = 90).

Overall, Figures 6.1 - 6.3 show that as temperature increased the maximum mean shell length decreased for all four populations investigated. Table 6.1 presents the von Bertalanffy growth parameters that were estimated using the Ford-Walford method to describe the growth patterns for the four snail populations. The growth coefficient (K) is a measure of the growth rate of a population. The higher the value of K, the more rapid the growth (expressed as an increase in the mean shell length). Figures 6.4 - 6.6 show the von Bertalanffy growth curves on pages 148 - 150.

From Table 6.1 it is apparent that for all populations the highest and lowest K values recorded were at 20°C and 30°C respectively, i.e. the growth was fastest at 20°C but less so at 30°C. At 20°C, *R. rubiginosa* had the highest K value (0.1316), followed by *P.*

acuta (0.1236), *L. natalensis* from the UKZN Pond (0.1128) and finally *L. natalensis* from the Greyville Pond (0.1017). At 25°C and 30°C, *P. acuta* had the highest K value, followed by *R. rubiginosa*, the UKZN Pond *L. natalensis* population and finally the *L. natalensis* population from the Greyville Pond.

Importantly, *R. rubiginosa* exhibited a growth coefficient (K) at 20°C that was 1.06 times larger than that of *P. acuta*, while those exhibited by *P. acuta* at 25°C and 30°C were 1.05 and 1.04 times greater than that of *R. rubiginosa* (Table 6.1). The growth coefficients for both *R. rubiginosa* and *P. acuta* were 1.10 - 1.29, 1.19 - 1.35 and 1.11 - 1.36 times larger than those of the two *L. natalensis* populations at 20°C, 25°C and 30°C respectively.

Table 6.1: Estimated growth parameters of the four snail populations maintained at the three temperatures. These parameters were calculated using the Ford-Walford method.

Temperature	**Snail Populations**	**K**	**L_∞**	**t_0**
20°C	*R. rubiginosa*	0.1316	19.00	-3.5289
	L. natalensis (UKZN Pond)	0.1128	18.00	-1.8652
	L. natalensis (Greyville Pond)	0.1017	18.50	-1.2566
	P. acuta	0.1236	12.00	-1.9005
25°C	*R. rubiginosa*	0.1234	18.75	-2.3598
	L. natalensis (UKZN Pond)	0.1034	16.50	-1.1934
	L. natalensis (Greyville Pond)	0.0960	17.00	-0.8667
	P. acuta	0.1299	10.50	-2.5512
30°C	*R. rubiginosa*	0.1014	18.50	-1.7535
	L. natalensis (UKZN Pond)	0.0910	16.25	-0.1989
	L. natalensis (Greyville Pond)	0.0779	17.50	-0.4134
	P. acuta	0.1059	10.25	-1.7686

K - the growth coefficient; L_∞ - the asymptotic length; t_0 - the time at which shell length is theoretically zero.

The asymptotic length (L_∞) was largest at 20°C and the smallest at 30°C, with the exception of *L. natalensis* (Greyville Pond) that displayed its smallest L_∞ at 25°C (Table 6.1). For each of the three temperatures, *R. rubiginosa* had the largest L_∞, followed by the UKZN Pond and Greyville Pond populations of *L. natalensis* and finally *P. acuta*. The L_∞ (Table 6.1) was always larger than the observed maximum shell length (Figures 6.1 - 6.3).

The time at which shell length is theoretically zero (t_0) displayed a similar pattern to the growth coefficient (K). From Table 6.1, it is seen that the highest and lowest t_0 values were at 20°C and 30°C respectively. For each of the three temperatures *R. rubiginosa* and *P. acuta* exhibited the highest t_0 values, while the lowest t_0 values were recorded for both the *L. natalensis* populations (Table 6.1).

The estimated growth parameters and the observed age for the four populations were fitted into the von Bertalanffy growth equation, resulting in the growth curves shown in Figures 6.4 - 6.6.

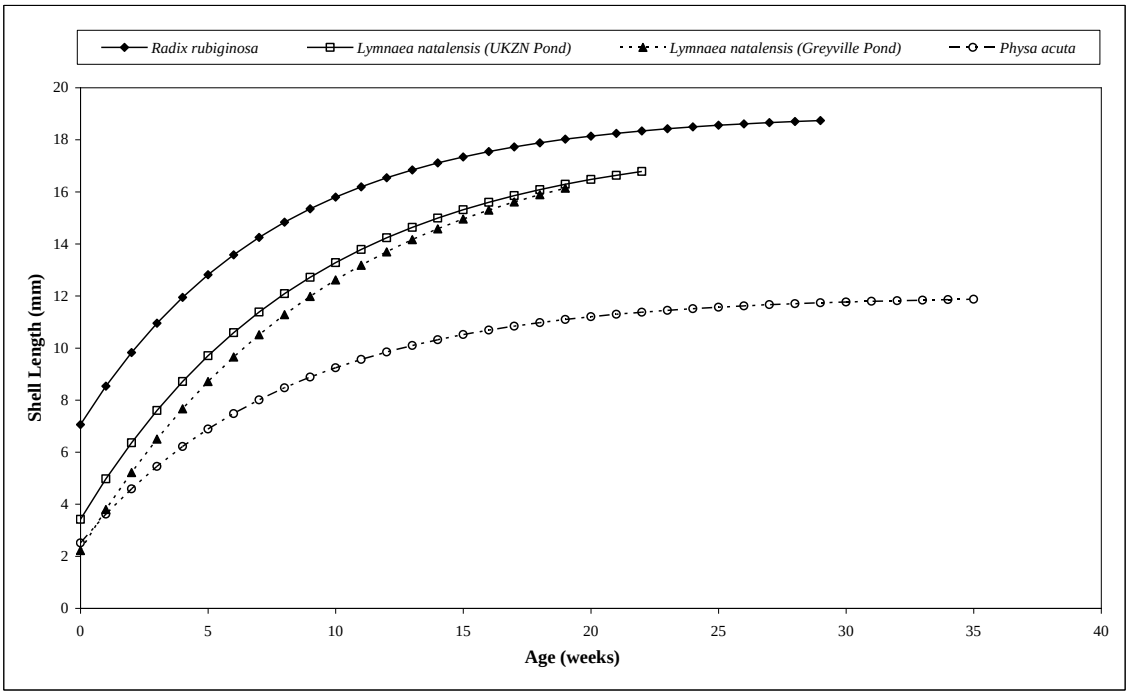


Figure 6.4: The von Bertalanffy growth curve for *R. rubiginosa*, *L. natalensis* (UKZN and Greyville Ponds) and *P. acuta* at 20°C.

Using the von Bertalanffy growth curve for 20°C (Figure 6.4), it was predicted that *R. rubiginosa* would reach the largest shell length of 18.74 mm followed by both the UKZN Pond (16.78 mm) and Greyville Pond populations (16.14 mm) of *L. natalensis* and finally *P. acuta* (11.87 mm). From Figure 6.1, the observed maximum shell lengths attained by *R. rubiginosa*, *L. natalensis* (UKZN Pond), *L. natalensis* (Greyville Pond) and *P. acuta* were slightly lower at 18.20 mm, 16.25 mm, 15.65 mm and 11.80 mm respectively.

At 25°C the maximum predicted shell lengths (Figure 6.5), for *R. rubiginosa*, the UKZN Pond and Greyville Pond populations of *L. natalensis* and *P. acuta* were 18.11 mm, 14.23 mm, 13.63 mm and 10.30 mm respectively. Figure 6.2 showed the observed maximum shell length attained by *R. rubiginosa*, *L. natalensis* (UKZN Pond), *L. natalensis* (Greyville Pond) and *P. acuta* at 25°C were 17.40 mm, 13.95 mm, 13.35 mm and 10.20 mm respectively.

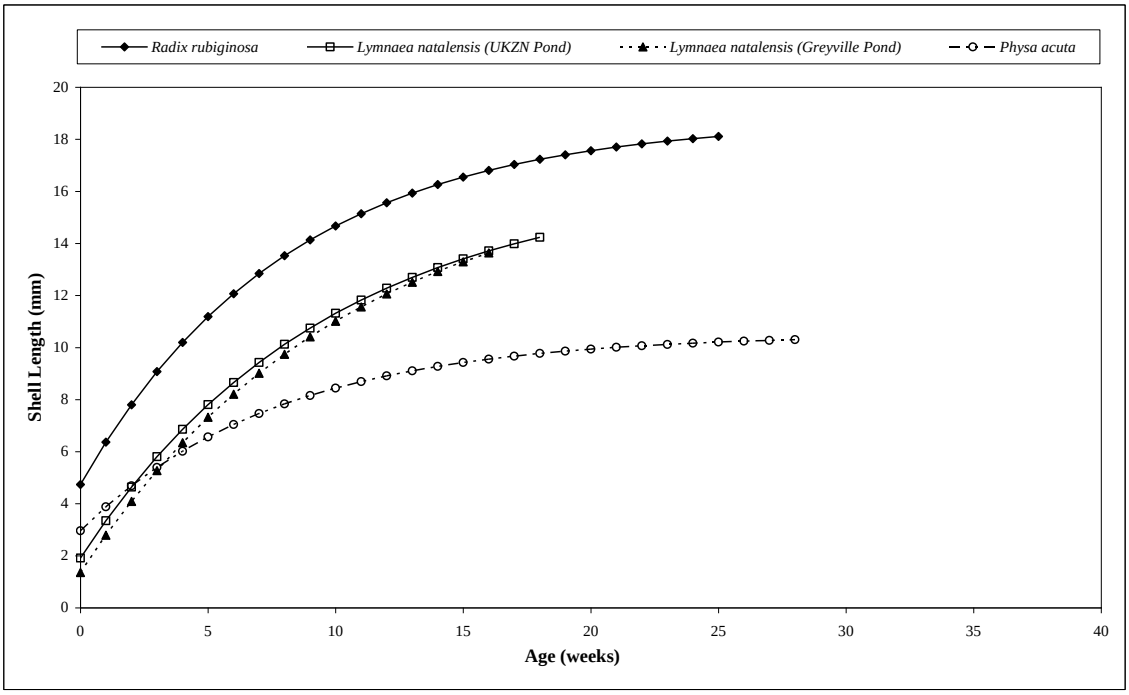


Figure 6.5: The von Bertalanffy growth curve for *R. rubiginosa*, *L. natalensis* (UKZN and Greyville Ponds) and *P. acuta* at 25°C.

At 30°C, the maximum predicted shell length using the von Bertalanffy growth curve for *R. rubiginosa*, the UKZN Pond and Greyville Pond populations of *L. natalensis* and *P. acuta* were 16.66 mm, 10.90 mm, 11.81 mm and 9.42 mm respectively. From Figure 6.3, the observed maximum shell lengths attained at this temperature by *R. rubiginosa*, *L. natalensis* (UKZN Pond), *L. natalensis* (Greyville Pond) and *P. acuta* were 16.10 mm, 11.25 mm, 11.65 mm and 9.20 mm respectively. For all three temperatures the maximum predicted shell lengths using the von Bertalanffy growth equation for all four populations (Figures 6.4 - 6.6) were larger than the observed maximum shell lengths (Figures 6.1 - 6.3).

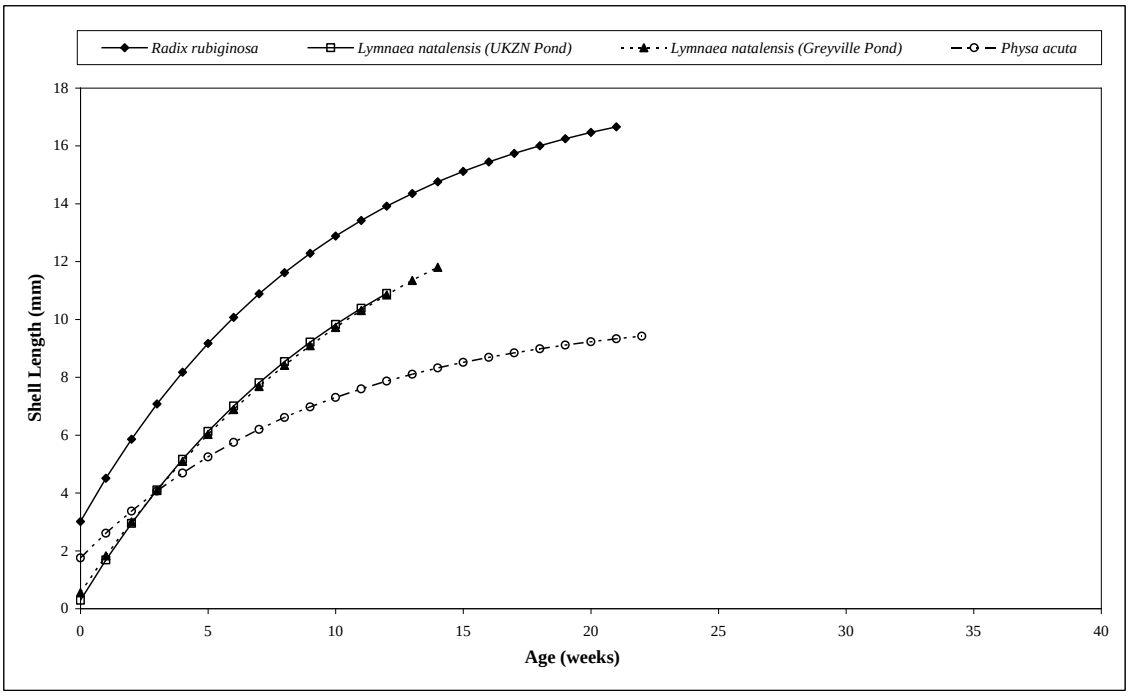


Figure 6.6: The von Bertalanffy growth curve for *R. rubiginosa*, *L. natalensis* (UKZN and Greyville Ponds) and *P. acuta* at 30°C.

6.3.2 Survival Rate

Age specific survival rates (l_x) for the four snail populations maintained at the three temperatures are presented in Figures 6.7 - 6.9 and statistical comparisons are summarised in Tables 6.2 and 6.3.

Table 6.2 summarises the statistical differences between the age specific survival rates of the four snail populations. For *R. rubiginosa*, there was no significant difference in survivorship between 20°C and 25°C ($p = 0.098$) and also between 25°C and 30°C ($p = 0.113$). There was however, a significant difference in survivorship between 20°C and 30°C (Table 6.2).

