Aplexa marmorata (Guilding, 1828) (Basommatophora: Physidae) – an invasive freshwater snail in South Africa.

By

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ABSTRACT

Invasions of ecosystems by alien species is a worldwide problem. Man, with his constant travelling, introduces organisms to places they have never occurred in before. The introductions may be accidental or deliberate. Some of the introduced organisms become invasive and some of these also become pests. Two aquatic pulmonate snails, *Physa acuta* (Physidae) and *Lymnaea columella* (Lymnaeidae), were introduced to South Africa probably through the aquarium industry in the 1940s and have now spread to most of the country's freshwater systems.

A third invasive pulmonate, and second physid provisionally called *Aplexa* cf. *marmorata*, has recently been found in South African freshwaters. Comparison between *A.*. cf. *marmorata* found in Durban and *P. acuta* from Pietermaritzburg as an example of the genus *Physa*, confirmed that they belong to different genera and are therefore different species. Features compared were the shell, radula, foot, mantle, male genitalia and sperm morphology. *Aplexa* cf. *marmorata* is characterized by its foot having a pointed posterior end with a dark mid-dorsal stripe while that of *P. acuta* does not have these features. The mantle edge of *A.*cf. *marmorata* has short triangular dentations while that of *P. acuta* has long finger-like projections. *Aplexa* cf. *marmorata* does not have an externally visible preputial gland whereas *P. acuta* does. The penis of *A.*cf. *marmorata* has a lateral opening while that of *P. acuta* has a sub-terminal outlet. TEM sections of the spermatozoon of *A.*cf. *marmorata* showed that it has a maximum of two glycogen helices around the mid-piece while *P. acuta* is known to have three. A study of the population dynamics of *A.*cf. *marmorata* in Durban showed it to produce three overlapping generations within a 14 month period whereas *P. acuta* has been shown to produce as many as eight over a similar time period.

Further comparisons between South African *A.* cf. *marmorata* and similar material from the West Indies, Nigeria and St Lucia (KwaZulu-Natal) showed that they shared the same features with the specimens collected in Durban and are therefore considered to belong to the same species, *Aplexa marmorata* (Guilding, 1828). This species is indigenous to the Caribbean and northern parts of South America. The picture is however complicated by the fact that Dr L Paraense, doyen of the Brazilian school of freshwater malacology, does not recognize the genus *Aplexa* and redescribed this species under the name *Physa marmorata* in 1986.

PREFACE

The experimental work described in this dissertation was carried out in the Department of Biology, University of Natal, Durban, from June 1997 to July 1999, under the supervision of Professor C.C. Appleton.

This study represents original work by the author and has 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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DEDICATION

This work is dedicated to my son, Siko Ntando, who understood when I left him home to study.

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CHAPTER 1

FRESHWATER SNAILS AS INVADERS IN SOUTHERN AFRICA

1.1 DEFINITION OF AN INVASIVE ORGANISM

Invasions are defined as "introductions via human activity, either accidental or deliberate, which have been *successful*" (A.F.G. Tonin, unpublished data, 1986; Bruton & van As, 1986). Barrett & Richardson (1986) defined an invasion as "the successful founding of a colony in a region where none existed before and which is followed by rapid spread by the invading species." The introduction of alien animals to new areas reached its peak during the colonial era (Roots, 1976).

The usual sequence of introductory events, *viz*, accidental transport, invasion and establishment, can only be successful for species with wide ecological tolerance (van Bruggen, 1964). Similarly, Ehrlich (1986) and de Moor (1992) describe invasive species as those that can easily cross physical barriers such as mountains, waterbodies and harsh climate and, with or without the help of human beings, establish themselves before multiplying rapidly and dispersing to new habitats. In fact, most of the species that have become invasive are those that are able to cross barriers because of their relationship with *Homo sapiens* (Elton, 1958). Before humans became the main distributors of plants and animals, geographically separated regions shared very few species (Mooney & Drake, 1990). Inter-continental travel and the wide dispersal of man have eliminated continental isolation of species and has led to introductions of species to places where they never occurred before because of natural barriers (Ashton & Mitchell, 1989; Brackenbury, 1989). Even very isolated places have been invaded. A recent illustration of this was the discovery of the land snail *Helix aspersa* and the slug *Deroceras laeve* on Easter Island, 2000 km from the nearest continental landmass and thus the most remote island in the world (Naranjo-García & Appleton, 1998).

The life-history style adopted by an introduced species is an important factor determining the success or failure of the introduction (Bruton, 1986). Clearly, invaders must be able to adapt themselves to a different climate, soil and vegetation and compete with the indigenous fauna for available niches. Species with wide geographical ranges might inhabit more different habitats than species with restricted distributions. In many cases it seems that invaders are in fact pre-adapted to their new environment(s) (Roots, 1976). Invaders are usually abundant in their

original range and are non-selective feeders or have a broad diet (Baker & Stebbins, 1965; Pimm, 1989; de Moor, 1992). They probably come from a region with the same climatic conditions as the invaded habitat (Hengeveld, 1989; Coope, 1986). They are probably r-strategists because they establish themselves quickly, lack natural enemies and competitors in the invaded area, have year-round breeding capabilities and are adept at finding an empty niche (Moyle, 1986). Their generation time is usually short and they are genetically variable (Hengeveld, 1989), although some appear to rely on reproduction by self-fertilisation. This presumably occurs in the slug *D. laeve*, since all specimens collected on Easter Island by Naranjo-García & Appleton (1998) were aphallic. They are ecologically and behaviourally distinct from native species. The importance of ecological parameters in determining invasive success, *viz.* strong resistance to conditions prevailing in the invaded region, inter-specific competition, community structure and food chains, was emphasized by Elton (1958). To these we can add predation and parasitism (Roots, 1976).

It is not a general rule for an invading species to behave like an invader in its place of origin. For example, *Pinus radiata* (Monterrey pine) does not show invasive behaviour in California, which is its place of origin, but it has become a strong invader in South Africa, Australia and New Zealand (di Castri, 1989).

Successful invasion occurs mostly in disturbed or modified habitats (de Moor, 1992). Ecological resistance was noted to be lower in disturbed, less complex environments and on small islands where biotic diversity is low (Elton, 1958). These environments are regarded as being more susceptible to invasion by introduced species. Fox & Fox (1986) noted a negative relationship between community richness and susceptibility to invasion, meaning that species-rich communities are less prone to invasion. Bruton (1990) challenges this idea and notes that the African Great Lakes, which are stable, complex, diverse ecosystems, are vulnerable to major disturbances such as the introduction of alien species, overfishing and pollution. These lakes can be regarded as "land-locked islands" because of their isolation and their fauna and flora may be as vulnerable to extinction as on oceanic islands. In summary, most undisturbed communities have a low invasibility to almost all invaders while the reverse is true for disturbed ones.

Man-made changes are the most important factors influencing the distribution and abundance of introduced snails in Africa. Construction of new highways and deforestation were thought to be important factors that might cause the introduced snails to spread. Artificial modification of aquatic habitats usually leads to a reduction in the heterogeneity of the environment, loss of many

microhabitats and hence a decrease in species diversity (de Moor, 1992). A more homogenous environment is then created which can be invaded by species adapted to the newly created aquatic habitat (Harrison & Shiff, 1966). Disturbance of natural systems is often associated with the success of introduced species as was the case in Florida, USA, where clearing, altered burning cycles and large drainage projects have turned previously stable ecosystems into unstable ones (Mooney & Drake, 1986). Naturally unstable habitats like river bends are susceptible to invasion (Macdonald & Jarman, 1984) and may therefore serve as entry points into the system for introduced species.

The "invasibility" of habitats or communities is thus an important characteristic of environments exposed to alien organisms and must influence the aliens' survival and spread. Fox & Fox (1986) regard "disturbance" as a pre-requisite for invasion. All communities are probably open to invasion to some extent but those disturbed by various agencies such as cultivation of land (Elton, 1958), and disturbance by glaciation, erosion or fire (Williamson & Brown, 1986), are undoubtedly the most susceptible (Hengeveld, 1986).

Competition between invaders and native organisms may give an impression that the ecosystem is "adapting" to the invading species as was the case during the spread of the fern, *Salvinia molesta,* over Lake Kariba, Zimbabwe (Arthington & Mitchell, 1986). In fact, Holdgate (1986) was of the opinion that invasion depends more on the interactions between the invader and the invaded habitat once dispersal has taken place than on the process of dispersal itself.

Biological invasion is an ongoing process. Sailer (1983) estimates that about 11 exotic species of insects and other arthropods become established in the USA annually. About five species of higher plants colonize eastern Australia each year (Groves, 1986). Forty percent of the birds and higher plants found in the Hawaiian islands are invaders (Mooney & Drake, 1990). Over 50% of the flora of New Zealand consists of invaders and unfortunately such large scale invasion causes the extinction of indigenous species.

1.2 BIOLOGICAL INVASIONS

Biological invasions have received considerable attention recently as SCOPE (the Scientific Committee on Problems of the Environment) established an international programme to investigate the ecology of these invasions (Mooney & Drake, 1986; Drake *et al.*, 1989; Mooney & Drake, 1990). One of the key issues debated by participants was whether or not invasive organisms shared common attributes that facilitated their success. Invasions of ecosystems by exotic species may be natural or "artificial" i.e. accidental, usually via the sport fishing and aquarium industries or deliberately as biological control agents (Appleton & Brackenbury, 1997). Natural invasions are those that have taken place without the help or influence of man (Ashton & Mitchell, 1989). They happen by chance but are characteristic of the earth's biota. An example is the invasion of South America by mammals after the appearance of the Panamanian land bridge (Marshall & Williams, 1981). It can therefore be argued that there is no difference between natural and "artificial" invasions but that they are occurring faster nowadays than previously.

"We are in the era when intentional and accidental introductions of alien fauna or flora may be documented, whereas their impact may be measurable only in the distant future" (de Moor, 1992). The planned introductions of exotic species into new habitats in the past, many of which have led to environmental disasters, were done to fill so-called "vacant niches". The impact an introduction of an alien species has on the ecosystem is not restricted to closely related taxa but extends across broad taxonomic boundaries so that the whole community structure is likely to be affected.

The spread of invasive organisms into places faraway from their original habitats is of increasing global concern (Scope, 1983). "We must make no mistake: we are seeing one of the great historical convulsions in the world's fauna and flora" (Elton, 1958). Many invading species have been shown to have altered the structure and functioning of the communities and ecosystems into which they were introduced or become established in habitats which had already been modified or disturbed by man (Herbold & Moyle, 1986; Moyle, 1986) and one suspects that they must all have done this to a greater or lesser extent. Commonly altered properties of ecosystems include productivity, soil structure and nutrient cycling (Vitousek, 1986) and some introductions have led to serious economic and health problems (de Moor & Bruton, 1996). Introduction of alien species has led to reductions in numbers of indigenous species, local extinctions and in some cases the extinction of endemic species (Scope, 1983; de Moor, 1992).

Although terrestrial in habit, it is pertinent to record here that four carnivorous snail species were introduced to the Western Cape during the period 1959 to 1963 by the Department of Agriculture (Dr. M. Walters, Plant Protection Research Institute, Pretoria, *in litt.* to C. C. Appleton, April 1999). These were: *Euglandina rosea, Gonaxis kibwiziensis*, *Gonaxis* sp. and *Gulella wahlbergi.* They were intended as biological control agents against invasive helicid snails such as *Theba pisana* and *Cochlicella* spp. Fortunately, none of these four seems to have survived. This is especially fortunate in respect of *E. rosea* since this species was also introduced to a number of Polynesian islands in an attempt to control invasive *Achatina fulica.* Instead of preying on the achatinid, *E. rosea* fed on the endemic tree-dwelling genus *Partula* and is credited with eating several species to extinction.

Similarly, the estuarine mud-snail *Velacumantus australis* was translocated from the east coast of Australia to the west coast where it has become established in the Swan River estuary, Perth, and transmits the dermatitis-producing avian schistosome, *Austrobilharzia terrigalensis*, to swimmers each summer (Appleton, 1989).

Introduced species that do not have their native predators present in the invaded area are able to out-compete indigenous species in reproduction (Macdonald & Jarman, 1984). Many of these invaders are of major importance to agriculture, forestry, fisheries etc. and some are intermediate hosts or vectors of parasites affecting man and/or his livestock, e.g. the snail *Lymnaea columella* which is a proven intermediate host of *Fasciola* spp. Magzoub & Kasim (1980) claim that invasive *Physa acuta* were successfully infected with *Schistosoma haematobium* in a laboratory in Arabia. There is no evidence, however, from any areas endemic for schistosomiasis in Africa, that snails of families other than the Planorbidae, transmit the parasite (Brown, 1995).

The malaria epidemic in South America between 1938 and 1939, where more than 20 000 people are believed to have died, can be used to show the impact an invader can have (Elton, 1958). The reason why *Anopheles gambiae*, the mosquito species that was introduced from Africa, became a major epidemiological problem, was because its larvae bred in open waterbodies and the adult stages rested in human habitations, unlike the indigenous anopheline species. Not all introduced species have become invasive however and the question as to why some do and others do not, is the subject of much debate.

1.3 HOW ARE INVADERS INTRODUCED?

Although some alien species have been deliberately introduced into new habitats, e.g. most plants used in commercial agriculture and most parasitoids and predaceous insects used in biological control, many invaders have been introduced accidentally. One of the major impacts of invasive aquatic animals is through the introduction of alien parasites and diseases that threaten natural communities and aquaculture (Bruton & van As, 1986). In South Africa, Wells *et al.* (1986) noted that most invasive plants were introduced intentionally and it is likely that many introduced animals were transported with them. Certainly many of our freshwater invasives were introduced via the sport fishing industry (i.e. fish and their parasites) and via the aquarium trade (medusae, snails and weeds) (de Moor & Bruton, 1988). Whereas most sport fish species were introduced intentionally, their accompanying parasites, other invertebrates and weeds were accidental introductions. Recently several insects were introduced to South Africa and Namibia as biological control agents of invasive water weeds such as *Salvinia molesta* and *Eichhornia crassipes* (Arthington & Mitchell, 1986; Mill, 1997).

1.4 THE SOUTH AFRICAN SITUATION

Many alien aquatic animals and plants were introduced into southern Africa through colonial colonisation of the subcontinent (e.g. de Moor & Bruton, 1988; Rayner & Appleton, 1992). The majority of the European and North American species were introduced in the 19th century and the South American and African aliens were probably introduced during the 20th century. According to Henderson & Wells (1986), Macdonald & Richardson (1986) and Wells (1986a), the ecosystems in southern Africa most affected by invasive plants are the riparian zones of rivers but the degree of invasion and prevalence of invasive species vary from area to area due to geographical differences.

Many South African freshwater systems are disturbed to some extent by the building of impoundments (e.g. dams and weirs), abstraction, agricultural runoff, bank erosion, siltation etc. and are therefore prone to invasion. Bruton & Merron (1985); Ashton *et al.* (1986) and de Moor & Bruton (1988) have catalogued the introduced freshwater animals in South Africa and assessed their "invasive" status. They record six molluscs (five gastropods and one bivalve). Two of the gastropods and the bivalve are marine. The remaining three gastropods are freshwater pulmonates: *Lymnaea columella, Helisoma duryi* and *Physa acuta.* Two of these, *L. columella*

and *P. acuta,* have become invasive and are actively spreading across the continent while *H. duryi* has only been reported from artificial waterbodies (Appleton, 1996). Appleton (1996) and Appleton & Brackenbury (1998) have since updated the list of freshwater gastropods introduced into South Africa but which have not necessarily become invasive, and noted the presence of several prosobranchs, *Pomacea* spp. (Family Ampullariidae) as well as a second member of the Family Physidae, *Aplexa* cf. *marmorata*. Like *Helisoma* duryi, the ampullariids have not spread beyond artificial waterbodies such as ornamental ponds but *A. marmorata* has, during the past 15 years, been found in a variety of waterbody types. These are all in the extreme eastern part of South Africa and range from artificial ponds to natural pans and backwaters in rivers. It seems to be spreading and is likely to become the country's third invasive freshwater pulmonate snail (Brackenbury & Appleton, 1997). *Aplexa* cf. *marmorata* is the subject of this study.

1.5 OBJECTIVES

1.5.1 GENERAL OBJECTIVE

The main aim of the study was to characterise the alien freshwater species provisionally called *Aplexa* cf. *marmorata* which has recently been found in South African freshwater systems.

1.52 SPECIFIC OBJECTIVES

1. To differentiate between *A.* cf. *marmorata* and *Physa acuta*, a successful invader.

2. To evaluate the potential of *A.* cf. *marmorata* to become a successful invader.

Morphological aspects that are of taxonomic value such as shell, foot, mantle, penial complex and egg capsules of the two physids were compared in trying to differentiate the two species. A detailed study of the sperm morphology of *A.* cf. *marmorata* was also carried out to facilitate a comparison with that of *P. acuta* (Brackenbury, 1989).

Annual population fluctuations of *A.* cf. *marmorata* and the response of this species to environmental disturbances at two sites within the Durban Metropolitan area were monitored. Egg production at three different constant temperatures in the laboratory was studied.

CHAPTER 2

AQUATIC PULMONATES AS INVADERS

2.1 INTRODUCTION

Before discussing the Family Physidae, which includes *Aplexa* cf. *marmorata*, it is necessary to present some characteristic features of the sub-class Pulmonata to which the Physidae belongs. Pulmonates are either terrestrial (Stylommatophora) or freshwater (Basommatophora) though a few of the latter are found in the marine inter-tidal zone (Brown, 1967; Fretter & Peake, 1978; Wethington & Dillon, 1993). Typically they have lost their gills and the roof of the mantle cavity has become richly vascularized to form a "lung" which allows them to breathe air. Most are simultaneous hermaphrodites. The freshwater forms are secondarily adapted to water since they can also extract dissolved oxygen from the water via their skin (Crowl & Covich, 1990). This cutaneous respiration has surely helped equip them to colonize a wide range of aquatic habitat types.

Few or even single species may often be found in high densities under extreme physical conditions, for example in temporary pools. Conversely, in lakes or on tropical islands, many closely related species may be found co-existing with each other but with each species being represented by only a few individuals. Either way, they are amongst the most common and conspicuous animal groups of the freshwater biota (Bondesen, 1950; Wethington & Dillon, 1993) and show adaptations to different environments (Crowl & Covich, 1990). They have short life cycles, maturing at a young age and having a short reproductive period.

2.2 THE PULMONATE RADULA

The radula is secreted by the radula gland and lies on the radula membrane which in turn covers the odontophoral cartilage (Roller *et al.*, 1984) and projects from its posterior end (Runham, 1969). The odontophore is the supportive base for the radula and is reinforced by cartilages and moved by a set of muscles. The radula membrane holds rows of teeth that are being formed continuously at its posterior end (Isarankura & Runham, 1968; Bullock, 1989; Gittenberger & Goud, 1994). Worn teeth drop off at the anterior (working) end and are usually swallowed by the

snail. Abrasion and reduction of the denticle cusp height begin soon after the teeth move anteriorly to the working surface and become involved in feeding.

The radula is replaced at a rate of 1-5 rows of teeth per day (Runham & Isarankura, 1966) but this will depend to some extent on the species as well as on temperature, age, "depth of sleep" (this relates to hibernating stylommatophorans), but not to starvation or aestivation (Gittenberger & Goud, 1994). For example, in *Lymnaea stagnalis* the radula is replaced at a rate of 2.98 rows/ day and 5.02 rows/day in *Agriolimax reticulatus* (Runham & Isarankura, 1966). Radula replacement is most rapid in newly hatched snails (Isarankura & Runham, 1968) but decreases with age (shell size), proportionally to temperature.

The pulmonate radula has transverse rows of teeth comprising a central tooth with several to many lateral and marginal teeth on either side. The transition between the lateral and marginal teeth is not always clear (Bor *et al.*, 1994) but, counting outwards from the central tooth, the first tooth with a basal plate decreasing disproportionally in size is considered to be the last lateral and the adjoining one is the first marginal.

2.3 EGGS AND EGG CAPSULES

Egg masses or capsules are often structurally complex and energetically costly to produce. They may provide embryos with calcium needed for cell adhesion, embryo formation or proper physiological functioning under osmotic stress (Taylor, 1973). They are beneficial in confining embryos until they are able to move away from the danger of being fed upon by suspension or deposit feeders (Pechenik, 1986). Encapsulation of the eggs may also protect the developing embryos from bacterial attack and predation and the capsular fluid itself is nutritive in some species.

The eggs of freshwater pulmonates are laid as capsules containing up to 40 or 50 eggs (Bondesen, 1950; Rudolph & White, 1979). The capsules of all freshwater pulmonates have a characteristic end part, the *existus terminalis* or terminal tail. The capsule may be more-or-less like a spout, tube or tapering thread. The egg mass of pulmonates typically rests on the substratum with the initial point downwards. During oviposition, the snail turns away from the capsule, i.e. the sinistral physids turn to the right and the capsule turns clockwise.

As fertilized eggs of, for example *Lymnaea,* pass down the oviduct, they receive various coatings which form the egg capsule (Duncan, 1957, 1959). Initially the egg is bounded only by an internal membrane (Bondesen, 1950). The egg itself then secretes a delicate plasmatic membrane, the primary envelope or vitelline membrane, around itself and as it passes through the efferent duct of the reproductive system, it is surrounded by albumen and a "1st order" tertiary envelope secreted by the female tract (Duncan, 1957; Rudolph & White, 1979).

The Families Physidae and Lymnaeidae have a 2nd order tertiary envelope, the external membrane, surrounding the internal membrane. The external membrane has a fine lamellar structure and is continued in the egg strings or *fila ovi,* found only in egg capsules of the two families. Similarly, in these two familes a gelatinous, slimy outer layer, the *pallium gelatinosum,* covers the egg capsules at deposition.

Pulmonate snails are amongst the most obvious invasive aquatic invertebrates in South Africa (Ashton *et al.*, 1986). As noted earlier, only two pulmonate species have become invasive in South African freshwaters so far but malacologists should be on the alert for introduced exotic freshwater species (Brown, 1995). The two already established are *Lymnaea columella* and *Physa acuta* and the state of knowledge on each is summarised below.

2.4 *Lymnaea columella* (Say, 1817) (Lymnaeidae)

Lymnaea columella, is an amphibious snail of North and Central American origin. It was not listed by Connolly (1939) in his monograph of South African non-marine molluscs so that the first report from South Africa, 1942 at Somerset West near Cape Town (Brown, 1994), is probably close to the true date of introduction. Like other aquatic invertebrates, it was probably imported into the country accidentally with fish or aquatic plants (Brown, 1980). Since then it has colonised most of the major river systems draining the subcontinent (van Eeden & Brown, 1966; de Kock *et al.*, 1989). According to the records of the National Freshwater Snail Collection held in the Zoology Department, Potchefstroom University, *L. collumella* is the third most widely distributed freshwater snail in South Africa after two indigenous species, *Lymnaea natalensis* and *Bulinus tropicus* (de Kock *et al.*, 1989). It also occurs in Kenya and Egypt (Madsen & Frandsen, 1989; Brown, 1994) and was recently found in Lake Kariba on the Zambezi River in Zimbabwe (Anon., 1998). Little is known of its ecology in Africa but Appleton

(1974) showed that in South Africa it lives for approximately eight months and breeds in winter. It is an intermediate host for the common liver fluke, *Fasciola hepatica*.

2.5 *Physa acuta* (Drapanaud, 1805) (Physidae)

Like *L. columella*, this species was introduced into South Africa in 1942 (Hamilton-Attwell, 1970; Brown, 1994). The first report was from KwaZulu - Natal where it was collected in the Umsinduzi River, Pietermaritzburg, in 1954 (K. N. de Kock, pers. comm.) but it has spread over much of the country during the intervening approximately 50 years (Hamilton-Attwell *et al.*, 1970; de Kock *et al.*, 1989). This distribution pattern shows concentrations of records around the major urban areas suggesting that it has been introduced more than once. It is now found in various artificial and natural habitat types and often dominates the macroinvertebrate fauna, e.g. the Umsinduzi-Umgeni system between Pietermaritzburg and Durban. It also occurs commonly in polluted water and has therefore been nicknamed "the sewage snail". According to Alexandrawicz (1986), its spread across Europe was associated with that of polluted and heated water due to industrial pollution. It is of no economic importance but sometimes it may be infected with bird schistosomes, which can cause "swimmer's itch" (Frandsen *et al.*, 1980) though this probably happens only in its native North America.

Physa acuta has a worldwide distribution today (Smith & Kershaw, 1979) but is, as noted above, presumably of North American origin (Brown, 1980) although van Bruggen (1966) suggested it was introduced to South Africa from Europe. Whatever the route of introduction, this invasive species has spread to many African rivers and lakes from South Africa to Morocco. Working in the Umsinduzi River, Pietermaritzburg, Brackenbury & Appleton (1993) showed that *P. acuta* was able to produce six identifiable cohorts within a 12 month period, most of them in response to disturbances due to floods. This and its other invasive attributes have been reviewed by Appleton & Brackenbury (1998).

A third aquatic pulmonate, *Aplexa* cf. *marmorata*, also belonging to the Physidae, has been reported from several localities in South Africa since 1985 (Appleton *et al.*, 1989; Brown, 1994) and seems to be spreading in the eastern half of the country. As mentioned earlier, this snail is the subject of this study.

CHAPTER 3

The Family Physidae in Africa

3.1 INTRODUCTION

Although the Family Physidae is not indigenous to Africa, representatives of two physid genera, *Physa* and *Aplexa,* have been introduced (see Chapter 2); both have become invasive and are probably still spreading (Hamilton-Attwell *et al.*, 1970; de Kock et al, 1989; Madsen & Frandsen, 1989; Brown, 1994; Appleton & Brackenbury, 1998). They do not however seem to have been reported from the same habitat suggesting that they may have different ecological requirements. Since it is possible that they may occur sympatrically in some places, it is useful to provide descriptions of both genera and compile a list of morphological criteria by which they can be separated (see below). As pointed out by Appleton & Brackenbury (1998), neither of the two physid species occurring in Africa has been adequately characterised or identified – in fact a number of authors have expressed dissatisfaction with the state of physid systematics in general (Te, 1980, Taylor, 1988, Brown, 1994). As noted by Clampitt (1970), the morphology of the male genitalia is important for specific diagnoses within the Physidae.

3.2 *Physa* Draparnaud, 1801

3.2.1 DISTRIBUTION

According to Te (1973), the genus *Physa* predominates in North America and, following introductions to Europe and Asia, is the dominant physid genus throughout the Northern Hemisphere. *Physa acuta* was introduced to Africa, probably via Europe from North America, and is known from many countries on the continent though not so far from West Africa south of the Sahara (Kristensen & Ogunowo, 1992). The first known African record for *P. acuta* was from Somerset West near Cape Town, South Africa in 1942 (Hamilton-Attwell, 1970; Brown, 1994). Brown (1994) has however also commented on the uncertainty over the correct identification of this widespread species. *Physa acuta* was also introduced into Australia (Smith & Kershaw, 1979) and Malaysia. Listed below is a review of features differentiating *P. acuta* from *A.* cf. *marmorata.*

3.2.2 SHELL

The shell is ovate-oblong (Te, 1978; Paraense, 1987; Shi-Kuei & Beetle, 1995, Appleton, 1996). Its texture is smooth and shiny, but not glossy as in *Aplexa* (Te, 1978), and light brown in colour (Smith & Kershaw, 1979). It has round, convex whorls and the body whorl is greatly expanded (Richards, 1964; Paraense, 1987) with protruding shoulders (Te, 1978; Barbosa, 1995). The spire is elevated and sharply conical (Smith & Kershaw, 1979; Paraense, 1987). Sutures are deep (Richards, 1964; Kristensen & Ogunowo, 1992) and well impressed (Paraense, 1987). The aperture is ovate-lunate (Smith & Kershaw, 1979).

3.2.3 MANTLE AND FOOT

The mantle edge processes are finger-like (Richards, 1964) and extend partly over the body whorl of the shell (Richards, 1964; van Bruggen, 1966; Clarke, 1973).). The foot does not have the central black stripe and neither does the body wall have the small yellow-green "vacuoles" that are both characteristic of *Aplexa* (Richards, 1964; Paraense, 1986) (see below).

3.2.4 MALE GENITALIA

The spermatheca is pyriform or pear-shaped (Paraense, 1987; Barbosa, 1995). The preputium has a distinct preputial or accessory gland (Richards, 1964; Te, 1973, 1978; Kristensen, 1987; Kristensen & Ogunowo, 1992; Barbosa, 1995; Shi-Kuei & Beetle, 1995; Appleton, 1996) (Fig. 3.4). The penial duct has a sub-terminal outlet (Richards, 1964; Paraense, 1987; Barbosa, 1995).

3.2.5 EGGS

The egg capsules of South African *P. acuta* are oval in shape and concave on the free side. They have a transparent, colourless capsular membrane which encloses a clear viscous fluid in which the eggs are embedded (Brackenbury, 1989). The clutch size ranges from 5-86 eggs/capsule.

Physa acuta may lay its eggs on the shells of its conspecifics and up to six such capsules have been reported from a single individual's shell (Brackenbury, 1989). Usually however, the egg capsules are laid in high densities on hard, submerged surfaces such as rock and compacted mud close to the water/bank interface.

3.3 *Aplexa* Fleming, 1820

Included here are characters reported by Paraense (1986) and other authors for *Physa marmorata* which Dr. Paraense and Dra. S. Thiengo equate with the South African material (see later).

