AN EVALUATION OF A NON-VECTOR SNAIL *MELANOIDES TUBERCULATA* (Müller) AS AN AGENT FOR THE BIOLOGICAL CONTROL OF *BULINUS TRUNCATUS* (Audouin) A SNAIL HOST OF URINARY SCHISTOSOMIASIS.

BY

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To the glory of **God Almighty** for providing the wisdom

To my dear wife **Adubea** for her encouragement and care

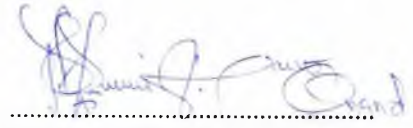
And to our son **Ohene** who at six is learning to write his thesis

"I know that, whatsoever God doeth, it shall be for ever: nothing can be put to it, nor any thing taken from it: and God doeth it, that men should fear before Him."

Ecclesiastes 3 : 14&15

DECLARATION

The work embodied in this thesis was carried out by the author exclusively unless otherwise indicated. Whenever the work of others is included, references are made to the source of the information. This thesis has not, in its present form or otherwise been submitted to this or any University for a degree, diploma or other qualification.



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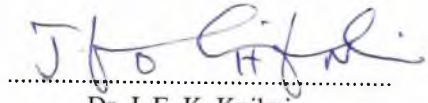

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Dr. J. E. K. Kpikpi
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Abstract

Preliminary studies carried out in the Weija Lake in Ghana gave indications of competitive interactions between *Melanoides tuberculata* and three snail hosts of schistosomiasis ie *Bulinus truncatus, Bulinus globosus* and *Biomphalaria pfeifferi*. Subsequent laboratory investigations involving *Melanoides tuberculata* and *Bulinus truncatus* carried out under four different conditions ie sandy gravel (Normal and Heat treated) and sandy clay loam (Normal and Heat treated) showed that *Melanoides tuberculata* is able to suppress the growth rate of *Bulinus truncatus* to varying extents depending on the type and condition of the sediment.

Of the two sediments used, it was noted that *Melanoides tuberculata* competes more effectively on sandy gravel than on sandy clay loam mixture as the adverse effects of the presence of this snail on the rate of weight of *Bulinus truncatus* using sandy gravel sediment was found to be more severe than the results obtained when the sandy clay loam was used.

Normal sediments were found to enhance the competitive advantage of *Melanoides tuberculata* over *Bulinus truncatus* more than the Heat treated sediments. In fact Heat treated sediments tended to reverse the competitive interactions in favour of *Bulinus truncatus* at least in some cases. The intensity of the competitive effects of *Melanoides tuberculata* over *Bulinus truncatus*was also found to be directly proportional to the ratio of *Melanoides tuberculata* to *Bulinus truncatus.* Thus M15B5 (ie 15 *M. tuberculata* to 5 *Bulinus truncatus*) combination had a greater effects on *Bulinus truncatus* than M10B10 (ie 10 *M. tuberculata* to 10 *Bulinus truncatus*) and M5B15 (ie 5 *M. tuberculata* to 15 *Bulinus truncatus*) respectively.

When the feeding behaviour and apparatus of the two snails were considered critically, to ascertain the possible influence of food on this ecological interaction it was established that, the radula of the two snails are different morphologically and also function differently. Food selection or preference among the two snail species was also found to be different (P(T=t)=0.001), though some similarities do exist . This suggests that, the basis of the observed competition between *Melanoides tuberculata* and *Bulinus truncatus* might not under normal circumstances be due to food However, the competitive ability of *Melanoides tuberculata* is directly or indirectly linked under normal circumstances to food stress.

The findings from these studies suggest the need to extend our biological control studies to include other environmental factors which might be critical in determining the outcome of the competitive interactions between the host and the non host snails of schistosomiasis

CHAPTER 1

INTRODUCTION

1.1 Water as a natural resource :

One of the basic natural resources is water. Although it occupies about three quarters of the earth's surface only 3% is freshwater (Pereira, 1973). The rest is saltwater which is usually unsuitable for the requirements of human life. This points out the inadequacy of freshwater for permanent supplies. Not only is the distribution of water uneven over the entire land surface, what is available is usually not wholesome for consumption mainly due to human activities and negligence. Its importance to life cannot be over emphasized since it plays a major role in the domestic, industrial and agricultural lives of mankind. In the past relative to the human population, freshwater has been considered abundant and hence its main values have been over looked (de Graft-Johnson, 1977). This however is not the situation now and every effort should be made to preserve our freshwater bodies since the conservation of water will help to meet the needs of the growing population (Ameka, 1987).

Conservation in some of the areas where freshwater is either inadequate or unevenly distributed has brought about the building of dams. This has tremendous prospects, however it has its own attendant problems which include those associated with the building and engineering of the dams themselves (Little, 1969) and lack of proper siltation and sedimentation of these dams (Barning and Banson, 1969). These aside there are social and health issues as a result of the dam construction.

The most common being the displacement and resettlement of the people who live along the banks of the dammed rivers (Moxon, 1984) and disease prevalence. An example is the case of the formation of the Weija Lake in Ghana. This involved over 2,000 people in six communities (Nukunya and Boateng, 1979). This adversely affects the socio-economic status of the communities. Usually the building of such dams creates new niches for organisms bringing about competition for use of the water by other organisms, for example aquatic macrophytes some of which may be noxious (de Graft Johnson, 1977). This leads to the slowing down of water currents which tend to favour the breeding and proliferation of some disease vectors like freshwater snails e.g. schistosomiasis transmitting snails like *Bulinus* sp. and *Biomphalaria* sp. etc. This is the reason why areas in Ghana where the dams have been built have seen an upsurge in the transmission of Schistosomiasis disease.