Table 6.2: Analysis of the mean age specific survival rate (l_x) within the four snail populations, for each of the three temperatures (n = 90). Differences in survival rates within populations were analysed using the Mann-Whitney-U test. Probability values are two-tailed and significance was determined at $p < 0.05$.

Temperature		*R. rubiginosa*	*L. natalensis* (UKZN Pond)	*L. natalensis* (Greyville Pond)	*P. acuta*
20°C	25°C	0.098	0.349	0.169	0.014
	30°C	0.004	0.002	0.021	<0.001
25°C	20°C	0.098	0.349	0.169	0.014
	30°C	0.113	0.010	0.238	0.008
30°C	20°C	0.004	0.002	0.021	<0.001
	25°C	0.113	0.010	0.238	0.008

Table 6.2 also shows that the UKZN Pond *L. natalensis* population displayed similar within population survivorship patterns at 20°C and 25°C ($p = 0.349$), while the Greyville Pond population displayed similar patterns at 20°C and 25°C ($p = 0.169$) and at 25°C and 30°C ($p = 0.238$). For the invasive physid, *P. acuta*, there was a significant difference in age specific survival rate at all three temperatures (Table 6.2).

Figure 6.7 represents graphically the age specific survival rates of the four snail populations maintained at 20°C. The first mortality was in *L. natalensis* populations from the Greyville Pond three weeks after the start of the cohort while the UKZN Pond population had its first mortalities four weeks after the start of the experiment. First mortalities for *P. acuta* and *R. rubiginosa* occurred after six and seven weeks respectively (Figure 6.7).

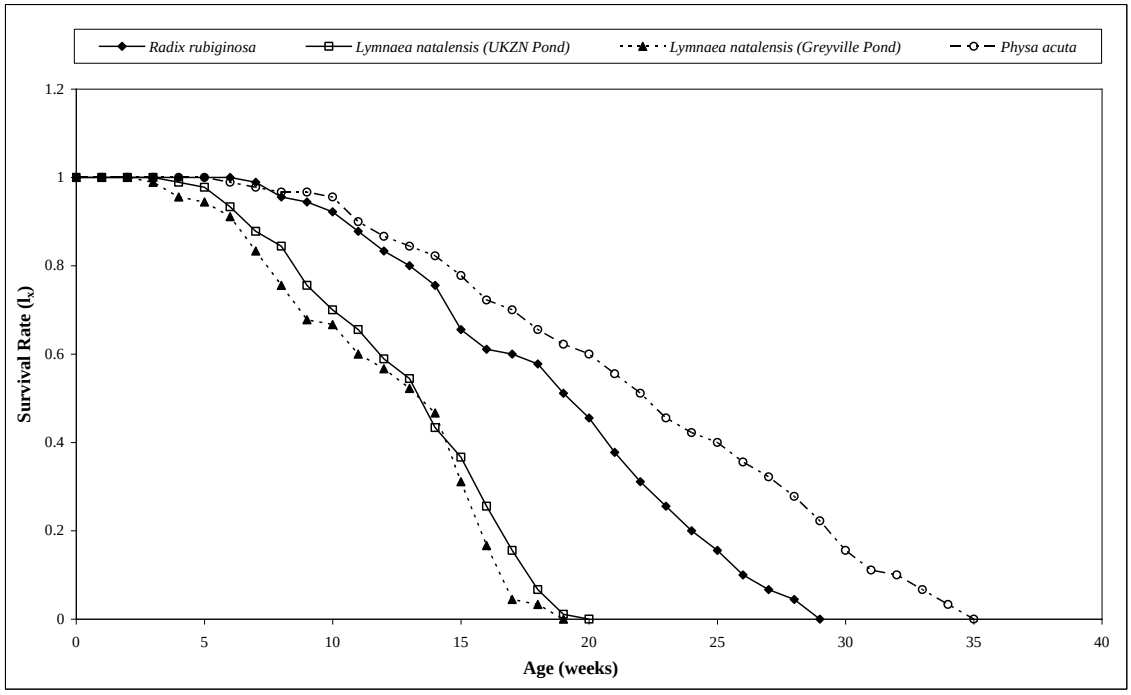


Figure 6.7: Age specific survival rates (l_x) for *R. rubiginosa*, *L. natalensis* (UKZN and Greyville Ponds) and *P. acuta* at 20°C.

Physa acuta displayed the greatest longevity at 35 weeks, followed by *R. rubiginosa* at 29 weeks (Figure 6.7) and *L. natalensis* from the UKZN and Greyville Ponds at 20 and 19 weeks respectively. The 50% mortality level (LT_{50}) for both *L. natalensis* populations was shorter (13.50 weeks) in comparison to the LT_{50} values for *R. rubiginosa* (19 weeks) and *P. acuta* (22 weeks). In addition, the duration of mortality was shorter for both the *L. natalensis* populations (16 weeks) compared with *R. rubiginosa* (22 weeks) and *P. acuta* (29 weeks).

Table 6.3 summarises statistical differences between the age specific survival rates for the four snail populations at the three temperatures.

Table 6.3: Analysis of the mean age specific survival rate (l_x) for the four snail populations, maintained at the three temperatures (n = 90). Differences in survival rates between populations were analysed using the Mann-Whitney-U test. Probability values are two-tailed and significance was determined at $p < 0.05$.

Snail Populations		**20°C**	**25°C**	**30°C**
R. rubiginosa	*L. natalensis* (UKZN Pond)	0.001	0.007	<0.001
	L. natalensis (Greyville Pond)	0.001	0.001	0.003
	P. acuta	0.033	0.297	0.585
L. natalensis (UKZN Pond)	*R. rubiginosa*	0.001	0.007	<0.001
	L. natalensis (Greyville Pond)	0.878	0.269	0.375
	P. acuta	<0.001	0.001	0.001
L. natalensis (Greyville Pond)	*R. rubiginosa*	0.001	0.001	0.003
	L. natalensis (UKZN Pond)	0.878	0.269	0.375
	P. acuta	<0.001	<0.001	0.005
P. acuta	*R. rubiginosa*	0.033	0.297	0.585
	L. natalensis (UKZN Pond)	<0.001	0.001	0.001
	L. natalensis (Greyville Pond)	<0.001	<0.001	0.005

Survivorship at 20°C showed no significant difference between the two *L. natalensis* populations ($p = 0.878$). All other populations displayed a significant difference in survival rate (Table 6.3).

At 25°C the first mortalities occurred in the UKZN and Greyville Pond *L. natalensis* populations at three and four weeks respectively (Figure 6.8). First mortalities for *R. rubiginosa* and *P. acuta* occurred five weeks after the start of the experiment.

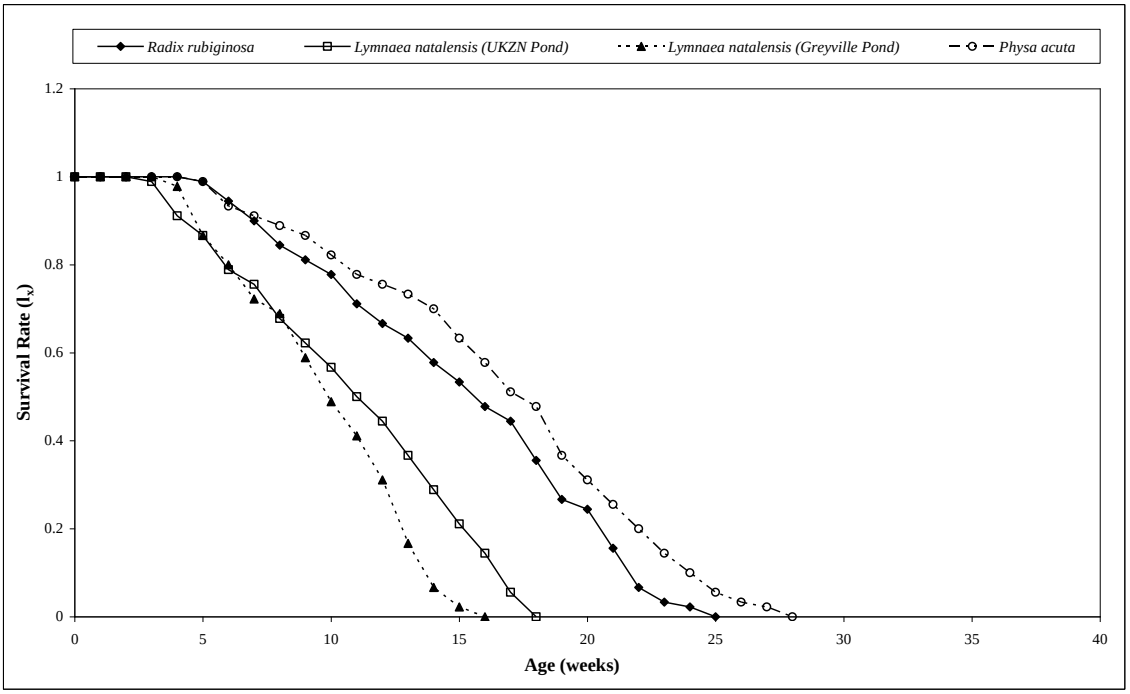


Figure 6.8: Age specific survival rates (l_x) for *R. rubiginosa*, *L. natalensis* (UKZN and Greyville Ponds) and *P. acuta* at 25°C.

Physa acuta again displayed the greatest longevity at 28 weeks, followed by *R. rubiginosa* at 25 weeks (Figure 6.8). *Lymnaea natalensis* from both the UKZN and Greyville Ponds had the shortest longevity at 18 and 16 weeks respectively. At 25°C *P. acuta* displayed the highest LT_{50} at 17.50 weeks (Figure 6.8). *Radix rubiginosa* had a shorter LT_{50} value of 15.50 weeks while the UKZN and Greyville Pond *L. natalensis* populations had LT_{50} values at 11 and 10 weeks respectively.

The duration of mortalities was again shortest in both *L. natalensis* populations, 12 weeks for the Greyville Pond population and 15 weeks for the UKZN Pond population. *Radix rubiginosa* had a duration of mortalities of 25 weeks while *P. acuta* displayed the longest at 28 weeks.

The analysis of differences in survivorship between populations maintained at 25°C (Table 6.3), indicated no significant difference between *R. rubiginosa* and *P. acuta* (p = 0.297). Also, the two *L. natalensis* populations had survival rates similar to each other (p = 0.269) and lower than *R. rubiginosa* and *P. acuta*.

Age specific survival rates at 30°C are shown in Figure 6.9. The first occurrence of mortality was by *L. natalensis* (UKZN Pond) two weeks after the start of the experiment. The remaining three snail populations exhibited their initial mortalities at three weeks for both the Greyville Pond *L. natalensis* population and *P. acuta* and four weeks for *R. rubiginosa* (Figure 6.9).

Longevity patterns at 30°C were similar to those at 20°C and 25°C. *Lymnaea natalensis* from both the UKZN and Greyville Ponds displayed the shortest longevity at 12 and 14 weeks respectively (Figure 6.9) while *P. acuta* and *R. rubiginosa* had greater longevity at 22 and 21 weeks respectively.

At 30°C the *L. natalensis* populations again displayed the lowest LT_{50} values. For the UKZN Pond population this was seven weeks and eight weeks for the Greyville Pond population (Figure 6.9). A LT_{50} value of 14 weeks was recorded for *R. rubiginosa*, marginally higher than that recorded for *P. acuta* (13 weeks). Also in Figure 6.9, *L. natalensis* from both the UKZN and Greyville Ponds had the shortest duration of mortalities (10 weeks for the UKZN Pond population and 11 weeks for the Greyville Pond population). *Radix rubiginosa* had mortalities over a period of 17 weeks while *P. acuta* did so for 19 weeks. From Figures 6.7 - 6.9 it is evident that the lowest survival rate was shown by the two *L. natalensis* populations, while *P. acuta* displayed the highest survival rate. This was to be expected given the highly invasive characteristics of this physid.

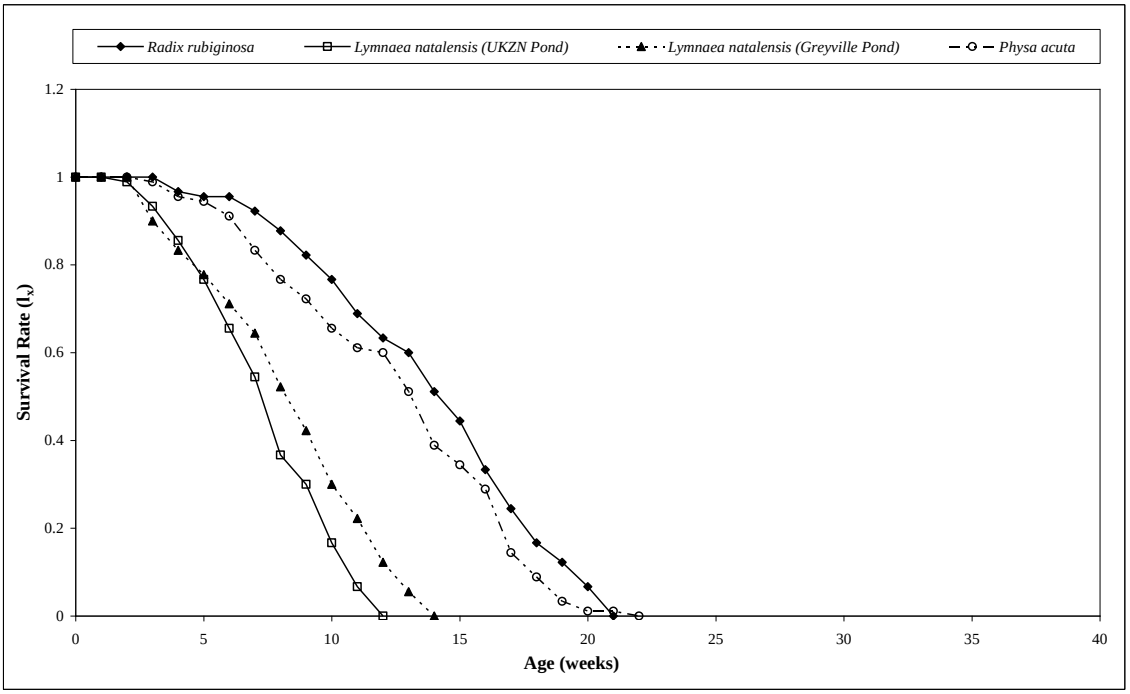


Figure 6.9: Age specific survival rates (l_x) for *R. rubiginosa*, *L. natalensis* (UKZN and Greyville Ponds) and *P. acuta* at 30°C.