3.3.1 DISTRIBUTION

The genus *Aplexa* is circum- boreal in distribution, i.e. it occurs in the Northern Hemisphere, and has been presumed to be monotypic (Te, 1978). It predominates in Central and South America (Te, 1973) but is also present in West Africa where it has become invasive after being introduced at least 60-70 years ago, probably from the West Indies (Ranson & Cherbonnier, 1951; Brown, 1994) or perhaps from Brazil via the slave trade in the 17th and 18th centuries (Appleton *et al.* 1989).

3.3.2 THE SHELL

The shell of *Aplexa* is smooth and glossy (Brown, 1980; Kristensen, 1987; Brown, 1994) with an acute spire (Te, 1978; Shi-Kuei & Beetle, 1995) but is considerably higher and more conical in shape than *Physa* (Fig. 3.1). The shell is thin with convex whorls without prominent shoulders (Te, 1978; Paraense, 1986; Barbosa, 1995). The body whorl is narrower than *Physa* and has shallower sutures (Richards, 1964; Appleton, 1996) bordered by a whitish band of growth lines (Paraense, 1986).

3.3.3 MANTLE AND FOOT

Richards (1964) described the mantle margin as plain or serrated while Paraense (1986) described it as having short, triangular dentations. On the right hand side the mantle extensions cover nearly half the right surface of the body whorl and on the left they cover the ventral surface up to the suture line (Paraense, 1986). The foot is spatulate in shape, rounded in front with an elongate, pointed tail. This tail has a central black stripe running along its length (Paraense, 1986; Appleton, 1989). The skin contains numerous small, yellow-green "vacuoles" that give the body wall a characteristic tint (Richards, 1964; Paraense, 1986). The precise nature of these "vacuoles" is not known.

3.3.4 MALE GENITALIA

The spermatheca has on oblong, curved body and is more-or-less constricted in the middle (Paraense, 1986; Barbosa, 1995). The preputium in *Aplexa* is thin-walled with two longitudinal folds or pilasters and the copulatory organ lacks and a preputial or accessory gland (Richards, 1964; Paraense, 1986; Kristensen, 1987; Shi-Kuei & Beetle, 1995). The penial canal opens laterally (Richards, 1964; Paraense, 1986).

3.3.5 EGGS

Eggs are laid within a gelatinous envelope (Richards, 1964) and the surrounding capsule is a slimy C-shaped ribbon with rounded ends (Paraense, 1986). The clutch size of *A. marmorata* ranged between 7 and 35 eggs/capsule (this study). No eggs were found attached to the shells of other snails.

3.4 DISTINGUISHING SOUTH AFRICAN *Aplexa* cf. *marmorata* FROM *Physa acuta*

Because of ongoing debate over the correct identification of the species of *Aplexa* occurring in South Africa (Appleton *et al.* 1989; Brown, 1994), Dr. D.S. Brown (The Natural History Museum, London) has suggested that it be called *Aplexa* cf. *marmorata* until the problem is resolved. This name is used here. *Aplexa* cf. *marmorata* can be distinguished from *P. acuta* by means of several characters:

- the shell is narrower and more conical than *P. acuta* and it is smooth and glossy whereas that of *P. acuta* is smooth and relatively dull (Fig. 3.1).
- the whorls of *A.* cf. *marmorata* are not shouldered and have shallow sutures whereas *P. acuta* has definite shoulders and deep sutures.
- the spire of *A.* cf. *marmorata* is not as sharp (acute) as in *P. acuta.*
- the body wall of *A.* cf. *marmorata* has the green pigmentation that is characteristic of *Aplexa* (Richards, 1964) while that of *P. acuta* does not.
- the mantle of *A.* cf. *marmorata* has small, triangular dentations which do not project over the shell whereas *P. acuta* has finger-like dentations that cover the whole shell when the snail is moving (Appleton *et al.*, 1989) (Fig. 3.2) – this shows *A.* cf. *marmorata.*

Figure 3.1: Smooth and glossy shell of *A.* cf. *marmorata.*

Figure 3.2: Triangular mantle dentations, M, of *A.* cf. *marmorata.*

- the preputium of *A.* cf. *marmorata* has two longitudinal pilasters while that of *P. acuta* has a glandular swelling, i.e. a preputial gland, that is visible externally (Figs. 3.3 & 3.4).
- in *A.* cf. *marmorata*, the spermatheca has an oblong body and is constricted in the middle while that of *Physa* is pyriform or pear-shaped (Fig. 3.5).
- the penial canal opens laterally in *A.* cf. *marmorata* while *P. acuta* has a subterminal outlet.

3.5 GENITAL ANATOMY AND SHELL SHAPE OF PHYSIDS FROM AFRICA AND THE CARIBBEAN.

3.5.1 INTRODUCTION

During this study, snails of the Family Physidae from six localities in Africa and the Caribbean: (i-ii) the islands of Guadeloupe (Séo) and St Lucia in the West Indies, (iii) Nigeria and (iv-vi) St Lucia and Durban (Botanic Gardens and Bluff Nature Reserve) in KwaZulu- Natal, South Africa were dissected. This was to enable the author to become familiar with the genital anatomy of the Physidae.

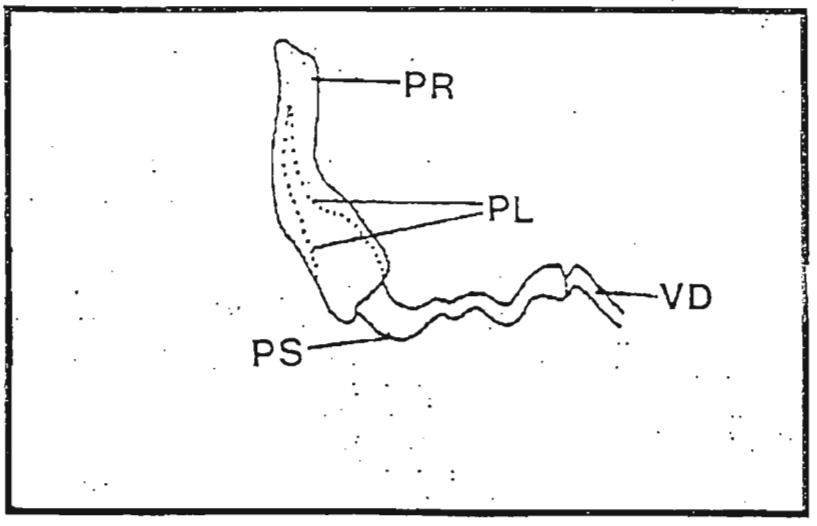
3.5.2 MATERIALS AND METHODS

The reproductive systems were dissected from representative specimens from all six localities. Histological sections were also prepared of the penis and preputial gland, where this was evident, and stained in Haematoxylin and counterstained in Eosin (Appendix 1). Duplicate sections were stained with Alcian Blue to determine whether or not the "glandular cells" of Paraense (1987) were in fact mucus-producing cells as he suggests (Appendix 2).

Shells of snails from each of the six localities were cleaned in a saturated solution of oxalic acid (Appendix 3) and examined for microsculpture and drawn using a Leica MS5 stereo-microscope fitted with a drawing tube.

Figure 3.3: Penis of *A.* cf. *marmorata* (P = prepuce, PL = preputial pilasters, PS = penial sheath, VD = vas deferens) (X10).

Figure 3.4: Penis of *P. acuta* with the preputial glandular swelling showing on the outside (G = glandular tissue, P = preputium, VD = vas deferens) (X10).


PR
PL
VD
PS

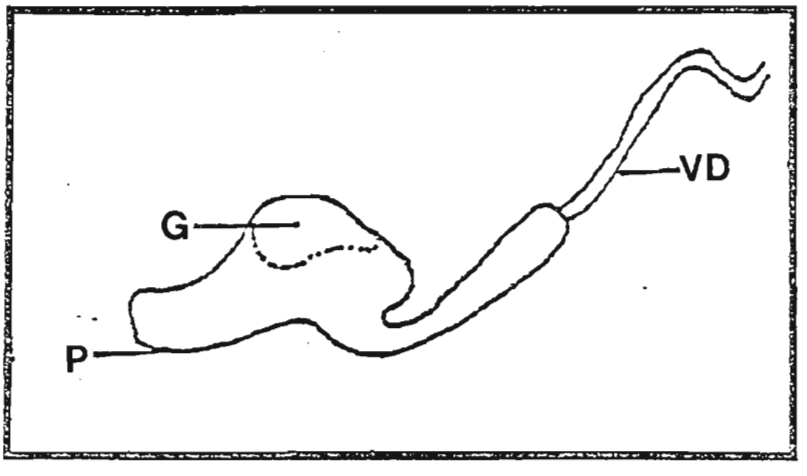
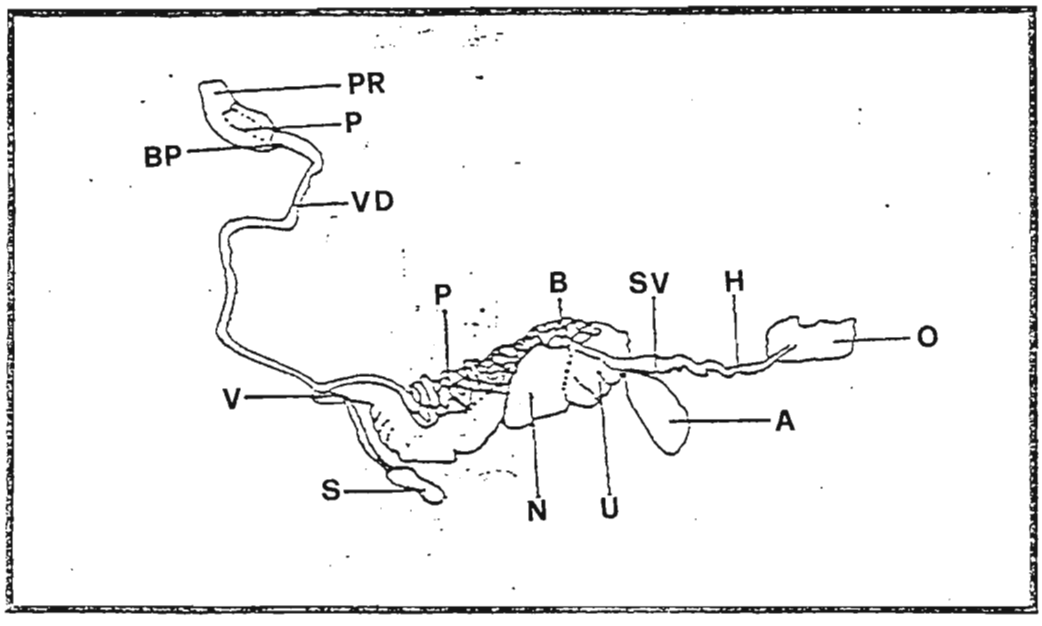

VD
G
P

Figure 3.5: Reproductive system of *A.* cf. *marmorata* (A = albumen gland, B= copulatory bursa, BP= bulb of penial sheath, H= hermaphrodite duct, N= nidamental gland, O = ovotestis, P= prostate gland, PL= penial pilasters, PR= preputium, S = spermatheca, SV= seminal vesicle, V= vagina, VD = vas deferens, U= uterus (X10).


PR
P
BP
VD
P
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SV
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A
S
N
U

3.5.3 RESULTS

Only the specimens from Seo in Guadeloupe had an externally visible preputial gland comparable with that illustrated in Figure 3.4 for *Physa acuta*. In the other five samples examined, this "glandular" tissue was not visible externally but the sections showed that it was distributed diffusely within the preputium wall (see Chapter 4 for detailed discussion of this work). Figures 3.6, 3.7 and 3.8 show outlines of the shells from the West Indies, Nigeria, St Lucia (South Africa) and Durban. Typically the shells were smooth, glossy and slender but narrower than those of *Physa acuta*. The spires were conical in shape, except for those from Nigeria which all had the two earliest whorls broken (Fig. 3.7). The upper part of the shell was acute-angled while the lower half was oval in shape. All had shallow sutures bordered by a band of growth lines. The columellar fold merged with the parietal callus.

3.6 CONCLUSIONS

Using the presence/absence of a discrete preputial gland as a generic character (Richards, 1964; Te, 1973; Kristensen, 1987; Appleton, 1989; Kristensen & Ogunowo, 1992; Barbosa, 1995, Shi-Kuei & Beetle, 1995), it was concluded that the snails from Guadeloupe belonged in *Physa*, perhaps *P. cubensis* (this study), while the others belonged in *Aplexa.*

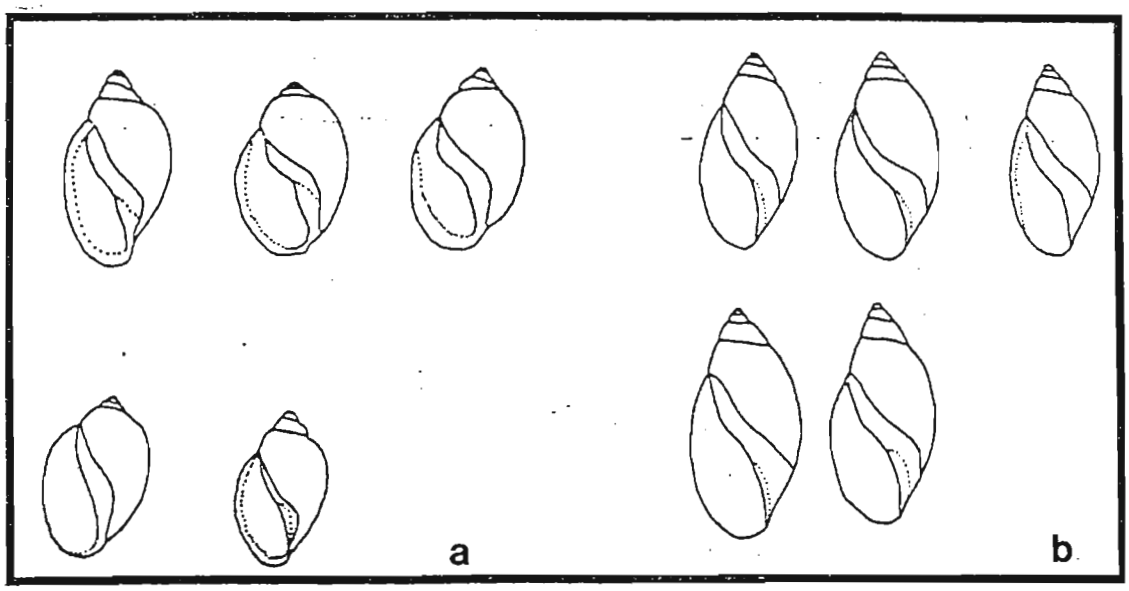
Shells from Guadeloupe were more globose and wider than the rest of the shells. The mean shell width/ shell length ratio (n = 6) was found to be 0.58 compared to 0.55 found for *P. cubensis* (Paraense, 1987). Shells from St. Lucia in the West Indies and St. Lucia in KwaZulu- Natal had slender body whorls and high spires. Shells from the Botanic Gardens and Bluff Nature Reserve also had slender body whorls but their spires were not as high as the ones from the West Indies and St. Lucia in KwaZulu- Natal. The spires of all shells from Nigeria in West Africa were broken. All these shells conform to the description given by Paraense (1987) for *Physa marmorata.*

Figure 3.6: a) Shells of *P. acuta* from Séo in Guadeloupe with a broad body whorl (X10).

b) Shells of *A. marmorata* from St. Lucia in the West indies (X10).

Figure 3.7: a) Shells of *A. waterloti* from Nigeria, West Africa (X10).

b) Shells of *A. marmorata* from St. Lucia, KwaZulu- Natal (X10).


a
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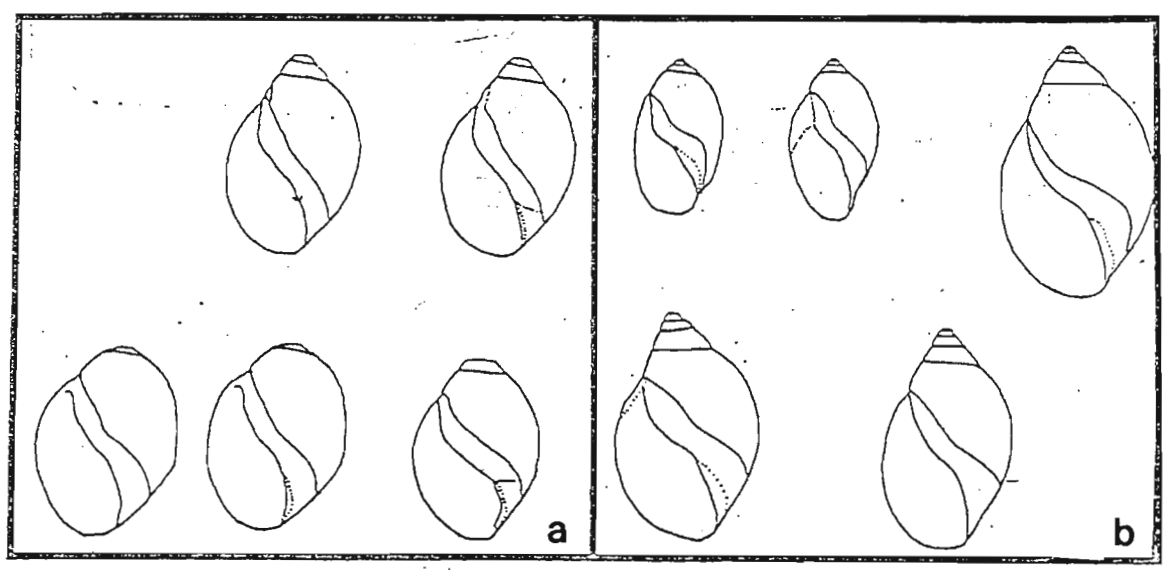
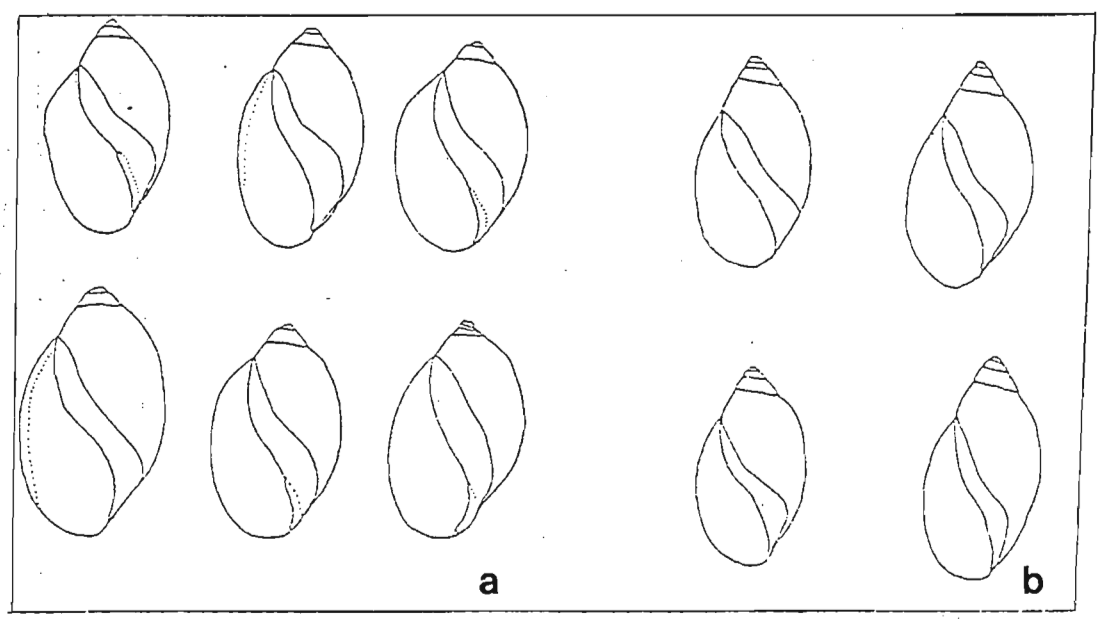

a
b

Figure 3.8: Shells of *A. marmorata* from (a) The Botanic Gardens (X10).
(b) The Bluff Nature Reserve, Durban (X10).


a
b

CHAPTER 4

WHAT IS *Aplexa* cf. *marmorata*?

4.1 HISTORICAL ACCOUNT

According to Te (1978), Baker (1928) showed differences between *Physa* from North America and Europe. Te (1978) further separated the former, on anatomical grounds, into its own genus, *Physella*, with two subgenera, *Physella* s.s. and *Physodon*. Thiele (1931-35) and Zilch (1959-60) both used shell and anatomical characters to recognise not only the two traditional American genera, *Aplexa* and *Physa*, but also several subgenera proposed by earlier workers (*Aplexa* s.s. and *Stenophysa* under *Aplexa*, and *Physa s.s.*, *Alampetista, Costatella* and *Petrophysa* under *Physa*. Starobogatov (1967) elevated the two traditional genera (*Aplexa* and *Physa*) to subfamily rank using anatomical features (Physinae with four genera: *Physa, Physella, Petrophysa* and a new genus *Afrophysa*, and Aplexinae with three genera: *Aplexa, Stenophysa* and a new genus *Sibirenauta*).

Hubendick (1978) listed only *Physa* and *Aplexa* in the Physidae, without any subdivisions and doubted that they merit to be separated as different genera. Te (1980) concluded that the Family Physidae can be grouped into four genera and two subfamilies containing a total of 48 species. The subfamily Aplexinae consisted of the genera *Aplexa* and *Stenophysa* while the subfamily Physinae consists of the genera *Physa* and *Physella*.

Aplexa is the predominant genus in Central and South America (Te, 1974, 1978) but has also been collected from West African countries like Ghana, Burkina Faso, Togo and Nigeria (Brown, 1994) and Te (1973) identified this population as *Aplexa* (*Stenophysa*) *marmorata*. Appleton, Brackenbury & Tonin (1989) have reviewed the generic and specific name changes this West African species has undergone. Specimens from Burkina Faso were originally described as *Physa (Aplecta) waterloti* by Germain (1911) and Ranson & Cherbonnier (1951) retained the name. In 1973, Te moved the species to the genus *Aplexa* and equated it with the West Indian *A.* (*Stenophysa*) *marmorata*. The West African snails were referred to as *A. (Stenophysa) waterloti* by Brown (1980) but Paraense (1986) called them *Physa marmorata*.

Many years previously however, Clessin (1886) had described *Physa mossambiquensis* from 'Moçambique' as the type locality and he noted that these specimens resembled South and Central American species more than East African ones. In discussing this species, Connolly (1925) mentioned that J. Thiele had provided him with details of the radula of *P. mosambiquensis*, the six cotypes of which are housed in the Berlin Museum, lodged under ZMB Moll.8484. These shells have been loaned to Prof. Appleton for this study by Dr. M. Glaubrecht who noted that no radula preparations could be found. These shells are described in section 4.9.

Specimens conforming to the species from South and Central America have been collected during the past 15 years from several sites in KwaZulu - Natal and Northern Province. The KwaZulu-Natal sites are:

Durban:

- Canals flowing through the market gardens in the Durban suburb of Newlands (29°48'S/30°57'E)
- Artificial pond in a nursery in Pinetown
- Ponds at Durban North sewage works (T.E. Crouch, personal communication to C.C.Appleton,1996)
- Lake in the Bluff Nature Reserve, Durban (29°56'S/31°59'E) by G.B. Wilken (University of Natal).
- It has also been collected from the Botanic Gardens, Durban, for purposes of this study.

This species appears to be spreading as it has also been found in three remote localities in northern Kwa-Zulu Natal, (P.A. Reavell, 1998-9, pers. comm.):

- Suni Ridge Game Farm, Hluhluwe (28°1'S/32°15'E). This is a spring-fed, clear water, deep borehole with marginal vegetation and permanent water.
- Western shores of St. Lucia Reserve (28°5'S/32°26'E). This habitat is a large hippo pool with bird hides. The water is clear and permanent with short marginal vegetation.
- Side of N2 road between Empangeni and Mtunzini near the turnoff to Amanzimnyama Mission (28°52'S/31°46'E). The habitat is spring fed with clear water in a shallow pan. It has marginal vegetation and only dries up during extremely severe drought.

- On the South Coast, 7 specimens were collected from a small, spring-fed grassy pool with slow-flowing muddy water close to the Highway near Hibberdene (30°32'S/30°35'E) (E. Saathoff, 1995, pers. comm).

The first two of these localities are particularly remote and no explanation can be given as to how the snails reached them.

In the Northern Province it was collected from an artificial pond in the grounds of the Siegfried Annecke Research Institute, Tzaneen (23°50'S/30°10'E) by D.L. Theron in the 1980s.
In Mpumalanga Province, the species has been collected in a backwater of the Sabie River near Lower Sabie Camp, Kruger National Park (K.N. de Kock, Potchefstroom University, pers. comm. to C.C. Appleton, 1998).

4.2 CHARACTERISATION OF *Aplexa* cf. *marmorata* FROM SOUTH AFRICA

Taking into account the inadequacy of physid systematics (Te, 1980; Taylor, 1988; Brown, 1994), it is useful here to describe or characterise the South African *A.* cf. *marmorata* as thoroughly as possible before commenting on its identity.

4.2.1 MATERIALS AND METHODS

Characterization of *A.* cf. *marmorata* required the use of a variety of techniques that are described in a series of appendices (1-7). Outlines of shells cleaned in a saturated oxalic acid solution (Appendix 3) were drawn using a Leica MS5 stereomicroscope fitted with a drawing tube (see Chapter 3); cleaned radulae (Appendix 4) and whole spermatozoa, the latter teased from the spermoviduct (hermaphrodite duct) were viewed under a Hitachi S520 Scanning Electron Microscope (Appendix 5). Sections of the penial complex were cut for histological study at 3 µm and stained
a) with Haematoxylin and Eosin to show general tissue arrangement and structure and
b) with Alcian Blue to demonstrate mucous secretions and/ or mucus-secreting cells (Appendices 1 & 2). The fine structure of the mature *A.* cf. *marmorata* spermatozoon was elucidated using a Jeol 1010 Transmission Electron Microscope (Appendix 6). An attempt was also made to karyotype these snails (Appendix 7) but the diploid chromosome number could not be adequately determined. All dissections were done on snails that had been narcotised by keeping them for 12

hours in a petri-dish of water to which a few menthol crystals had been added. This killed the snails with the headfoot protruding maximally from the shell, relaxing organs such as the spermoviduct and penial complex and making dissection easier.

4.2.2 THE SHELL

As noted in Chapter 3 (Plates 2b; 3a & b), the shell of *Aplexa* cf. *marmorata* is smooth, shiny and has a conical spire. Compared with *Physa acuta*, the only other physid found in the country, the body whorl is more slender, the sutures are shallower and are bordered by white bands of growth lines. The upper half of the shell is acute-angled while the lower half is oval. The outer lip is sharp with an outer callus. The columellar fold gradually merges with the narrow parietal callus. Length x width measurements of shells examined (n = 112) ranged from 7.4 x 3.5 mm to 12.3 x 6.5 mm (mean in mm). Ratios between the different shell dimensions were:

Shell width/shell length: 0.47 – 0.59 (mean ± S. D. = 0.5 ± 0.029);
Spire length/shell length: 0.26 – 0.42 (mean ± S. D. 0.32 ± 0.031);
Aperture length/shell length: 0.65 – 1.13 (mean ± S.D. 0.71 ± 0.063).

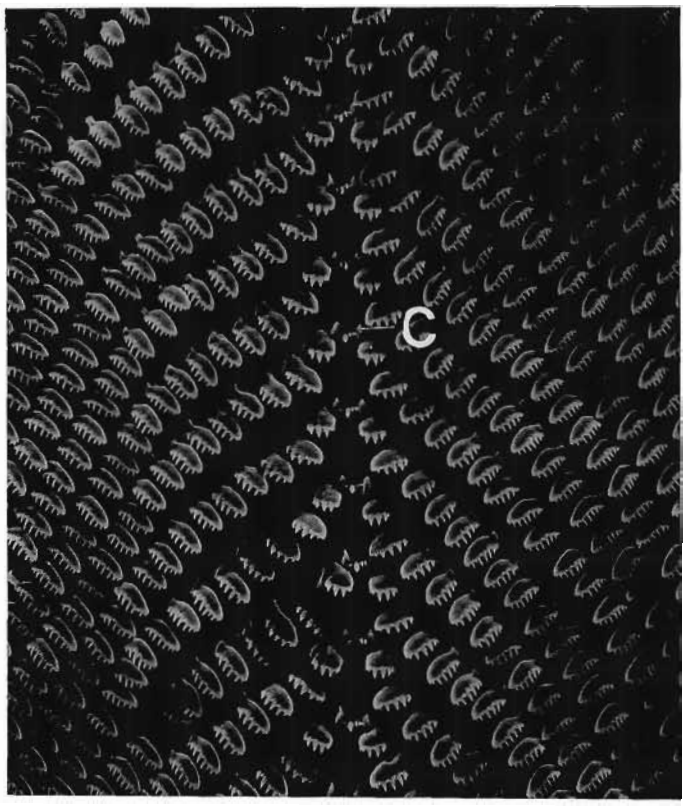
4.2.3 THE RADULA

The radulae were dissected from 11 snails after they were narcotised in a menthol crystal solution for twelve hours (Appendix 4). The teeth of *A.* cf. *marmorata* are arranged in V-shaped rows across the radula (Fig. 4.1).