1.2 Historical discoveries of the agent of schistosomiasis :

Schistosomiasis is an insidious debilitating and usually a chronic parasitic disease which is known to be a serious public health problem in more than 70 countries throughout the world. It is known to have infected over 200 million people with a total of 600 million people at risk (W H O, 1993). The first known incidence of the disease was found in Egypt as early as 1500BC and was then known as haematuria or â â â disease (Abdel - Wahab, 1979). However it was not until 1910 that a direct evidence of the presence of the disease was

unearthed by Sir Armund Ruffer, a professor of pathology at the Cairo Medical School who found calcified eggs of the parasite among the kidney tubules of two Egyptian mummies of the 20th dynasty [1250 to 100BC] (Ruffer, 1910).

In 1851 a German physician Theodor Bilharz discovered the unknown causative agent of haematuria during an autopsy at Kasr El Ainy hospital in Cairo and he named it *Distomum haematobium.* It was later described as *Bilharzia haematobium* by Meckel von Hemsbach in his thesis in 1856. However in 1858, the agent was named *Schistosoma haematobium* by Weinland who was not aware of Meckel von Hemsbach's thesis. Cobbold in 1859, pointed out that on anatomical grounds (ie the presence of the gynaecophoral canal), the parasite was clearly not of the genus *Distomum* and so referred to it as *Bilharzia haematobium* after the discoverer and also in support of the findings of Meckel von Hemsbach. In 1889, in Paris, the International Commission of Zoological Nomenclature (ICZN) validated the name *Schistosoma* suppressing *Bilharzia* as a generic name. This was again confirmed by the International Zoological Congress in 1948 (Abdel - Wahab, 1979).

In 1902, Sir Patrick Manson put forward the suggestion that there were two species of *Bilharzia*, one with lateral spined ova depositing its eggs in the rectum only and the other in the bladder. Sambon then formally named the second species after Sir Patrick Manson as *Schistosoma mansoni* (Abdel - Wahab, 1979). Fujii in 1947 discovered *Schistosoma japonicum* infections (Jordon & Wedde, 1969). In all five species of *Schistosoma* are

medically important in different parts of the world. These are *S. japonicum, S. haematobium, S. mansoni, S. intercalatum, S. mekongi.* *S. japonicum* is present in eastern Asia extending westward to Thailand particularly along the Mekong catchment area. *S. haematobium* is common in Africa, Arabia, Southwest Asia, Cyprus, Portugal and India. *S. mansoni* is common in Africa and South America.

1.3 The life cycle :

In 1884 and 1885, Sonsino in Egypt established the likelihood of molluscs acting as intermediate hosts of the disease. By 1901, he had also established the connection of water with the infection. The skin as the route of infection was pointed out by Leiper in 1915, who also not only confirmed the involvement of freshwater snails in the transmission as found by Sonsino in 1901 but also further indicated that the different parasites are spread by different snails species.

The life cycle is characterised by alternation of generations with the sexual generation (the adult schistosome) taking place in the definitive vertebrate host (man), and the asexual (larval) stages in the intermediate host snails. Adult schistosomes are found at different sites depending on the type of species one is dealing with. Adults of *S. haematobium* are found in the vesical plexus and sometimes in the portal veins and its mesenteric tributaries. Adults of *S. mansoni* however are commonly found in the inferior mesenteric veins and its tributaries. At these sites copulation and oviposition begin.

1.4 The eggs and sporocysts :

The eggs when laid by the adult in their respective sites, secrete enzymes which enable them to home in to the lumen of the intestines or the urinary bladder by passing through the venules and tissues. From these sites they are shed to the outside environment through urine or faeces depending on the type of infection one has. Some of the eggs stay trapped in the various organs in the tissue where they embryonate in six days and live for another fifteen days. During this period they secrete antigenic materials usually toxic to the surrounding tissues. This elicits immune reactions which lead to the formation of granulomas. These eggs eventually are destroyed by macrophages through phagocytosis over a period of weeks or months depending on the species involved.

The eggs which are deposited in the external environment, on reaching a freshwater body hatch to produce a miracidium which is relatively short lived and free-swimming. This larva on encountering the proper host snail penetrates into it to continue its development. Thus the miracidium is the infective stage to the host snail. Penetration usually takes 3 to 15minutes and could be through the head- foot, the tentacles or the mantle collar. The mechanisms of penetration are not well understood but it is believed that both muscular effort and lytic substances are combined to achieve this. On penetration, the miracidium develops into a mother sporocyst which further produces a large number of daughter sporocysts. These then migrate from the area of penetration to the hepatopancreas of the snail where development continues for several weeks leading to the formation of a cercaria which are later shed by the snail in the surrounding water body.

1.5 Cercaria and schistosomulum :

Cercaria is the infective stage to the definitive host (man). On leaving the snail they penetrate the skin and mucus membrane of man. This is accomplished by enzyme action (Stjernholm & Warren, 1974) on the acellular non fibrillar ground substance of the dermis and the subepithelial basement membrane. Penetration leads to the formation of a schistosomulum (Sher & Moser, 1981) which is different from the cercaria by a) the separation of the tail b) the loss of penetration glands and c) the acquisition of a characteristic seven layer plasma membrane. Schistosomulae home to the lungs through the blood stream and the lymphatics to the tissues of the portal vein where they mature, mate and start a new cycle.

1.6 The Schistosomes :

These are digeneric trematodes which belong to the phylum Platyhelminthes, the family Schistosomatidae and the genus *Schistosoma*. The parasites are dioecious trematodes which need both definitive and intermediate hosts to complete their development. They are also heteroxenous which means they require two hosts to complete their development There are in all 16 species but not all are of medical importance. Five species are important in different parts of the world. These are *S. haematobium, S. mansoni, S. japonicum, S. mekongi* and *S. intercalatum.* Of these five, *S. haematobium, S. mansoni, S. japonicum* are of a greater threat to public health due to their high prevalence, wide distribution and pathogenicity. *S. intercalatum* is of less epidemiological importance.