From Table 6.3 it was evident that similar patterns of survivorship occurred between the four populations maintained at 25°C and 30°C. At 30°C, no significant difference was found between the survival rate of *R. rubiginosa* and *P. acuta* ($p = 0.585$). It was also at this, the highest temperature tested, that both *L. natalensis* populations displayed similar patterns of survivorship ($p = 0.375$).

6.3.3 Fecundity

Table 6.4 summarises statistical differences between the age specific fecundity for the four snail populations at each of the three temperatures. For *R. rubiginosa*, there were no significant differences in fecundity between 20°C and 25°C ($p = 0.136$) or at 25°C and 30°C ($p = 0.193$), but there was a significant difference between 20°C and 30°C.

Table 6.4: Analysis of the mean age specific fecundity (m_x) for the four snail populations at each of the three temperatures (n = 3). Differences in fecundity within populations and between temperatures were analysed using the Mann-Whitney-U test. Probability values are two-tailed and significance was determined at $p < 0.05$.

Temperature		***R. rubiginosa***	***L. natalensis* (UKZN Pond)**	***L. natalensis* (Greyville Pond)**	***P. acuta***
20°C	25°C	0.136	0.081	0.035	0.010
	30°C	0.010	<0.001	0.250	<0.001
25°C	20°C	0.136	0.081	0.035	0.010
	30°C	0.193	0.001	0.932	0.056
30°C	20°C	0.010	<0.001	0.250	<0.001
	25°C	0.193	0.001	0.932	0.056

For *L. natalensis* from the UKZN Pond there was no significant difference in age specific fecundity between 20°C and 25°C ($p = 0.081$) but fecundity did differ significantly between 20°C and 30°C as well as between 25°C and 30°C (Table 6.4). For *L. natalensis* from the Greyville Pond similar fecundity were exhibited between 20°C and 30°C as well as between 25°C and 30°C.

For *P. acuta* (Table 6.4) the fecundity at 25°C and 30°C were marginally similar ($p = 0.056$), while at 20°C and 25°C as well as at 20°C and 30°C, the fecundity for *P. acuta* were clearly influenced by temperature.

Figures 6.10 - 6.12 show the age specific fecundity for the four snail populations maintained at the three temperatures.

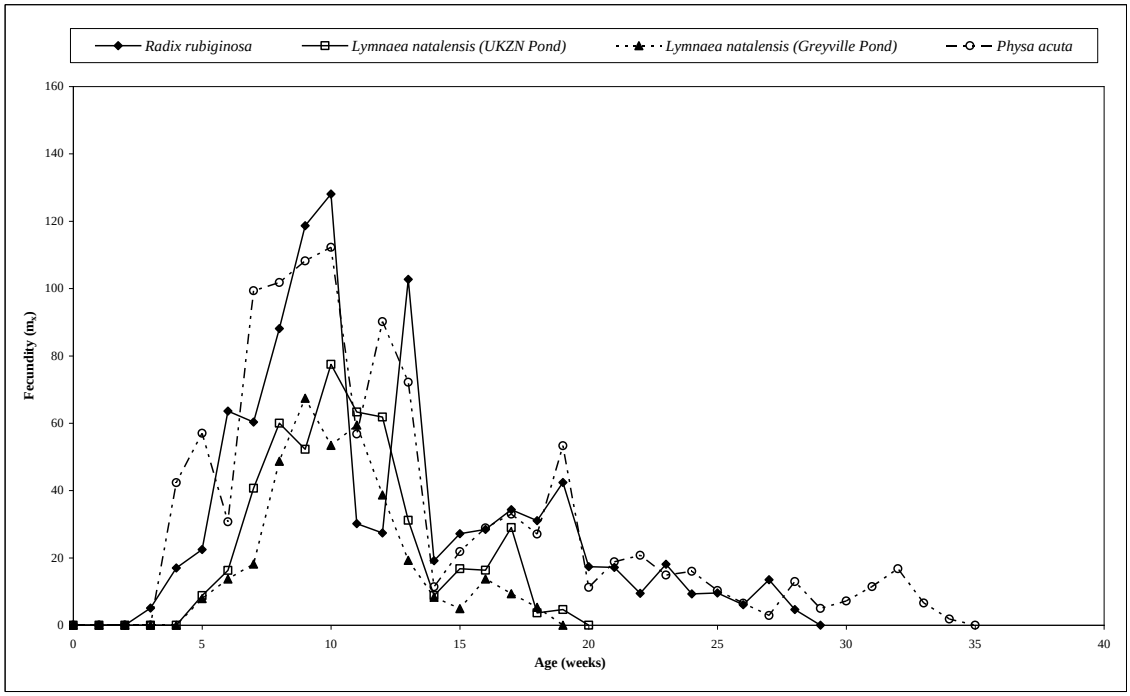


Figure 6.10: Age specific fecundity (m_x) for *R. rubiginosa*, *L. natalensis* (UKZN and Greyville Ponds) and *P. acuta* at 20°C.

At 20°C *R. rubiginosa* began oviposition three weeks after the start of the experiment (Figure 6.10). Egg production was high and fluctuated until the death of the last individual at 29 weeks. From Figure 6.10 it is seen that *R. rubiginosa* had five distinct peaks in fecundity at 10, 13, 19, 23 and 27 weeks. The most prominent fecundity peak occurred at 10 weeks (128.08 eggs per snail) while fecundity at 13 weeks was 102.74 eggs per snail. By 19 weeks *R. rubiginosa* had declined to the 50% mortality level (Figure 6.7) and the remaining reproductive peaks at 19 (42.39 eggs per snail), 23 (18.10 eggs per snail) and 27 (13.50 eggs per snail) weeks were much lower (Figure 6.10).

Also, a decrease in the fecundity at week seven (Figure 6.10) coincided with the first occurrence of mortalities in *R. rubiginosa* (Figure 6.7). The reduced fecundity at weeks 11 and 12 (Figure 6.10) could be explained by the increased mortalities recorded over

this period (Figure 6.7). Conversely, the gradual increase in the fecundity after week 14 until the reproductive peak at 19 weeks (Figure 6.10) coincided with fewer mortalities and a more stable survival rate (Figure 6.7).

Egg production at 20^{o}C for *P. acuta* began four weeks after the start of the experiment and continued until 35 weeks (Figure 6.10). Five fecundity peaks were observed for *P. acuta* at 10, 12, 19, 28 and 32 weeks. The highest reproductive peak for *P. acuta*, observed at 10 weeks (112.25 eggs per snail) was lower than the peak observed for *R. rubiginosa* at the same time. From Figure 6.10 it was further noted that three of the reproductive peaks for *P. acuta* occurred before the population reached the 50% mortality level at 22 weeks (Figure 6.7). The decrease in the fecundity at week six (Figure 6.10) coincided with the first occurrence of *P. acuta* mortalities (Figure 6.7).

Oviposition by *L. natalensis* began at five weeks after the start of the experiment and stopped at 20 and 19 weeks in the UKZN Pond and Greyville Pond populations respectively (Figure 6.10). The UKZN Pond population displayed three fecundity peaks at 8, 10 and 17 weeks with the most prominent at 10 weeks (77.50 eggs per snail). The Greyville Pond population also exhibited three peaks at 9, 11 and 16 weeks with the highest at 9 weeks (67.38 eggs per snail). Furthermore these reproductive peaks were attained before the two *L. natalensis* populations declined to the 50% mortality level at 13.50 weeks (Figure 6.7). It is also clear from Figure 6.10 that *R. rubigionsa* and *P. acuta* displayed higher fecundity and longer oviposition periods than either of the two *L. natalensis* populations.

Table 6.5 summarises the statistical differences in the age specific fecundity between the four snail populations at the three temperatures.

Table 6.5: Analysis of the mean age specific fecundity (m_x) for the four snail populations maintained at the three temperatures (n = 3). Differences in fecundity between populations were analysed using the Mann-Whitney-U test. Probability values are two-tailed and significance was determined at $p < 0.05$.

Snail Populations		**20°C**	**25°C**	**30°C**
R. rubiginosa	*L. natalensis* (UKZN Pond)	0.003	0.019	<0.001
	L. natalensis (Greyville Pond)	0.001	0.003	0.060
	P. acuta	0.028	0.155	0.249
L. natalensis (UKZN Pond)	*R. rubiginosa*	0.003	0.019	<0.001
	L. natalensis (Greyville Pond)	0.203	0.085	0.045
	P. acuta	<0.001	0.002	<0.001
L. natalensis (Greyville Pond)	*R. rubiginosa*	0.001	0.003	0.060
	L. natalensis (UKZN Pond)	0.203	0.085	0.045
	P. acuta	<0.001	<0.001	0.001
P. acuta	*R. rubiginosa*	0.028	0.155	0.249
	L. natalensis (UKZN Pond)	<0.001	0.002	<0.001
	L. natalensis (Greyville Pond)	<0.001	<0.001	0.001

At 20°C both the *L. natalensis* populations displayed similar patterns in age specific fecundity ($p = 0.203$) while *R. rubiginosa* and *P. acuta* exhibited fecundity significantly different from *L. natalensis* (Table 6.5).

At 25°C oviposition by *R. rubiginosa* began at three weeks after the start of the experiment and stopped at 25 weeks when the last mortality occurred (Figure 6.11). The three fecundity peaks observed at 6, 9 and 13 weeks occurred before the population had declined to the LT_{50} level of 15.50 weeks (Figure 6.8). The most prominent of these

reproductive peaks was at nine weeks (143.58 eggs per snail) and this exceeded the maximum rate of 128.08 eggs per snail observed at 20°C (Figure 6.10).

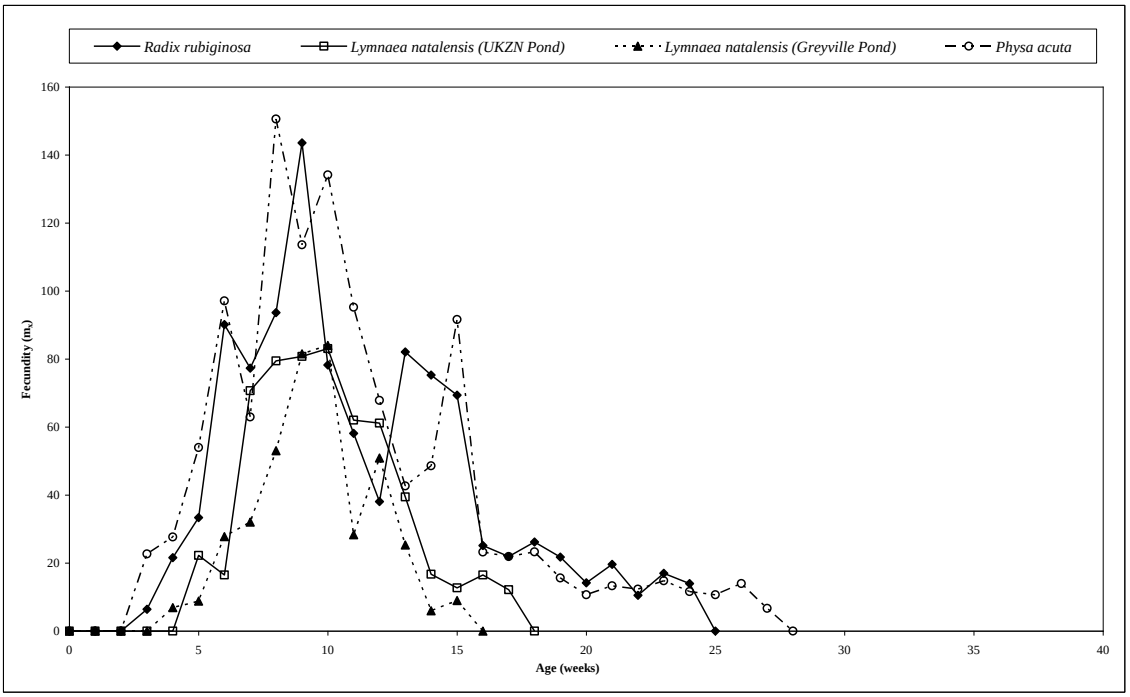


Figure 6.11: Age specific fecundity (m_x) for *R. rubiginosa*, *L. natalensis* (UKZN and Greyville Ponds) and *P. acuta* at 25°C.

Physa acuta started egg production at three weeks and stopped at 28 weeks. The fecundity fluctuated over this period with four peaks at 6, 8, 10 and 15 weeks. All these reproductive peaks were attained before the population declined to the LT_{50} level at 17.50 weeks (Figure 6.8). Among the four snail populations maintained at 25°C, *P. acuta* had the highest fecundity, recorded at eight weeks (150.57 eggs per snail). Smaller but less erratic fecundity were observed after 20 weeks (Figure 6.11). This could be attributed to a more stable older population with a gradual mortality rate (Figure 6.8).

The fecundity of both *L. natalensis* populations were lower and the duration of egg production was shorter in comparison to that of *R. rubiginosa* and *P. acuta* (Figure 6.11). Egg production started in both the UKZN and Greyville Pond *L. natalensis* populations at

five and four weeks respectively. Fecundity stopped at 18 weeks in the UKZN population and at 16 weeks in the Greyville population.

From Figure 6.11, it can be seen that the UKZN Pond populations had two peaks at 5 (22.24 eggs per snail) and 10 (83.09 eggs per snail) weeks while the Greyville population also had two fecundity peaks at 10 (84.01 eggs per snail) and 12 (50.90 eggs per snail) weeks. Again, all reproductive peaks occurred before the populations reached the LT_{50} level (Figure 6.8).

Table 6.5 shows that the fecundity curves displayed by *R. rubiginosa* and *P. acuta* at 25^{o}C were similar to each other ($p = 0.155$) while there was again no significant difference in the fecundity between the two *L. natalensis* populations ($p = 0.085$).