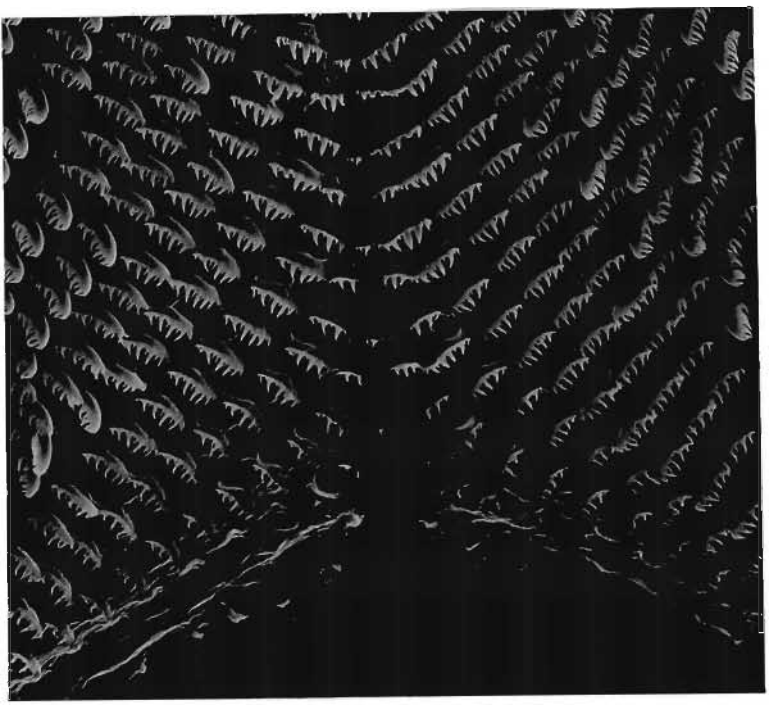
The central tooth has a wide base and a large, long central cusp with three smaller cusps on either side (Fig. 4.2). The first lateral tooth has three big cusps alternating with a single smaller cusp per tooth. From the 2nd lateral tooth to the marginal teeth, all the teeth look alike with large cusps alternating with small cusps. Figs. (4.3a to 4.3d) show the sequence of tooth development from newly formed to working to worn teeth from the same radula. The number of cusps was not clear on the worn marginal teeth.

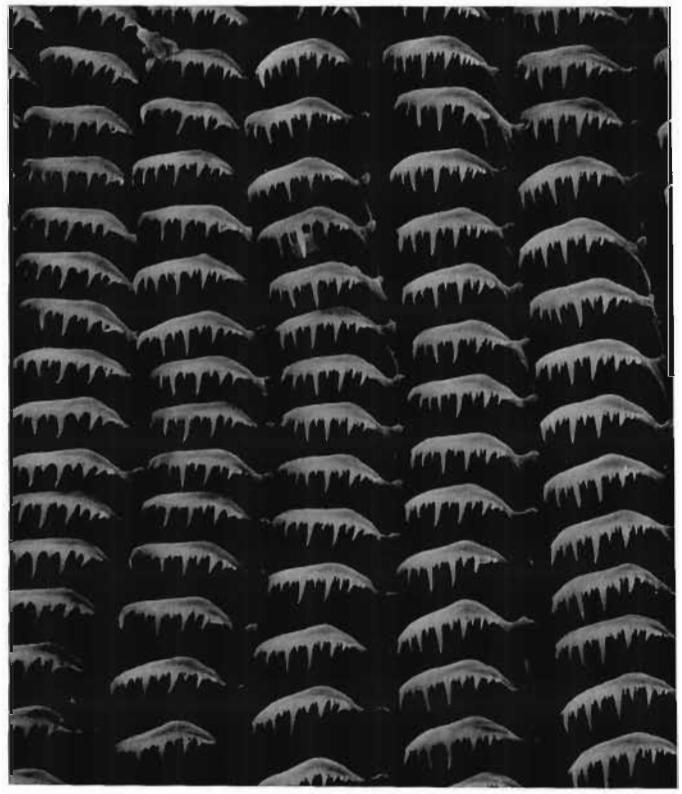
Figure 4.1: The radula of *A.* cf. *marmorata* showing the v-shaped rows of teeth. The central teeth, C, can be seen in the middle of each row.

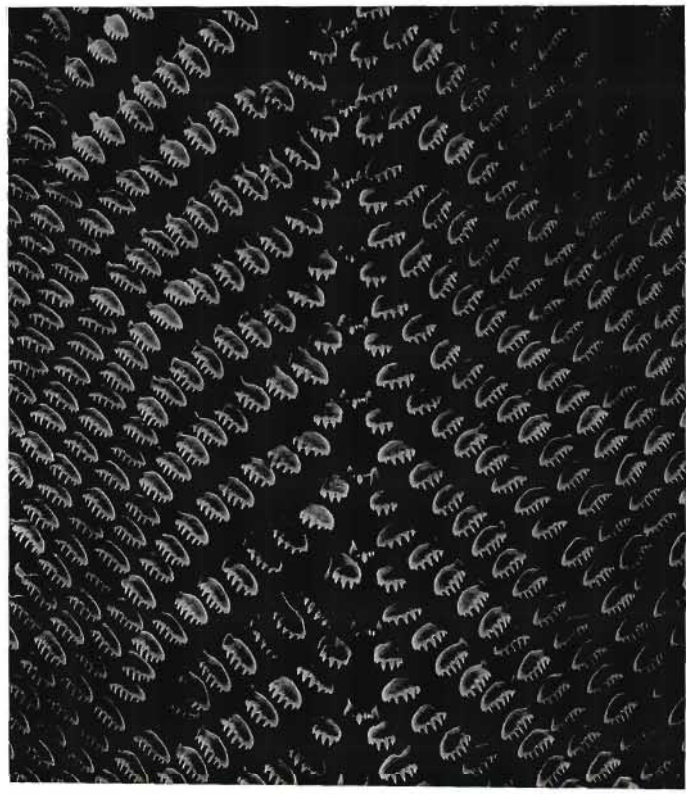
Figure 4.2: Central tooth, C, showing the wide base, a large central cusp with three smaller cusps on either side (15.0µm).


C

Figures 4.3: (a) Newly formed teeth (43 μm); (b) Newly formed working teeth (20 μm); (c) Worn-out working teeth (30 μm); (d) Normal working teeth (25 μm).







4.2.4 THE FOOT

In a freely moving animal, the foot is rounded anteriorly with a pointed tail and is thus spatulate in shape. The pigment melanin is concentrated in the anterior region and the triangular oral lappets and is also present as conspicuous, thin lines along the slender tentacles and as a mid-dorsal stripe on the narrow posterior end of the foot. The body has yellow-green "vacuoles" in the skin and these stain paper if the foot is crushed on it. The pigmentation and staining are stated by Richards (1964) to be characteristic of species of *Aplexa*, but not of *Physa*. Pigmentation is noted by Paraense (1987) to be absent in the foot tail of *Physa cubensis*.

4.2.5 THE MANTLE

The mantle collar does not protrude beyond the mantle edge. This mantle edge has short, triangular, serrated dentations that do not extend over the shell when the snail is moving. The right hand edge of the mantle is much longer than the left.

4.2.6 THE PENIAL COMPLEX

The penis has two preputial pilasters along its length but lacks the prominent preputial gland that is characteristic of *Physa* spp. Brown (1980; 1994) refers to the preputial gland of *Physa* as being "visible **externally** as a bulge". This wording is important since it does not preclude the gland being present **internally,** and therefore **not** visible as a bulge, in other genera such as *Aplexa.* This is in fact what the histological study showed the situation in *A.* cf. *marmorata* to be.

A comparison of the sections through the penises of both *A.* cf. *marmorata* and *P. acuta*, including the preputial gland in *P. acuta* (Appendix 1), showed that the tissue making up the body of this gland in *P. acuta* was also present in *A.* cf. *marmorata.* It was however scattered through the entire circumference of the wall of the preputium and not aggregated into a "gland" Fig. 4.4a shows the transverse section through the preputium of *A.*cf. *marmorata* and Fig. 4.4 b shows the clustered "glandular cells" on the preputium of *P. acuta.* Fig. 4.4 c is a line drawing of a transverse section of the penial complex of *A.* cf. *marmorata.*

The same cells that were present in the preputial gland of *P. acuta* were thus present as a more diffuse tissue in the wall of the preputium in *A.* cf. *marmorata,* not aggregated in one place in the

Figure 4.4a: Transverse section through the preputium of *A.* cf. *marmorata* (C = cavity of penis sheath, CM = circular muscle, G = glandular cell, I = inner penis sheath, L = lumen of penial canal; PC = penial canal, O = outer penis sheath) (X10).

Figure 4.4b: Transverse section through the penial complex of *P. acuta* (G = glandular cells) (X10).


G

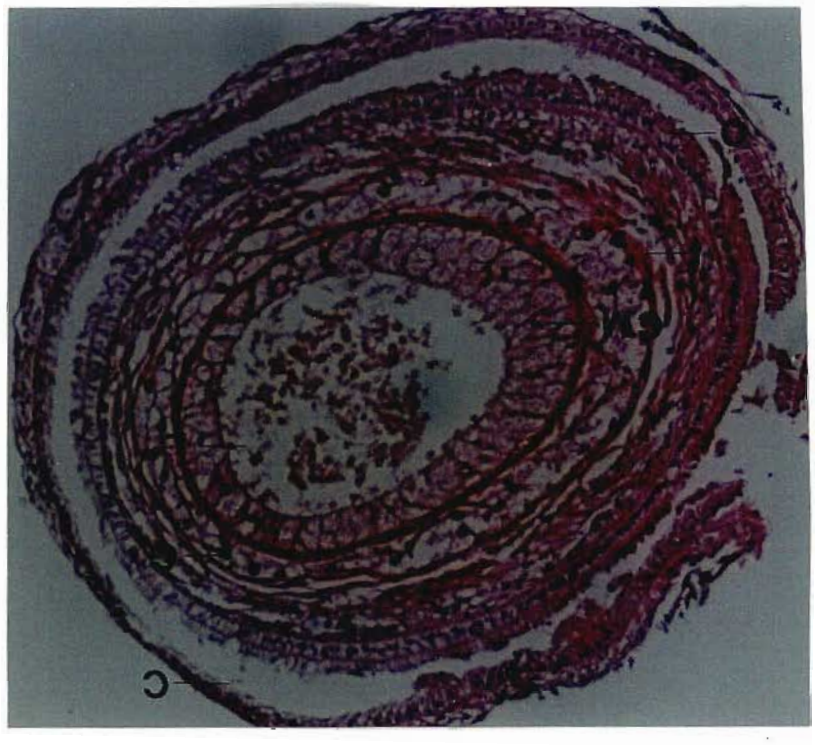
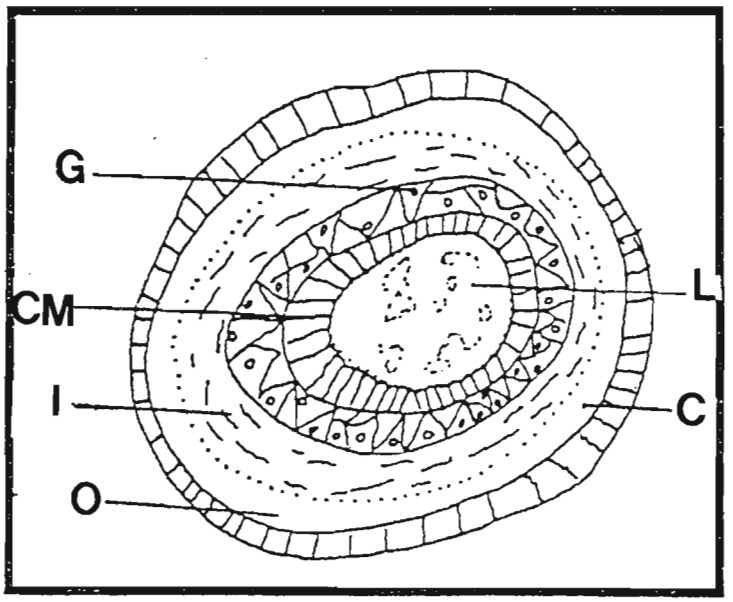

C

Figure 4.4 c: Line drawing of a transverse section through the penial complex of *A.* cf. *marmorata* showing diffuse distribution of the "glandular tissue" in the wall of the penial canal (C = cavity of penis sheath, CM = circular muscle, G = glandular cells, I = inner penis sheath, L = lumen of penial canal, O = outer penis sheath) (X10).


G
CM
I
O
L
C

form of a gland. Further, these cells do not open into the lumen as glandular cells may be expected to do – there were however some indications of ducts of gland cells passing between these cells from the outside. Staining with Alcian Blue, i.e. for mucus-producing cells, failed to demonstrate any evidence of mucin. In fact this "glandular" tissue was characterized by cells with prominent, large nuclei and resembled nerve tissue more than glandular tissue. Deposits of melanic pigment between some of these cells may also be indicative of nerve tissue. A layer of muscle was present on the outside. This tissue may therefore not be glandular as the terms *preputial/accessory gland* in the literature suggest but perhaps an innervated sensory structure surrounded by muscle and related to the regulation of copulation. Further study is needed to determine the true nature of this tissue in the Physidae.

The penial canal has a lateral opening and the spermatheca has an oblong body which is more or less constricted in the middle. The penial complex of *A.* cf *marmorata* from Durban thus has the same morphological features as what Te (1974) called *A. (Stenophysa) marmorata* from West Africa and Paraense (1986) called *Physa marmorata* from Brazil.

4.2.7 SPERM MORPHOLOGY

The use of scanning (SEM) (Appendix 5) and transmission electron microscopy (TEM) (Appendix 6) in the study of sperm morphology have proved to be useful techniques for determining phylogenetic relationships among prosobranch and opisthobranch gastropods. It has helped in the allocation of families to superfamilies, species to species groups and provided species-specific characters (Healy, 1989a & b; Hodgson & Bernard, 1988). Because species or genera may have sperm with a unique morphology, sperm structure can sometimes be used as a taxonomic character. In the present case, it was anticipated that it might help in characterising *A.* cf. *marmorata.*

(i) SCANNING ELECTRON MICROSCOPE

The SEM is a useful instrument that can be used to gain valuable information about the structure of any specimen and it makes observations of surface details quite easy. This is due to the fact that it shows the specimen in 3- dimensions. This instrument was used to study the spermatozoa of *Aplexa* cf *marmorata.*

Measurements of whole spermatozoa teased from the spermoviduct of an adult snail (11 mm) indicated a total length (head + tail) of 266 - 282 μm (n=2) (Figs. 4.5- 4.6).

The head appears twisted or shaped like a corkscrew and measures 3.68 x 0.5 μm at the base (n = 1). The anterior end is capped by a blunt-ending acrosome measuring 0.59 x 0.36 μm (n = 1) in the middle and which is bilaterally symmetrical. The head has a helical keel running transversely across it and it measures 0.82 μm in width (n = 1).

Just posterior to the head is the neck region from which the tail emerges. This is indicated by a slight constriction separating the head from the midpiece. The diameter of the tail at its basal point was 0.053 μm (n=1). Some of these micrographs (Figs.4.7 & 4.8) show evidence of two glycogen helices around the proximal part of the midpiece. The points where these helices end could not be clearly seen.

Figure 4.9 shows however the annular ring that separates the mitochondrial derivative region from the end-piece and Fig.4.10 shows that the tip of this end-piece is blunt. The annular ring measured 0.079 x 0.038 μm (n=1).

The distal end of the tail of the *A.* cf *marmorata* was seen to have a tendency to coil on itself like a spring (Figs.4.11 & 4.12). This has not been reported for other snail species and may be a distinguishing feature of sperm of this species.

(ii) TRANSMISSION ELECTRON MICROSCOPE

Based on the study of the mature spermatozoa of *Physa acuta* by Brackenbury & Appleton (1991) and Appleton & Brackenbury (1997), sections of the sperm of *A.* cf *marmorata* were viewed under TEM and described in terms of the same five morphological divisions:

4.2.7.1. Acrosome

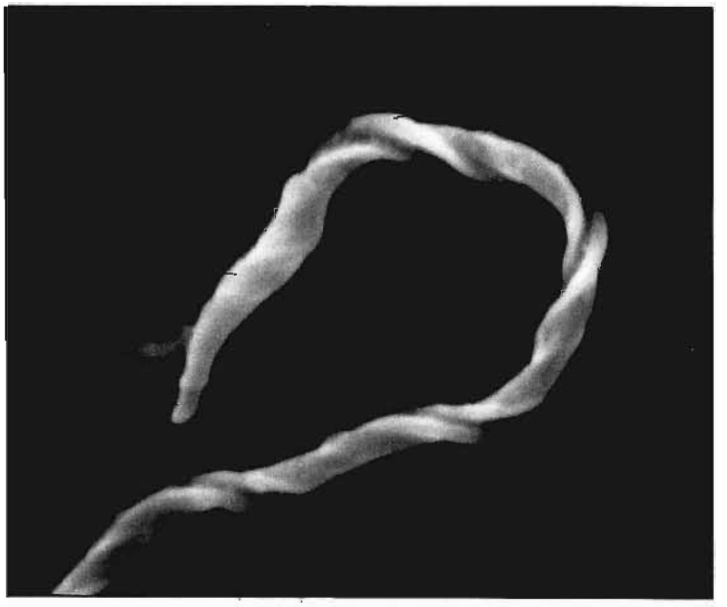
4.2.7.2. Nucleus

4.2.7.3. Mid-piece (neck region, glycogen helix region and mitochondrial derivative region)

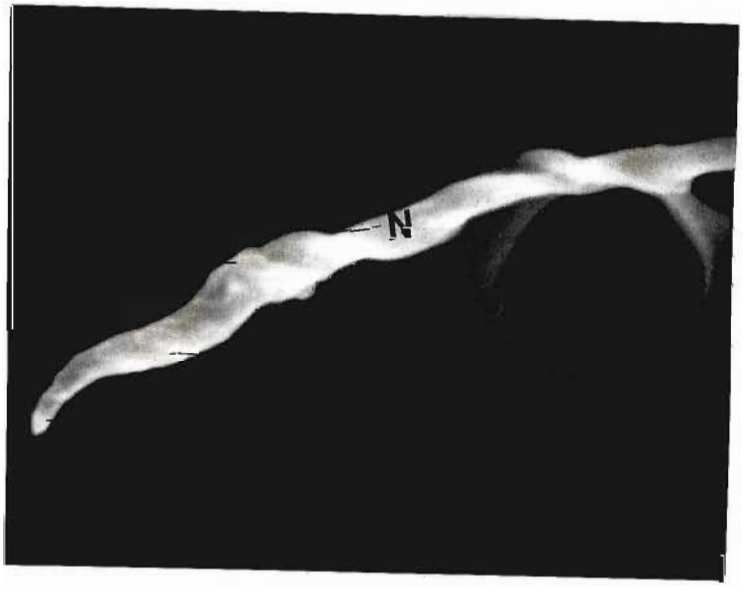
4.2.7.4 Glycogen piece

4.2.7.5. End piece

Figures 4.5 & 4.6: Heads of mature spermatozoa of *A.* cf. *marmorata* (A = acrosome, G= double glycogen helices, H = head, K = helical keel, N = neck region).

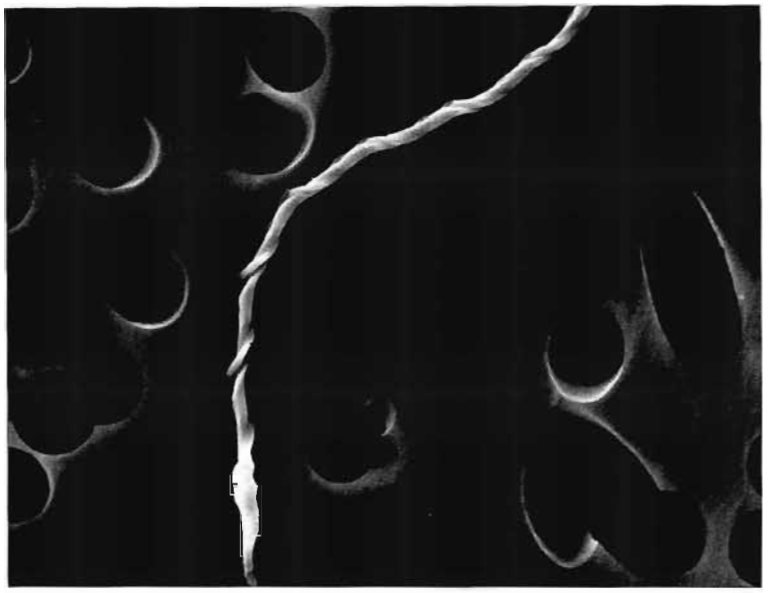


1000 nm



1000 nm

Figures 4.7 & 4.8: Heads, H, and double glycogen helices, G, becoming less prominent along the length of the spermatozoon.



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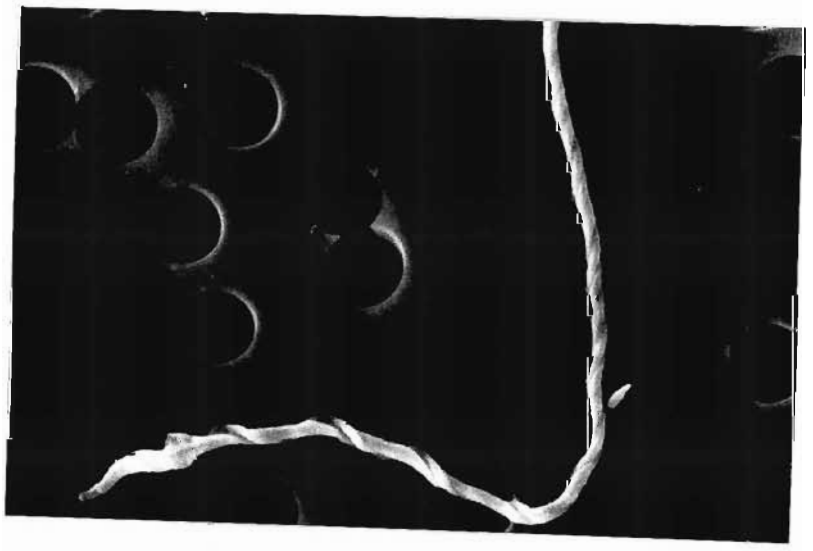
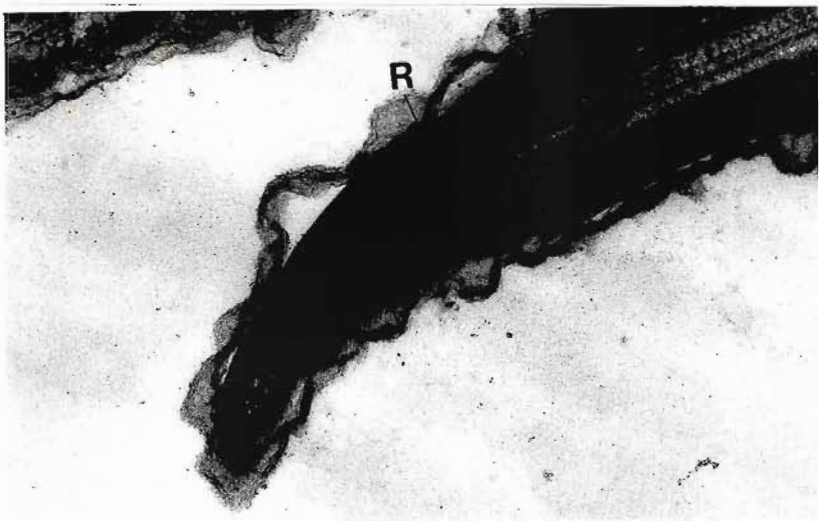


1000 nm

Figure 4.9: Annular ring, R, separating the mitochondrial derivative from the end piece.

Figure 4.10: Tip of the tail of spermatozoon showing an annular ring, R, and a blunt end.



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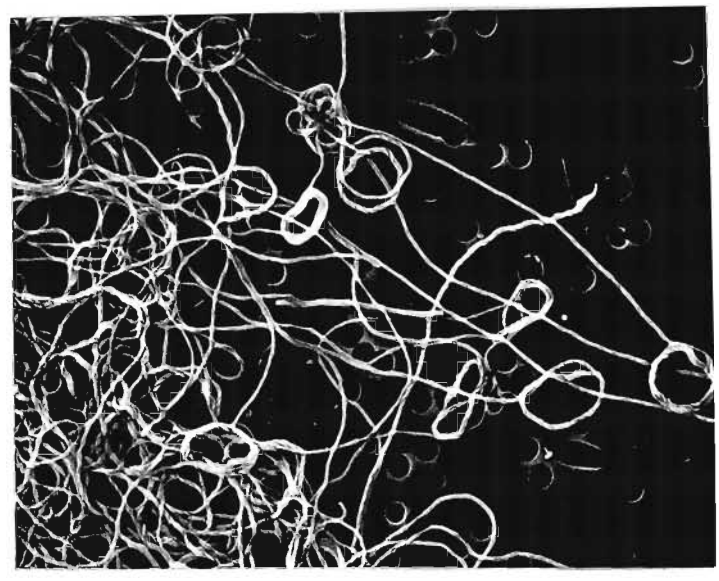
Figures 4.11 & 4.12: Spermatozoon of *A.* cf. *marmorata* showing the coiled tail.



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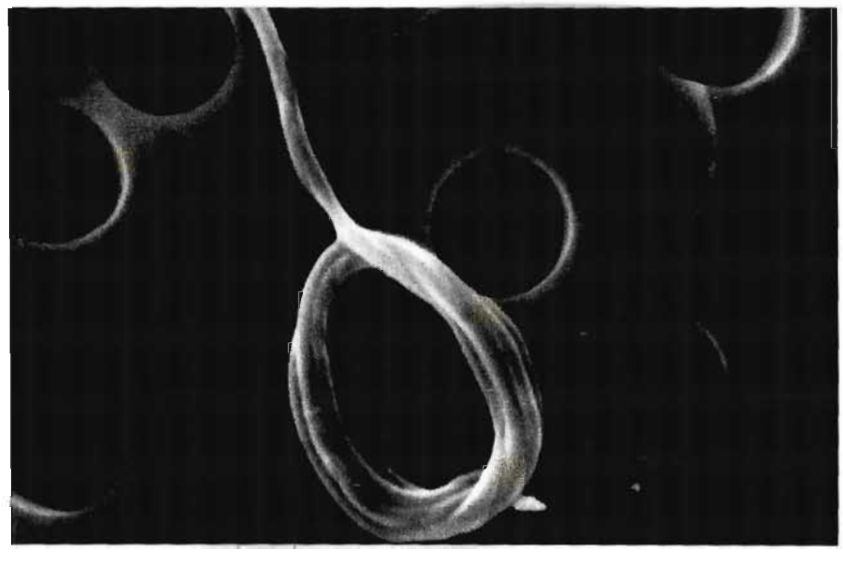


1000 nm

4.2.7.1 THE ACROSOME

The acrosome is found at the anterior tip of the head and caps the nucleus. It is a semi-transparent structure made up of semi - dense material and has a blunt end (Fig. 4.13). The acrosomal vesicle was not visible.

4.2.7.2 THE NUCLEUS

Posterior to the acrosome is an extremely electron-dense nucleus. The tip of the nucleus is rounded and it widens towards the neck region. It has two laterally-projecting helical keels (Fig. 4.14). A deep implantation fossa or a basal invagination can be seen at the base of the nucleus and the axoneme is embedded in it. A loose plasma membrane surrounds the head (acrosome + nucleus) and its distal end can be seen to enclose a quantity of perinuclear material.

4.2.7.3 THE MIDPIECE

i) NECK REGION

Fig. 4.14 shows the proximal end of the midpiece inserted into the implantation fossa via a basal body of the axoneme. At the core of the neck region are the centriole and peripheral microtubules which are in a 9 + 2 arrangement. These microtubules are derived from the semi-electron dense "plug" of the basal body and extend through the midpiece. The coarse fibres are also visible but the banding pattern and crescent shape reported by Brackenbury & Appleton (1991) for *Physa acuta* were both unclear.

ii) THE GLYCOGEN HELIX REGION

The glycogen helix region is characterised by the presence of glycogen-containing helices wound around the axoneme. The maximum number of helices found in mature *A.* cf. *marmorata* spermatozoa was two (Figs. 4.16 & 4.17). In this double helix, the glycogen compartments protrude markedly from the body of the midpiece, in other words, the radial depth of the helices is considerable. These compartments are surrounded by two paracrystalline layers and two

Figure 4.13 (a): Line diagram of a whole mature spermatozoon of *A.* cf. *marmorata* showing its morphological features (A= acrosome, E = End piece, G = Glycogen piece, 2H = double helix region, 1H = single helix region, M = mitochondrial derivative, N = nucleus, NK = neck region.