1.7 The snail intermediate host :

Freshwater (aquatic and amphibious) snail intermediate hosts transmit schistosomiasis. Of the aquatic snails, the genus *Bulinus* transmits *S. haematobium* and the genus *Biomphalaria* transmits *S. mansoni*. Of the amphibious snails, the genus *Oncomelania* transmit *S. japonicum* and the hydrobiid snails of the genus *Neotricula* transmit *S. mekongi*.

1.8 The clinical symptoms :

The symptoms of the disease are related to the following a) the stages of infection b) previous exposure c) worm burden and d) the host response. Common symptoms include cercarial dermatitis, acute schistosomiasis (Katayama fever) and tissue changes as a result of deposition of eggs. These symptoms aside, it is known that nutritional imbalances as well as retardation of growth is common among victims (McGarvey et al., 1992)

Cercarial dermatitis is caused primarily by skin penetration by the cercariae. This is believed to occur in a host who has already been sensitised (or have mounted an immune reaction against the parasite) by exposure to a previous infection (Colley et al., 1972). A second exposure therefore causes a rapid humoral and cellular immune response to be elicited which lead to this symptom. This kind of symptom is common with *S. haematobium* and *S. mansoni* infections but not *S. japonicum*. This dermatitis leads to maculopapular rash which usually lasts for about 36 to 48 hours.

Heavy primary infections and initiation of egg production leads to Katayama fever which is characterised by high fever, hepatosplenomegaly, lymphadenopathy, eosinophilia and dysentery. Increased deposition of eggs in the tissue of the host causes an immune reaction (T cell mediated hypersensitivity to parasite eggs) which subsequently leads to the formation of granulomas which inflames, thickens and causes the walls of the urinary bladder and the intestines to become fibrotic. Some of these granulomas are formed in the periportal tissues of the liver which subsequently lead to enlargement and acute liver disease.

Egg deposition can also occur in the lungs, the brain, the spinal chord, the pancreas and the myocardium. Those trapped in the brain cause seizures and also cerebral atrophy. In the spinal chord, inflammation is caused which leads to focal lesions. Pulmonary infections could also occur, usually this is common with heavily infected *S. haematobium* worms. This leads to pulmonary hypertension and right - sided heart failure. There are also reported cases of chronic bacteraemia infections associated with schistosomiasis. The most common being *Salmonella* species (Garcia and Bruckner, 1993).

1.9 Some of the factors which promote the disease occurrence :

Over the past decades many attempts have been made to eradicate the disease yet the prevalence of the disease continues to increase in the endemic areas. This is due to the fact that different factors continue to militate against the various control methods and strategies.

All the three organisms involved in the disease [the parasite, the intermediate host snail and the definitive host (man)] exhibit some behavioural characteristics which tend to promote the occurrence of the disease.

1.9.1 The Parasite :

The parasite is known unequivocally to stimulate protective immunity in its host causing the immune mechanisms of the host to see the parasite as self. This prevents any attack that would otherwise have been marshalled against it. Some of these strategies are as follows. The parasites of a primary infection induce a kind of immunity (concomitant immunity) which protects these parasites but attacks the parasites of a secondary infection. Thus the parasites of the primary infection tend to create a barrier which acts against continual re-infection and prevent overcrowding in the host. To achieve this, certain characteristics are exhibited by the parasites which include the following.

a) Surface masking :- the parasites are able to use host antigenic determinants to coat their body surface in order to block the recognition of the worm immunogens by the host. This is a form of molecular mimicry. The parasites are also able to suppress antibodies by this mimicry which leads to the production of antireceptor antibodies.

b) Stage specific immunogen presentation :- this is another strategy used by the parasite to evade the immune mechanisms of the host. At each stage of development in the host the parasites exhibit different antigens . This tends to confuse the immune system of the host leading to a non target antibody production which is not able to eliminate the parasite.

c) Active suppression of immune responses :- the parasite is able to actively suppress both specific and non specific immune responses of the host. This is achieved by inducing the formation of certain antibodies which effectively shield the relevant immunogens from the immune response of the host. They can also cause the production of antibodies against self determinants of the host.

d) Formation of complexes :- the parasites are capable of releasing some immunogens or antigens which circulate in the blood leading to the formation of complexes which mislead the immune response directed towards the parasites. Another possible factor is the unique anatomic compartmentalization of the parasite (ie the comparmentalization of the immune responses relative to resistance eg while antibody and cells are able to localize within the skin resulting in a selective attack on the schistosomulae, the rapid blood flow or other factors prevent effective localization of immune response directed against adult worms localized within the blood stream of the mesenteries (Abdel- Wahab, 1979).

1.9.2 The host snail :

Other mechanisms exhibited by the snail host have also contributed immensely to the presence of the disease in the communities in which it is endemic. The host snails are hermaphroditic thus just one mature snail is enough to cause an explosion of snails in a new water body provided all other factors remain conducive for growth and reproduction. Notwithstanding, it has been found that these aquatic snails can survive out of water (Oliver; 1955). This was supported by the investigations of Chu et al (1967a) who also pointed out

that not only can these snails survive outside water but they are actually able to live under controlled humidity on paper in the air and also on dry mud. Thus in areas where the disease is endemic, unprolonged drought cannot eradicate the snails since they can re-appear in the water from aestivation to intensive breeding, Chu et al (1967b).

Thomas and Tait (1984) also found out that these snails can survive long periods of aestivation in sediments, detritus or vegetation such as *Ascroceras, Commelina , Pistia, Salvinia* and *Azolla.* This behaviour of the snails makes it difficult to totally eradicate them since they can hide from view to conceal their presence in a water body which is temporarily dry. The extensive transport of freshwater plants either privately or commercially and exchanges between botanical gardens mostly result in snail species passing natural barriers.

1.9.3 The definitive host :

Activities and practices of the human host to a large extent promote the transmission of the disease and also its continuous occurrence in the endemic areas. These practices are paramount to the disease prevalence such that the disease is referred to as a 'man made disease ' indicating that its onset is mostly man dependent. The single most prominent underlying factor here is one of lack of education and knowledge of the disease among the people in the area where the disease is endemic. For example, in a survey carried out in one of such communities in Kenya where most children were infected, 55% of the mothers of these children did not know the cause of the disease. Only 19% of these mothers knew that the disease was water related, 24% thought it comes as a result of drinking dirty water. 2% of them thought it was food related and believed it came by eating hot pepper and too much sugar (Stephenson et al, 1986).