At 30^{o}C *R. rubiginosa* began egg production four weeks after the start of the experiment and stopped at 20 weeks (Figure 6.12). Three fecundity peaks were observed at 7 (43.22 eggs per snail), 11 (72.82 eggs per snail) and 15 (26.67 eggs per snail) weeks. The two largest peaks occurred before *R. rubiginosa* reached the 50% mortality level at 14 weeks (Figure 6.9). The relatively steady fecundity from four to six weeks (Figure 6.12) was associated with few mortalities and a stable survival rate over that period (Figure 6.9).

Fecundity in *P. acuta* began at three weeks and stopped at 20 weeks (Figure 6.12). Peaks in fecundity were observed at 5 (35.89 eggs per snail), 10 (80.58 eggs per snail), 14 (31.00 eggs per snail) and 17 (15.31 eggs per snail) weeks. The larger reproductive peaks occurred before the population declined to the LT_{50} level at 13 weeks (Figure 6.9) and the fecundity peaks occurring after this period were much lower (Figure 6.12). Among the four snail populations maintained at 30^{o}C, *P. acuta* had the highest fecundity, recorded at 10 weeks (80.58 eggs per snail).

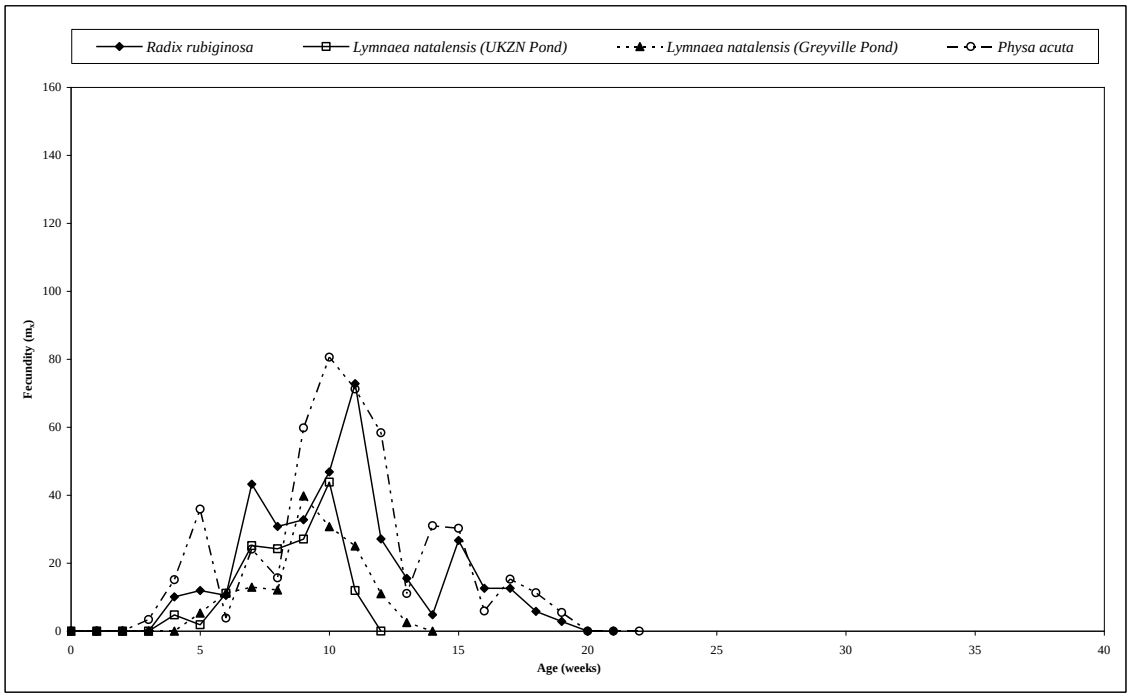


Figure 6.12: Age specific fecundity (m_x) for *R. rubiginosa*, *L. natalensis* (UKZN and Greyville Ponds) and *P. acuta* at 30°C.

Egg production by *L. natalensis* from the UKZN Pond began four weeks after the start of the experiment and stopped at 12 weeks while the Greyville Pond population began oviposition at five weeks and stopped at 14 weeks (Figure 6.12). Prominent fecundity peaks were exhibited at 10 weeks (43.86 eggs per snail) for the UKZN Pond population and at nine weeks (39.76 eggs per snail) for *L. natalensis* from the Greyville Pond population. The decreases in fecundity in both *L. natalensis* populations declined after the populations had fallen to the 50% mortality level (Figure 6.9).

From Figure 6.12 it is evident that all snail populations at 30°C had lower egg production and shorter fecundity durations than at 20°C and 25°C. Also, both *L. natalensis* populations had lower fecundity and shorter durations of egg production than either *R. rubiginosa* or *P. acuta*.

It can be seen from Table 6.5 that at 30°C there was no significant difference in fecundity between *R. rubiginosa* and *P. acuta* ($p = 0.249$) or between *R. rubiginosa* and the

Greyville Pond *L. natalensis* population ($p = 0.060$). It was also noted that at 20°C and 25°C there was no significant difference in fecundity between the two *L. natalensis* populations (Table 6.5) but at 30°C these populations differed slightly from each other as indicated by the marginally significant probability value of $p = 0.045$.

6.3.4 Life History Parameters

The life history parameters calculated for the four snail populations maintained at the three temperatures are summarised in Table 6.6. These parameters include the gross reproductive rate, net reproductive rate, intrinsic rate of natural increase, mean generation time and finite rate of increase.

(a) Gross Reproductive Rate (GRR)

All snail populations had the highest gross reproductive rate (GRR) at 25°C while the lowest values were at 30°C. *Physa acuta* had the highest GRR, followed by *R. rubiginosa* and then *L. natalensis* (Table 6.6). This pattern in GRR was evident at all temperatures. From Table 6.7 it was also noted that the effects of temperature on GRR were significant within the snail populations except *P. acuta* which displayed a similar GRR at 20°C and 25°C ($p = 0.397$).

Multiple comparisons between GRR for the four snail populations were analysed using Tukey HSD (Tables 6.8 - 6.10). Strongly significant differences in GRR *p*-values were recorded between the populations. Exceptions to this pattern however were both *L. natalensis* populations at 20°C and 30°C (Tables 6.8 and 6.10).

Table 6.6: Life history parameters of the four snail populations maintained at the three temperature treatments (n = 3). The values are based on a time interval of one week and are presented as means (± standard deviation).

Temperature	Snail Populations	GRR	R_o	r_m	T	λ
20°C	*R. rubiginosa*	951.306	749.833	0.994	6.657	2.703
		(±35.529)	(±10.911)	(±0.005)	(±0.018)	(±0.013)
	L. natalensis (UKZN Pond)	491.025	316.378	0.712	8.081	2.039
		(±14.570)	(±13.930)	(±0.009)	(±0.070)	(±0.019)
	L. natalensis (Greyville Pond)	368.271	228.756	0.667	8.145	1.949
		(±5.866)	(±5.626)	(±0.014)	(±0.155)	(±0.026)
	P. acuta	1109.520	903.522	1.066	6.383	2.906
		(±107.159)	(±59.839)	(±0.031)	(±0.121)	(±0.088)
25°C	*R. rubiginosa*	1037.518	715.644	1.050	6.259	2.858
		(±7.657)	(±9.689)	(±0.003)	(±0.023)	(±0.007)
	L. natalensis (UKZN Pond)	573.442	319.600	0.765	7.543	2.149
		(±32.294)	(±6.45)	(±0.019)	(±0.201)	(±0.041)
	L. natalensis (Greyville Pond)	413.730	216.167	0.759	7.087	2.137
		(±19.487)	(±7.601)	(±0.027)	(±0.289)	(±0.058)
	P. acuta	1186.765	898.578	1.218	5.584	3.381
		(±46.144)	(±38.959)	(±0.025)	(±0.082)	(±0.084)
30°C	*R. rubiginosa*	360.181	255.711	0.781	7.104	2.183
		(±19.661)	(±13.578)	(±0.019)	(±0.113)	(±0.04)
	L. natalensis (UKZN Pond)	149.963	51.656	0.594	6.632	1.812
		(±17.394)	(±7.837)	(±0.032)	(±0.097)	(±0.058)
	L. natalensis (Greyville Pond)	150.620	59.567	0.544	7.469	1.724
		(±16.091)	(±15.836)	(±0.041)	(±0.059)	(±0.071)
	P. acuta	478.406	294.689	0.917	6.206	2.503
		(±4.387)	(±4.745)	(±0.032)	(±0.230)	(±0.080)

GRR – gross reproductive rate; R_o – net reproductive rate; r_m – intrinsic rate of natural increase; T – mean generation time; λ – finite rate of increase.

Table 6.7: Multiple comparisons using Tukey HSD. Life history parameters within the four snail populations were analysed for differences at each of the three temperatures (n = 3). Probability values are two-tailed and significance was determined at $p < 0.05$.

Snail Populations	Temperature		GRR	R_o	r_m	T	λ
R. rubiginosa	20°C	25°C	0.011	0.025	0.002	0.001	0.001
		30°C	<0.001	<0.001	<0.001	<0.001	<0.001
	25°C	20°C	0.011	0.025	0.002	0.001	0.001
		30°C	<0.001	<0.001	<0.001	<0.001	<0.001
	30°C	20°C	<0.001	<0.001	<0.001	<0.001	<0.001
		25°C	<0.001	<0.001	<0.001	<0.001	<0.001
L. natalensis (UKZN Pond)	20°C	25°C	0.011	0.918	0.062	0.007	0.043
		30°C	<0.001	<0.001	0.001	<0.001	0.001
	25°C	20°C	0.011	0.918	0.062	0.007	0.043
		30°C	<0.001	<0.001	<0.001	<0.001	<0.001
	30°C	20°C	<0.001	<0.001	0.001	<0.001	0.001
		25°C	<0.001	<0.001	<0.001	<0.001	<0.001
L. natalensis (Greyville Pond)	20°C	25°C	0.023	0.377	0.020	0.001	0.013
		30°C	<0.001	<0.001	0.005	0.012	0.006
	25°C	20°C	0.023	0.377	0.020	0.001	0.013
		30°C	<0.001	<0.001	<0.001	0.111	<0.001
	30°C	20°C	<0.001	<0.001	0.005	0.012	0.006
		25°C	<0.001	<0.001	<0.001	0.111	<0.001
P. acuta	20°C	25°C	0.397	0.988	0.002	0.002	0.001
		30°C	<0.001	<0.001	0.002	0.407	0.003
	25°C	20°C	0.397	0.988	0.002	0.002	0.001
		30°C	<0.001	<0.001	<0.001	0.007	<0.001
	30°C	20°C	<0.001	<0.001	0.002	0.407	0.003
		25°C	<0.001	<0.001	<0.001	0.007	<0.001

GRR – gross reproductive rate; R_o – net reproductive rate; r_m – intrinsic rate of natural increase; T – mean generation time; λ – finite rate of increase.

Table 6.8: Multiple comparisons using Tukey HSD. Life history parameters between the four snail populations were analysed for differences at 20°C (n = 3). Probability values are two-tailed and significance was determined at $p < 0.05$.

Snail Populations		**GRR**	**R_o**	**r_m**	**T**	**λ**
R. rubiginosa	*L. natalensis* (UKZN Pond)	<0.001	<0.001	<0.001	<0.001	<0.001
	L. natalensis (Greyville Pond)	<0.001	<0.001	<0.001	<0.001	<0.001
	P. acuta	0.038	0.001	0.004	0.050	0.003
L. natalensis (UKZN Pond)	*R. rubiginosa*	<0.001	<0.001	<0.001	<0.001	<0.001
	L. natalensis (Greyville Pond)	0.111	0.037	0.052	0.873	0.170
	P. acuta	<0.001	<0.001	<0.001	<0.001	<0.001
L. natalensis (Greyville Pond)	*R. rubiginosa*	<0.001	<0.001	<0.001	<0.001	<0.001
	L. natalensis (UKZN Pond)	0.111	0.037	0.052	0.873	0.170
	P. acuta	<0.001	<0.001	<0.001	<0.001	<0.001
P. acuta	*R. rubiginosa*	0.038	0.001	0.004	0.050	0.003
	L. natalensis (UKZN Pond)	<0.001	<0.001	<0.001	<0.001	<0.001
	L. natalensis (Greyville Pond)	<0.001	<0.001	<0.001	<0.001	<0.001

GRR – gross reproductive rate; R_o – net reproductive rate; r_m – intrinsic rate of natural increase; T – mean generation time; λ – finite rate of increase.

Table 6.9: Multiple comparisons using Tukey HSD. Life history parameters between the four snail populations were analysed for differences at 25°C (n = 3). Probability values are two-tailed and significance was determined at $p < 0.05$.

Snail Populations		**GRR**	**R_o**	**r_m**	**T**	**λ**
R. rubiginosa	*L. natalensis* (UKZN Pond)	<0.001	<0.001	<0.001	<0.001	<0.001
	L. natalensis (Greyville Pond)	<0.001	<0.001	<0.001	0.002	<0.001
	P. acuta	0.001	<0.001	<0.001	0.008	<0.001
L. natalensis (UKZN Pond)	*R. rubiginosa*	<0.001	<0.001	<0.001	<0.001	<0.001
	L. natalensis (greyville Pond)	0.001	0.001	0.987	0.058	0.994
	P. acuta	<0.001	<0.001	<0.001	<0.001	<0.001
L. natalensis (Greyville Pond)	*R. rubiginosa*	<0.001	<0.001	<0.001	0.002	<0.001
	L. natalensis (UKZN Pond)	0.001	0.001	0.987	0.058	0.994
	P. acuta	<0.001	<0.001	<0.001	<0.001	<0.001
P. acuta	*R. rubiginosa*	0.001	<0.001	<0.001	0.008	<0.001
	L. natalensis (UKZN Pond)	<0.001	<0.001	<0.001	<0.001	<0.001
	L. natalensis (Greyville Pond)	<0.001	<0.001	<0.001	<0.001	<0.001

GRR – gross reproductive rate; R_o – net reproductive rate; r_m – intrinsic rate of natural increase; T – mean generation time; λ – finite rate of increase.

Table 6.10: Multiple comparisons using Tukey HSD. Life history parameters between the four snail populations were analysed for differences at 30°C (n = 3). Probability values are two-tailed and significance was determined at $p < 0.05$.