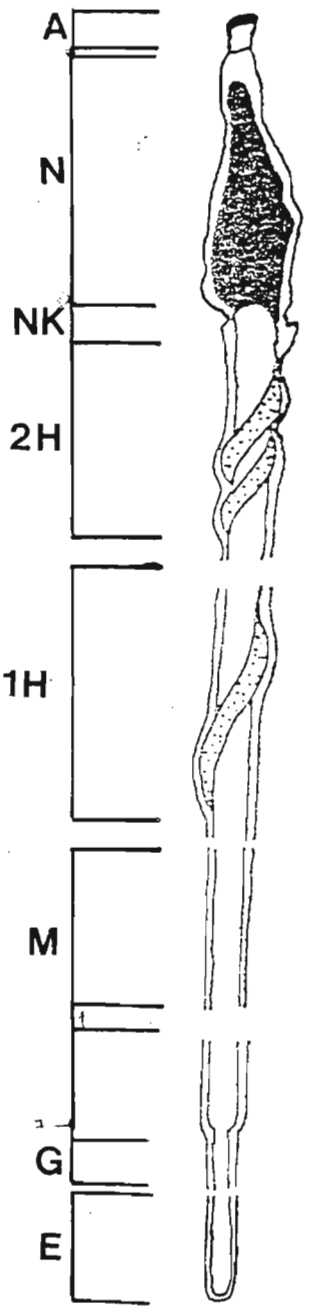
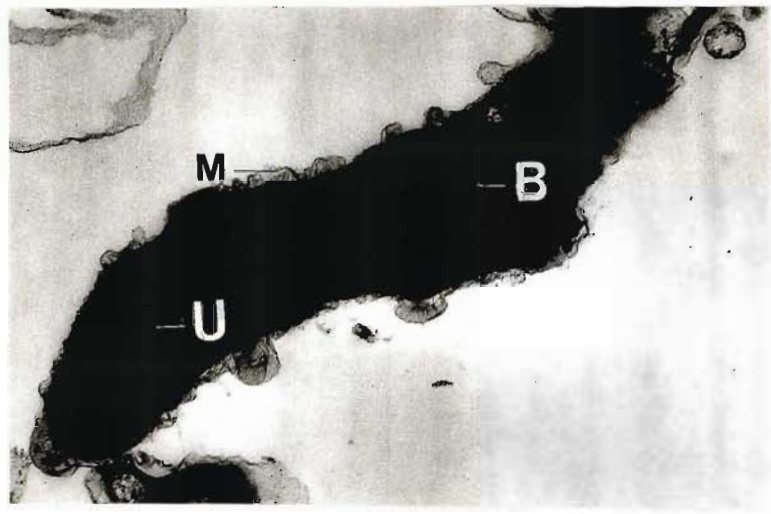

A
N
NK
2H
1H
M
G
E

Figure 4.13 (b): Longitudinal section through the head of a mature spermatozoon of *A.* cf. *marmorata* (A = acrosome, B = basal body of axoneme, M = plasma membrane, U = nucleus).

Figure 4.14: Longitudinal section through the head and body of a mature spermatozoon of *A.* cf. *marmorata* (B = basal body, C = coarse fibres, E = centriole microtubules, F = peripheral microtubules, G = glycogen helices, I = mitochondrial matrix, K = helical keels, M = plasma membrane, U = nucleus, Y = paracrystalline layers).



100 nm

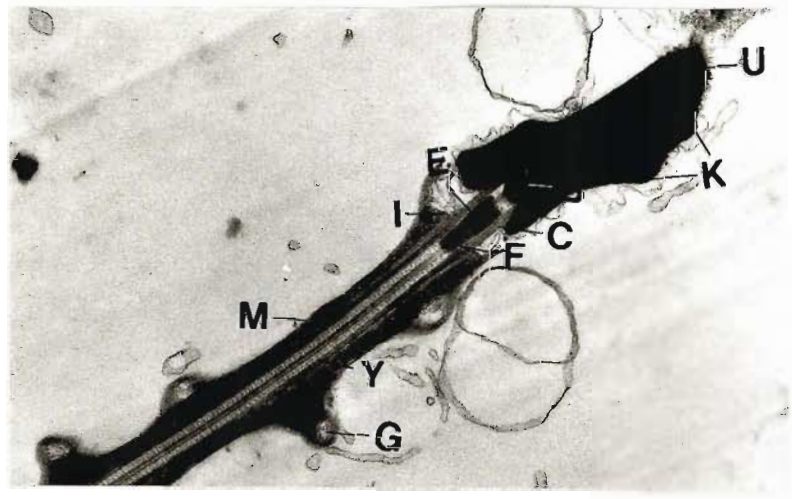


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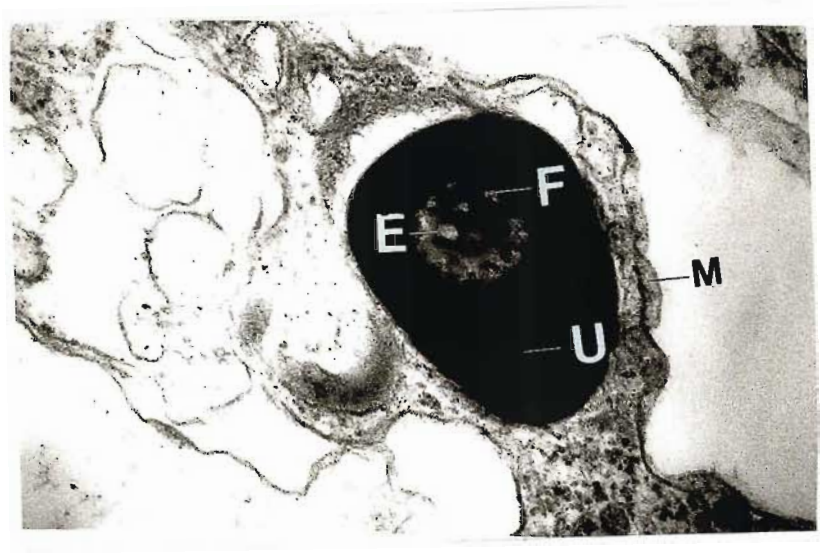
Figure 4.15: Transverse section through the nucleus cut at its base at the beginning of the neck region (E = centriole microtubules, F = peripheral microtubules, M = plasma membrane, U = nucleus).

Figure 4.16: Transverse section through the midpiece showing the double glycogen helices (E = centriole microtubules, F = peripheral microtubules, X = axoneme, G = glycogen compartment).



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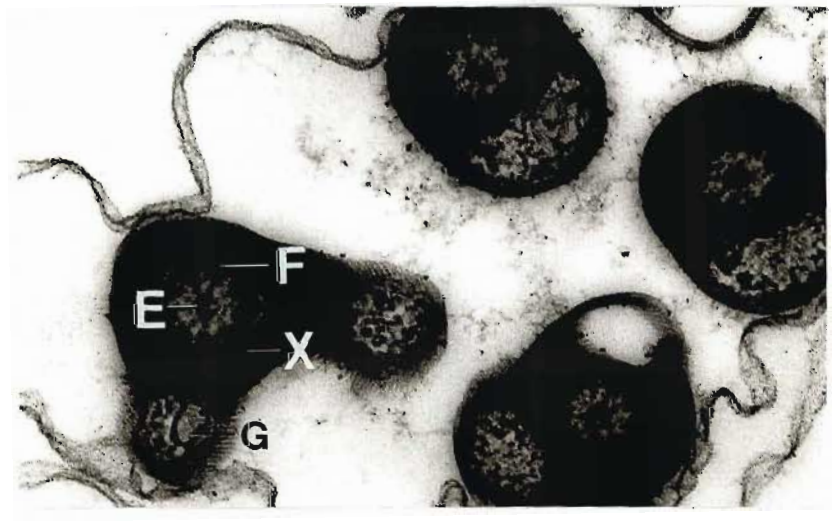
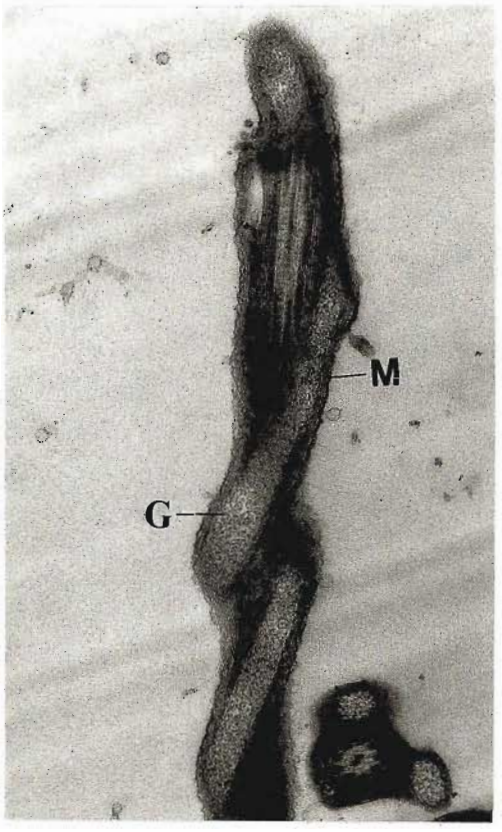


100 nm

Figure 4.17: Longitudinal section of the spermatozoon showing the double glycogen helices (G = glycogen helix, M = plasma membrane).



100 nm

mitochondrial matrix layers. The 9+2 arrangement of the microtubule inside the axoneme can be clearly seen in cross sections through the midpiece.

a) DOUBLE HELIX REGION

The double helix sections show a deep radial depth whereas the equivalent sections of *P. acuta* illustrated by Brackenbury & Appleton (1991) have only a shallow radial depth. In *P. acuta*, it is the sections in the triple helix region that have a deep radial "depth". This is important since it is well recognised that the radial depth of glycogen helices decreases with increasing distance from the neck region, in other words, the initial helices sections will have the greatest depths. Figure 4.18 shows sections that closely resemble the triple helix region found in *P. acuta* by Brackenbury & Appleton (1991).

b) THE SINGLE HELIX REGION

Distal to the double helix region is the single helix region (Figs. 4.19 & 4.20). The internal structures here are the same as in the double helix region except that one of the mitochondrial matrix layers is lost. Also, the radial height of the glycogen compartments is greatly reduced compared with that in the double helix region.

c) MITOCHONDRIAL DERIVATIVE REGION

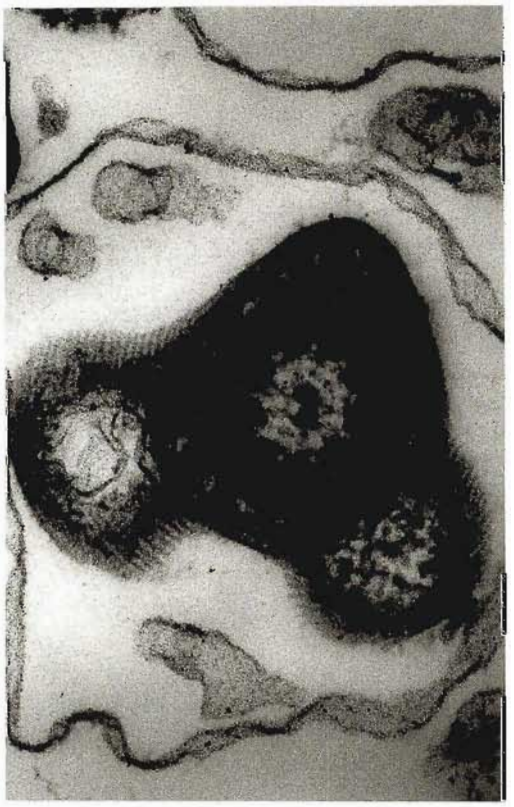
This region is characterised by the absence of any glycogen helices (Fig. 4.21). Transverse sections show in detail the 9+2 microtubule pattern of the axoneme; the nine pairs of peripheral microtubule doublets, each with two subtubules, an A- subtubule connected to a B – subtubule, and two centriole microtubules. Around the axoneme is a thick mitochondrial matrix layer and two paracrystalline layers. A loosely attached plasma membrane surrounds the whole structure.

4.2.7.4 GLYCOGEN PIECE

The transition between the mitochondrial derivative region of the midpiece and the glycogen piece of the tail is abrupt and clearly defined. The annular ring separating these two structures is not clearly visible (Fig. 4.22). The mitochondrial matrix layer seen in Fig. 4.21 has been replaced by a layer of glycogen that encapsules the axoneme. Fig. 4.22 shows a transverse section of the

Figure 4.18: Sections that closely resemble the triple helix region of *Physa acuta.*

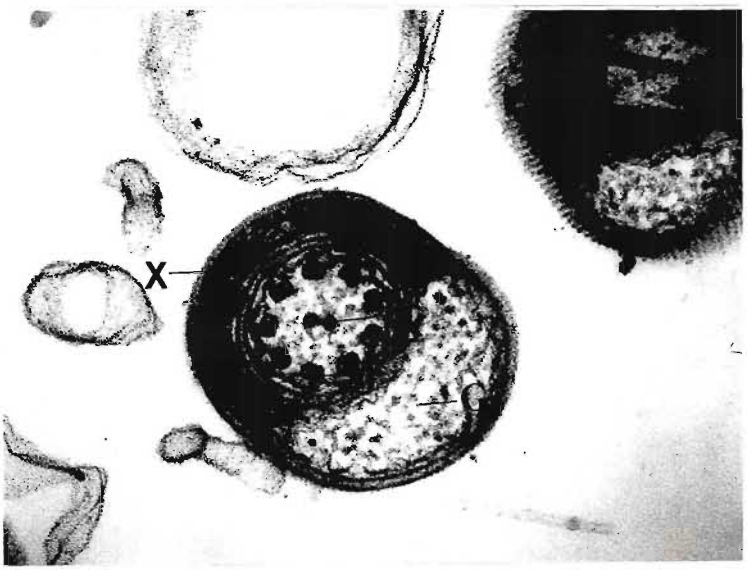
100 nm



100 nm

Figure 4.19: Transverse section through the midpiece showing a single glyycogen helix (E = centriole microtubules, F = peripheral microtubules, G = glycogen compartment, X = axoneme).

Figure 4.20: Longitudinal section of spermatozoon showing the single glycogen helix (G = glycogen helix, M = plasma membrane).



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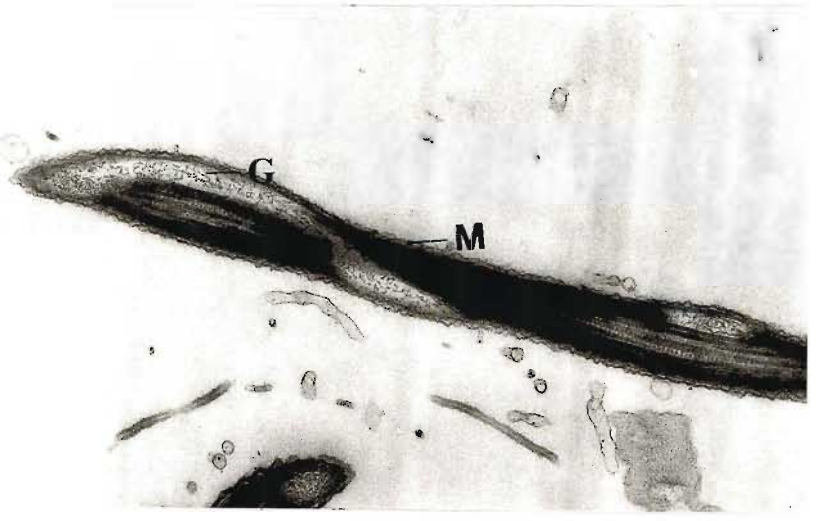
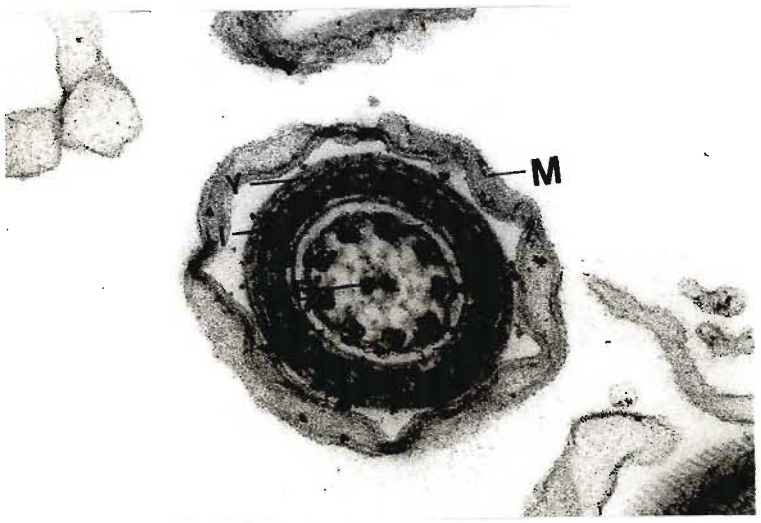


100 nm

Figure 4.21: Transverse section through the mitochondrial derivative region (E = centriole microtubules, F = peripheral microtubules, I = mitochondrial matrix, M = plasma membrane, X = axoneme, Y = paracrystalline layers).

Figure 4.22: Transverse section through the glycogen piece (E = centriole microtubules, F = peripheral microtubules, GS = glycogen sheath, M = plasma membrane, X = axoneme).



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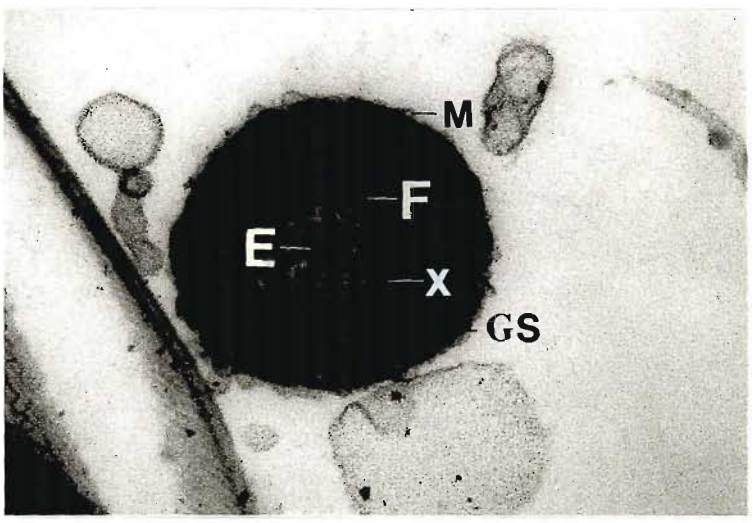


100 nm

glycogen piece of the tail of the sperm of *A.* cf *marmorata.* The 9 + 2 arrangement of microtubules is still visible within the axoneme and the whole structure is surrounded by a loosely attached plasma membrane.

4.2.7.5 END PIECE

This is the last (most distal) region of the mature spermatozoon (Fig. 4.23) and no longer has a glycogen sheath - only the axoneme with its microtubules remains and it is surrounded by the plasma membrane. A structure that looks like a glycogen ring observed in the neck region of *Bulinus tropicus* by Brackenbury & Appleton (1991) is seen at the tail tip of *A.* cf *marmorata* (Fig. 4. 9).

4.3 EGG CAPSULES

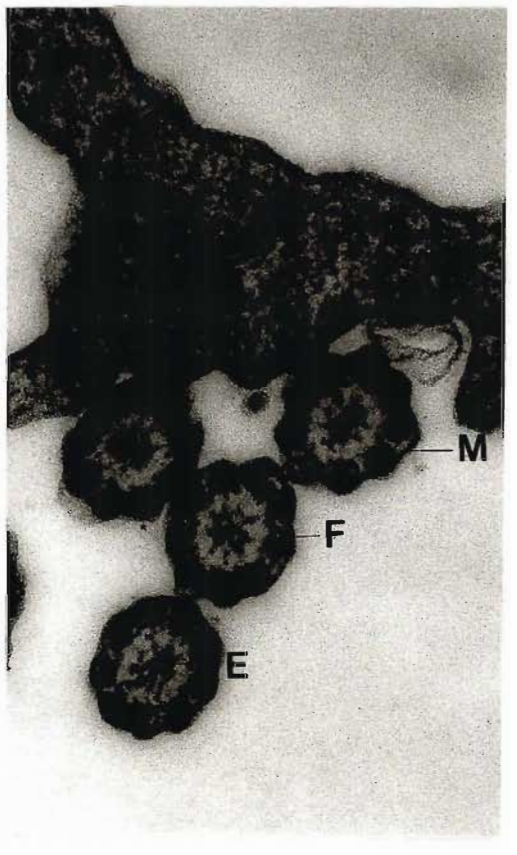
The egg capsules of *A.* cf *marmorata* were elongate, convex, thick-walled with a bumpy "outline" (Fig. 4.24). The terminal tail was short but clearly visible. The egg capsules ranged between 4 - 6 mm in size. The clutch size ranges between 7 to 35 eggs per capsule. Figure 4.25 shows line diagrams of egg capsules of *A.* cf *marmorata.*

4.4 IDENTIFICATION

Specimens from the two Durban localities sampled during this study (see Chapter 6) were sent to Dra S Thiengo and Dr L Paraense, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil, and Dr J-P Pointier (Université de Perpignan, France) for examination. All three malacologists are familiar with Neotropical pulmonates and Dr. Paraense is the author of several papers on the Physidae. Following dissection, particularly of the male reproductive system, both Dra Thiengo and Dr Paraense identified the material as *Physa marmorata* – a species indigenous to Brazil. Dr Pointier however was of the opinion that they are not *marmorata*, largely because (J-P Pointier, *in litt.*, 28.1.99) the columellar dentations of the mantle are not as marked as in *marmorata*, the penial complex is bigger and the penis sheath/preputium ratio is much less (1.07, 1.11 and 1.13 for three specimens dissected) than the 2.00 given for *P. marmorata* by Paraense (1986).

It must be mentioned here that while the genus *Aplexa* is recognized by malacologists of the European and North American school of freshwater malacology, the Brazilian school does not

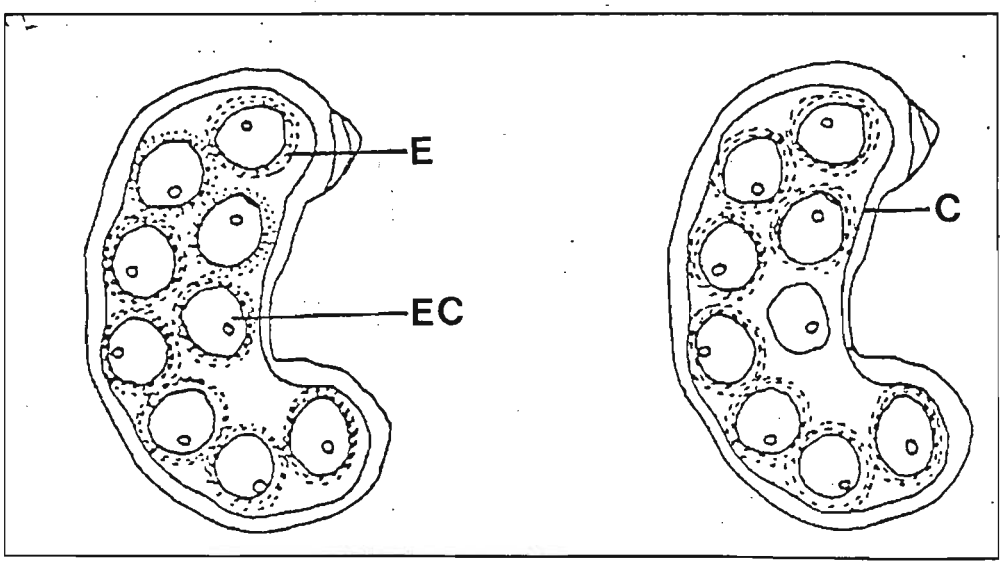
Figure 4.23: Transverse section through the end pieces of 4 spermatozoa (E = centriole microtubules, F = peripheral microtubules, M = plasma membrane).



100 nm

Figure 4.24: Egg capsules of *A.* cf. *marmorata* (C = capsular membrane, E = egg case, EM = embryo).

Figure 4.25: Outline drawings of egg capsules of *A.* cf. *marmorata* (C = capsular membrane, E = egg case, EC = egg cell) (10X).


E
EC
C

recognize it, hence their identification of the present material as *Physa marmorata* and not *Aplexa* cf *marmorata*, as Te (1973) and Brown (1980), call it. This opinion is based (S. Thiengo, *in litt.*,10. 12. 98) on Paraense's 1987 observation that the type species of *Aplexa*, the European *A. hypnorum* (L.), has a smooth mantle edge, i.e. without digitations or dentations, and its penial canal has a terminal outlet. Following Thiengo (1998), *P. marmorata* does not have a preputial gland but it does have mantle digitations while the related *Physa cubensis* has both. The penial canal has a lateral outlet. In essence, Brazilian malacologists consider *Aplexa* to be a European genus and their Neotropical counterparts to belong to *Physa.*

In my opinion the South African material equates with the Brazilian *Physa marmorata.* The question that remains is whether or not I should accept the genus *Aplexa.* Traditionally, freshwater malacologists working in Africa have followed the systematics of European malacology as updated by Brown (1980, 1994). I believe that it is wisest to accept *Aplexa* as a valid genus for South American physids until such time as good reasons are advanced for its rejection. Those put forward by the Brazilian school do not seem to me to be adequate. I therefore propose that the snail under study should be called *Aplexa marmorata* (Guilding, 1828). There is however one further complication and that is *Physa mossambiquensis* Clessin, 1886.

4.5 WHAT IS *Physa mossambiquensis* CLESSIN, 1886?

Clessin (1886) described *Physa mossambiquensis* from six specimens collected at Tete, Mocambique, by Wilhelm C. H. Peters between 1842 and 1848. These specimens are now lodged in the Berlin Museum, Germany, under accession number ZMB Moll. 8484 (Kilias, 1961). The relationship between these specimens, the *Aplexa marmorata* currently being found in the eastern parts of South Africa and what Brown (1994) calls *Aplexa* (*Stenophysa*) *waterloti* from several countries in West Africa was discussed by Appleton *et al.*(1989).

Clessin (1886) noted that *P. mossambiquensis* from Moçambique resembled South and Central American species more than they did East African species. Connolly (1939) provided an English translation of Clessin's (1886) Latin description of the shells of *P. mossambiquensis* as follows: "The shell is narrow ovate, smooth, glossy, transparent, corneus yellow and hardly rimate. The spire is short and acute. The shell has four elongated, little convex, rapidly increasing whorls. The sutures are little impressed (shallow). Aperture is narrow and pyriform. The columella is

thin and little contorted. The shell has growth lines or striolae. The type specimen measured 7.5 x 4 mm in size".

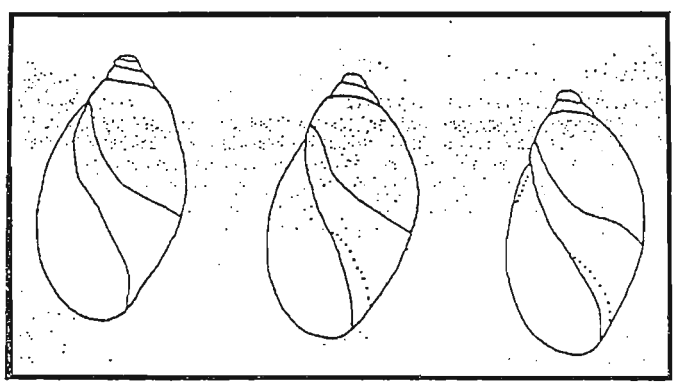
Following Connolly's (1925) comment that he was given details of the radula of *P. mossambiquensis* by J. Thiele (*in litt.*), it must be assumed that the specimens were collected alive. According to Dr. M. Glaubrecht (Berlin Museum) (in litt. to C.C. Appleton, 1999) however there are no radula preparations belonging to this species in the museum's collection and only the shells remain. He kindly arranged for the loan of these shells for inclusion in this study.

Figure 4.26 shows outline drawings of the shells of *Physa mossambiquensis*, one of which Dr Glaubrecht noted has Byne's Disease. Although these shells are small, 5.9- 8.4 mm in height with a mean of 7.15, relative to those of *A. marmorata* from South Africa and indeed the material from Nigeria and St Lucia (West Indies) that was included in this study, I cannot find any differences between them.

For these reasons, I consider that they should all be identified as *Aplexa marmorata* Guilding, 1828.

*Byne's Disease is a condition caused by the reaction of weak acids (e.g. malic and acetic acids) with the calcium carbonate of the shell. These acids gradually dissolve the shell and, over a period of time, rendering it chalky and destroying surface features such as microsculpture. These acids often come from the containers in which shells are stored, viz. paper boxes, tissue paper, cotton wool, wooden drawers and cabinets, especially of oak. Suggested remedies are to dilute the acids with distilled water though, if the shell is severely affected, it may be destroyed; alternatively, remove the deposit by sonication and then spray the shell with silicone oil (RN Kilburn [Natal Museum] & M Glaubrecht [Berlin Museum], pers. comm., May 1999).

Figure 4.26: Shells of *Physa mossambiquensis* (10X).



Te (1974) called the West African physid *A.* (*Stenophysa*) *marmorata* but Brown (1980, 1994) refers to it as *A.* (*Stenophysa*) *waterloti*. The name *Aplexa (Stenophysa) marmorata* (Guilding, 1828) should take precedence because it was the first name given to these snails. From its shell, I do not think that *P. mossambiquensis* is different from *Aplexa* cf *marmorata* from South Africa. Coupled with all the above-mentioned characteristics showing that this species is not *Physa*, I consider the species under study should be called *Aplexa marmorata.*

4.6 CONCLUSION

Several of the above characters of *A.* cf. *marmorata* can be used in its classification at genus level (Table 1). The body surface of both species have tiny dots of yellow-green pigment and these are characteristic of *Aplexa* according to Richards (1964), so is the absence of a visible preputial gland. The presence of a visible preputial gland is characteristic of *Physa.* The foot is spatulate with an acuminate tail which has a distinct dark stripe down its length, like that illustrated for *P. marmorata* by Paraense (1986).