Other factors include lack of basic necessities of life and also certain cultural practices and beliefs of the people. For example among the Gongola people of Nigeria where the disease is not only endemic but has a prevalence of 40%, the occurrence of haematuria is incorporated into the local custom and is considered to signify the coming of age of the males and haematuria is considered a manly urine (Akogun; 1991). In some of the areas where the disease is endemic, the beliefs of the people tend to expose them to infection . Some of these beliefs lead to ritual ablutions which are very dangerous (Stephenson et al; 1986). All other human activities (domestic, commercial, agricultural, recreational, constructional, etc) which bring human beings into close and prolonged contact with freshwater bodies also expose same to infection.

All over the world there are examples of the spread of schistosomiasis as a result of development of man- made lakes or water resources (WHO, 1993). For example the records of the International Commission on Large Dams show that between 1951 and 1986 the annual rate of construction of dams increased from 209 to 357 with a large part of it in Africa (WHO,1993), this means an increase in the prevalence of the disease. In Ghana, the construction of a dam over the Volta river created the world's largest man- made water impoundment. As a result the prevalence of *S. haematobium* infections which was 5 - 10% in the Volta basin before the construction increased to 90%.

Irrigation also plays a major role in the increase in transmission of this disease, for example in Cameroun, irrigation caused a rise in shistosomiasis from 15% in 1950 to 30% in early 1960's and to 40% recently (WHO, 1993). Another factor is the construction of small

reservoirs, eg in Ghana, between 1958 and 1960, 104 small dams were constructed in the north east of the country. This resulted in the tripling of the prevalence of S. haematobium infection in the local community from 17% to 51% in 38 survey areas. In Mali, the effect was even greater, the prevalence increased from 13% to 67%.

Aquaculture and fisheries also play major roles, for example in the Nyanza Province of Kenya some 10,000 fish ponds dug to increase fish production resulted in an increase of water borne diseases including schistosomiasis by 30% among school children near these ponds. Refugees have also been noted to be contributors to the spread of schistosomiasis (WHO, 1993).

1.10 Schistosomiasis in Africa and Ghana:

Schistosomiasis is a major problem in most African countries. In some of the countries eg Algeria, Mauritius, Morocco, Tunisia and Tanzania (Zanzibar), urinary schistosomiasis is endemic while in others such as Burundi, intestinal schistosomiasis is common. In a third group of countries, both forms of the disease exist. This group includes countries like, Botswana, Egypt, Ghana, Madagasca, Malawi, Mali, Nigeria, Sudan and also Zimbabwe (WHO, 1993).

The first publication on schistosomiasis in Ghana was in 1920 when Macfie noted the occurrence of bilharzia eggs in the urine of a male African lunatic. However as far back as 1895 the annual reports of the then colony had reports of schistosomiasis (Eddington, 1957). Since then the prevalence of the disease has been widespread in Ghana with urinary schistosomiasis being the most prevalent (WHO,1993). A recent questionnaire survey on urinary schistosomiasis carried out in 1989 as part of Ghana guinea worm education project revealed that out of 17,320 villages visited, 5,947 (34.3%) had urinary schistosomiasis.

Children are known to be at a higher risk in Ghana. As far back as 1950, in the western Ashanti (Goaso), 90% of the children examined were found to be infected (Eddington, 1957). This was confirmed by Gothe et al, (1965) who did not only find 90% infection among children but also noted that the reason for the high infection was due to the fact that parents of the infected children refuse to send them for medical treatment because of their belief that, the symptoms of the disease do vanish with growth and maturity.

Both *S. mansoni* and *S. haematobium* infections are known to exist in Ghana. Prior to 1951, extremely little was known about the disease *S. mansoni*. When reported it was found to be in foci in areas like Tarkwa in the southwestern part of the country and also at Bawku, Zesilla, Navrongo, Weija, Nyive and Atikpui (Grant, 1965). It was also reported in other centres in the northwestern part of the country (Odei, 1964). These observations were due to the presence or occurrence of the host snails like *B. globosus, B. truncatus* (Odei, 1964; McCullough, 1959, 1962) *B. rohlfsi* (Klumpp and Chu, 1977; Klumpp et al, 1985), *B. pfeifferi* (Amankwa et al, 1994).

Factors influencing schistosomiasis spread in Ghana include the increasing human population density, the geological changes with particular references to the surface water supply (Grant, 1965), water development projects such as lake Volta, the Tono irrigation project (Amankwa et al, 1994), the Weija reservoir and other smaller water projects throughout the country (WHO, 1993). Other lesser factors include the movement of populations, the types of settlements, water body characteristics and the human activities such as swimming, washing, fishing, farming, ritual washing etc (Grant, 1965). Developmental activities which play a major role include the construction of roads, railways, bridges, reservoirs, irrigation schemes and quarries.

In the past, treatment was by injection with Astiban (a trivalent antimonial preparation). This was used successfully to treat *S. haematobium* infection among school children in Agogo (Gothe et al,1965). Recently because of the high prevalence of *S. mansoni* in the Lower Volta District, systematic mass treatment with praziquantel was carried out in all schools in the area which led to a significant reduction in prevalence. However inadequate and irregular supplies of antischistosomal drugs and lack of reliable transport persist as major problems (WHO, 1993).

1.11 Control of schistosomiasis :

There are two possible ways of tackling a disease system such as schistosomiasis

a) to eradicate the disease which aims at a complete cessation of transmission.

b) to reduce the prevalence or morbidity of the disease to a level at which serious pathological changes associated with advance stages of the disease will not occur (WHO, 1983). This approach is believed at the moment to be more feasible.