Snail Populations		GRR	R_o	r_m	T	λ
R. rubiginosa	*L. natalensis* (UKZN Pond)	<0.001	<0.001	<0.001	0.014	<0.001
	L. natalensis (Greyville Pond)	<0.001	<0.001	<0.001	0.051	<0.001
	P. acuta	<0.001	0.013	0.003	<0.001	0.001
L. natalensis (UKZN Pond)	*R. rubiginosa*	<0.001	<0.001	<0.001	0.014	<0.001
	L. natalensis (Greyville Pond)	1.000	0.829	0.296	<0.001	0.392
	P. acuta	<0.001	<0.001	<0.001	0.024	<0.001
L. natalensis (Greyville Pond)	*R. rubiginosa*	<0.001	<0.001	<0.001	0.051	<0.001
	L. natalensis (UKZN Pond)	1.000	0.829	0.296	<0.001	0.392
	P. acuta	<0.001	<0.001	<0.001	<0.001	<0.001
P. acuta	*R. rubiginosa*	<0.001	0.013	0.003	<0.001	0.001
	L. natalensis (UKZN Pond)	<0.001	<0.001	<0.001	0.024	<0.001
	L. natalensis (Greyville Pond)	<0.001	<0.001	<0.001	<0.001	<0.001

GRR – gross reproductive rate; R_o – net reproductive rate; r_m – intrinsic rate of natural increase; T – mean generation time; λ – finite rate of increase.

(b) <u>The net reproductive rate</u> (R_o)

All snail populations exhibited the lowest net reproductive rates (R_o) at 30°C (Table 6.6). At 20°C *R. rubiginosa*, *P. acuta* and *L. natalensis* (Greyville Pond) displayed the highest R_o values while the UKZN Pond *L. natalensis* population had its highest R_o value at 25°C. The high R_o values calculated could be attributed to the longer survival (Figures 6.7 and 6.8) and higher fecundities (Figures 6.10 and 6.11) recorded at both 20°C and 25°C. Within temperature treatments, *P. acuta* had the highest R_o followed by *R. rubiginosa* and then *L. natalensis* (Table 6.6).

As shown in Table 6.7, *P. acuta* ($p = 0.988$) and both *L. natalensis* populations ($p = 0.918$ for the UKZN Pond and $p = 0.377$ for the Greyville Pond) exhibited R_o values that were not significantly different at 20°C and 25°C. The net reproductive rates of *R. rubiginosa* however were significantly different at all temperatures (Table 6.7).

Multiple comparisons of R_o between the four snail populations (Tables 6.8 - 6.10) displayed a trend similar to that recorded for GRR. Strongly significant differences in R_o were recorded between all snail populations maintained at the three temperatures. There were however, no significant differences between the two *L. natalensis* populations at 30°C ($p = 0.829$).

(c) Intrinsic rate of natural increase (r_m)

Since the intrinsic rate of natural increase (r_m) summarises the reproductive capacity of the snails in terms of longevity, survival and fecundity, comparisons of the respective r_m values enabled an assessment of the effects of the various temperatures on the reproductive capacity.

The optimum temperature was 25°C (Table 6.6). At this temperature all four snail populations were expected to increase as indicated by r_m. From Table 6.6 the highest mean r_m values recorded were 1.218 (*P. acuta*), 1.050 (*R. rubiginosa*), 0.765 (*L. natalensis* from the UKZN Pond) and 0.759 (*L. natalensis* from the Greyville Pond). At 30°C all four populations had the lowest r_m values and they therefore exhibited their weakest performance at this temperature.

From Table 6.7 it is seen that temperature significantly influenced the intrinsic rate of natural increase. Only *L. natalensis* from the UKZN Pond displayed a similar r_m at 20°C and 25°C ($p = 0.062$). The multiple comparisons of r_m between the four snail populations (Tables 6.8 - 6.10) were significantly different for each temperature. Only *L. natalensis* (both populations) had statistically similar r_m values at each of the three temperatures (Tables 6.8 - 6.10). Overall, the invasive *P. acuta* had the highest mean r_m followed by

R. rubiginosa, *L. natalensis* from the UKZN Pond and lastly the Greyville Pond population (Table 6.6).

(d) Mean generation time (T)

Physa acuta (6.383) and the two *L. natalensis* populations (8.081 and 8.145 for both the UKZN Pond and the Greyville Pond respectively) exhibited their longest mean generation times (T) at 20°C while *R. rubiginosa* (7.104) had its longest at 30°C (Table 6.6). The shortest T was at 30°C for the UKZN Pond *L. natalensis* population and at 25°C for all remaining snail populations. *Physa acuta* had the shortest T at 20°C and 25°C followed by *R. rubiginosa* and thereafter both populations of *L. natalensis*. At 30°C however, *P. acuta* still had the shortest T, but was now followed by *L. natalensis* from the UKZN Pond, *R. rubiginosa* and lastly *L. natalensis* from the Greyville Pond.

Table 6.7 shows that the mean generation time for *P. acuta* was similar at 20°C and 30°C ($p = 0.407$) while *L. natalensis* (Greyville Pond) exhibited similar values at 25°C and 30°C ($p = 0.111$). Significant temperature influences were recorded for all the remaining snail populations. Comparisons of T between the four snail populations are presented in Tables 6.8 - 6.10. At 20°C (Table 6.8), the mean generation time was similar for *R. rubiginosa* and *P. acuta* ($p = 0.050$) and also for the two *L. natalensis* populations ($p = 0.873$). At 25°C (Table 6.9), the two *L. natalensis* populations were again similar ($p = 0.058$) while *R. rubiginosa* and the Greyville Pond *L. natalensis* population exhibited no statistical difference at 30°C (Table 6.10).

(e) Finite rate of increase (λ)

Table 6.6 shows that the pattern for the finite rate of increase (λ) was similar to that of the intrinsic rate of natural increase (r_m). All four snail populations had the highest λ values at 25°C. Of these *P. acuta* had the highest λ value, indicating that 3.381 individuals of the next cohort will replace one individual of the original cohort. The multiplication value for *R. rubiginosa* was 2.858 followed by *L. natalensis* that exhibited the lowest

finite rate of increase, 2.149 and 2.137 for the UKZN and Greyville Ponds respectively (Table 6.6). Also, all four snail populations had the lowest λ values at 30°C.

Table 6.7 shows that significant differences in the finite rate of increase were recorded for all populations. This indicated that λ the finite rate of increase was significantly influenced by temperature. Multiple comparisons of λ between the four snail populations (Tables 6.8 - 6.10) displayed a similar pattern to r_m. No significant differences were recorded for the *L. natalensis* populations at 20°C (0.170), 25°C (0.994) or 30°C (0.392). The λ values for the two *L. natalensis* populations were significantly different when compared to *R. rubiginosa* and *P. acuta* (Tables 6.8 - 6.10).

6.4 Discussion

The key findings of Chapter 6 are summarised in Table 6.11.

Table 6.11: Summary of the results for the age specific growth, survival, fecundity and life history parameters for each of the four snail populations.

	Summary of Key Results
Growth	• As temperature increased, the maximum mean shell length decreased for all four snail populations. • When compared to *L. natalensis*, *R. rubiginosa* exhibited a higher longevity and growth coefficient (K) at each of the three temperatures. • *Physa acuta* had the highest longevity for all temperatures, being slightly higher than that of *R. rubiginosa*. • At 20°C, *R. rubiginosa* had a higher K than *P. acuta*, while at 25°C and 30°C, *R. rubiginosa* had a slightly lower K than *P. acuta*. • The asymptotic length (L_∞) decreased with increasing temperature. • *Radix rubiginosa* had the highest L_∞ at each temperature. • The longevity, K and L_∞ for both the *L. natalensis* populations were similar at all temperatures.
Survival	• Overall, *P. acuta* exhibited the highest values for longevity, LT_{50} and the duration of mortality, being only slightly higher than *R. rubiginosa*. • It was only at 30°C that *R. rubiginosa* had a higher LT_{50} value than *P. acuta*. • Both *R. rubiginosa* and *P. acuta* exhibited the longest time until first mortality, LT_{50} and longevity values when compared to the two *L. natalensis* populations. • There was no significant difference between the two *L. natalensis* populations for survivorship.
Fecundity	• For each of the four snail populations, the highest fecundity peak was attained before the population declined to the LT_{50} level. • *Radix rubiginosa* and *P. acuta* displayed higher fecundity and longer oviposition periods compared to the two *L. natalensis* populations. • At 20°C, *R. rubiginosa* had a higher reproductive peak than *P. acuta*. • At 25°C and 30°C *P. acuta* had the highest reproductive peak, but more importantly, there was no significant difference in the fecundity for *R. rubiginosa* and *P. acuta* at these temperatures. • The two *L. natalensis* populations were similar at 20°C and 25°C, but not at 30°C.

Summary of Key Results

- All four snail populations had the highest and lowest GRR at 25°C and 30°C respectively.
- At each of the three temperatures *P. acuta* had the highest GRR, followed by *R. rubiginosa* and then *L. natalensis.*
- Strongly significant differences in the GRR *p*-values were recorded between the four populations at all temperatures, with the exception for the two *L. natalensis* populations at 20°C and 30°C.

- Multiple comparisons of R_o between the four snail populations displayed a trend similar to that recorded for GRR.
- Strongly significant differences in R_o were recorded between all snail populations maintained at the three temperatures.
- There were however, no significant differences between the two *L. natalensis* populations at 30°C.

- At the optimum temperature of 25°C, *P. acuta* had the highest mean r_m value followed by *R. rubiginosa, L. natalensis* (UKZN Pond) and *L. natalensis* from the Greyville Pond.
- There was no significant difference between the two *L. natalensis* populations for r_m.

- At 20°C and 25°C *P. acuta* had the shortest mean generation time followed by *R. rubiginosa* and thereafter the two *L. natalensis* populations.
- Importantly, at 20°C, the mean generation times for *R. rubiginosa* and *P. acuta* were similar.

- The λ values for the four snail populations followed a trend similar to r_m.

6.4.1 Growth

The quantification of age and growth is a vital component in understanding the life history traits of organisms. This is especially important for an introduced species. Knowledge of the individual growth rates and age structure of an introduced population is required to help determine its success and degree of establishment, as well as to predict its impact on the indigenous populations. Growth rate information can also be used to compare dynamics among environments, to describe trends over time, to determine the general status of a population and to assess the adaptation of a species to different environmental conditions (Kwak *et al*., 2006; Pedicillo *et al*., 2008).

Temperature affects growth and also its underlying processes (von Bertalanffy, 1960; Hochachka and Somero, 1984; Gillooly *et al*., 2001). For poikilotherms, rising temperature leads to increasing rates of biochemical processes, physiological processes and life history characteristics (Woods *et al*., 2003). Most poikilotherms are larger when they develop in or acclimate to colder temperatures compared with conspecifics exposed to warmer temperatures (Ray, 1960; von Bertalanffy, 1960; Körner and Larcher, 1988; Atkinson, 1994; Woods *et al*., 2003) and this can have important consequences for fitness (Roff, 1992; Stearns, 1992; Caley and Schwarzkopf, 2004).

Figures 6.1 - 6.3 show that when compared to the indigenous *L. natalensis*, *R. rubiginosa* exhibited the larger shell length at all three temperatures as well as having a greater longevity and a higher growth coefficient (Table 6.1). The large shell size of *R. rubiginosa* may present an advantage in competitive interactions over *L. natalensis*. The rapid growth of *R. rubiginosa* might allow it to achieve and maintain a size advantage over the indigenous *L. natalensis*. In addition, despite *P. acuta* having the smallest shell size across all temperatures (Figures 6.1 - 6.3), it had the fastest growth coefficient at 25^{o}C and 30^{o}C (Table 6.1).

The estimated growth parameters for *P. acuta* are in accordance with what is known about the species' biology, i.e. *P. acuta* is an invasive, long lived and rapidly growing species. In addition, due to its faster life history (high growth coefficient and smaller body size), this species tends to devote proportionally more of its resources to increased reproductive output (Read and Harvey, 1989; Gunderson, 1997; Denney *et al*., 2002). These traits are to be anticipated given the invasive characteristics of *P. acuta*.

The growth coefficient (K) is a measure of the growth rate of a population. The higher the K value, the more rapid the growth (expressed as an increase in the mean shell length). All four snail populations exhibited the highest and lowest growth coefficients at 20^{o}C and 30^{o}C respectively (Table 6.1). As K increased, the rate at which the asymptote was approached also increased. From Table 6.1, it was apparent that K decreased as the temperature increased.

The asymptotic length (L_∞) also decreased with increasing temperature (Table 6.1). For each of the three temperature treatments, *R. rubiginosa* had the highest L_∞. This parameter also indicated a competitive superiority for *R. rubiginosa* over the indigenous *L. natalensis*.

6.4.2 Survival Rate

The most important characteristics of the survival rate are the time elapsed before any individuals died (100% survival), the 50% mortality level (LT_{50}) and the time elapsed until all the individuals of a cohort were dead (0% survival). The survivorship curves (Figures 6.7 - 6.9) corresponded to the Type II, diagonal survival curves, following the classification of Slobodkin (1962). From these curves it was evident that mortality occurred during the early life of the individual and that there was a gradual reduction in mortality as the individual approached adulthood. The three age specific survival characteristics listed above all decreased as the temperature increased from 20°C to 30°C (Figures 6.7 - 6.9).

The within population variation (Table 6.2) indicated that survival was significantly influenced by temperature for *P. acuta*. This adaptability could contribute to its invasive success as it tended to maximise the survivorship characteristics at each temperature. *Radix rubiginosa* displayed similar within population survivorship patterns between 20°C and 25°C, as well as between 25°C and 30°C (Table 6.2). This indicated that the survival rates for *R. rubiginosa* were not influenced by temperature and that *R. rubiginosa* could be adapted to survive over a wider temperature range than *P. acuta*. But *P. acuta* is widely distributed in South Africa over a wide altitudinal range.