Table 1 was used by Paraense (1987) to differentiate between *Physa marmorata* from the Caribbean Island of St. Vincent and *P. cubensis* from Cuba in respect of a number of conchological and genital characters. I have extended it to include *A.* cf. *marmorata* from South Africa.

Table 1: Comparison between *P. cubensis*, *P. marmorata* and *A.* cf. *marmorata*.

	Physa cubensis	*Physa marmorata*	*Aplexa* cf. *marmorata*
Whorls	Roundly convex, moderately shouldered	Flatly convex, not shouldered	Flatly convex, not shouldered
Spire	Lower, wider	Higher, narrower	Lower, wider
Suture	Well impressed	Shallow	Shallow
Columellar plait	Well marked	Low	Well marked
Shell width/shell length ratio	Mean 0.55	Mean 0.47	Mean 0.50
Spire length/ shell length ratio	Mean 0.31	Mean 0.39	Mean 0.32
Aperture length/ shell length	Mean 0.69	Mean 0.62	Mean 0.71
Penial canal	Sub-terminal outlet	Lateral outlet	Lateral outlet
Bulb of penial sheath	Somewhat narrower than proximal end of sheath	Much wider than proximal end of sheath	Much wider than proximal end of sheath
Preputial gland	Present	Absent	Absent
Penial sheath/ preputium ratio	Mean 1.49	Mean 2.08	Mean 0.61

As might be expected from the comment by Clampitt (1970) however, the male genital anatomy provided the most useful diagnostic characters for separating *A.* cf. *marmorata* from the genus *Physa* as exemplified by *P. acuta*. *Aplexa* cf. *marmorata* lacks an externally visible preputial gland and its penial duct was found to have a lateral outlet – both are characteristic of what Paraense (1986) called *Physa marmorata* from Brazil. The preputium is shouldered and is much wider than the narrow portion of the penial sheath.

The structure of the penial complex of *A.* cf. *marmorata* from South Africa thus conforms to the type B of Te (1974), the example of which was *A.* (*Stenophysa*) *marmorata* from both the West Indies and West Africa (Ghana, Burkina Faso, Togo and Nigeria). This same author relates the snails from the two areas by suggesting that the West African population was introduced there

from the West Indies. Te (1974) therefore named the West African physid *A.* (*Stenophysa*) *marmorata* though Brown (1980, 1994) and Kristensen & Ogunowo (1992) adopted a more conservative approach and retained the specific name *waterloti*, calling it *Aplexa waterloti* (Germain, 1911).

In view of the evidence gathered in the present study, I believe that not only the West African snails, but also those now being found in the eastern parts of South Africa (currently called *A.* cf. *marmorata*) and the isolated record of *Physa mossambiquensis* from Mocambique, should also be included in *A. (Stenophysa) marmorata* (Guilding, 1828) since this takes precedence over *waterloti.*

Sperm morphology may also be useful as a diagnostic character here but at this stage too few physid species have been examined and, as noted earlier, the systematics of the family need clarification. Nevertheless, the fine structure of the sperm of *A.* cf. *marmorata* resembles that of *Physa acuta* as described by Brackenbury & Appleton (1991) but with several potentially important differences. These are listed in Table 2.

Table 2: Morphological differences between the mature spermatozoa of *A.* cf. *marmorata* (this study) and *Physa acuta* (Brackenbury & Appleton, 1991).

Morphological Character	*A.* cf. *marmorata*	*Physa acuta*
Total length (μm)	266 – 282	365*
Max. no. of glycogen helices around the midpiece	2	3
Shape of the tip of end piece	Blunt	Tapered

*Tuzet & Mariaggi (1951) gave a total length of 400 μm for *P. acuta* sperm.

Neither the loss of the plasma membrane along the midpiece nor the presence of a terminal ring at the junction between the glycogen helix and the mitochondrial derivative regions as described by Ackerson & Koehler (1977) for an unidentified species of *Physa*, could be verified for *A.* cf. *marmorata*. There is however no doubt that the plasma membrane of the midpiece is, if present, much more tightly applied to the axoneme that on either the head or the glycogen piece (Compare Figs. 4.7, 4.8 & 4.14). This needs to be investigated further.

CHAPTER 5

POPULATION FLUCTUATIONS OF *Aplexa marmorata* IN SOUTH AFRICA

5.1 INTRODUCTION

Because *Aplexa marmorata* appears to be spreading in the eastern parts of South Africa and is likely to become the country's third invasive freshwater pulmonate snail, it is important that as much as possible is known about its biology and the way it responds to ecological disturbances. For this reason a sampling programme was started with the aims of (i) monitoring its annual population fluctuations and (ii) assessing its response to environmental disturbances. This is particularly important in view of the success of *Physa acuta* as an invader and the discovery by Brackenbury & Appleton (1991) that *P. acuta* responded to flood disturbances by immediately producing a new cohort (generation) and where repeat floods occurred, producing seven cohorts within a single year.

5.2 MATERIALS AND METHODS

Aplexa marmorata was sampled fortnightly at two sites within the Durban Metropolitan Area: an ornamental pond in the Botanic Gardens and a small lake in the Bluff Nature Reserve. Neither site experiences any regular direct human contact although people do visit them. These sites are ecologically very different and are described below. The reason for choosing the Botanic Gardens site was that it is protected from disturbances such as floods or drying out whereas the Bluff Nature Reserve site is known to dry out if no rain falls.

5.2.1 BOTANIC GARDENS SITE

The Durban Botanic Gardens are about 5km from the city centre ($29^0$15'S : $31^0$06'E) (Fig. 5.1). The actual site was a man-made pond measuring 18.1 x 1.7 m and with concrete walls and floor (Fig. 5.2). It is approximately 1.2m deep. Water lilies (*Nymphaea* sp.) provided surfaces for snail shelter, feeding and egg-laying. In addition to *A. marmorata*, four other snail species were collected: *Pomacea* cf. *lineata*., *Melanoides tuberculata*, *Lymnaea columella* and *Helisoma duryi*. The pond is kept full (of municipal water) and the surface kept clean of dead leaves etc. by the Gardens' staff..

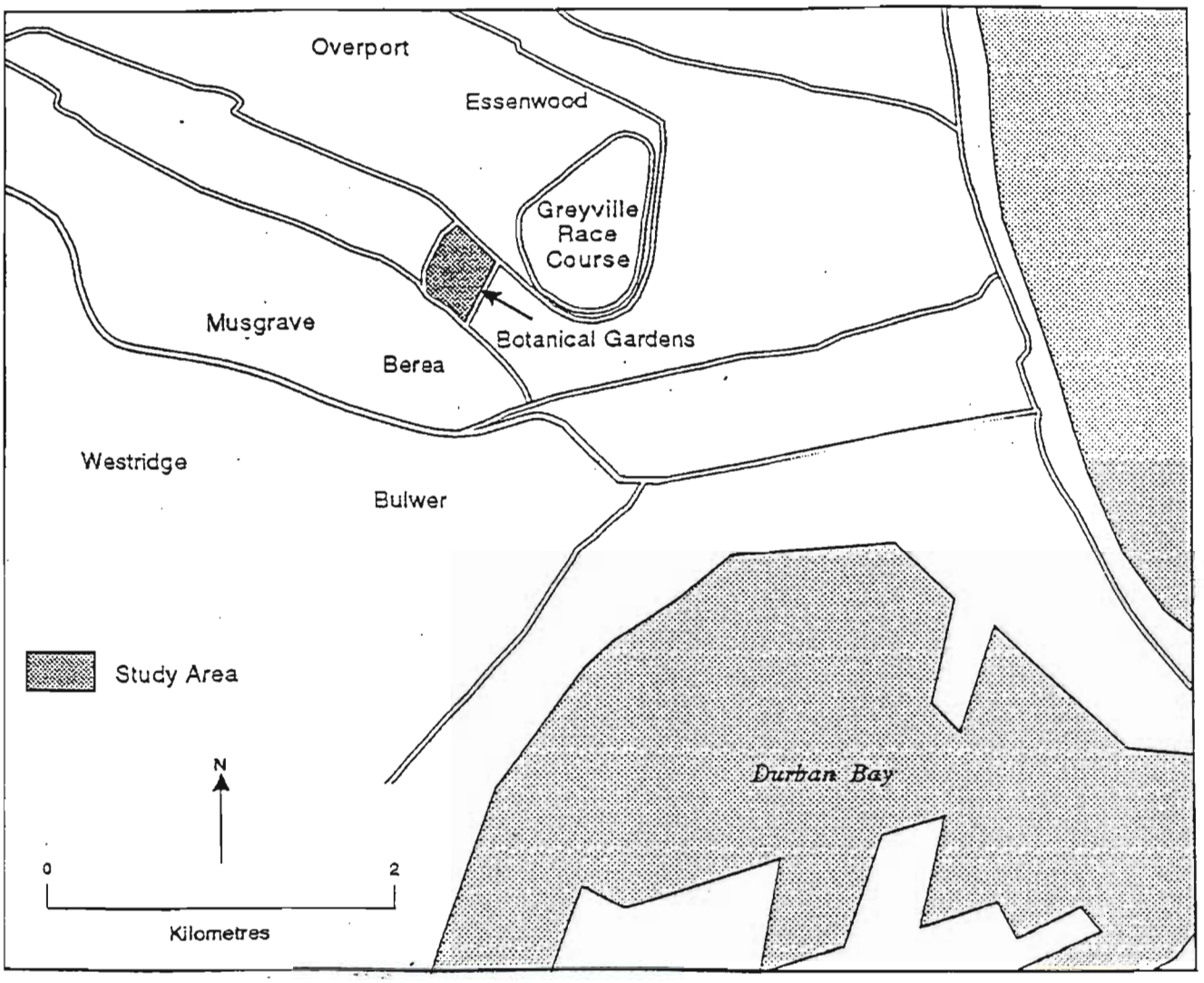


Figure 5.1: Map showing the geographical location of the Botanic Gardens.

Figure 5.2: Botanic Gardens sampling site.

5.2.2 BLUFF NATURE RESERVE SITE

This sampling site extended along 13.3 m of the shoreline of a small lake in Bluff Nature Reserve ($29^0$56'S : $31^0$59'E) (Figs 5.3 & 5.4). The site has reeds (*Phragmites* sp.) and bulrush (*Typha* sp.) growing in it and the floating fern *Azolla* sp. covers most of the water surface. *Aplexa marmorata* was first collected from this site by GB Wilken (University of Natal) in June/July 1989 and it was the first natural waterbody found to harbour the snail in South Africa (Appleton *et al.*, 1989). This lake depends on rainfall for its water and its shoreline therefore rises and recedes according to the prevailing rainfall pattern.

5.2.3 SAMPLING TECHNIQUE

Samples were collected for 30 minutes on each occasion over a period of 14 months. A metal scoop net with a 5 mm^2 wire mesh fitted into a 300 mm^2 frame on a long aluminium handle was used throughout and by the same person each time. All snails collected were taken to the laboratory where they were identified and their shell heights measured to the nearest 0.1mm using a Vernier Caliper, i.e. distance from the apex of the spire to the ventral margin of the aperture. The number of *A. marmorata* present in each sample was recorded and densities expressed as the number of snails collected per unit time (30 minutes). All snails were returned to their respective habitats the following day.

Egg capsules found attached to the undersides of *Nymphaea* sp. leaves at the Botanic Gardens site were counted and clutch sizes recorded. Unlike the situation reported by Brackenbury (1989) for *P. acuta* in the Umsinduzi River, Pietermaritzburg, no eggs were found laid on the shells of *A. marmorata* by its conspecifics.

5.2.4 TEMPERATURE, pH AND CONDUCTIVITY

Water temperature, pH and electrical conductivity were measured fortnightly at both sites using a mercury-in-glass thermometer, a Beckman 32 pH meter and a Crison micro CM 2201 Conductivity meter respectively.

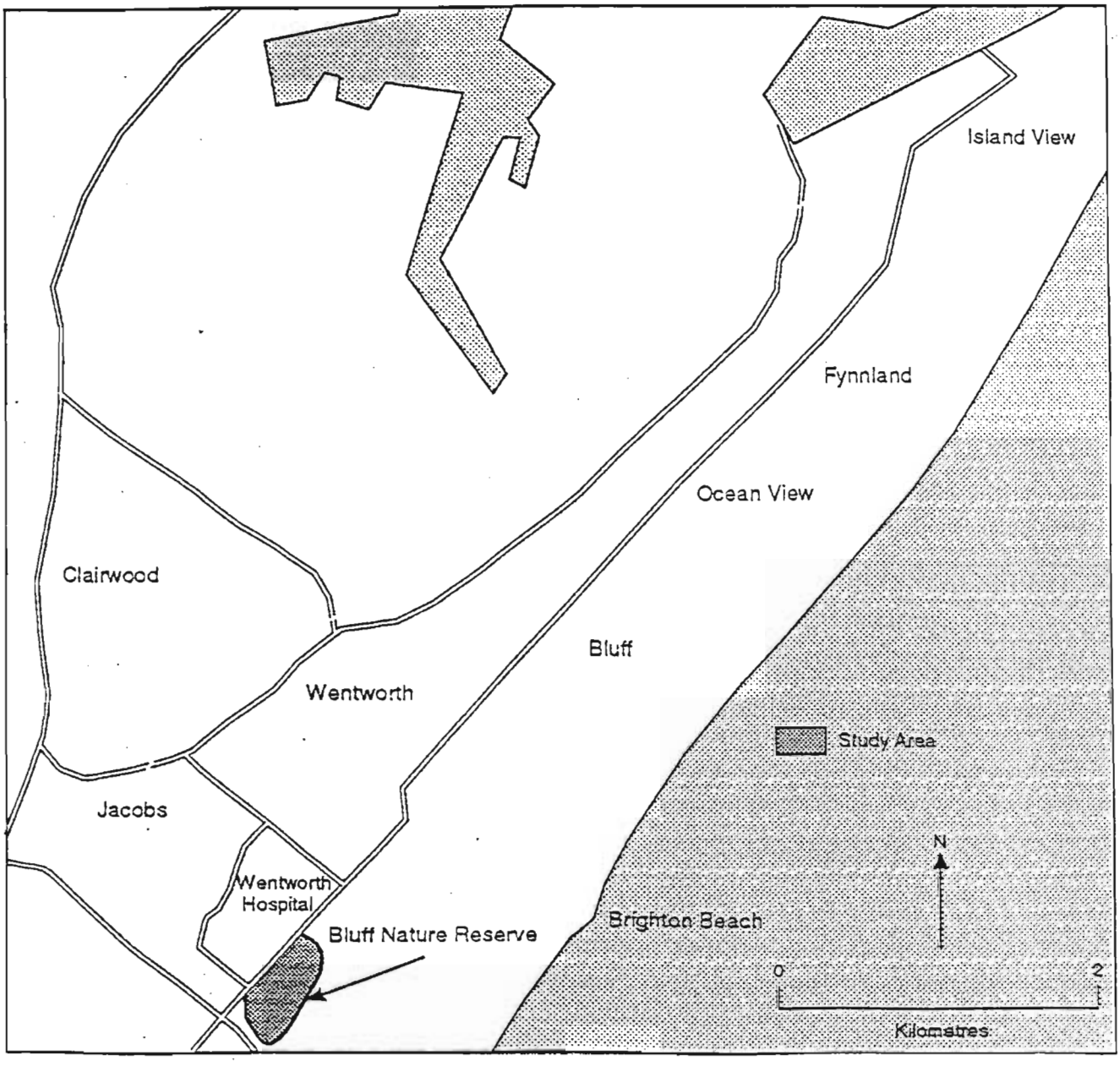


Figure 5.3: Map showing the geographical location of the Bluff Nature Reserve.

Figure 5.4: The Bluff Nature Reserve sampling site.

5.2.5 STATISTICAL ANALYSIS

The ELEFAN computer programme (Wetherall, 1986) was used to determine from successive samples the number of cohorts occurring in the 14-month sampling period. Percentage frequencies of the samples collected from the Bluff Nature Reserve and the Botanic Gardens were calculated (Appendices 8 & 9 respectively).

5.3 RESULTS

The results from both sites show that *A. marmorata* is a long-lived species with an estimated generation time of 14-15 months. The size-frequency distributions of successive samples are shown in Figs 5.5 & 5.6, plotted using the ELEFAN programme (Wetherall, 1986). ELEFAN also calculates growth curves for the individual cohorts based on the observed values of $L\infty$ (Figs. 5.5 & 5.6).

5.3.1 BOTANIC GARDENS SITE

The model indicates a pattern of three overlapping cohorts at this site during the 14 - month sampling period. Cohort 1 was present as mature snails >8mm shell height in November 1997when sampling began and had reached 17 mm by early March 1998 after which it disappeared. Cohort 2 was first collected in May/June 1998, represented by snails of shell height 4-9 mm and persisted until December 1998 when sampling stopped. By this time it had attained a shell height of >10mm. Cohort 3 appeared in October 1998 and had reached a shell height of 4-9mm by the end of the sampling programme.

The egg production data are shown in Fig. 5.7 and indicate four peaks in oviposition which can be allocated to the different cohorts as follows: a small peak in early January 1998 by cohort 1, a major peak in March/April and a minor one in July 1998, both by cohort 2, and a small peak by cohort 3 in October 1998. The two peaks allocated to cohort 2 lie either side of mid-winter. It is possible that the late March peak was a response by the snails to a drop in water level two weeks earlier when the pond was partially drained and not immediately refilled. This coincided with the loss of the largest snails. A feature of this habitat was however its relative stability in terms of water level and consequent lack of disturbances.

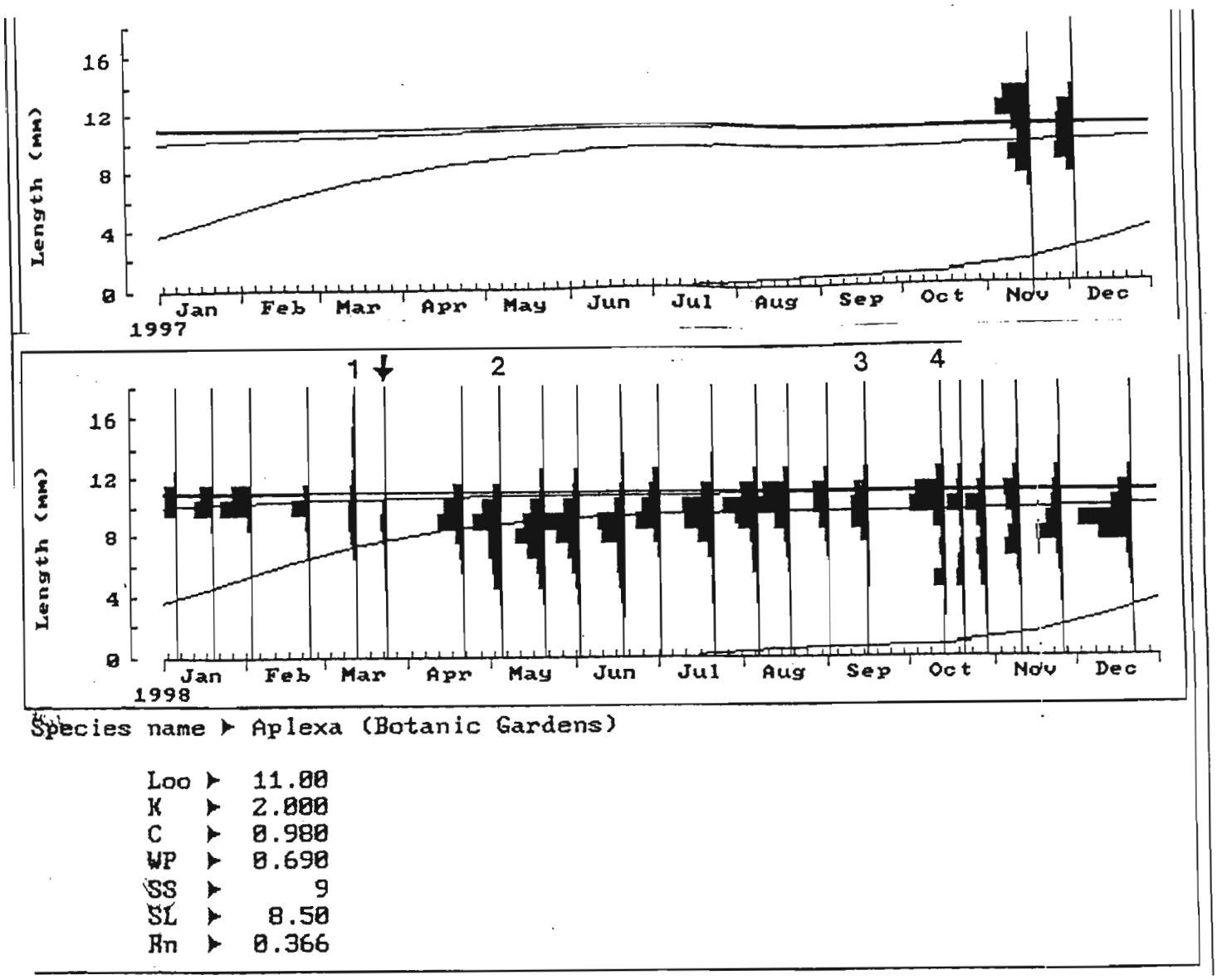
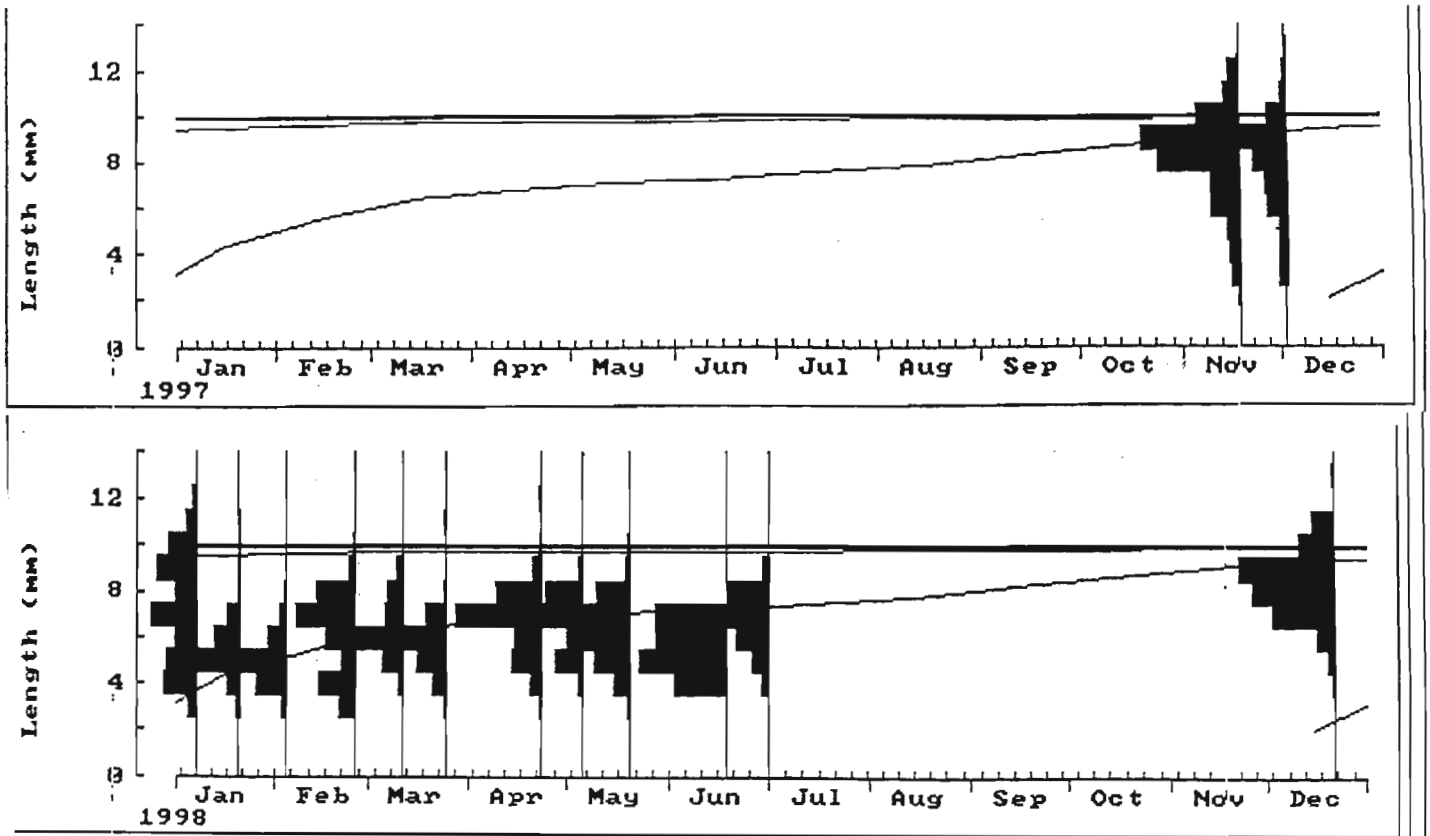


Figure 5.5: ELEFAN graph showing the number of generations of snails collected in the Botanic Gardens. The line running across the graph shows the mean shell heights. (C = amplitude of seasonal growth oscillation in the von Bertalanffy Growth Function, VBGF; K = curvature parameter of VBGF; L_∞ = asymptotic length or mean length; Rn = "goodness of fit index" of the ELEFAN 1 routine; SL = starting length; SS = starting sample; WP = winter point in the seasonalised VBGF).



Species name ▶ Aplexa (Bluff)

Loo	▶	10.00
K	▶	2.500
C	▶	0.700
WP	▶	0.400
SS	▶	4
SL	▶	4.50
Rn	▶	0.565

Figure 5.6: ELEFAN graph showing the snail population in the Bluff Nature Reserve. The line running across the graph shows the mean shell heights. (C = amplitude of seasonal growth oscillation in the von Bertalanffy Growth Function, VBGF; K = curvature parameter of VBGF; L_∞ = asymptotic length or mean length; Rn = "goodness of fit index" of the ELEFAN 1 routine; SL = starting length; SS = starting sample; WP = winter point in the seasonalised VBGF).

The water was always clear (turbidity = 1.6 Nephelometric Turbidity Units [NTU]) and its temperature (measured at 11h00) ranged from a low of 17-18 ^{0}C in winter (June-July) to a high of 29-30^{0}C in summer (December-January) (Table 3). Figures 5.8 & 5.9 are graphs showing the water temperature taken during the sampling period at the Botanic Gardens and the Bluff Nature Reserve respectively. Conductivity values ranged from 129 μS.cm^{-1} in early January to the relatively high value of 694 μS.cm^{-1} and pH varied between 6.3 and 7.54 (Table 3).