This has been tackled using different approaches including chemotherapy (which deals with treating the infected definitive host with schistosomicidal drugs), snail control (which involves biological, chemical and bioengineering approaches). All these various approaches are aimed at breaking the transmission cycle. This is because schistosomiasis has an elaborate transmission cycle and the spread of the disease is based on the successful completion of the cycle. Thus any attempt to break any of the links in the cycle is likely to halt the transmission.

1.11.1 Chemotherapy :

A wide range of compounds have, in the past, been used in the treatment of schsistosomiasis. These include trivalent antimony compounds (Astiban) (Gothe et al, 1965), thioxanthone compounds, hycanthone compounds, metrifonate (an organophosphorous cholinestrase) niridazole etc. Presently, oxamniquine, praziquantel and also metrifonate (WHO, 1983), are the anthelminthic drugs being used. Through the few operational experiences with chemotherapy, it has been revealed that in order to maintain a low level of morbidity using chemotherapy in areas where the disease is endemic, repeated treatment with chemotherapy at a frequency of twice a year (which is rather uneconomical) is required (Wilkins, 1989).

In view of this it was concluded that morbidity reduction through chemotherapy should be undertaken alongside preventive measures aimed at reducing the level of exposure to cercariae infected water (Wilkins, 1989). Although this can be achieved through water supply and sanitation backed by intensive health education, this may not significantly contribute to a reduction of water contact due to recreational or occupational reasons which tend to expose the young (Jordan,1977). Water and sanitation programmes therefore need to be supplemented by the provision of snail- free water bodies which would be useful for recreational and domestic use. Ecological studies on the intermediate hosts are thus extremely relevant either to optimally apply existing control measures or to develop alternative measures of snail control (Madsen, 1992a).

The primary objective of chemotherapy if the appropriate drugs are taken in the correct dosage, is to reduce and prevent morbidity. Praziquantel has transformed the treatment of schistosomiasis since its introduction. This drug is effective in a single dose against all species of the parasite and thus plays a major role in patients with mixed infections and also those who do not respond to other drugs adequately. This drug is effective in all forms of schistosomiasis especially in intestinal schistosomiasis due to *S. japonicum*, *S. intercalatum* or *S. mekongi* where these parasites are not responsive to oxamniquine. This drug initially was suspected to be carcinogenic but has been found to be neither mutagenic, teratogenic nor embryotoxic (WHO, 1983). However it is known to promote the clastogenicity of benzene (Anwar, 1994).

Metrifonate is an organophosphorous compound originally used as an insecticide. It is selective and effective for the treatment of *S. haematobium* infections. It is neither teratogenic nor embryotoxic. Oxamniquine is a tetrahydroquinoline derivative with selective and variable schistomicidal activity against *S. mansoni.* The use of drugs has its own attendant problems. Notable among these are :

a) the potential of the parasite to develop resistance to the available drugs.

b) the problem of reinfection following treatment and the requirement of subsequent treatment.

c) the cost of drugs and skilled manpower needed to diagnose and deliver treatment.

There are indications that although chemotherapy is a useful interim control measure, alternative approaches are desirable in the long term. One such alternative approach currently attracting attention (but yet to be tried clinically) is vaccine development (Waine et al,1993; Tanner and Evans, 1994; Taylor, 1994).

Although a significant reduction of schistosomiasis infections may be possible by the use of effective drugs through reducing disease severity and contamination of the environment and also controlling the transmission, chemotherapeutic treatment as a sole method of controlling transmission is rather impossible. In the past decade, a combination of snail control and chemotherapy was believed to be the quickest and most cost effective way to reduce prevalence and intensity of infection and transmission (Webbe and Lambert, 1983). However the current trend is to integrate schistosomiasis control activities on to the general health care delivery system (Dias et al., 1995)

1.11.2 Snail Control :

This can be achieved principally through the application of chemicals , manipulation of the environment and also biological means. The most reliable method of achieving great reduction in snail population is the use of molluscicides (McCullough, 1986).

1.11.2.1 Molluscicides :

This plays a role in the effective control of schistosomiasis. Although a number of molluscicides have been used, only one is commercially available, ie niclosamide (Madsen and Christensen, 1992). Due to the rising cost of synthetic molluscicides there is an increased interest in plants with molluscicidal properties (Okunji and Iwu, 1988; Webbe and Lambert,1983). A large number of plants have shown promising effects eg the plant *Phytolacca dodecandra, Ambrosia maritima* and *Anacardium occidentalis* (Webbe and Lambert,1983). Others include *Swartzia madagascariensis* and *Tetrapleura tetraptera* (Madsen and Christensen, 1992) and *Callophyllum* species (Ravelonjato et al, 1992).

Other investigations into molluscicides are aimed at the bioefficacy of a bacterial insecticide *(Bacillus thuringiensis)* as a biological control agent against the snail vector of schistosomiasis. A concentration of SAN 415 of *Bacillus thuringiensis* is known to be effective in killing the snail hosts while a concentration of 500ppm is effective in causing a complete loss of egg production. Thus *Bacillus thuringiensis* is suspected to be an effective biological control agent (Osman et al, 1992; Osman and Mohamed, 1991).

Although snail control using molluscicides is promising, it has its own attendant problems. These include the following ;

a) the high cost of synthetic molluscicides which make the use of those chemicals in areas where the disease is endemic (usually developing countries) almost impossible.

c) the fact that most molluscicides are not target specific and tend to have adverse effect on non target organisms some of which may be potentially beneficial species.

d) the difficulties encountered in treating running or large stagnant water bodies.

e) the need for repeated applications which may not only be costly but also may lead to increased killing of more non target species.

f) although most of these molluscicides are applied in concentrations which are not lethal to man, there is the possibility that some of the synthetic chemical can lead to bio- accumulation and bio-magnification in humans who use these water bodies for domestic purposes. This can cause other health problems.

Various approaches have been considered to help curb these problems including the focal application of molluscicides at transmission sites where the snail populations exhibit k-characteristics at certain times of the year. This will not only avoid the wasting of chemical but also time (Thomas, 1986).