At 20°C, 25°C and 30°C, the cohorts of *P. acuta* survived approximately 1.21, 1.12 and 1.05 times longer than *R. rubiginosa* (Figures 6.7 - 6.9). In addition, LT_{50} values at 20°C and 25°C were 1.16 and 1.13 times longer in *P. acuta* than *R. rubiginosa* (Figures 6.7 and 6.8). At 30°C however, *R. rubiginosa* exhibited a LT_{50} value 1.08 times longer than that of *P. acuta* (Figure 6.9). The similarity in the survival rates of these species is quite

evident and Table 6.3 shows that the survivorship curves of *R. rubiginosa* and *P. acuta* were similar at 25°C and 30°C.

In addition, the survivorship curves for the two *L. natalensis* populations were similar to each other, but significantly different from both *R. rubiginosa* and *P. acuta* (Table 6.3). This was apparent at all three temperatures. From Figures 6.7 - 6.9 it is evident that the cohorts of *R. rubiginosa* and *P. acuta* lived 1.53 - 1.75, 1.39 - 1.75 and 1.50 - 1.83 times longer than those of the *L. natalensis* populations at 20°C, 25°C and 30°C respectively. So too the LT_{50} values of *R. rubiginosa* and *P. acuta* were 1.41 - 1.63, 1.41 - 1.75 and 1.63 - 2.00 times longer than the *L. natalensis* populations at 20°C, 25°C and 30°C respectively.

It was also evident from the survivorship curves (Figures 6.7 - 6.9) that *R. rubiginosa* and *P. acuta* exhibited the highest age specific survival characteristics (duration until first mortality, LT_{50} and longevity) at each of the three temperatures when compared to the *L. natalensis* populations. This is advantageous to both *R. rubiginosa* and *P. acuta* in that they are longer lived and therefore more competitive in terms of size, accessing resources (food and oviposition sites) and exhibiting an extended duration of oviposition in comparison to the indigenous *L. natalensis*.

6.4.3 Fecundity

Life histories are constrained by trade offs between survival and reproduction yielding combinations that should maximise the reproductive output (De Kock, 1973; Roff, 1992; Stearns, 1992; Charnov, 1993; Denney *et al*., 2002). Analysis of the species' reproductive data should allow identification of a mechanism that can affect invasion success.

From Figures 6.10 - 6.12, it was evident that higher temperatures shortened longevity and lowered fecundity. All populations exhibited the highest and lowest fecundity at 25°C and 30°C respectively (Figures 6.10 - 6.12).

Both *R. rubiginosa* and *P. acuta* displayed longer durations of oviposition than the indigenous *L. natalensis*, presumably because of their greater longevity (Figures 6.7 - 6.9). When the maximum fecundity of any of the four snail populations was related to its survival rate, it was clear that peaks in reproductive activity occurred when a high proportion of the cohort was still alive, i.e. before the cohorts declined to the 50% mortality level (LT_{50}). This was evident at all temperatures. It was also noted that the LT_{50} values for *R. rubiginosa* and *P. acuta* were reached much later in comparison to those of *L. natalensis* and this could contribute to the higher fecundity displayed by *R. rubiginosa* and *P. acuta*.

The within population variation (Table 6.4) indicated that fecundity was significantly influenced by temperature for *P. acuta*. This adaptability could again contribute to its invasive success as it laid the most eggs at each test temperature. *Radix rubiginosa* displayed similar within population oviposition patterns between 20°C and 25°C, as well as between 25°C and 30°C (Table 6.4). This indicated that the fecundity shown by *R. rubiginosa* was not influenced by temperature and that it could reproduce efficiently over a wider range in temperature than the two *L. natalensis* populations.

At 20°C, the reproductive peak of *R. rubiginosa* was 1.14 times higher than that of *P. acuta* (Figure 6.10). At 25°C and 30°C however, *P. acuta* had reproductive peaks 1.05 and 1.11 times higher than *R. rubiginosa* (Figures 6.11 - 6.12). The similarity in fecundity is quite evident and it is further noted in Table 6.5 that the survivorship curves of *R. rubiginosa* and *P. acuta* were similar to each other at 25°C and 30°C. From Table 6.5, both *L. natalensis* populations exhibited similar fecundity at 20°C ($p = 0.203$) and 25°C ($p = 0.085$) and were only marginally different at 30°C ($p = 0.045$). It is clear that the two *L. natalensis* populations were significantly different from *R. rubiginosa* and *P. acuta* (Table 6.5) with the exception that *R. rubiginosa* and the Greyville *L. natalensis* population were only marginally different at 30°C ($p = 0.060$).

Importantly, at 20°C the reproductive peaks of both *R. rubiginosa* and *P. acuta* were 1.45 - 1.90 times greater than those of *L. natalensis* (Figure 6.10). At 25°C and 30°C the

reproductive peaks exhibited by both *R. rubiginosa* and *P. acuta* were 1.71 - 1.81 and 1.66 - 2.03 times greater than *L. natalensis* respectively (Figures 6.11 and 6.12). It was therefore evident that *R. rubiginosa* and *P. acuta* were reproductively superior across a wide temperature range when compared to *L. natalensis*.

6.4.4 Life History Parameters

Two types of reproductive rates, namely, gross reproductive rate (GRR) and the net reproductive rate (R_o), were obtained from the life tables.

(a) Gross Reproductive Rate (GRR)

The gross reproductive rate (GRR) represented the sum of eggs produced per snail over the entire duration of the study. All snail populations had their highest and lowest GRR at 25°C and 30°C respectively (Table 6.6). *Physa acuta* exhibited the highest GRR for all temperature treatments and this rate was 1.17, 1.14 and 1.33 times greater than that of *R. rubiginosa* at 20°C, 25°C and 30°C respectively. This was anticipated given the success of *P. acuta* as an invader in South Africa (Hamilton-Attwell *et al*., 1970; De Kock *et al*., 1989; Brackenbury and Appleton, 1993; Appleton and Brackenbury, 1998; Appleton, 2003).

In addition, the reproductive peaks of both *R. rubiginosa* and *P. acuta* were 1.94 - 3.01 times greater than those of *L. natalensis* at 20°C and 1.81 - 2.87 and 2.39 - 3.19 times greater at 25°C and 30°C (Table 6.6). This has important implications in that the high reproductive yield of *R. rubiginosa* could lead to a competitive superiority over the indigenous lymnaeid *L. natalensis*. However, the GRR as a reproductive parameter does not incorporate mortality during the reproductive period. Therefore the more appropriate parameter to assess the reproductive capacity would be the net reproductive rate (R_o).

(b) The net reproductive rate (R_o)

The optimal life history strategy for a species has been defined as the one that maximises lifetime reproduction and is determined by maximizing age specific survival and fecundity (Roff, 1992; Stearns, 1992; Arendt, 1997). The net reproductive rate (R_o) represents the actual mean replacement per generation and includes the effect of the cohort survival rate (Birch, 1948; Shiff, 1964). In this study, all snail populations had the lowest R_o at 30°C (Table 6.6). *Lymnaea natalensis* from the UKZN Pond had its highest R_o at 25°C while the remaining snail populations exhibited their highest R_o values at 20°C. This characteristic of highest R_o values at 20°C was attributed to the extended longevity, increased survival and higher oviposition rates displayed by the populations (Figures 6.7 - 6.10).

From Table 6.6, it was noted that R_o for *P. acuta* was higher than *R. rubiginosa* at all three temperatures (1.20, 1.26 and 1.15 times at 20°C, 25°C and 30°C respectively). In addition, R_o for both *R. rubiginosa* and *P. acuta* was 2.37 - 3.95 times greater than *L. natalensis* at 20°C and 2.24 - 4.16 and 4.29 - 5.70 times greater at 25°C and 30°C respectively (Table 6.6). This parameter also highlights the reproductive and competitive superiority of *R. rubiginosa* and *P. acuta* over the indigenous *L. natalensis*.

(c) Intrinsic rate of natural increase (r_m)

All organisms tend towards unlimited increase and it has long been known (Lotka, 1925) that in unrestricted conditions, the number of individuals approaches consistency as the rate of increase approaches its final value (Shiff, 1964; De Kock, 1973; Prinsloo and van Eeden, 1976). Under natural conditions increase will become restricted and the organism will live according to a life pattern, depending on the environmental circumstances. This pattern, however, is not only dependent upon environmental factors, but is also affected by an innate attribute on the part of the organism itself (Shiff, 1964).

Life history parameters are important as indices used to assess how a species' population growth might respond to selected conditions. In addition, these parameters can also be used as bioclimatic indices to assess the invasive potential of an introduced population (Southwood and Henderson, 2000). The intrinsic rate of natural increase (r_m) is a key population parameter based on the comparative summary of the longevity, age specific survival rate and fecundity under a particular set of environmental conditions (Shiff, 1964; De Kock, 1973; Prinsloo and van Eeden, 1976).

The rate r_m was initially used as a measure of population growth (Lotka, 1943) but was later described by Andrewartha and Birch (1954) as the maximal rate of increase attained. It is also a useful measure of the relative favourability of experimental conditions in studies on population dynamics and limiting factors (Birch, 1948; Leslie and Park, 1949; Evans and Smith, 1952; Shiff, 1964).

The intrinsic rate of natural increase has a number of component variables that influence its magnitude (Birch, 1948). The length of the non-reproductive period in relation to the duration of oviposition and age specific fecundity is one such variable. The earlier in an animal's life that an egg is laid, the greater is its contribution to the values of r_m and R_o. Another important determinant of r_m is the survival pattern, i.e. the shape of the l_x curve. When r_m is small its value may depend significantly on oviposition in late adult life but when it is large it is determined mostly by oviposition rates in early adult life (Birch, 1948). In addition, r_m and R_o have been shown to be associated in terms of the mean generation time (T). If r_m decreases while R_o remains constant, T will be correspondingly longer (Shiff, 1964).

The intrinsic rate of natural increase (r_m) can also be a good indicator of the temperature at which the increase of a population is most favourable, because it reflects the overall effects of temperature on longevity, survival rate and fecundity of the population. In this study, all snail populations had the lowest r_m values at 30°C while the highest values were recorded at 25°C. This suggests that the optimum temperature was 25°C and that the populations would be under ideal conditions at this temperature. The high r_m values

attained at 25°C were further attributed to the high oviposition rates early in the adult life of the individual (Figure 6.11) and the shorter mean generation times (Table 6.6).

Differences in r_m values reflect the influence of temperature on the potential rate of population increase of the populations as shown in Table 6.7. The multiple comparisons between r_m values of the four snail populations (Tables 6.8 - 6.10) were also significantly different at each temperature. Only the two *L. natalensis* populations had statistically similar r_m values at each of the three temperatures.

Table 6.6 shows that the highest mean r_m value recorded was 1.218 for *P. acuta* and this was anticipated given its rapid recolonisation of the Umsindusi River, KwaZulu-Natal, South Africa (Brackenbury and Appleton, 1993). The intrinsic rate of natural increase for *R. rubiginosa* (1.050) was higher than the corresponding value for *L. natalensis* from the UKZN Pond (0.765) and from the Greyville Pond (0.759).

(d) Mean generation time (T)

If two or more populations have the same R_o but their r_m values are different then this could be attributed to differences in their mean generation time (T) since r_m is inversely but strongly related to T. A shortening of T will in effect increase r_m (Birch, 1948; Cole, 1954; Shiff, 1964; Lewontin, 1965). It therefore follows that an accurate estimate of the mean generation time cannot be obtained until the value of r_m is known (Birch, 1948).

When a population with a high r_m at the optimal temperature lives in an environment in which the temperature approaches this optimum, the population will expand rapidly until such time as other factors begin to restrict the increase (Shiff, 1964). Therefore, if r_m is lower, i.e. if conditions are suboptimal, R_o may still be high but the population will take longer to achieve this stable state and the mean generation time will be longer.

Physa acuta and both *L. natalensis* populations exhibited their longest mean generation times (T) at 20°C while *R. rubiginosa* had its longest T at 30°C (Table 6.6). It was

evident that all snail populations exhibited their highest r_m values at 25°C, i.e. conditions were optimal for population increase. This trait eventually resulted in the shortest mean generation time being recorded for *P. acuta*, *R. rubiginosa* and the Greyville Pond *L. natalensis* population at 25°C.

This life history trait has important implications for an assessment of the invasivness of a species. At an optimal temperature of 25°C, *P. acuta* required 5.584 weeks to attain the R_o of 898.578 (Table 6.6). *R. rubiginosa* required 6.259 weeks to attain its R_o of 715.644 while the UKZN and Greyville *L. natalensis* populations required 7.543 and 7.087 weeks to attain R_o values of 319.600 and 216.167 respectively (Table 6.6).

The reproductive superiority of *P. acuta* in comparison to the other snail species is evident. This is to be expected given the widespread distribution of *P. acuta* and its invasive characteristics. From Table 6.6 it is also evident that *R. rubiginosa* displays a tendency for rapid population growth over a short mean generation time. This has important implications for potential invasiveness.

(e) Finite rate of increase (λ)

This parameter indicates the number of individuals of the future cohort that will replace one individual of the existing cohort. Since the finite rate of increase (λ) is the natural antilogarithm of the innate capacity of increase (r_m), λ values displayed similar trends to r_m (Table 6.6).

All four snail populations had their highest λ values at 25°C. Of these, *P. acuta* had the highest λ, indicating that 3.381 individuals of the future cohort will replace one individual of the existing cohort. The multiplication value for *R. rubiginosa* was 2.858 followed by *L. natalensis* which had the lowest, 2.149 and 2.137 for the UKZN and Greyville populations respectively (Table 6.6). Finally, all four snail populations had their lowest λ values at 30°C.

In summary, life history parameters reflect the schedule of resource allocation to growth and reproduction which the organisms then adjust to mortality so that reproductive output is maximised (Roff, 1992; Stearns, 1992; Charnov, 1993). For most organisms, it is size rather than age that determines survivorship (Gross, 1981; McGraw and Wulff, 1983; Werner, 1988) and fecundity (McGraw and Wulff, 1983; Aarssen and Clauss, 1992). The growth rate is therefore an important factor to consider since it defines the relationship between size and age (Gotthard *et al*., 1994).

When compared to the indigenous *L. natalensis*, *R. rubiginosa* exhibited the largest shell length at all three temperatures, as well as having a higher longevity and growth coefficient (K). When compared to the invasive *P. acuta*, it was evident that *R. rubiginosa* exhibited longevities and K values that either exceeded or conformed closely to that of the physid. At 20^{o}C *R. rubiginosa* had a higher K than *P. acuta*, indicating that at this temperature, *R. rubiginosa* was superior to *P. acuta* in terms of growth (Table 6.1). At 25^{o}C and 30^{o}C however, *P. acuta* had the highest K followed marginally by *R. rubiginosa*.