Figure 5.7: Graph of egg capsules collected at the Botanic Gardens over a 12 month period

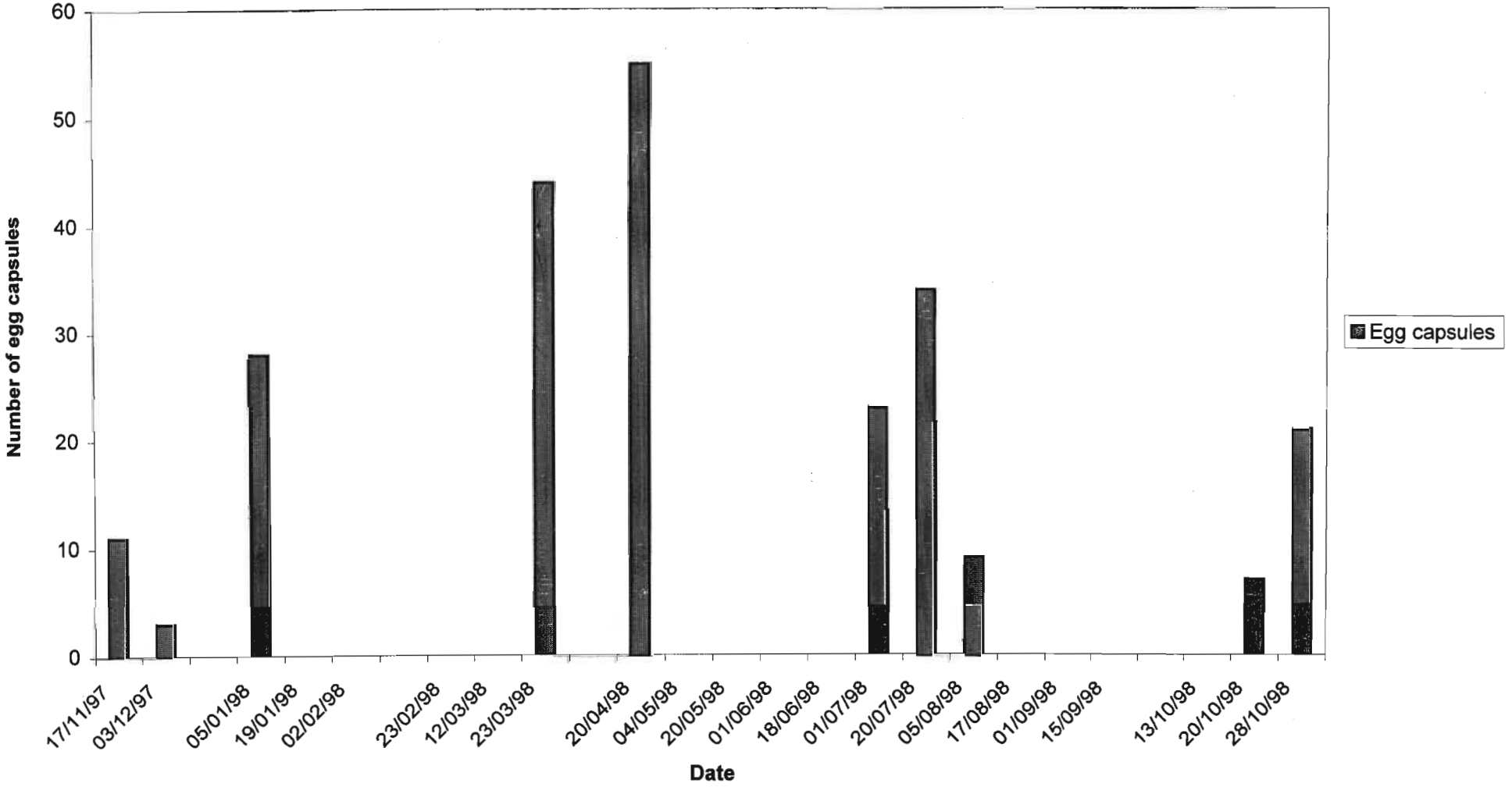


Table 3: Temperature, conductivity and pH at Botanic Gardens site

Date	Temperature (°C)	Conductivity ($\mu S.cm^{-1}$)	pH
17/ 11/ 97	30.5	160	9.13
03/ 12/ 97	28	78.5	8.2
05/ 01/ 98	30	118	7.38
10/ 01/ 98	26	126	7.31
02/ 02/ 98	28	107	6.9
23/ 02/ 98	28	148	6.48
12/ 03/ 98	29	197	6.63
23/ 03/ 98	27	172	7
20/ 04/ 98	25	135	7.61
04/ 05/ 98	22	248	6.29
20/ 05/ 98	19	250	6.9
01/ 06/ 98	15	273	7.52
18/ 06/ 98	14	151	7.39
01/ 07/ 98	15	203	6.34
20/ 07/ 98	21	165	6.36
05/ 08/ 98	18.5	137	7.91
17/ 08/ 98	20	167	6.69
01/ 09/ 98	21	184	6.01
15/ 09/ 98	22	176	6.87
13/ 10/ 98	25	155	7.18
20/ 10/ 98	26	214	8.39
28/ 10/ 98	26	216	6.97
10/ 11/ 98	25	185	6.99
25/ 11/ 98	25	233	6.7
22/ 12/ 98	25	199	6.78

5.3.2 BLUFF NATURE RESERVE SITE

The size-frequency data for this site are shown in Fig. 5.6. Again, three cohorts were found. Cohort 1 corresponds to #1 in Fig. 5.5 at the Botanic Gardens site; it was present when sampling started and had all but disappeared by late January 1998 though a few large specimens persisted until April. Cohort 2 appeared in January 1998 but disappeared due to a drastic drop in water level for 5½ months between July and early December 1998. After the water level rose again in late December following rains in November, mature snails apparently representing cohort 3 were present. No juveniles were present in this sample and because physids are not vegetation-dependent, the snails may have moved with the receding water level. The adults presumably migrated from deeper parts of the lake that did not dry out or might have aestivated. During the dry season, no empty shells were collected from the sampling area, evidence to prove that the snails did not die during that period. The deep waters of the lake could only be reached by a boat and because none was available for this study, sampling did not occur in that part of the lake.

Water at the lake was not turbid (4.4 NTU) and Table 4 shows that winter temperatures (measured at 11h00) were several degrees lower than in the Botanic Gardens pond, 14-15^{0}C, while summer temperatures were similar, 28-31^{0}C. Conductivity readings ranged from a very low level of 51-79 μS.cm^{-1} to maxima of 248-273 μS.cm^{-1}. These are considerably lower than in the Botanic Gardens site. pH readings ranged 6.05 to 7.54 and like the Botanic Gardens site, these conform to what would be expected in many natural waterbodies. No seasonal pattern in conductivity or pH was evident at either of the two sites.

Table 4: Temperature, conductivity and pH at Bluff Nature Reserve site

Date	Temperature (°C)	Conductivity ($\mu S.cm^{-1}$)	pH
18/ 11/ 97	20	203	6.8
02/ 12/ 97	30	694	7.3
06/ 01/ 98	29	129	7.05
20/ 01/ 98	28	339	6.99
03/ 02/ 98	33	361	7.54
24/ 02/ 98	27	307	7.14
11/ 03/ 98	29	546	6.96
24/ 03/ 98	29	548	6.85
22/ 04/ 98	23	493	6.05
05/ 05/ 98	24	281	6.14
19/ 05/ 98	20	355	7.5
17/ 06/ 98	17	-	-
30/ 06/ 98	17	317	6.33
22/ 07/ 98	18	471	6.3
21/ 12/ 98	30	268	6.83

Figure 5.8: Water temperature at the Botanic Gardens sampling site

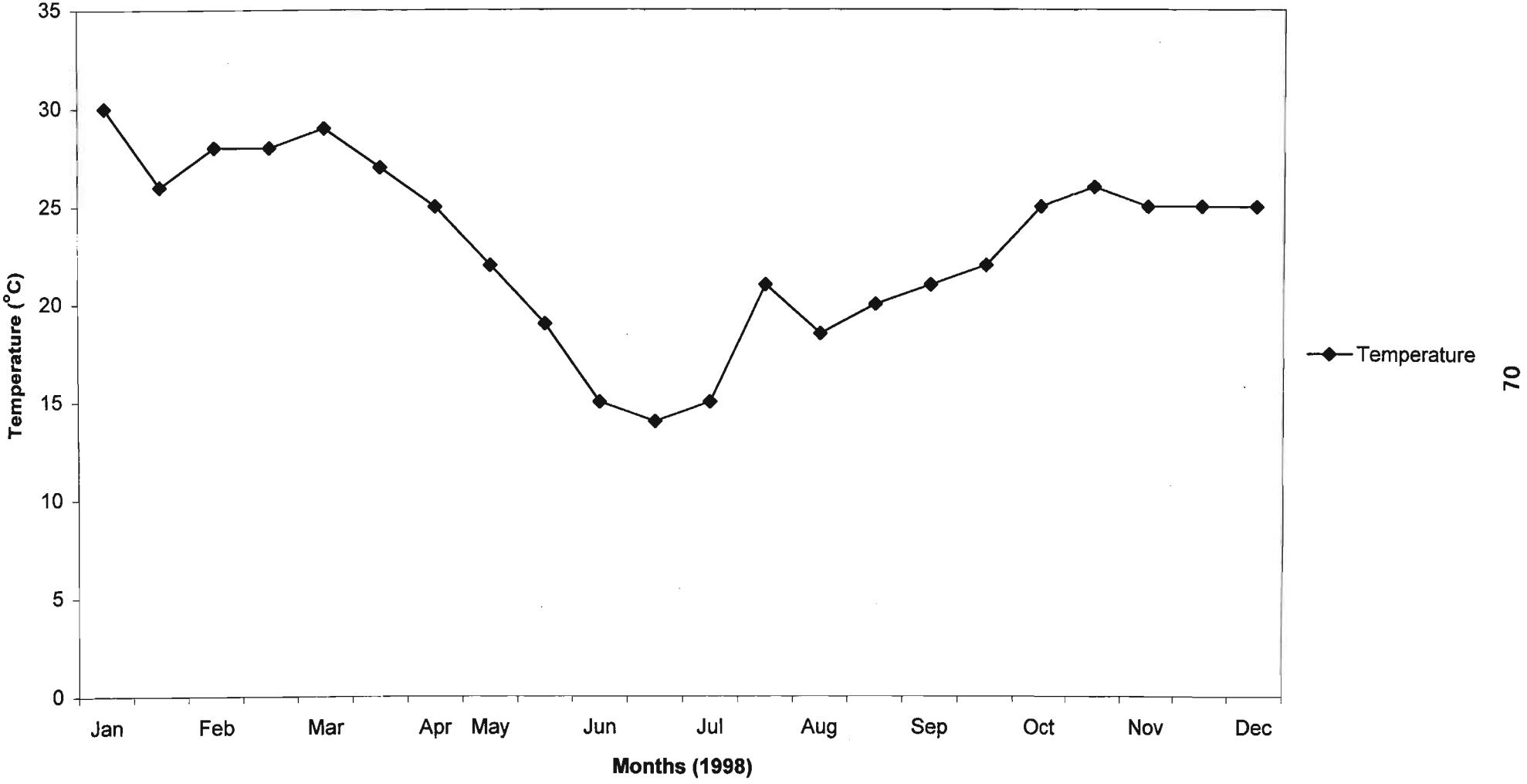


Figure 5.9: Water temperature at the Bluff Nature Reserve sampling site (The sampling area dried up between July and December therefore no samples were collected).

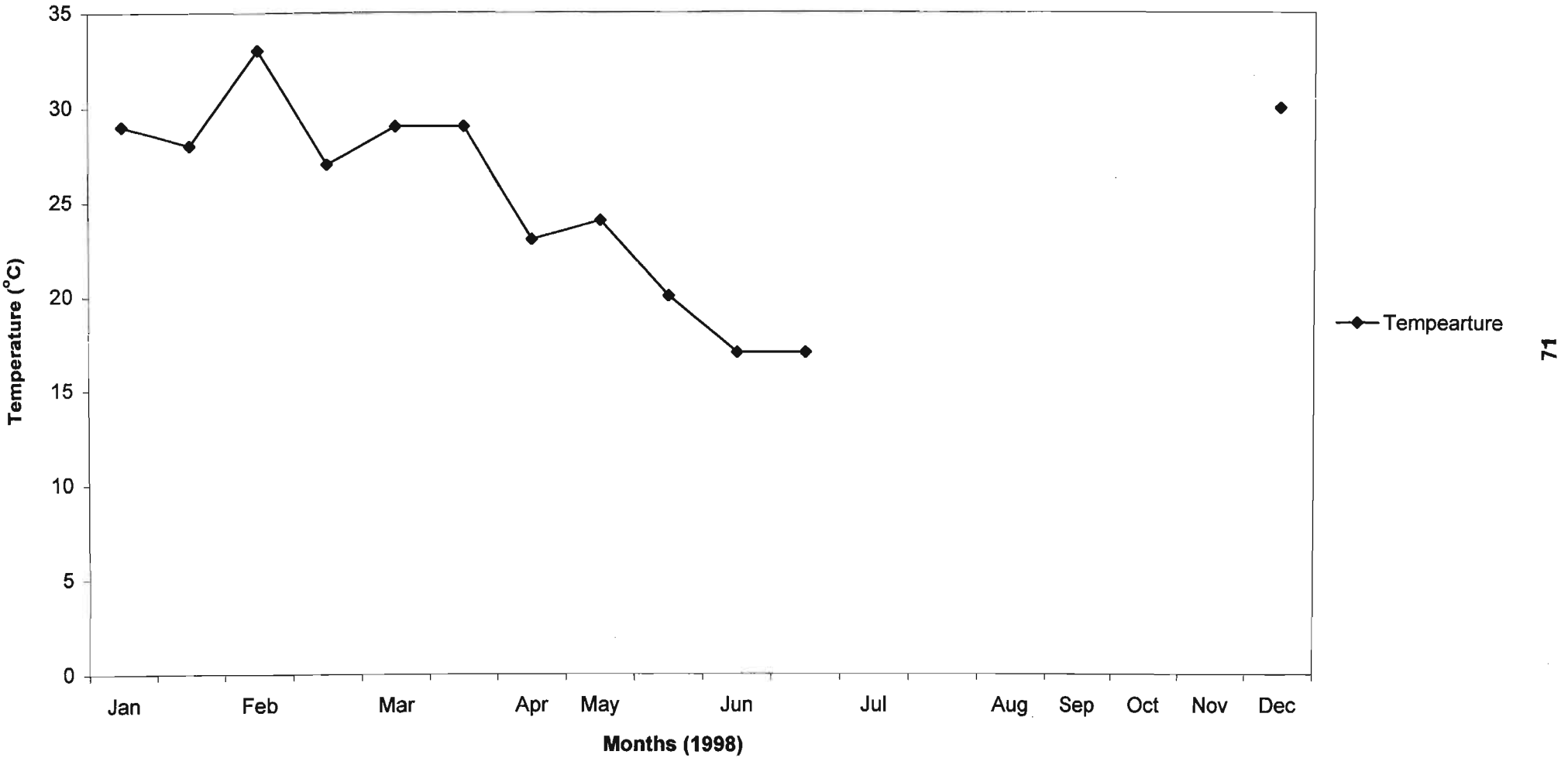


Table 5: The population density of *Aplexa marmorata* at Bluff Nature Reserve sampling site.

Date	Total No. of snails sampled in 30 minutes
18/ 11/ 97	249
02/ 12/ 97	217
06/ 01/ 98	206
20/ 01/ 98	264
03/ 02/ 98	316
24/ 02/ 98	237
11/ 03/ 98	347
24/ 03/ 98	298
22/ 04/ 98	305
05/ 05/ 98	216
19/ 05/ 98	262
17/ 06/ 98	16
30/ 06/ 98	19
22/ 07/ 98	0
21/ 12/ 98	109

Measurements of population density ranged from zero during the dry period of June to November to between 264 and 316 snails/ 30 minutes during March and April, rather earlier than the highest numbers at the Botanic Gardens site (Table 6). Figures 5.10 & 5.11 show the graphs of the population densities at the Bluff Nature Reserve and the Botanic Gardens respectively.

Figure 5.10: The population density of Aplexa at Bluff Nature reserve

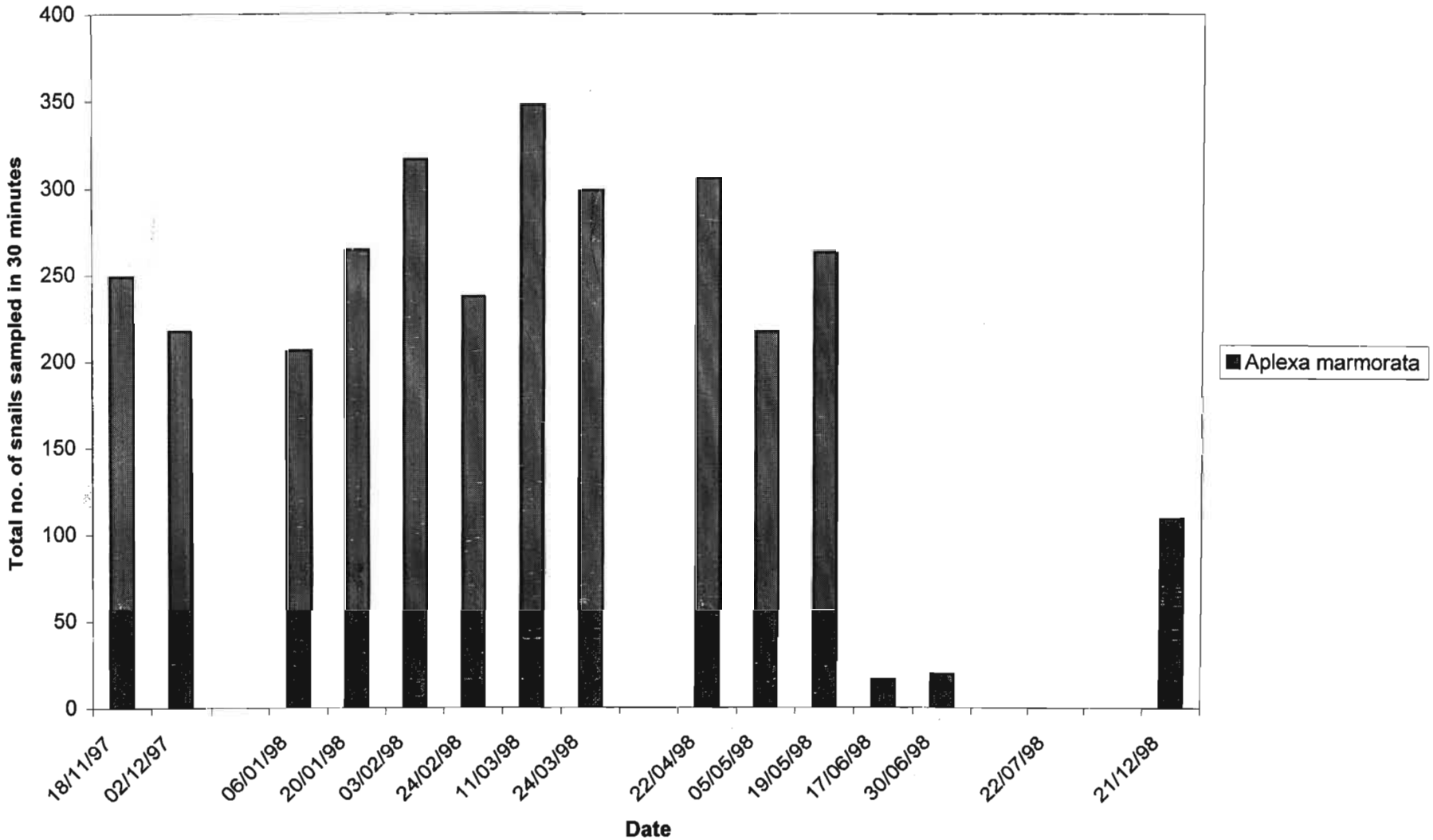


Figure 5.11: The population density of Aplexa at the Botanic Gardens sampling site

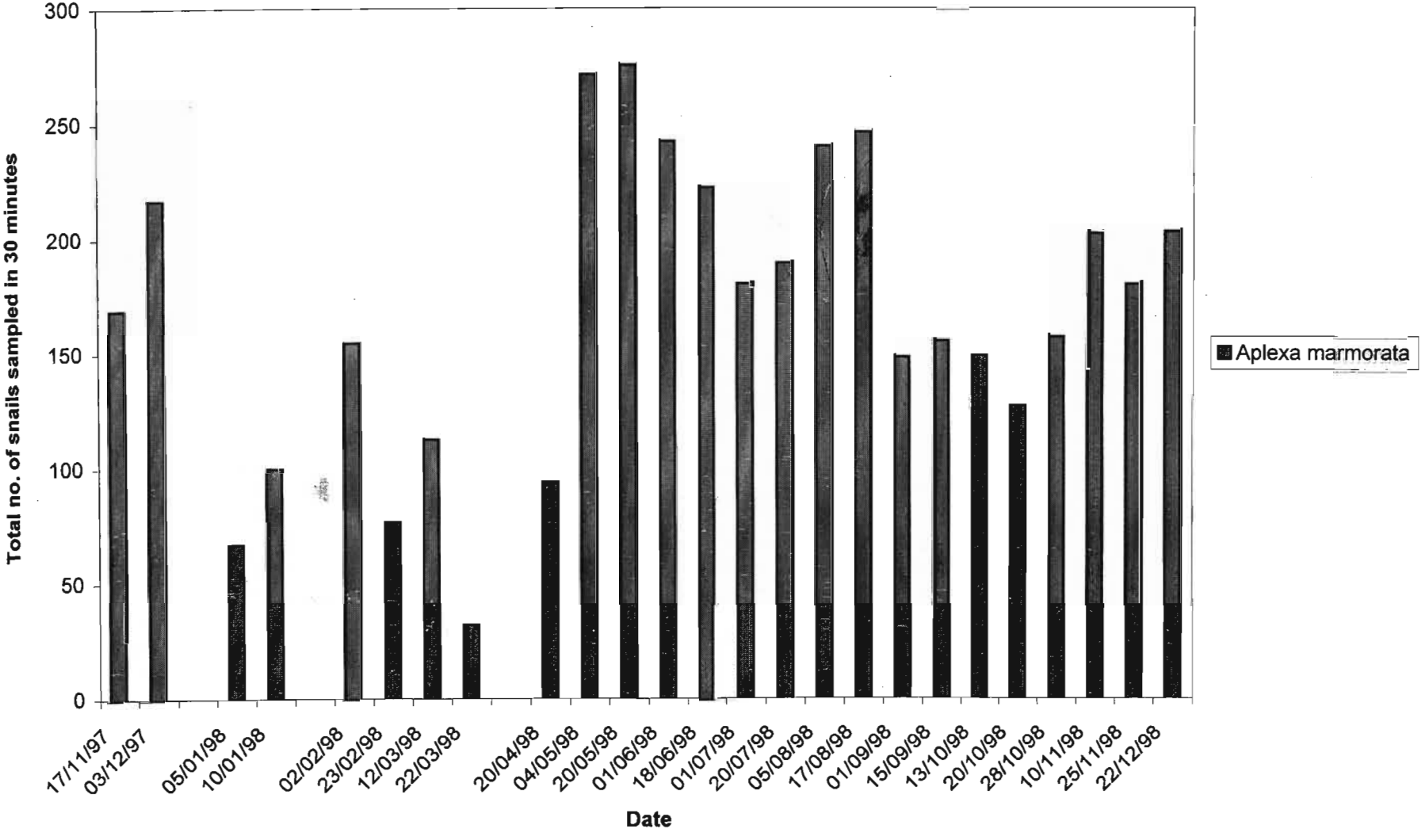


Table 6: The population density of *Aplexa marmorata* at the Botanic Gardens sampling site

Date	Total No. of snails sampled in 30 minutes
17/ 11/ 97	169
03/ 12/ 97	217
05/ 01/ 98	67
10/ 01/ 98	100
02/ 02/ 98	155
23/ 02/ 98	77
12/ 03/ 98	113
22/ 03/ 98	32
20/ 04/ 98	94
04/ 05/ 98	272
20/ 05/ 98	276
01/ 06/ 98	243
18/ 06/ 98	223
01/ 07/ 98	181
20/ 07/ 98	190
05/ 08/ 98	241
17/ 08/ 98	247
01/ 09/ 98	149
15/ 09/ 98	156
13/ 10/ 98	149
20/ 10/ 98	127
28/ 10/ 98	158
10/ 11/ 98	203
25/ 11/ 98	181
22/ 12/ 98	204

5.3.3 OTHER FRESHWATER SNAILS

While *A. marmorata* was the most abundant snail at the Botanic Gardens site, three other alien and one indigenous gastropod species, *Melanoides tuberculata*, were taken in samples on virtually every occasion (Table 7). *Pomacea* cf. *lineata* (Spix, 1827) and *Melanoides tuberculata* (Müller, 1774) are prosobranchs and the others, *Lymnaea columella* (Say, 1817) and *Helisoma duryi* (Wetherby, 1879), are pulmonates. No explanation can be given for the sudden decline in numbers of *Pomacea* cf. *lineata*, *M. tuberculata* and *H. duryi* during May and June and it is noticeable that *A. marmorata* numbers did not decline at this time. It may be relevant that temperatures were falling below 20^0C and the dramatic 81% lowering of conductivity from 273 to 51 $\mu S.cm^{-1}$ in mid-June coincided with the virtual disappearance of *Pomacea* cf. *lineata.* Numbers only recovered four months later, in late October. This drop in conductivity could have affected its osmoregulation – i.e. as an osmotic shock. Numbers of *M. tuberculata* fell to very low levels during winter and had not recovered by December 1998 when sampling stopped.

The only other snail sampled with *A. marmorata* in Bluff Nature Reserve was *L. columella.* Very few specimens were collected during each sampling period.

Table 7: Species composition and density (number of snails /30 minutes) at Botanic Gardens sampling site.

Date	*Aplexa*	*Pomacea* cf. *Lineata*	*Melanoides tuberculata*	*Lymnaea columella*	*Helisoma duryi*
17/ 11/ 97	169	7	178	0	21
03/ 12/ 97	217	17	23	3	21
05/ 01/ 98	67	35	90	0	9
10/ 01/ 98	100	16	75	0	43
02/ 02/ 98	155	49	77	0	31
23/ 02/ 98	77	57	94	2	39
12/ 03/ 98	113	58	27	4	22
22/ 03/ 98	32	69	177	0	49
20/ 04/ 98	94	59	29	3	49
04/ 05/ 98	272	48	84	2	45
20/ 05/ 98	276	54	16	0	77
01/ 06/ 98	243	57	5	6	15
18/ 06/ 98	223	3	11	0	3
01/ 07/ 98	181	7	41	4	25
20/ 07/ 98	190	2	3	8	3
05/ 08/ 98	241	2	0	4	13
17/ 08/ 98	247	0	1	5	6
01/ 09/ 98	149	3	0	12	10
15/ 09/ 98	156	0	0	8	6
13/ 10/ 98	149	0	0	4	21
20/ 10/ 98	127	17	0	4	0
28/ 10/ 98	158	11	0	8	15
10/ 11/ 98	203	22	9	5	13
25/ 11/ 98	181	15	0	4	11
22/ 12/ 98	204	49	0	15	18

5.4 DISCUSSION

In the Botanic Gardens, *A. marmorata* was found to have three generations. Egg capsules were collected at the Botanic Gardens between November 1997 to January 1998. They were again collected between March and April 1998. More eggs were collected in October 1998. Snails bigger than 12.5 mm were collected in March (#1 in Fig. 5.5). Two weeks later the pond was cleaned and the largest snails died and this signified the end of the first generation (downward arrow in Fig. 5.5). In May, a large number of snails smaller than 6 mm was collected (#2 in Fig. 5.5). This signified the beginning of the second generation, which survived up to the end of September (#3 in Fig. 5.5). In October, a large number of snails smaller than 6 mm was collected again and this was the beginning of the third generation (#4 in Fig. 5.5).

This pattern of three overlapping cohorts which was recorded for *A. marmorata* at both sampling sites differs markedly from *P. acuta* which produced seven cohorts over a similar period of time in the Umsindusi River, Pietermaritzburg (Brackenbury & Appleton, 1989). Apart from the record from the Sabie River in Mpumalanga Province, all habitats colonized by *A. marmorata* in South Africa are lentic. It would therefore be interesting to see how this snail would cope if its habitat was subjected to floods. The rapid re-colonization of the Bluff lake sampling site by adult *A. marmorata* after the November 1998 re-filling was not unexpected because physids are known to be very mobile members of the benthos of lakes in their native North America (Clampitt, 1970) and could, as suggested above, simply migrate into the newly inundated areas from refuges in deeper water.

Clampitt (1970) conducted a comparative study on two freshwater pulmonate snails, *Physa gyrina* Say and *Physa integra* Haldeman in Iowa, U.S.A., investigating the local distributions of each species and their causes. *Physa integra* was found on rocky shores and in vegetated off-shore areas of lakes and was absent from ponds while *P. gyrina* was found in ponds, in rocky lake shore areas and intermediate type habitats but always in shallow water.

All the localities where *A. marmorata* has been collected in South Africa, are standing water. This species has not been collected in streams or running water. Brackenbury (1989) found that *Physa acuta* is tolerant of fast current velocities of up to 0.6 $m.s^{-1}$ while *Bulinus tropicus* and other indigenous pulmonate snails are not known to occur in water with a velocity of greater than 0.3 $m.s^{-1}$ (Appleton, 1978). *Physa acuta* recolonised its habitat in the Umsindusi River after the

1987 floods that eroded the river channel, removing all marginal vegetation and associated fauna (Brackenbury & Appleton, 1993). The ponds and lakes where *A. marmorata* has been found in South Africa have not been subjected to floods during the period of this study so it is not known whether this snail can withstand flooding of its habitats.

Since little field information is available for *A. marmorata,* data on North American physids will have to be used for comparative purposes. Reproduction in *P. gyrina* (DeWitt, 1954) and *Physa fontinalis* (Duncan, 1959) under natural conditions is directly related to temperature. Growth almost ceases for *P. fontinalis* at temperatures below 7°C (Duncan, 1959) while it ceases for *P. gyrina* at 10-12°C (DeWitt, 1955). When the water temperature rose above 7°C, the growth of *P. fontinalis* was renewed and the female system matured (Duncan, 1959). The male system was found to function only in winter and snails did not oviposit at this time even when they were kept at temperatures of 15-20°C. In Durban, where this study was conducted, temperatures do not drop as low as 7°C in winter. Snails between 7-11 mm were collected during the winter, from May to July but no eggs were laid during this period.

In field populations of both *P. gyrina* and *P. integra*, Clampitt (1970) found that growth and reproductive activity were greatest in spring, slight in winter and there was considerable mortality in summer. Under stable laboratory conditions, *P. gyrina* was found to live up to 22 months (DeWitt, 1955).

Brackenbury & Appleton (1991) conducted a study on the effect of controlled temperatures on gametogenesis in *P. acuta* at 15°C, 25°C and 28°C. At 15°C, *P. acuta* attained sexual maturity after 34 days with a mean shell height of 6.1 mm and egg production continued for 54 weeks. At 25 °C, the species attained sexual maturity after 20 days with a mean shell height of 5.1 mm and egg production lasted for 15 weeks. At 28°C, the species attained sexual maturity after 18 days with a mean shell height of 4.7 mm and egg production lasted for 9 weeks.