To drastically reduce cost, plant molluscicides are recommended for use instead of the synthetic ones. The most interesting approach which is currently receiving attention is the incorporation of the molluscicides in formulations and substances which release chemical attractants, arrestants and phagostimulants specific to the host snails (Thomas et al, 1980, 1983, Kpikpi, 1990, 1991 ; Kpikpi and Thomas,1992 ,1993; Kpikpi and Ansah, 1994). This is very promising and could be practised alongside other snail control measures to selectively remove the host snails.

1.11.2.2 Environmental control of the snail host :

Several environmental measures abound which could be employed to reduce the population of the snails host. Notable among these are, stream canalization, seepage control, canal lining, canal relocation with deep burial of snails, proper drainage in irrigation schemes, removal of vegetation, earth filling, improved agricultural practices and also increases in the velocity of movement of the water (Madsen and Christensen, 1992; Thomas and Tait, 1984). These measures though very promising are very expensive to implement hence attention is being turned towards biological control.

1.11.2.3 Biological Control :

Presently this is in the experimental phase (Madsen and Christensen, 1992), although it has been the subject of much discussion for the past 30years (Frandsen, 1987). In recent years it has attracted much attention because biological control methods can very easily be fitted into the primary health care system with great success. Several species have been tried as possible potential competitors or predators including parasites and pathogens. However most of these studies have been laboratory based (McCullough, 1981).

1.11.2.3.1 Microbial pathogens , parasites, parasitoids and predators :

Bacillus thuringiensis is one microbial pathogen which has been used in biological control methods to control schistosomiasis. It is known to have a molluscicidal effect against *B truncatus* and *B. alexandrina* (Osman and Mohamed, 1991). Trematode antagonism is one of the strategies through which parasites have been investigated in biological control measures (Lie et al, 1970, 1971,1974) Some of the trematodes (parasites) used are *Echinostoma malayanum* against *S. spindale,* a combination of *Echinostoma malayanum* and *Trichobilharzia brevis* against *Lymnaea rubiginosa*, *E. audyi* and *Fasciola gigantica* in *L. rubiginosa*, *Echinostoma malayanum* and *S. spindale* in *Indoplanorbis exustus.*

These investigations were aimed at using these trematodes to induce in the various snail habitats a high infection rate that will block or suppress the transmission of the target species, to cause the introduced trematode to be dominant in order to eliminate or subordinate target species from the snails and completely prevent re-infection by the target species (Lie et al, 1970, 1974). Other trematodes like *Ribeirora marini guadeloupensis* are known to be able to sterilize the host snail *B. glabrata.* Although these strategies have proved successful in some of the areas where the disease is endemic several field trials have failed (Lie et al, 1974).

Some vertebrate predators especially fish, prawns, and birds are known to contribute to the reduction of the snail host populations. Several fish species have been found to be malacophagous eg *Serranochromis* sp, *Astatoreochromis alliuadi, Tillapia melanopluera* and also *Claria* sp (McCullough, 1981; Slootweg, 1989). Other fishes used include *Giophagus brasiliensis* (Weinzettl and Jurberg, 1990), *Haplochromis ishmaeli.H xenognathus, H sauvagei* and *Macropleurodus bicolor* (Slootweg, 1987), *Heteropneustes fossili* (Zakaria, 1964)

The Malaysian prawn *Macrobranchium rosenbergii* has also been investigated as a possible predator (Lee et al, 1982) as well as the crayfish *Procambarus clarkii* (Hofkin et al, 1992). Some insects have also been used as biological control agents, some of these are, *Aspisoma sp* (Vadim, 1989), *Sepedon scapularis* (Diptera : Sciomyzidae) (Maharaj et al, 1992), and also the flesh fly Sarcophaga (*Parasarcophaga misera*) (Parashar and Rao,1988). Most of these organisms have contributed to the reduction of snail populations but there is a lack of sound evidence of their efficacy in the field.

1.11.2.3.2 Inter-molluscan competition :

Inter-molluscan competition is currently considered to be the most promising approach to the control of the snail host (WHO, 1984; Madsen, 1990). In recent years there has been a lot of research findings on this type of competition involving both prosobranch and pulmonate snails which have supported this argument. Not only have the competitor snails been found to have a direct effect on the target snail number, they are also known to adversely affect the miracidal penetration by acting as a miracidal sponge thereby showing a decoy effect (Frandsen, 1987).

The acceptance and feasibility of this approach is based on the principle of competitive exclusion or displacement which points out that if two species which are sufficiently similar in their biological profile and niches coexist in the same water body, one of them (preferably the stronger and in this case the introduced) will eventually eliminate the weaker (the target) species. In cases where total elimination does not occur at least a considerable reduction in the population of the target species is expected (McCullough, 1981). The most extensively studied competitors are *Marisa cornuarentis* (L), *Helisoma duryi* (Wetherby), *Thiara granifera* (Lamarck) and *Melanoides tuberculata* (Muller), (Madsen and Christensen, 1992).

Merisa cornuarientis has been under investigation for about three decades. Investigations carried out by Demian and Lutfy (1965) pointed out that young and adult *M. cornuarientis* do not only feed on *B alexandrina* but also consume their egg masses. In another investigation involving a combination of *M. cornuarientis*, *Lanistes carinatus* and *Pila ovata* against *B. glabrata*, it was noted that the adults of all these three ampullarid species consumed all the eggs of *B. glabrata* presented to them with *P. ovata* adults consuming significantly more . This therefore suggests that , further investigations of the role of ampullarids as biological agents for pulmonate snails in Sub- Saharan Africa should focus more on *Pila ovata*, (Stryker et al, 1991).