The large shell sizes of *R. rubiginosa* may present an advantage in competitive interactions over *L. natalensis*. The rapid growth displayed by the species allowed it to achieve and maintain a size advantage over the indigenous *L. natalensis*. While the asymptotic length (L_{∞}) decreased with increasing temperature (Table 6.1), *R. rubiginosa* had the highest L_{∞} at each temperature. This parameter (L_{∞}) also indicated a competitive superiority of *R. rubiginosa* over *L. natalensis*.

Survival and reproduction are also measures of the success of a species and affect the numerical stability of its populations. Knowledge of mortality and reproductive rates allows for inferences to be made on factors that are important in the evolution of a species' life history (Butler, 1984).

It was evident from the survivorship curves that *R. rubiginosa* and *P. acuta* exhibited the highest age specific survival characteristics (time until first mortality, LT_{50} and longevity)

at each of the three temperatures in comparison to the two *L. natalensis* populations. This presents an advantage to both *R. rubiginosa* and *P. acuta*, in that since they are longer lived, they might be more competitive in terms of size, accessing resources (food and oviposition sites) and exhibiting an extended duration of oviposition in comparison to the indigenous *L. natalensis*. At 20°C and 25°C *P. acuta* had a higher LT_{50} level than *R. rubiginosa*. At 30°C however, *R. rubiginosa* had a slightly higher LT_{50} than *P. acuta*, indicating that it has a higher maximum temperature tolerance which might contribute significantly to its potential spread.

Both *R. rubiginosa* and *P. acuta* exhibited longer durations of oviposition and higher fecundities at the three temperatures in comparison to the two *L. natalensis* populations. At 20°C *R. rubiginosa* had a higher reproductive peak than *P. acuta* but at 25°C and 30°C *P. acuta* had the higher reproductive peak. More importantly, there was no significant difference in the fecundity between *R. rubiginosa* and *P. acuta* at these temperatures. This implies that *R. rubiginosa* has the ability to equal and at lower temperatures even exceed the reproductive potential of *P. acuta*. This assessment of the exceptional fecundity of *R. rubiginosa* suggests a likelihood for rapid spread.

Selection favours members of any population that leave the greatest number of descendents (Shiff, 1964). Overall, the invasive *P. acuta* had the highest mean r_m followed by *R. rubiginosa*, *L. natalensis* from the UKZN Pond and finally the Greyville Pond *L. natalensis* population. This pattern was evident for all temperatures. Taking into account the similarities between the age specific survival and fecundity of *R. rubiginosa* and *P. acuta* coupled with high r_m values and shorter generation times, it seems clear that *R. rubiginosa* has a propensity for rapid spread and that if this happens, it could impact on the indigenous *L. natalensis* and lead to its extirpation in some situations.

7

General Discussion and Conclusions

The discovery of an alien freshwater snail in any country is a cause for concern, particularly, if it has the potential to (a) spread and (b) to be of economic importance. But it is frequently non-biologists who are in the front line when it comes to intercepting these aliens as, for example, they inspect aquarium supplies at points of entry or work as SASS practitioners sampling rivers. Guidelines are needed to enable these staff to recognize such snails and Chapter 4 of this study does this by identifying simple characters that allow the alien *Radix rubiginosa* (Michelin, 1831) to be separated from the indigenous *Lymnaea natalensis* Krauss, 1848.

A suite of conchological characters, shell length, length of last body whorl and aperture width, provide simple though variable criteria for the separation of *R. rubiginosa* and *L. natalensis*. *Radix rubiginosa* had larger, more broadly ovate shells with longer (higher) body whorls than either of the two populations of *L. natalensis* that exhibited smaller, elongated shells with shorter (lower) body whorls. Also, *R. rubiginosa* had a narrower aperture width compared to the larger, wider aperture of the UKZN Pond *L. natalensis* population. The Greyville *L. natalensis* population was found to have narrower apertures than both *R. rubiginosa* and *L. natalensis* (UKZN Pond).

The morphology of the radula and the reproductive anatomy of *R. rubiginosa* and *L. natalensis* from both the UKZN and Greyville Ponds showed little variation and were observed to be homoplastic. The species did however vary in the relative numbers of radula teeth in each field and this serves as an additional useful diagnostic character.

Mantle pigmentation however offered a more definitive distinguishing character. Both *L. natalensis* populations had similar mantle pigmentation patterns. The entire mantle was gray to black in colour but interspersed with unpigmented spots that were numerous in the region above the kidney and towards the mid-region of the mantle. *Radix rubiginosa* displayed a distinctly different mantle pigmentation pattern. The mantle surface of *R. rubiginosa* was mottled black with patches of pale white to yellow. There were also large unpigmented fields and stripes that were not observed in *L. natalensis*. In addition, the mantle was interspersed with numerous unpigmented spots that were most frequent in the region above the kidney and towards the mid-region of the mantle.

Having found characters to conveniently separate the alien *R. rubiginosa* from the indigenous *L. natalensis*, it became increasingly important to assess the invasiveness of this introduced species and its likely impact.

In general, the species that become successful invaders are predicted to be species that, in their native ranges, display characteristics that enable them to successfully survive conditions encountered during transport, introduction, establishment and integration (Suarez and Tsutsui, 2008). Two main attributes of biological invasions are thus: invasiveness, the traits that enable a species to invade a habitat and invasibility, the habitat characteristics that determine its susceptibility to the establishment and integration of an invasive species (Lonsdale, 1999; Alpert *et al*., 2000; Marco *et al*., 2002).

Numerous hypotheses address the reasons behind successful biological invasion (Richardson and Pyšek, 2006) and most attribute it to characteristics of the invader or characteristics of the invaded ecosystems (Catford *et al*., 2009). Table 7.1 presents examples of some of these hypotheses.

Table 7.1: Examples of research on hypotheses of species invasiveness and ecosystem invasibility.

Hypothesis / Trait	Definition	Reference
Introduction Effort Hypothesis	The invasiveness of a species can be increased by repetitive and extensive introductions.	Williamson, 1996; Richardson, 1998; Lockwood *et al*., 2005; Gravuer *et al.*, 2008
Residence Time Hypothesis	Invasions experience a lag and that species introduced earlier are more likely to become invasive.	Richardson, 1998; Rejmánek, 2000
Enemy Release Hypothesis	A species may proliferate in non-native ecosystems because they leave behind their co-evolved predators, disease and parasites that regulate their populations in the native ecosystems.	Keane and Crawley, 2002; Fromme and Dybdahl, 2006; van der Velde *et al*., 2006; Wilson *et al*., 2009
Ecosystem Invasibility	Besides characteristics of species themselves, certain habitat aspects, such as close proximity to the metropolitan area, a high disturbance regime, or low native species diversity, can make an ecosystem prone to invasion. In habitats rich in native species, niches are already occupied and resources are fully utilised leaving little available for exploitation by invaders.	Elton, 1958; Richardson, 2004
Invasional Meltdown Hypothesis	The risk of new establishments by non-indigenous species increases when the habitat is already invaded by another introduced species. A species that is already established in a community could facilitate the invasion of a second species.	Simberloff and Von Holle, 1999; Bruno *et al.*, 2003
Species Traits	Certain intrinsic traits of the non-indigenous species may enhance establishment and invasiveness.	Rejmánek and Richardson, 1996; Kolar and Lodge, 2001

With reference to the "Introduction Effort Hypothesis", Madsen and Frandsen (1989) in their review of introduced freshwater snails worldwide, concluded that the aquarium trade was a source of multiple introductions and responsible for the introduction and

distribution of several of the common invasive species, including *Physa acuta* Draparnaud, 1805 (Appleton, 2003). In addition, Duggan *et al*. (2006) stated that a clear relationship existed between the frequency of occurrence of exotic species in the pet and aquarium industry to the increased likelihood of non-indigenous introduction and establishment. According to the "Invasional Meltdown Hypothesis", a species that is already established in a community could facilitate the invasion by a second alien species. If this is the case with two non-indigenous species, "invasional meltdown" may occur, with every new introduced species making it easier for subsequent ones to establish. This results in an accelerating accumulation of introduced species (Bruno *et al*., 2003).

In the context of this study, the "Introduction Effort" and "Invasional Meltdown" hypotheses are important since the Amatikulu Hatchery trades with Asia for both tropical fish and plants and may explain the many Asian exotic snails at the Hatchery. A survey of the snail species occurring at this study site showed that five of the six snail species present were introduced (Table 4.2). With reference to the above hypotheses, three invasive snails, *Tarebia granifera* Lamarck, 1822, *Physa acuta* Draparnaud, 1805 and *Lymnaea columella* Say, 1817 already occur in KwaZulu-Natal and could facilitate the establishment and spread of *R. rubiginosa*.

The "Enemy Release Hypothesis", states that invasive species are able to succeed in their new range (achieving higher population densities and broader ecological ranges than in their native range) because they have been released from the pressures that kept their population in check in their native ranges, such as predation, disease and parasitism (Keane and Crawley, 2002; van der Velde *et al*., 2006). As previously noted *R. rubiginosa* is the intermediate host for several trematodes over much of southeast Asia, i.e. *Fasciola gigantica* (Srihakim and Pholpark, 1991; Malone, 1997), *Trichobilharzia* sp. (Nithuithai *et al*., 2004), *Schistosoma incognitum* (Bunnag *et al*., 1983) and various echinostomes (Charoenchai *et al*., 1997). Introduced populations could however evolve lower investment in resistance or could down-regulate their immune system as a response to the absence of predators, disease and parasites (Fromme and Dybdahl, 2006). This

response might have consequences for the success of introduced species as with no or few natural "enemies" the introduced species will have a greater success at establishing and spreading in the new area (Keane and Crawley, 2002; Wilson *et al*., 2009).

Characteristics of the introduced species are critical to both their success and impact (Lodge, 1993) and identification of these traits allows recommendations to be made on how to evaluate the invasive potential of these new introductions (Kolar and Lodge, 2001). Factors that have been suggested as predictors of invasive success include abundance and range in the native habitat, a broad physiological tolerance (euryhalinity and eurythermy), rapid growth, large size, early sexual maturity and life history parameters such as short generation times, high fecundity and a high intrinsic rate of natural increase (Rejmánek and Richardson, 1996; Williamson and Fitter, 1996; Barrat-Segretain *et al*., 2002; Moyle and Marchetti, 2006; Keller *et al*., 2007; Suarez and Tsutsui, 2008). Therefore, the extent to which a species can spread, as well as its success in a given environment, is thought to be determined mainly by those factors which can limit growth, reproduction and survival (Sastry, 1979; Borcherding, 1995).

Data presented in Chapters 5 and 6 clearly show that in terms of the rates of embryonic development, growth, survivorship, fecundity and life history parameters (GRR, R_o, r_m, T and λ) at the three test temperatures, there is (i) little difference between the two introduced species *R. rubiginosa* and *P. acuta* and (ii) that they consistently perform better than the two populations of the indigenous *L. natalensis* which are similar to each other.

The temperatures at which snails lay eggs and the relationship between embryonic development, growth, survivorship, fecundity and life history parameters to temperature have important implications for the potential of an introduced species to spread and the extent of that spread (Harris and Charleston, 1977). Further, following Zukowski and Walker (2008), the increase in abundance and distribution of introduced species generally results in a decline of indigenous species.

As anticipated, *P. acuta* had a higher growth coefficient, longer survivorship, higher fecundity (higher hatching rate, fewer egg abnormalities, longer duration of oviposition), a shorter incubation period, greater life history parameters (GRR, R_o, r_m and λ) and wider temperature tolerances than the other species tested. These attributes undoubtedly contribute to its success as an invader. On the basis of this argument, its high adaptability to changing environmental factors such as temperature, is in agreement with the fact that *P. acuta* is now more widespread in South Africa than the indigenous species *L. natalensis*.

This has important implications for *R. rubiginosa*, since this snail displayed attributes and a temperature tolerance similar to the invasive *P. acuta*. This implies that *R. rubiginosa* also has the ability to colonize a wider geographical and altitudinal range than *L. natalensis*. Further, the superior reproductive ability of *R. rubiginosa* over *L. natalensis* is likely to create a situation that allows for the rapid spread of this species as well as impact on the indigenous *L. natalensis* that might render it vulnerable.

In Chapter 5, it was observed that *R. rubiginosa* had the largest egg capsules and the highest number of eggs per capsule (clutch size) of the three snail species investigated. Also, *R. rubiginosa* had the largest eggs and this may confer an advantage in that a larger amount of albumen is at the disposal of the embryo, allowing for more rapid development before hatching. *Physa acuta* produced the smallest eggs and smallest egg capsules but despite this characteristic it produced the second largest average clutch size after *R. rubiginosa*. As noted by Shiff (1964), selection favours members of any population that leave the greatest number of descendents. This characteristic is exhibited by both of the introduced species, *R. rubiginosa* and *P. acuta*, and may enhance the reproductive advantage of *R. rubiginosa* over the indigenous *L. natalensis*.

At each of the three temperatures, *R. rubiginosa* and *P. acuta* had higher mean hatching rates than the two *L. natalensis* populations (Table 5.2). In addition, *R. rubiginosa* and *P. acuta* exhibited frequencies of egg abnormalities that were 2.34 - 4.12, 2.36 - 3.46, 2.07 - 2.48 times lower than either of the populations of *L. natalensis* at 20°C, 25°C and 30°C

respectively (Table 5.2). The lower frequencies of egg abnormalities at each of the three temperatures in both *R. rubiginosa* and *P. acuta* further implies that a greater proportion of the eggs produced by these introduced species are likely to hatch.

Radix rubiginosa and *P. acuta* showed no significant difference in hatching rate or egg abnormalities at the three temperatures except that the number of eggs without development for *R. rubiginosa* showed a temperature influence (Table 5.3). This is important since it indicates that both *R. rubiginosa* and *P. acuta* have the ability to reproduce more efficiently over a wider range in temperature than the indigenous *L. natalensis*.