In the laboratory, *A. marmorata* was subjected to three different temperatures to determine the effect of temperature on gametogenesis. Ten specimens between 8 -10 mm were put in each of six 10- litre tanks and allowed to lay eggs at 18°C, 21°C and 25°C. The first day after the snails were put in the tanks at 21°C and 25°C, 5 –8 egg capsules with a clutch size of 7 -25 eggs per capsule were observed in each tank. Seven days later, more than 30 egg capsules were laid in each tank. The eggs started to hatch 14 days after they were laid. Nine days after hatching, the

young snails started to die. The experiment was repeated three times but each time the young snails died 9 to 14 days after hatching. Snails kept at 18°C laid fewer eggs than the specimens that were subjected to 21°C and 25°C. This may be due to the cooler temperature delaying the egg –laying and hatching processes. Table 8 shows the number of eggs found in each egg capsule laid by the snails two days after they were put under the three different temperatures.

Table 8: Number of eggs found in each egg capsule laid after 2 days in the laboratory at three different temperatures.

18°C		21°C		25°C	
14	6	7	14	20	14
12	15	11	17	13	22
8	20	9	15	17	15
19	12	18	8	21	7
4	11	12	7	11	18
16	18	18	13	12	25
7	6	16	8	15	19
10	9	10	16	11	8
	15	17	12	6	22
	12	22	9	19	16
	13	15	13	17	14
	9	11	5	14	6
		13	7	11	11
		20		16	16
		12		8	14
		7		10	17
		16		7	22
		21		9	9
					16
					18
					15

Snails at the Botanic Gardens survived the disturbance to their habitat, when the pond was cleaned, by laying large numbers of eggs. Large snails died but two weeks later, young snails appeared. Snails moved to deeper water at the Bluff Nature Reserve when drought set in. Large snails were collected when rains started falling.

In the Umsindusi River, Pietermaritzburg, Brackenbury & Appleton (1993) found that *P. acuta* responded to disturbances to its habitat, in this case floods, by reproducing very fast. Seven generations were produced by this species after successive floods within a three - month period.

Thomas & McClintock (1996) found for *Physella cubensis* (= *Physa cubensis* of Paraense, 1987) that increased water temperature increased the growth rates of embryos and juveniles and also influenced the body size at which sexual maturity was attained. This species avoided regions of the stream with high water velocities and was found in pools and riffles within the stream. In areas with high rainfall and therefore high water velocities, juveniles (1-5 mm) burrowed into the substratum or moved under rocks or immobile debris. As noted earlier, *Aplexa marmorata* has never been found in running streams in South Africa but only in stagnant water except in the Sabie River, Mpumalanga, where it was collected in a quiet backwater.

Temporary pond pulmonates are more resistant to desiccation than lake dwelling species (Cheatum, 1934). *Physa cubensis* survived drying of the pond by burrowing into the moist areas of the sediments. In Kenk's pond, juvenile *P. gyrina* was also noted to aestivate in dry bottom materials (DeWitt, 1955). This might have been due to the fact that less body surface area was exposed in juvenile than in adults. Adult *P. cubensis* subjected to dry conditions in the laboratory produced a dry mucous film across the shell aperture (Thomas & McClintock, 1996) but did not survive for more than 24 hours in the absence of moisture.

When the lake at the Bluff Nature Reserve dried out, snails moved to deeper waters and when the lake refilled, they moved back to the original shore of the lake. At the Botanic Gardens, *A. marmorata* did not aestivate because the pond was always filled with water.

CHAPTER 6

General Discussion

The introduction of organisms into ecosystems in which they did not exist previously is a worldwide problem. Some of these introduced organisms have become invasive, i.e. they have an ability to reproduce and colonize the new habitats but often only after a lag period of several years. Biological invasions have been given considerable attention as SCOPE formed an international programme to investigate the ecology of invasions (SCOPE, 1983). Invasions of habitats may be natural or artificial. Natural invasions occur without the influence of man (Ashton & Mitchell, 1989) while artificial invasions are due to human influences. In respect of the latter, the introduction of animals or plants for ornamental purposes, recreation, food or as biological control agents against pests, has been done intentionally (deliberately). Some introduced organisms have themselves become pests while others have become involved in the spread of diseases, e.g. *Lymnaea columella*, which is an intermediate host for fascioliasis. Some organisms are introduced accidentally, usually via sport fishing and aquarium industries.

As far as invaded habitats are concerned, there is little doubt that disturbed ecosystems are most prone to invasion. This disturbance may be by natural forces such as soil erosion, fire or by man, e.g. the cultivation of land which of course involves the introduction of alien species to formerly stable habitats. In invaded areas where there are no native predators, the invaders tend to dominate and out-compete indigenous species in respect of reproduction (Macdonald & Jarman, 1984) and competition for food and shelter may also lead to a reduction or even extinction of the indigenous organisms.

Man is undoubtedly the main vector of introductions of organisms to "foreign" habitats because of his constant travelling around the world. The occurrence of organisms in places far away from their habitats of origin has become a global concern (SCOPE, 1983) – often collectively called "globalization".

In South Africa, many of the alien aquatic animals and plants now in the country were probably introduced during the 1940s and 1950s or even before (Rayner & Appleton, 1992; de Moor & Bruton, 1998). The most affected aquatic habitats in South Africa are considered to be the riparian zones of rivers (Macdonald & Richardson, 1986). The building of dams and weirs, bank

erosion, agricultural runoff and siltation are some of the factors that have contributed to the disturbance of many of the country's freshwater systems and, as noted above, have facilitated invasion.

Possibly as many as eight species of freshwater gastropods, including *A. marmorata,* have been recorded in South Africa (Brown 1994; Appleton, 1996; Appleton & Brackenbury, 1998). Five of these are pulmonates and two of these, *Lymnaea columella* and *Physa acuta,* are now invasive and are still spreading (de Kock *et al.*, 1989). *Aplexa marmorata* seems to be the third species in this category. Several prosobranchs, all South American members of the Family Ampullariidae, have also been reported in the country. These and the other introduced pulmonates have however not, as far as is known, spread beyond aquaria and artificial waterbodies such as ornamental ponds. Vigilance is needed to prevent their being introduced into natural systems. This is emphasized by the recent interception of the Asian pulmonate *Gyraulus chinensis* at South African harbours (Brown *et al.*, 1994), and which may indicate that there are more alien species here than we know about.

The Family Physidae is known for variability or "plasticity" in both the morphology of both shells and soft parts and in its members' life styles (Brown & Devries, 1985; Crowl, 1990), and it is presumably this intraspecific variation that has resulted in the systematics of the family still being in a state of flux. As far as the physids that have been introduced to Africa are concerned, it is clear from the present study that the *Aplexa marmorata* currently being found in the eastern parts of South Africa is different from the better established *Physa acuta.* Use of a variety of techniques has shown that what I have called *Aplexa* cf. *marmorata* does indeed belong to the genus *Aplexa.* Differences among malacologists over the validity of the genus *Aplexa* and the redescription by Paraense (1986) of what seems to be *Aplexa marmorata* as *Physa marmorata* is a complicating factor here, though it does appear that most malacologists accept *Aplexa* as a valid genus of long standing, 171 years.

The presence of a preputial or accessory gland is the most frequently cited characteristic of the genus *Physa* and it is conspicuous in *P. acuta* but lacking in *A. marmorata.* This study has shown however that it is probably not a gland at all since it does not have the expected structure of a gland, viz. a layer of secretory epithelial cells enclosing a lumen, and its cells do not appear to be glandular. It needs further study to determine its function. Further, the demonstration of identical tissue distributed diffusely in the wall of the preputium of *A. marmorata* suggests that it may be

present in all physids and may be essential to some aspect of their reproductive activity – it has not been reported in, for example, the Planorbidae or Lymnaeidae (Wright, 1957; Pretorius & van Eeden, 1969).

Sperm morphology is proving a valuable taxonomic character in a variety of molluscan taxa (Healy, 1989a & b; Hodgson & Bernard, 1988). The finding that the sperm of *A. marmorata* has two glycogen helices wound around its midpiece in contrast to the three in the sperm of *P. acuta* (Brackenbury & Appleton, 1991) was unexpected. Clearly the situation in the *P. acuta* sperm will have to be confirmed and if correct, may point to sperm morphology being a useful tool for resolving physid systematics.

The conclusion that the West African physids (*Aplexa* or *Physa waterloti*) and *Physa mossambiquensis* should be included in *Aplexa marmorata* and the fact that *A. marmorata* is indigenous to the Caribbean islands (the type locality is the island of St Vincent) and northern South America including Brazil, lends support to the hypothesis put foreward by Appleton *et al.* (1989) that it was introduced to Africa by the slave trade during the 15th and 16th centuries.

The production of three overlapping generations or cohorts by *A. marmorata* in both a stable, artificial habitat (Botanic Gardens) and a natural, rainfall-dependent habitat (Bluff Nature Reserve) may be evidence that this species does not have the adaptiveness of *P. acuta* in overcoming reductions in density due to disturbance of the habitat. In addition, the egg production pattern recorded at the Botanic Gardens site suggests that *A. marmorata* has several well-defined egg-laying periods during the year – rather than laying eggs more-or-less continuously throughout the year. These observations may explain why, to date, A. *marmorata* has not been found in rivers in South Africa (except for a backwater in the Sabie River, Kruger National Park, see Chapter 4). It may be that the species cannot easily survive in riverine habitats, because it cannot regain its numbers quickly enough after disturbances, particularly the silt-laden floods that are such a feature of South African rivers, have washed large numbers of snails away.

Certainly the variability in physid life-strategies referred to earlier would seem to pre-adapt them to a range of habitats and climates. Also, *A. marmorata* is adept at avoiding predators such as sciomyzid fly (*Sepedon* spp.) larvae and the leech *Helobdella conifera* (Wilken & Appleton, 1991). This ability to detect and avoid predators may be because the digitated border of the

physid mantle seems to have a chemosensory function. As Frieswijk (1957), Townsend & McCarthy (1980) and Wilken & Appleton (1991) have observed, physids including *A. marmorata,* exhibit a vigorous shell shaking action followed by detachment from the substratum and (usually) escape when their mantle border touches a malacophagous leech such as *Glossiphonia* and *Helobdella*. In contrast, indigenous snails such as *Bulinus* cannot avoid this kind of predation.

The apparent rarity of *A. marmorata* in riverine or lotic habitats in South Africa suggests that it cannot tolerate flowing water above a certain speed. Many physids are lacustrine animals which suggests that they are adapted to standing water so that this critical flow speed may be low; *P. acuta* is of course an exception since it can tolerate speeds of up to $0.6 m.sec^{-1}$ (Brackenbury & Appleton, 1993). If *A. marmorata* cannot survive in flowing water it is unlikely to spread as widely as *P. acuta* has done because it will not be able to colonize large areas of the country's major river systems. It is however also true that during the past 15 years, *A. marmorata* has spread to a variety of natural and artificial lentic habitats at localities along the coastal strip of KwaZulu-Natal as well as isolated localities in the lowveld of Mpumalanga and Northern Province while another common introduced pulmonate, *Helisoma duryi*, has not spread beyond artificial waterbodies such as ornamental ponds.

Despite its apparent intolerance of flowing water, *Aplexa marmorata* has spread over quite a large part of South Africa, particularly KwaZulu-Natal. How it has achieved this spread is not known but it may well be more widely distributed than the records cited in this thesis suggest.

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APPENDIX 1

Sectioning and staining of the snail's penial complex

1. Snail tissue was placed into a tissue processing cassette and processed for 15 hours overnight in an automatic tissue- processing machine:

i) Two changes of formalin fixative	1 hour each
ii) Three changes of 95 % alcohol	1 hour each
iii) Three changes of 100 % alcohol	1 hour each
iv) Three changes of xylene	1 hour each
v) Four changes of wax	1 hour each

2. After 15 hours the fixed tissue was embedded in wax (paraplast +) on square plastic stubs.

3. Excess wax was trimmed off the wax block and the block was placed on ice to cool.

4. 3 μm sections were cut on the tissue microtome and floated in a cold water bath.

5. The sections were picked up on glass slides and baked on a hot plate.

6. The sections were stained with haematoxylin and eosin stains following this method:

i) Sections were dewaxed in two changes of xylene.

ii) Agitated or dipped in 2 changes of absolute (100 %) alcohol.

iii) Put in 95 % alcohol.

iv) Hydrated and washed in water.

v) Placed in Mayer's haematoxylin for 5-10 minutes.

vi) Put in lithium carbonate and washed with tap water.

vii) Agitated in 95 % alcohol.

vii) Placed in 0.5 % eosin for 5 minutes.

viii) Put in 95 % alcohol.

ix) Put in 2 changes of 100 % alcohol.

x) Put in 2 changes of xylene

xi) The slides were covered with coverslips.

APPENDIX 2

Mucin staining technique

1. Sections were treated as in Appendix 1.

2. Placed in haematoxylin for 10 minutes.

3. Washed in tap water and stained blue with lithium carbonate.

4. Washed in tap water and placed in Southgates' mucicarmine solution for 30 minutes.

5. Washed in tap water.

6. Dehydrated : 95 % alcohol

   2x100 % alcohol

7. Cleared : 2x xylene

8. Mounted onto slides.

Nuclei were stained blue, mucin was stained red and the cytoplasm and connective tissue were stained varying shades of pink.

APPENDIX 3

Cleaning of the shell

1. Put shell in saturated (10 %) oxalic acid solution for 2-4 minutes.

2. Clean the shell with a toothbrush.

3. Rinse in water and dry.

APPENDIX 4

Extraction of radula

1. Narcotise snails in menthol crystals solution for 6 hours.
2. Dissect out odontophore and radula.
3. Half fill the test tube with 10 % sodium hydroxide (NaOH) solution.
4. Heat the solution to 70 - 80°C.
5. Place odontophore in the solution for 5 - 10 minutes.
6. Rinse with freshwater.
7. Place radula in distilled water for 15 minutes.
8. Dehydrate twice in 70 % alcohol for 5 minutes.
9. Dehydrate twice in 100 % alcohol for 5 minutes.
10. Sonicate the radula for 10 minutes.
11. Put double -sided tape on stub.
12. Lay out radula on tape.
13. Let it dry.
14. Gold- coat for Scanning Electron Microscope (SEM).

APPENDIX 5

Scanning Electron Microscope (SEM) Protocol

1. Primary fixation	:	2-24 hours in buffered 2.5 % glutaraldehyde
2. Wash	:	3x5 minutes buffer
3. Post fixation	:	1x1 hour 0.5 % osmium tetroxide
4. Wash	:	3x5 minutes washes with distilled water
5. Dehydration	:	2x5 minutes 25 % alcohol
		2x5 minutes 50 % alcohol
		2x5 minutes 75 % alcohol
		2x10 minutes 100 % alcohol

6. **Critical point drying**

1. Primary fixation	:	8 hours in 3 % buffered glutaraldehyde.
2. Wash	:	2x30 minutes in 0.05M phosphate buffer.
3. Secondary fixation	:	4 hours in 2 % osmium tetroxide
4. Wash	:	1x30 minutes in 0.05M phosphate buffer.
5. Dehydration	:	2x30 % ethanol
		2x50 % ethanol
		2x70 % ethanol
		2x80 % ethanol
		2x90 % ethanol
		2x100 % ethanol

6. Transferred specimens into CPD baskets under alcohol to a Hitachi Critical Point Dryer HCP-1.

7. Examined specimens using a Hitachi S520 Scanning Electron Microscope.

APPENDIX 6

Transmission Electron Microscope (TEM) Embedding Protocol

1. Primary fixation	:	2-24 hours in buffered 2.5 % glutaraldehyde
2. Wash	:	3x5 minutes buffer
3. Post fixation	:	1x1 hour 0.5 % osmium tetroxide
4. Wash	:	3x5 minutes buffer
5. Dehydration	:	2x5 minutes 25 % acetone
		2x5 minutes 50 % acetone
		2x5 minutes 75 % acetone
		2x10 minutes 100 % acetone
6. Infiltration	:	1x4 hours (equal parts) resin + acetone
		18-24 hours whole resin
7. Polymerisation	:	Orientate specimens in moulds in whole resin.
		Polymerise for 8 hours at 70°C

8. Ultra-thin sections obtained using Reichert Ultracut E microtome.

9. Placed sections on glass slides.

10. Stained them with uranyl acetate for 10 minutes

11. Rinsed with distilled water and stained with lead citrate for 10 minutes

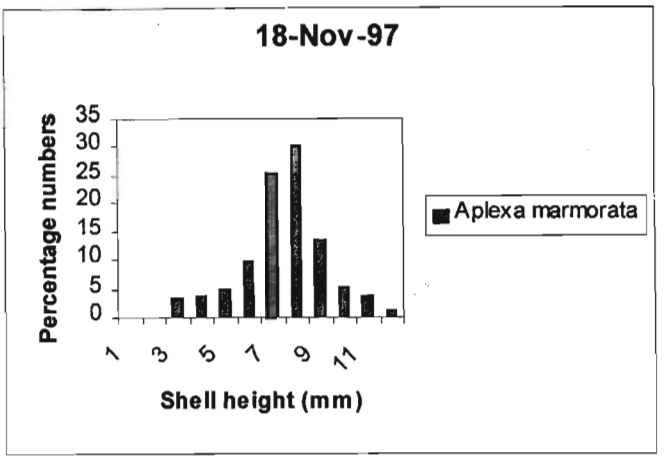
12. Viewed the sections using Jeol 1010 Transmission Electron Microscope

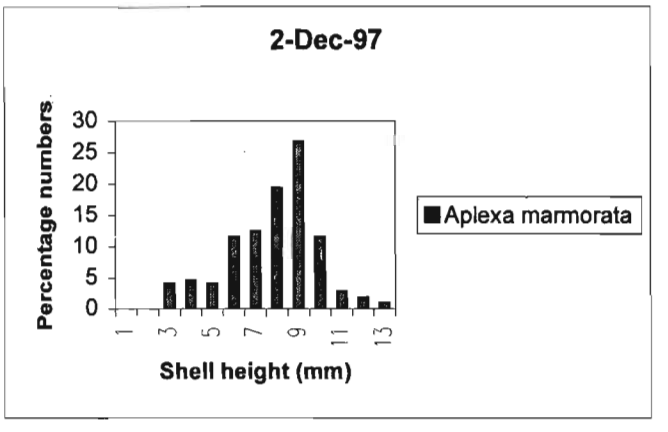
APPENDIX 7

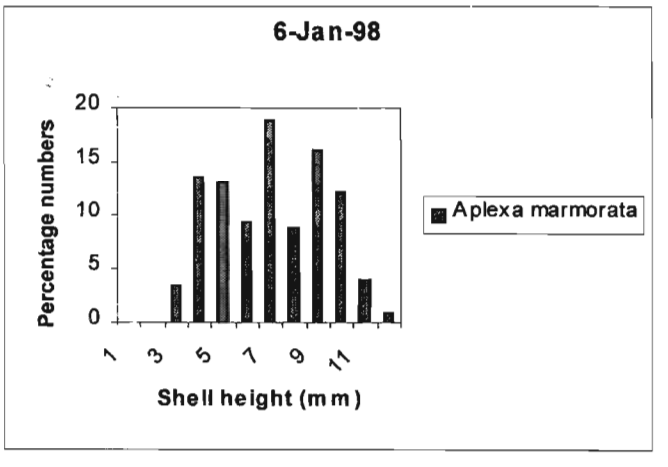
KARYOTYPE PROTOCOL

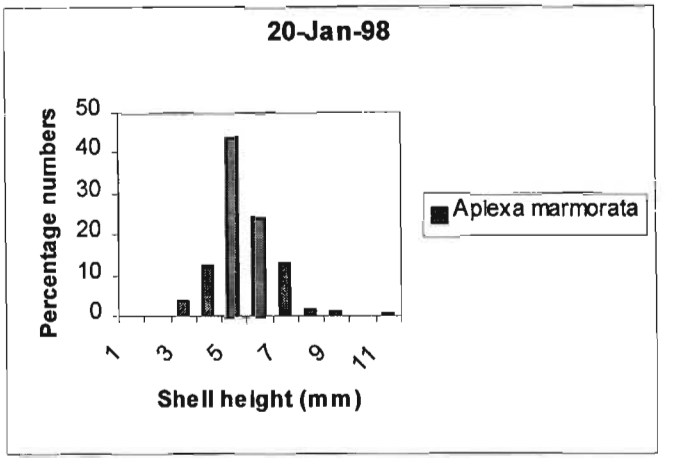
1. Remove gonads from snail following standard procedure for the organism.
2. Transfer the gonads to a petri- dish with 5 – 10 ml distilled water or 0.45 % saline or KCl 0.075 M.
3. Remove any tissue covering the gonads and tease them into small pieces with scissors.
4. Incubate the gonads in the hypotonic solution at room temperature for 15 – 30 minutes.
5. Drain the hypotonic solution and replace it with an equivalent amount of Carnoy fixative (methanol: glacial acetic acid, 3:1).
6. Incubate in the fixative for 1- 2 hours.
7. Transfer tissue fragments onto a microscope slide previously coated with albumin solution and smoked.
8. Coat one side of coverslip with grease.
9. Carefully lower the coverslip onto the slide in order to avoid air bubbles and squash the tissue, using the heel of your hand, through several layers of blotting paper. Avoid lateral slipping of the coverslip.
10. Stand slide in 70 % ethanol for 1 hour to allow coverslip to float off.
11. Stain the tissue in 5 % Giemsa in Sorenssen's phosphate buffer pH 6.8 for 5 – 10 minutes.
12. View under a microscope.

APPENDIX 8: Percentage histograms of snail samples from Bluff Nature Reserve





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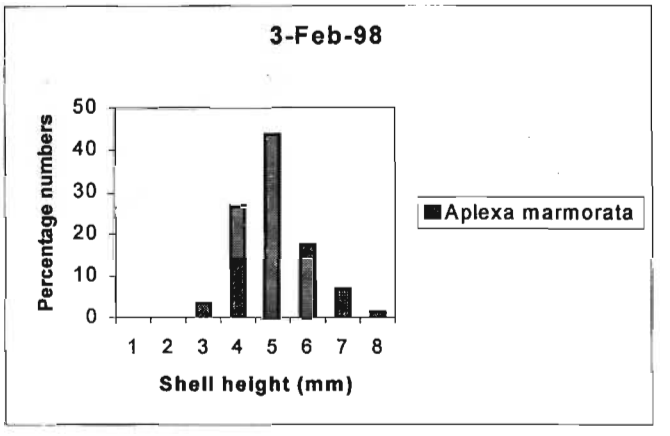
Shell height (mm)
Aplexa marmorata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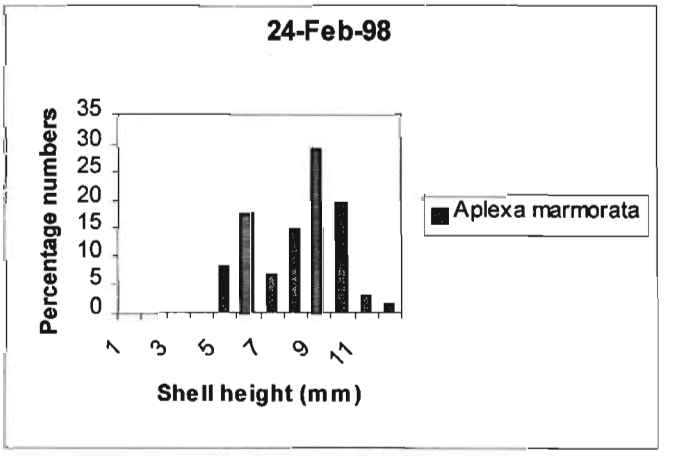
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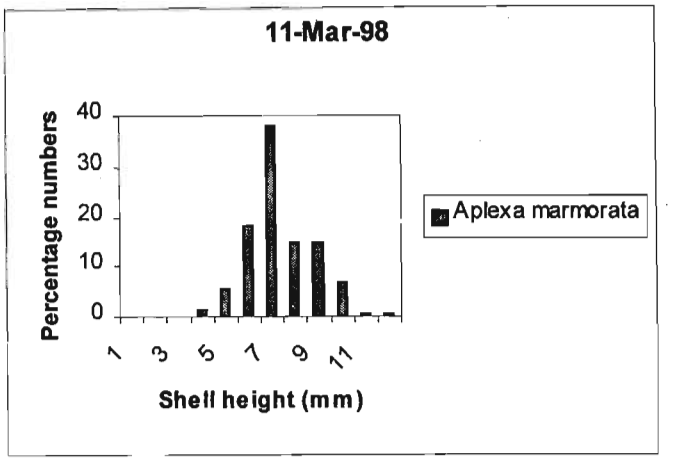
Shell height (mm)
Aplexa marmorata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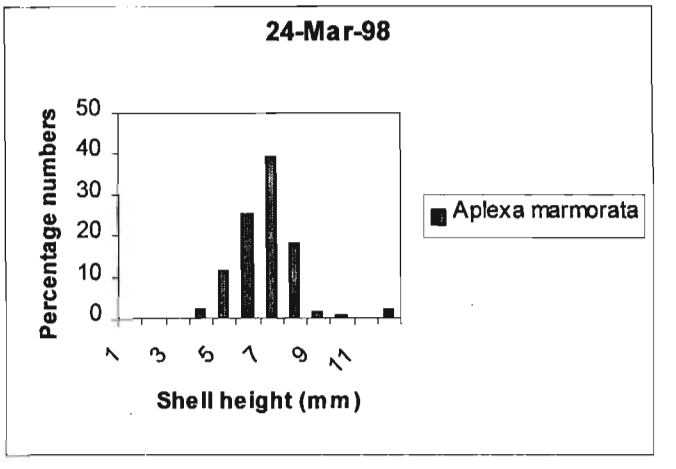
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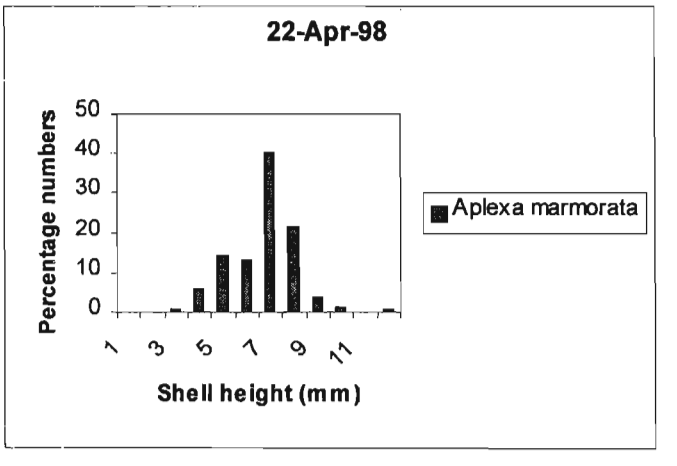
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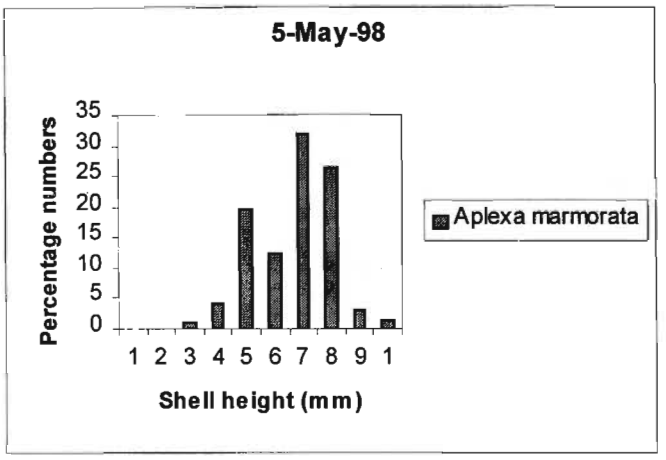
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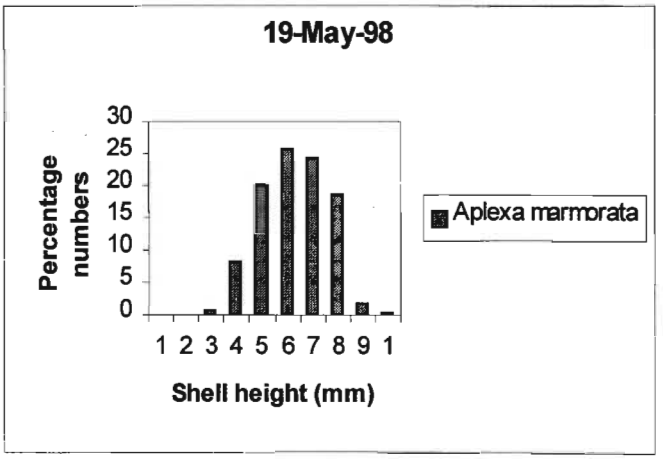
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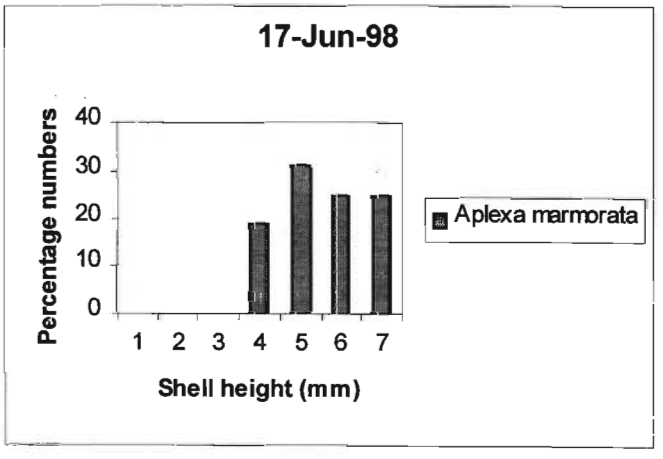
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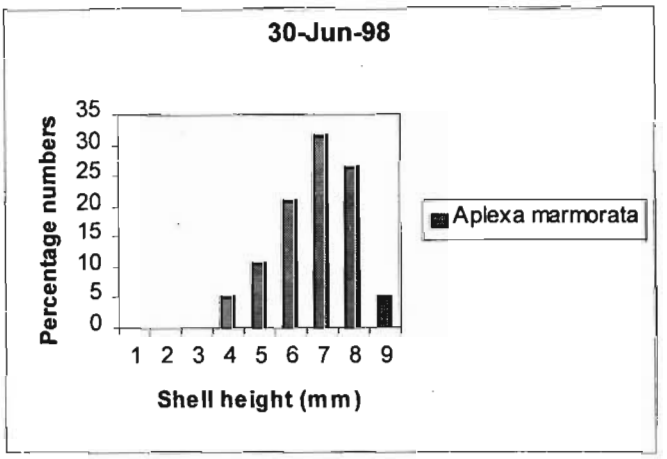
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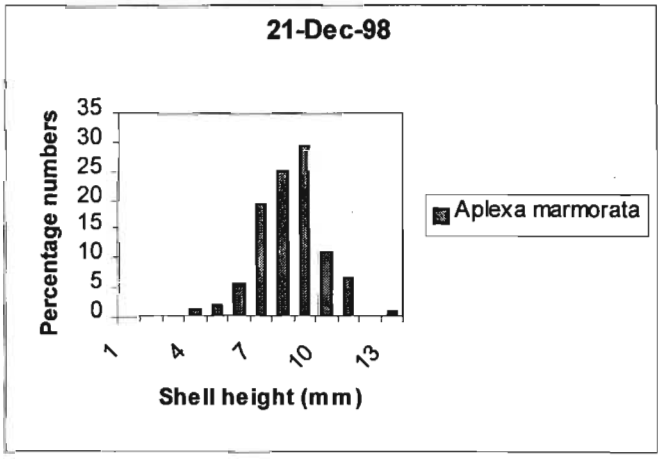
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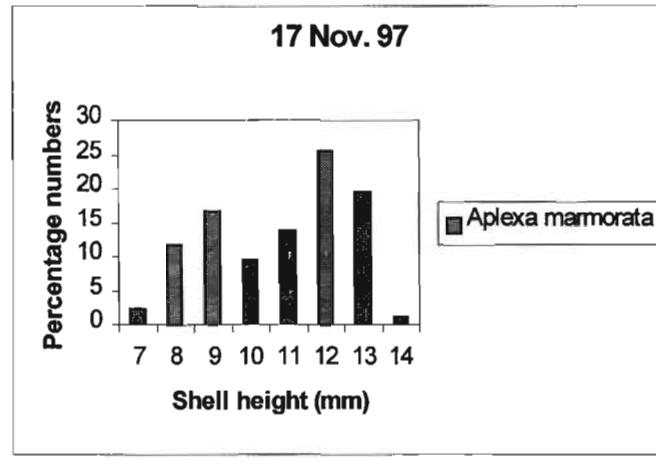
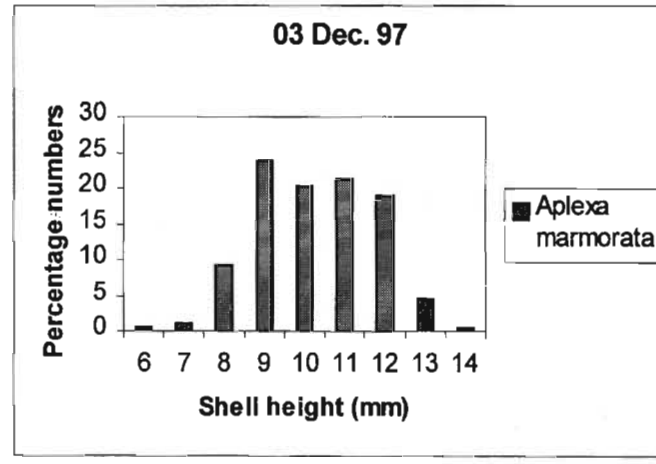
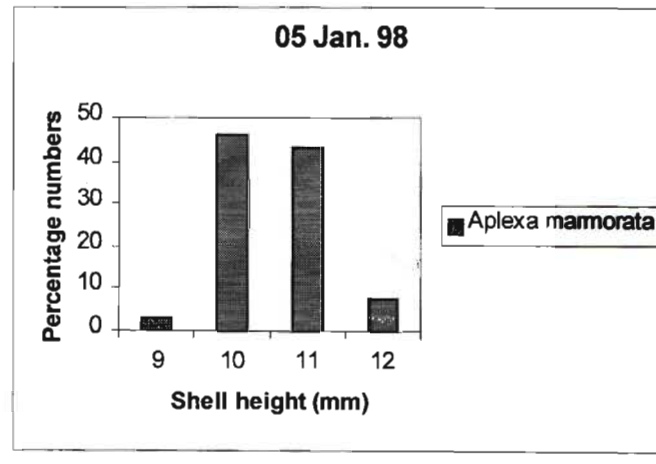
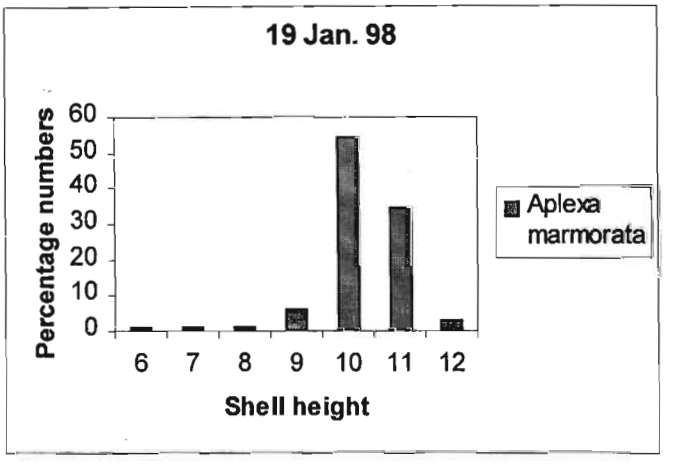
Shell height (mm)
Aplexa marmorata