Helisoma duryi was first tried in Pueto Rico in 1958 in the field with some success (Frandsen and Madsen, 1979). This snail has since been studied extensively in the laboratory for its possible use in biological control of the host snail of schistosomiasis (WHO, 1984). This species which is believed to have originated from Florida, has also been found in certain parts of Africa eg northern parts of Tanzania (possibly through the exchange or

transportation of aquatic plants) where it is known to have successfully reduced the population of *B. pfeifferi* (Madsen, 1983). It is known to have competed successfully with *B. glabrata* in St Lucia under simulated field conditions but failed when tried in the field (Christie et al, 1981). It is considered a potential biological agent against *B. truncatus* because it is known to prey massively on egg masses of *B. truncatus* (Joubert et al, 1990), thereby reducing the reproduction rate (Meyer- Larsen and Madsen, 1989). This effect was found to be stronger with the increase in the density of *Helisoma duryi*. Despite this success it failed to thrive in lentic habitats in South Africa (Joubert et al, 1990).

Thiara granifera (Thiaridae or Melaniidae) is indigenous in Southeast Asia , Southern U S and the Carribbean but not in Africa (Hicklin, 1988), has been found to be an effective antagonist of *Biomphalaria glabrata* under natural conditions (Perez et al, 1991). It competes and displaces *B. glabrata* but this observation has been found not to be a competition for food nor living space, but possibly due to some chemical released by *T. granifera* because during these investigations *B. glabrata* was found to have avoided *T. granifera* completely. Under laboratory conditions, *T. granifera* was found to effectively reduce the fecundity of *B. glabrata* especially when initial densities of the two species are equal or where the density of *T. granifera* was higher (Gomez et al, 1990).

Another snail species (*Melanoides tuberculata*) belonging to the family Melaniidae or Thiaridae which is indigenous in Southeast Asia and also common in Africa, has demonstrated the capacity to colonise many types of habitats. It has also been found to limit or exclude certain pulmonate species, particularly snail host of schistosomiasis without any observed adverse effect on the environment (Thomas and Tait, 1984; Pointier, 1989).

In an investigation in Martinique using this snail as a competitor, it caused a decline in the population of *B. glabrata* and *B. straminea.* This led to its introduction into other parts of Martinique, (Pointier, 1989). In St Lucia where it proved to be successful, the mechanism of the success is believed to be due to prolonged pressure for space as well as competition for food since the food niche of *Melanoides tuberculata* and *Biomphalaria* are quite similar comprising of organic matter of animal or plant origin, micro-algae and bacteria (Prentice, 1983).

Melanoides has shown signs of being a successful biological agent in many other investigations (Pointier, 1993; Mkoji et al, 1992; Pointier et al, 1991, 1993; Thomas and Tait, 1984). However the exact nature of this competition is not known. It has been suggested that *Melanoides* snails would be successful on organically rich, eroding types of substrate (ie one consisting of coarse gravel and stones) as host snails require depositing substrate (ie one consisting of fine silt, mud and detritus generally colonised by aquatic plants) and macrophytes for survival (Thomas and Tait, 1984). This suggests that the substrate nature and characteristics play a major role in the success of *Melanoides tuberculata.*

Since the introductions of exotic species into freshwater bodies has its dangers (Sturrock, 1984), it is necessary to avoid their use until enough investigations have been conducted to prove that they pose no public health threats. For this reason and also for the following : a) the fact that there is no evidence to date which shows that *Melanoides tuberculata* causes any adverse environmental impact b) the potential of *Melanoides tuberculata* as a cost effective bio-control agent of the snail host of *S. mansoni* in several types of habitats c) the unknown mechanisms by which *Melanoides tuberculata* has a competitive advantage over *B. glabrata* in certain kinds of habitats d) the abundance of *Melanoides*

tuberculata in Ghanaian freshwater bodies and e) the fact that much work has not been done on *Bulinus truncatus* in this regard using *Melanoides tuberculata*, the present study was initiated.

1.12 The objectives of the present study :

The present study was to evaluate the potential of *Melanoides tuberculata* as a possible biological control agent against *Bulinus truncatus* under laboratory conditions with the following objectives :

a) to find out the kind of ecological interaction existing between the two snails in their natural environment

b) to find out whether *Melanoides tuberculata* snails have any adverse effects on *Bulinus truncatus* snails?

c) to find out whether these effects (if any) are due to feeding overlaps (similarities in food preference) ?

d) to find out whether these effects (if any) are density dependent?

e) to find out whether these effects (if any) are accentuated or diminished by the nature and condition of the sediments?

CHAPTER 2

PRELIMINARY FIELD STUDY AND CULTURE OF SNAILS.

2.1 INTRODUCTION :

To effectively control schistosomiasis, a knowledge of the ecology and bionomics of the snail host is necessary. In most control methods for the elimination of host snails, the habitats are either eliminated or rendered inhabitable (Odei, 1965). To be able to do this effectively without threats to the other freshwater fauna and flora in the habitat, a good knowledge of the general ecological factors in the freshwater aquatic environment is required since this will affects the maintenance and distribution of host snails (Ferguson, et al.,1968).

Among the factors which condition the habitat and also influence the snails are temperature, light, pH, dissolved chemicals, oxygen, organic pollution, vegetation, aquatic macrophytes, season and climate, parasites, predators and other fauna (Odei, 1965). Thus in any ecological studies that deal with aquatic snails, especially in tropical Africa where freshwater systems have received little attention, these factors should be carefully studied to understand the ecology of the systems where schistosomiasis is transmitted.