For each of the three test temperatures there was no significant difference in hatching rate or the frequency of egg abnormalities between the two *L. natalensis* populations (Tables 5.4 - 5.6). Importantly these lymnaeids were significantly different from both *R. rubiginosa* and *P. acuta* for these attributes. In addition, there was no significant difference between *R. rubiginosa* and *P. acuta* for hatching rate and egg abnormalities at 25^{o}C and 30^{o}C. Despite hatching rates being significantly different at 20^{o}C, *R. rubiginosa* was similar to *P. acuta* for the occurrence of dwarf eggs, eggs without egg cells and polyvitelline abnormalities. This suggested that *R. rubiginosa* exhibited similar characteristics to *P. acuta*, more so at the higher temperatures.

Incubation periods decreased with increasing temperature (Tables 5.7 - 5.10). *Radix rubiginosa* had a marginally longer incubation period than *P. acuta*, which had the shortest period for all temperatures. Importantly, the incubation periods for *R. rubiginosa* and *P. acuta* were shorter than the two *L. natalensis* populations.

Temperature induces developmental responses in body size, with larger individuals developing at lower temperatures (Imai, 1937; Vaughn, 1953; Fischer *et al*., 2003). It was therefore suggested by Vaughn (1953) that the animals grew and developed more rapidly at higher temperatures, using larger amounts of energy but were of a smaller size. This was found to be the case in this study as all four snail populations had the largest

and smallest hatching sizes at 20°C and 30°C respectively (Tables 5.7 - 5.10). For freshwater snails, a sufficient quantity of nourishment is of importance for the growth and survival of embryos since the size of the snail at hatching depends largely upon the supply of albumen available to the developing embryo. It was noted that *R. rubiginosa* had both the largest egg size and the largest embryo size at hatching.

The growth rate was however not constant for successive developmental periods and was dependent on both the temperature and length of the incubation period. At higher temperatures, faster growth rates associated with accelerated development of the embryo led to shorter incubation periods. Both *L. natalensis* populations exhibited similar growth rates but these were low when compared to *R. rubiginosa* and *P. acuta* (Tables 5.7 - 5.10). In fact, *P. acuta* had higher growth rates than *R. rubiginosa* for most developmental stages. The exception was the early veliger stage where *R. rubiginosa* had higher growth rates.

In Chapter 6, an assessment of the growth coefficient (K) indicated that *R. rubiginosa* and both *L. natalensis* populations had their highest values at 20°C while *P. acuta* had its highest K value at 25°C. All four populations had their lowest K values at 30°C, i.e. hatchling growth was fastest at the lower temperature and less so at the higher temperature. *Radix rubiginosa* had a K value at 20°C that was 1.06 times greater than that of *P. acuta*, while those exhibited by *P. acuta* at 25°C and 30°C were 1.05 and 1.04 times greater than *R. rubiginosa* (Table 6.1). Importantly, this indicated that at 20°C *R. rubiginosa* had a growth advantage over *P. acuta*, while at 25°C and 30°C it matched the higher values exhibited by the physid. The K values for both *R. rubiginosa* and *P. acuta* were 1.10 - 1.29, 1.19 - 1.35 and 1.11 - 1.36 times larger than those of the two *L. natalensis* populations at 20°C, 25°C and 30°C respectively.

Both the maximum shell length and the asymptotic shell length (L_∞) were largest at 20°C and smallest at 30°C for all snail populations except *L. natalensis* (Greyville Pond) which displayed its smallest L_∞ at 25°C (Table 6.1). *Radix rubiginosa* had the largest shell length and L_∞ at each temperature followed by the two *L. natalensis* populations and

finally *P. acuta* (Figures 6.1 - 6.3). This suggests that *R. rubiginosa* enjoys a competitive superiority over *P. acuta* in terms of its rapid growth creating and maintaining a size advantage over the indigenous *L. natalensis* as well. This is of advantage to both the introduced species in that they are longer lived and more competitive in terms of size, accessing resources (food and oviposition sites) and exhibit an extended duration of oviposition in comparison to *L. natalensis*.

Measurements of age specific survivorship indicated that there was no significant difference between the two *L. natalensis* populations (Table 6.3). When compared to the indigenous populations, *R. rubiginosa* and *P. acuta* exhibited higher survivorship values for the time until first mortality, the 50% mortality level (LT_{50}) and longevity (Figures 6.7 - 6.9). *Physa acuta* exhibited the greatest longevity, LT_{50} and longest duration of mortality, being only marginally higher than *R. rubiginosa*. Importantly, the time until first mortality for *R. rubiginosa* was similar to *P. acuta* at 25^{o}C but slightly longer at 20^{o}C and 30^{o}C (Figures 6.7 - 6.9). In addition, at 30^{o}C *R. rubiginosa* had a higher LT_{50} when compared to *P. acuta*, indicating that it has a higher maximum temperature tolerance which might contribute significantly to its ability to spread. A comparison of survivorship indicated no significant differences between *R. rubiginosa* and *P. acuta* at 25^{o}C and 30^{o}C, i.e. at the higher temperatures survivorship of *R. rubiginosa* conformed closely to that of the already invasive physid, *P. acuta* (Table 6.3).

Higher temperatures were associated with short oviposition periods and lowered fecundity for all four snail populations (Figures 6.10 - 6.12). Although the two *L. natalensis* populations displayed similar age specific fecundity (Table 6.5), *R. rubiginosa* and *P. acuta* had higher fecundity and longer oviposition periods. It was also noted that the LT_{50} values for *R. rubiginosa* and *P. acuta* were attained much later compared to *L. natalensis* and this could contribute to the higher fecundity displayed by the two exotics.

Comparisons between these introduced species showed that at 20^{o}C *R. rubiginosa* had the higher reproductive peak while *P. acuta* did so at 25^{o}C and 30^{o}C. More importantly perhaps, there was no significant difference in fecundity between *P. acuta* and *R.*

rubiginosa at the higher temperatures (Table 6.5). This implies that *R. rubiginosa* has the ability to equal and at lower temperatures even exceed the reproductive potential of *P. acuta*. This assessment of the exceptional fecundity of *R. rubiginosa* suggests real potential for significant spread.

The higher gross reproductive rates (GRR) and net reproductive rates (R_o) recorded at 20^{o}C and 25^{o}C could be attributed to the extended longevity, increased survivorship and higher oviposition rates displayed by all populations at these temperatures. At each of the three temperatures *P. acuta* had the highest GRR and R_o, followed by *R. rubiginosa* and then finally the two populations of *L. natalensis*. This was anticipated given the success of *P. acuta* as an invader in South Africa (De Kock *et al*., 1989; Brackenbury and Appleton, 1993; Appleton, 2003; De Kock and Wolmarans, 2007). A higher reproductive yield is an attribute on the part of the introduced species that allows for maximum oviposition over a wide range in temperature and highlights the potential reproductive superiority of the introduced *R. rubiginosa* and *P. acuta* over the indigenous *L. natalensis*.

In this study, 25^{o}C was clearly the optimum temperature for all four snail populations since they all exhibited a maximum intrinsic rate of natural increase (r_m) at this temperature (Table 6.6). This is also the experimental temperature found to be suitable for planorbids such as *Biomphalaria* and *Bulinus* spp. (Shiff, 1964; De Kock, 1973; Brackenbury and Appleton, 1991). The maximum r_m exhibited at 25^{o}C was attributed to the high oviposition rates early in adult life and the shorter mean generation times. At 30^{o}C all populations had their lowest r_m values and therefore exhibited their weakest performance at this temperature. Multiple comparisons of r_m among the four snail populations were significantly influenced by temperature (Tables 6.8 - 6.10). Only *L. natalensis* (both populations) had statistically similar r_m values at each of the three temperatures. Overall, the invasive *P. acuta* had the highest mean r_m followed by *R. rubiginosa*, *L. natalensis* from (UKZN Pond) and finally the Greyville Pond population. Since λ is the natural antilogarithm of the innate capacity of increase (r_m), λ values displayed similar patterns to r_m values.

In the earlier chapters, it was noted that the introduction of *R. rubiginosa* is likely to go unnoticed due to its resemblance to *L. natalensis*. This is because the shell morphology of *L. natalensis* is notoriously variable and some of its variations resemble *R. rubiginosa*. This is reflected in recent research into the epidemiology of human and bovine fascioliasis (Chen and Mott, 1990; Mas-Coma and Bargues, 1997; Esteban *et al*., 1998; Mas-Coma *et al*., 1999; Marquardt *et al*., 2000) which has focused on the different "forms" of *L. natalensis* in Africa. This is further complicated by increasing evidence suggesting that the forms of what is widely called *L. natalensis* in Africa may in fact comprise more than one species (Brown, 1994).

Dondero and Lim (1976) and Mienis (1986) have commented that it is easy to breed *R. rubiginosa* in aquaria and this was also found to be the case in this study. The indigenous *L. natalensis* is not as easy to breed and this raises the question, "If *R. rubiginosa* spreads in South Africa, will it do so at the expense of *L. natalensis*?" The occurrence of *L. natalensis* in KwaZulu-Natal appears to have decreased already perhaps due to the competition with yet another introduced lymnaeid, *Lymnaea columella* Say, 1817 (C.C. Appleton pers. comm). If *R. rubiginosa* becomes established in the same area then this could increase pressure on the indigenous *L. natalensis* and eventually lead to its extirpation in some areas.

Importantly, analysis of the results from Chapters 5 and 6 indicated that the introduced *R. rubiginosa* and *P. acuta* exhibited a superior performance at all temperatures when compared to *L. natalensis*. *Radix rubiginosa* and *P. acuta* therefore exhibited a wider temperature tolerance than *L. natalensis*, which might reflect a capability for these introduced species to colonize a wider geographical area and altitudinal range in South Africa. This wide range for *P. acuta* has already been documented (De Kock *et al*., 1989; De Kock and Wolmarans, 2007) and this species is regarded as one of the most invasive freshwater gastropod species in the country. According to De Kock and Wolmarans (2007), *P. acuta* has the ability to establish and maintain populations over an altitudinal range of 0 - 2000m, virtually the whole sub-continent.

The potential of *R. rubiginosa* to also occupy a wide geographical and altitudinal range is however at variance with its natural distribution, where *R. rubiginosa* occurs (and serves as the intermediate host for *F. gigantica*) over the lowlands of much of south-eastern Asia. In Chapters 5 and 6 it was noted that at higher temperatures (25°C and 30°C), the performance of *R. rubiginosa* was similar to that of *P. acuta*. However, at 20°C *R. rubiginosa* had the ability to equal and even exceed the performance of *P. acuta* in respect of certain attributes (growth coefficient and fecundity). This apparent anomaly where a south-east Asian species (*R. rubiginosa*) is found to exhibit a similar performance and in certain attributes exceed the performance of a palearctic species probably introduced from Europe (*P. acuta*) was unexpected. The data show that the advantage of *R. rubiginosa* lay in the period of growth from hatching to maturity coupled with its fecundity. No explanation can immediately be offered for this eurythermal performance of *R. rubiginosa* extending to such low temperatures. Unfortunately, there is a dearth of information on the biology of *R. rubiginosa*.

The present series of experiments suggest that *R. rubiginosa* has the ability to colonize a range of South African environments similar to that of the invasive *P. acuta* and depending on its compatibility to the local strain of *F. gigantica*, it could exacerbate the fascioliasis problem in the country too. Here again it is likely to interact with *L. columella* though the role of this latter species in fascioliasis transmission in South Africa has not been investigated. *Lymnaea columella* has been shown to be susceptible to *F. gigantica* in Egypt (Ahmed and Ramzy, 1999; Dar *et al*., 2005), and so is likely to be susceptible here as well.

Finally, it should be appreciated that laboratory studies on embryonic development, growth, survivorship, fecundity and life history parameters cannot be directly extrapolated to natural populations. They do however provide a useful basis from which to predict the invasive potential of introduced species. It not only increases our understanding of the mechanics of biological invasions but it helps to identify which potentially invasive species should be targeted at points of entry. This implies the need for quantitative analyses of the processes involved in biological invasions (attributes that

might enhance the invasiveness of introduced species and identification of invasion pathways) and of the large scale biotic changes (biotic homogenization and the possible extirpation of indigenous species) following the invasions.

The conclusions that can be drawn from this study are:

(i) The shell length, length of last body whorl and aperture width are useful conchological characters in the separation of *R. rubiginosa* and *L. natalensis*. *Radix rubiginosa* had larger, more broadly ovate shells with longer (higher) body whorls than either of the two populations of *L. natalensis* that exhibited smaller, elongated shells with shorter (lower) body whorls. *R. rubiginosa* had a narrower aperture width compared to the larger, wider aperture of the UKZN Pond *L. natalensis* population. The Greyville *L. natalensis* population was found to have narrower apertures than both *R. rubiginosa* and *L. natalensis* (UKZN Pond). These differences in morphology in *L. natalensis* populations only approximately 5.5 km apart again focuses attention on the plasticity of shell shape in this indigenous lymnaeid.

(ii) The mantle surface of *R. rubiginosa* was mottled black with patches of pale white to yellow and large unpigmented fields and stripes; the mantle was also interspersed with numerous unpigmented spots that were most frequent in the region above the kidney and towards the mid-region of the mantle. The entire mantle of *L. natalensis* was gray to black in colour but interspersed with unpigmented spots that were numerous in the region above the kidney and towards the mid-region of the mantle.

(iii) *Radix rubiginosa* and *P. acuta* had a higher growth coefficient (K), longer survivorship, higher fecundity (higher hatching rate, fewer egg abnormalities, longer duration of oviposition), shorter incubation period, greater life history parameters (GRR, R_o, r_m and λ) and wider temperature tolerances than the two *L. natalensis* populations tested.

(iv) The high adaptability of *P. acuta* to changing environmental factors such as temperature, is in agreement with the fact that it is now more widespread in South Africa than the indigenous species *L. natalensis*.

(v) *Radix rubiginosa* displayed attributes and a temperature tolerance that were similar to and in certain cases even exceeded the performance of the invasive *P. acuta*.

(vi) *Radix rubiginosa* appears to have the potential to colonize a wider geographical and altitudinal range than *L. natalensis*, perhaps also *P. acuta*. Also, the superior reproductive ability of *R. rubiginosa* over *L. natalensis* is likely to present a situation that allows for its significant spread as well as a possible impact on the indigenous *L. natalensis* that might render it vulnerable.

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