APPENDIX 9: Percentage histograms of snail samples from the Botanic Gardens





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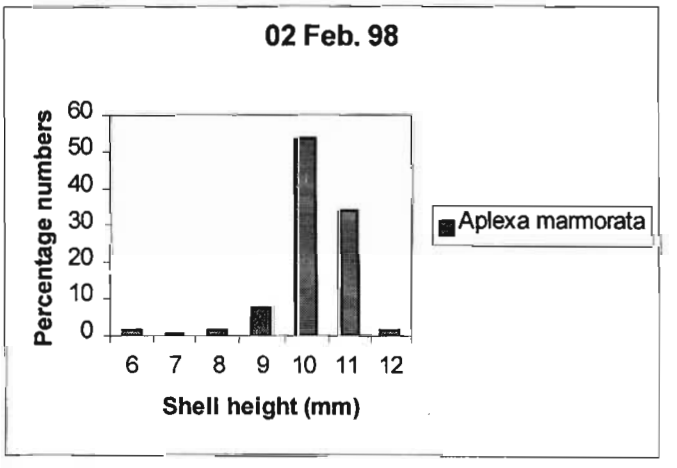
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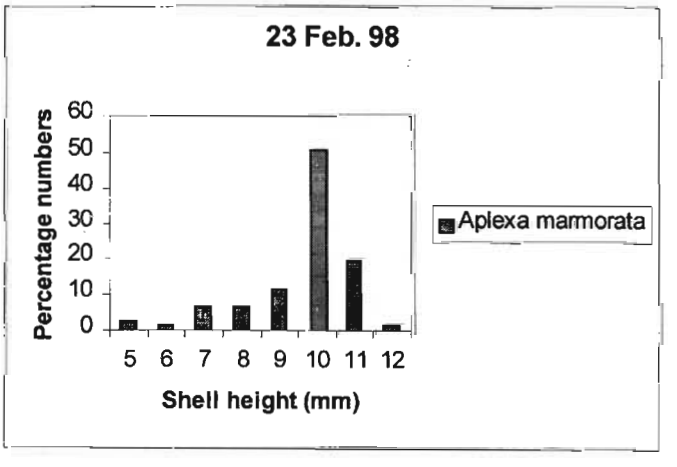
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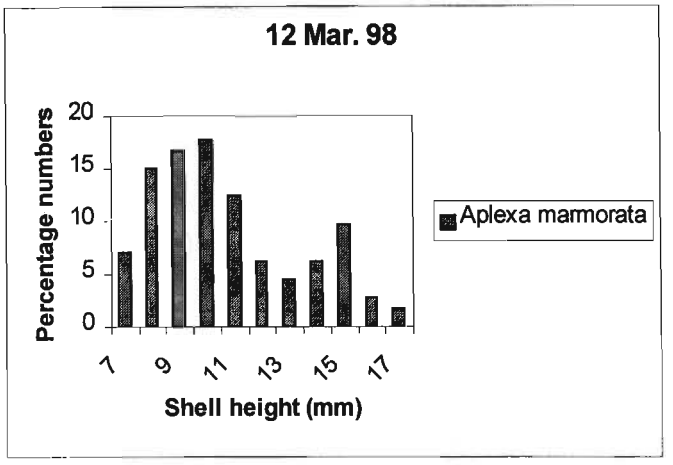
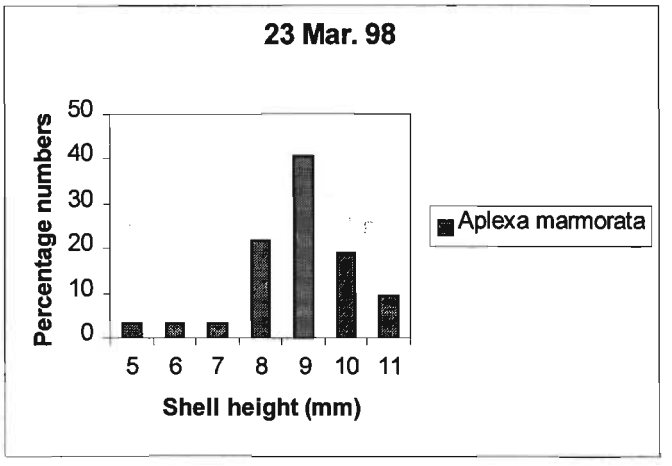
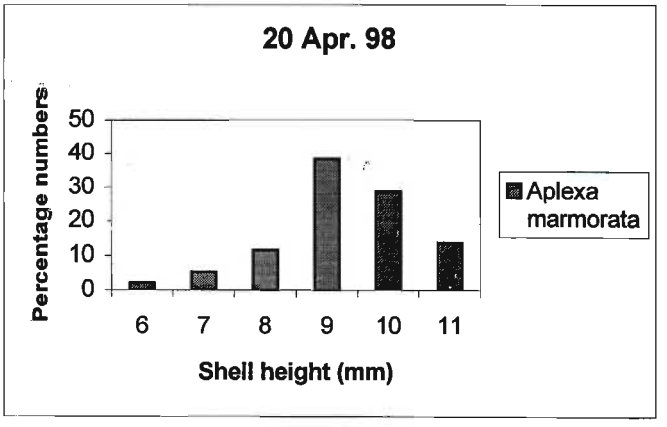
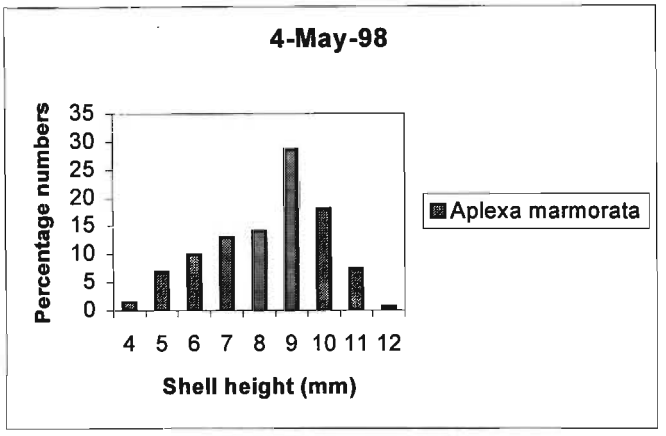
Shell height (mm)
Aplexa marmorata

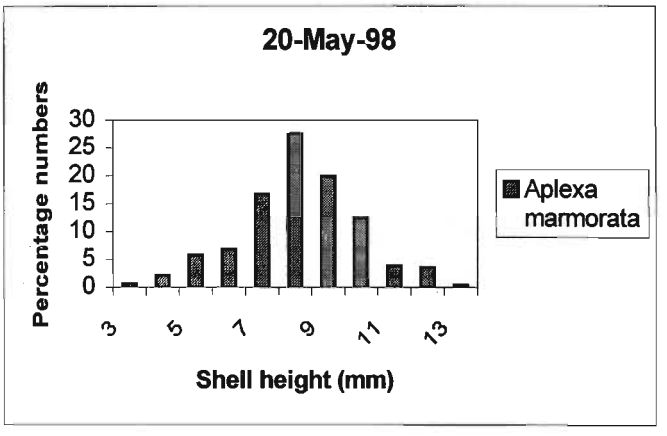
APPENDIX 9 (CONTINUED)

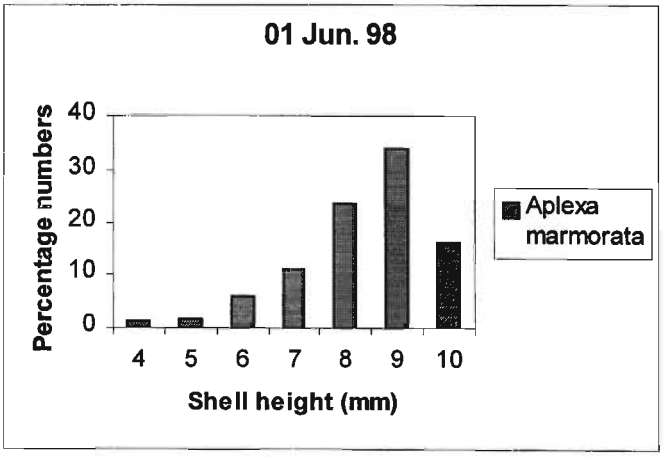


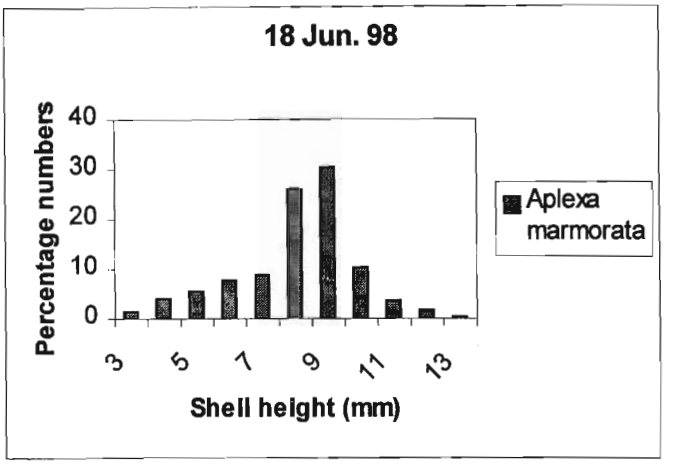





4-May-98
Percentage numbers
35
30
25
20
15
10
5
0
4 5 6 7 8 9 10 11 12
Shell height (mm)
Aplexa marmorata


20-May-98
Percentage numbers
30
25
20
15
10
5
0
3 5 7 9 11 13
Shell height (mm)
Aplexa marmorata


01 Jun. 98
Percentage numbers
40
30
20
10
0
4 5 6 7 8 9 10
Shell height (mm)
Aplexa marmorata


18 Jun. 98
Percentage numbers
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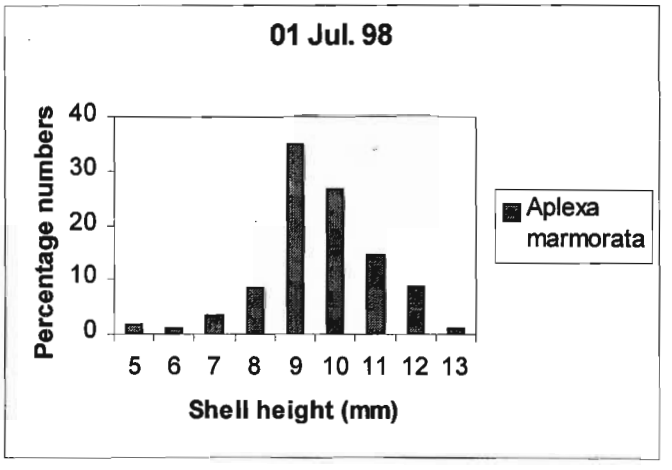
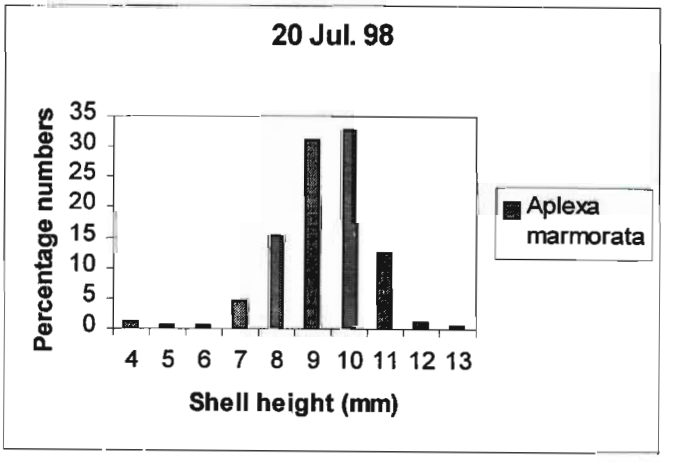
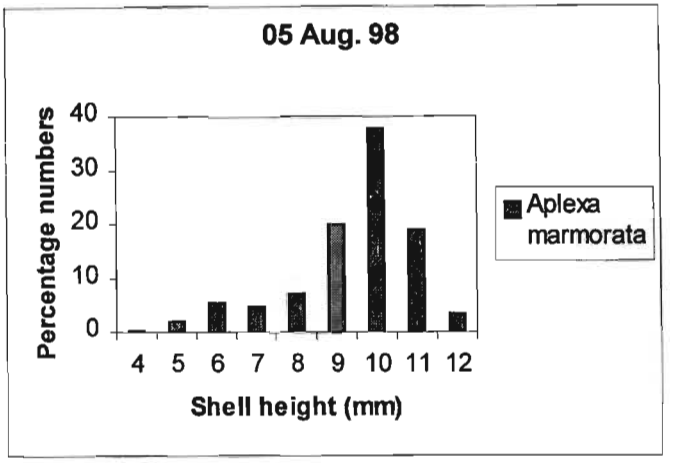
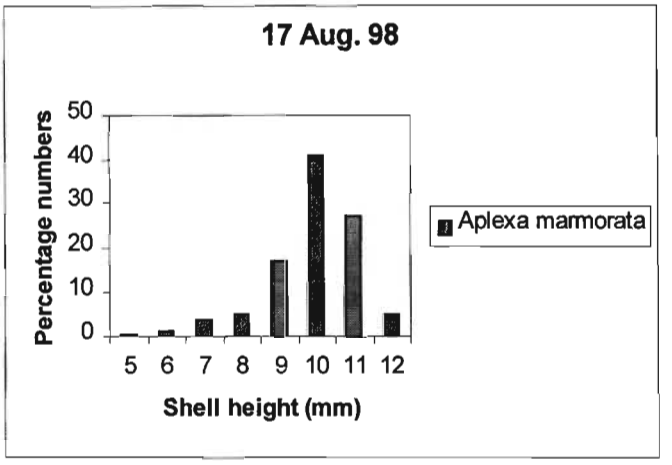
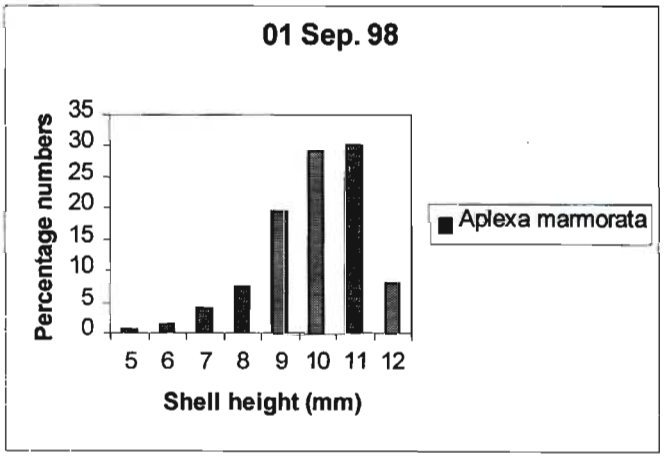
30
20
10
0
3
5
7
9
11
13
Shell height (mm)
Aplexa marmorata


01 Jul. 98
Percentage numbers
40
30
20
10
0
5 6 7 8 9 10 11 12 13
Shell height (mm)
Aplexa marmorata

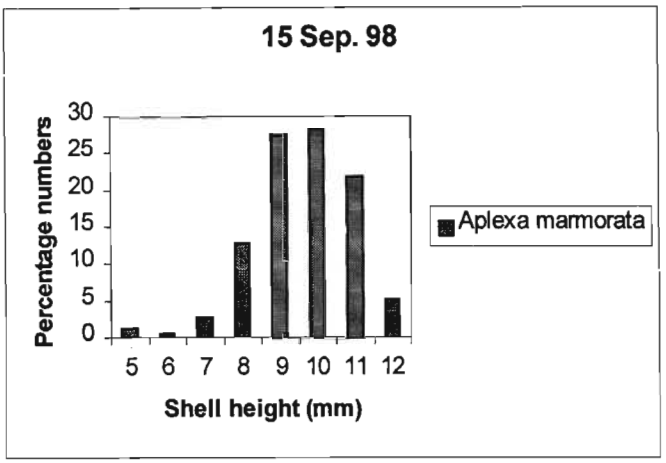

20 Jul. 98
Percentage numbers
35
30
25
20
15
10
5
0
4 5 6 7 8 9 10 11 12 13
Shell height (mm)
Aplexa marmorata

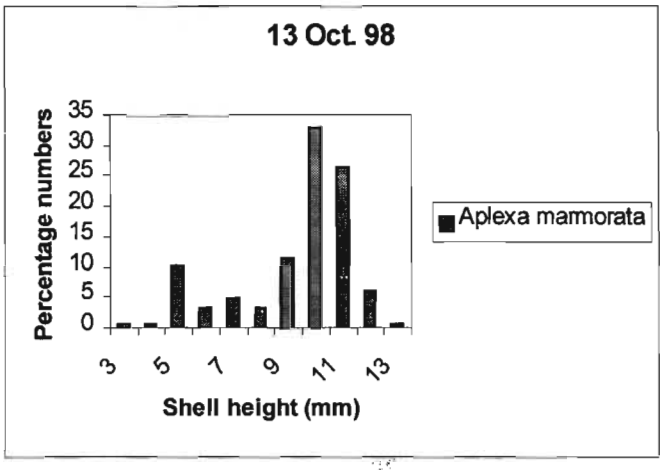

05 Aug. 98
Percentage numbers
40
30
20
10
0
4 5 6 7 8 9 10 11 12
Shell height (mm)
Aplexa marmorata

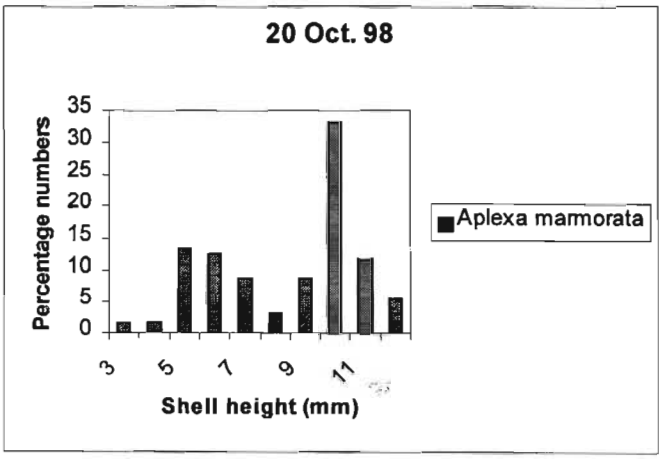

17 Aug. 98
Percentage numbers
50
40
30
20
10
0
5 6 7 8 9 10 11 12
Shell height (mm)
Aplexa marmorata

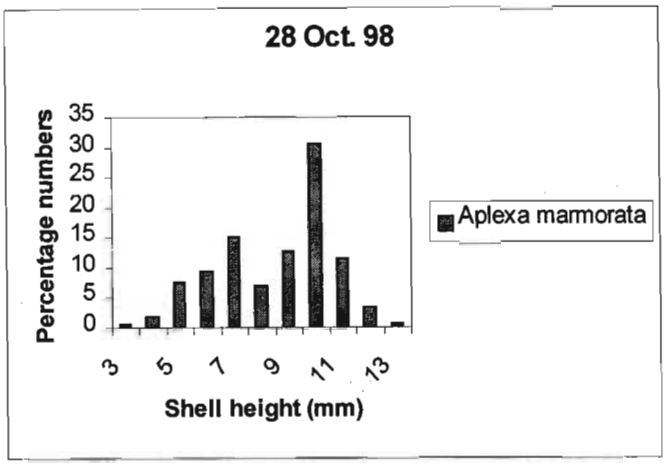

01 Sep. 98
Percentage numbers
35
30
25
20
15
10
5
0
5 6 7 8 9 10 11 12
Shell height (mm)
Aplexa marmorata

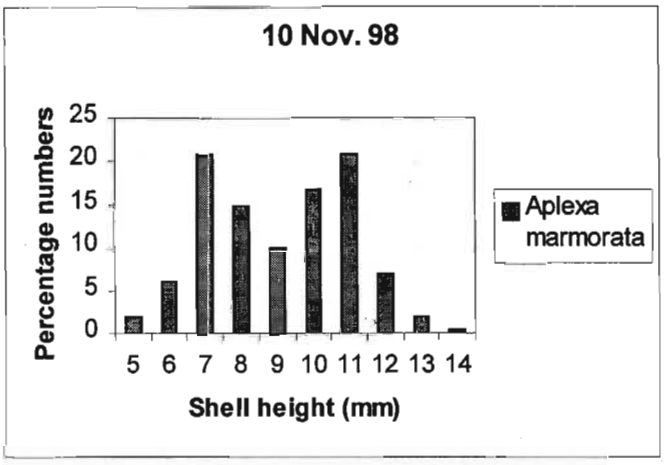
APPENDIX 9 (CONTINUED)

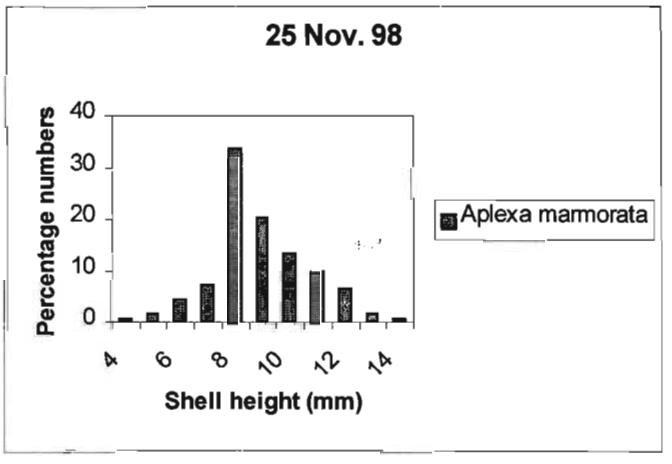


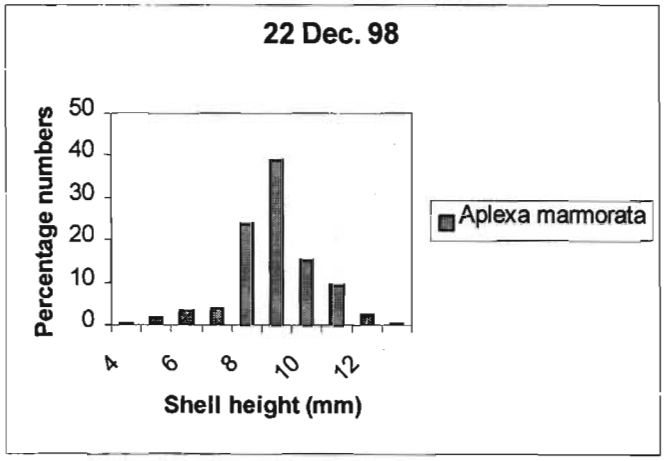





28 Oct. 98
Percentage numbers
35
30
25
20
15
10
5
0
3
5
7
9
11
13
Shell height (mm)
Aplexa marmorata


10 Nov. 98
Percentage numbers
25
20
15
10
5
0
5 6 7 8 9 10 11 12 13 14
Shell height (mm)
Aplexa marmorata


25 Nov. 98
Percentage numbers
40
30
20
10
0
4
6
8
10
12
14
Shell height (mm)
Aplexa marmorata


22 Dec. 98
Percentage numbers
50
40
30
20
10
0
4
6
8
10
12
Shell height (mm)
Aplexa marmorata