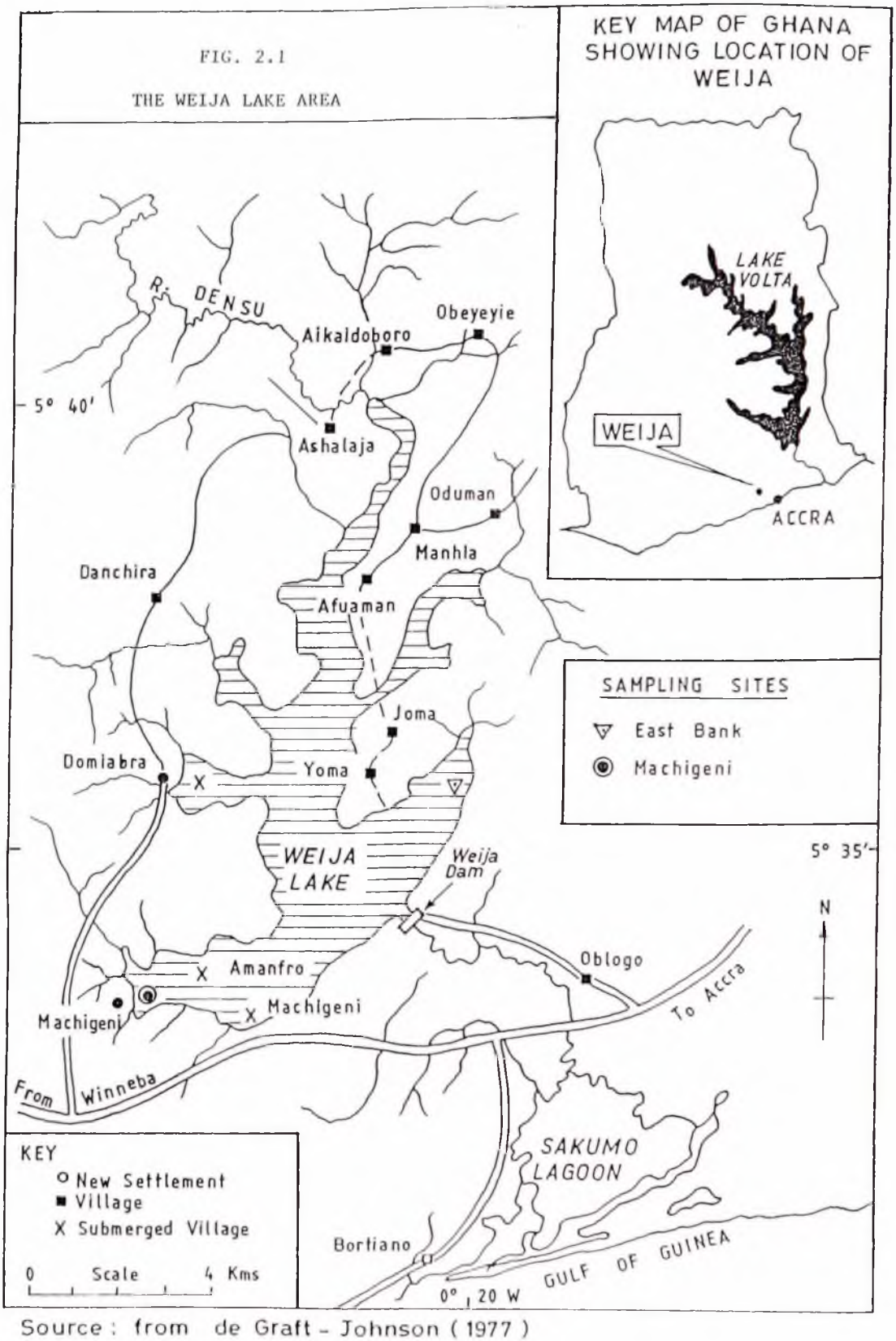
In this chapter, preliminary studies were carried out on the western banks of the Weija Lake prior to the main experiment to find out a) the kind of ecological interaction existing among the two snails in their natural environment b) the conditions prevailing in the natural

environment which should be maintained in the laboratory during the experiment. c) to obtain snails (brood stock) to establish snail cultures in the laboratory to be used for the experiment and d) to identify some of the factors which control the observed ecological interactions.

2.2 The site of the survey (the Weija dam and irrigation area).

This area which is part of the Accra plains is 17km to the west of the capital city Accra and is about 8 km from the sea (Figure 2.1). It is lacking in riverine forest but has a scanty natural tree population made up mainly of Borassus palm (deGraft - Johnson, 1977) . Principally it consists of a dense thicket interspersed with small patches of grass (Boateng, 1970; Rose Innes, 1977). The lack of woody species is believed to be as a result of edaphic and anthropogenic factors (Rose Innes, 1977).

The first dam which was constructed across the Densu river was destroyed and washed away. A new dam was started in 1973 and completed in 1978 (deGraft - Johnson, 1977) This was an earth and rock filled dam 374.90m long with a five gated spillway. The maximum height above the dam is 15.8m with a normal water surface elevation of the reservoir estimated at 14.33m reaching a maximum of 15.43m. The lake which was formed below the dam, covers an area of 3361.5 ha with an estimated storage capacity of 116.04 x $10^6 m^3$ (Dissan and Abban, 1979), it has a shoreline of nearly 48km. The Ghana water and Sewerage Corporation (G.W.S.C) depends on this water body for its supply of potable water.



Source : from de Graft - Johnson (1977)

to the western parts of Accra. In addition the lake water is used for irrigation of 1.110 hectares (4,200 acres) of land for the cultivation of crops mainly vegetables (Nukunya and Boateng, 1979), since rainfall in this area is usually low [average of 838mm per annum (Boateng, 1970)].

This site was chosen for the survey partly because of its nearness to the University of Ghana and also the fact that there is a history of schistosomiasis among the inhabitants of the villages located on the lake because of their practices and life style which often bring them into contact with the water body. These inhabitants are predominantly farmers, fishermen and cattle rearers (deGraft - Johnson,1977).

2.3 Sampling procedure :

Sampling was done along the west banks of the Weija Lake (Plate 2.1) . In all five sites were selected including two water contact sites. At each site a quadrat measuring (4 x 2)m was marked out using a tape measure and wooden pegs. Five scoops were made per quadrat using a hand net and the samples collected including the molluscan species were stored in labeled plastic bags and brought to the laboratory where all the molluscan species were sorted, counted and recorded.

2.4 Characteristics of the study site :

In addition to the sampling schedule, the physical, chemical and the biological characteristics of each of the sites was noted as these play a major role in the ecology of the snails.

The west banks of the Weija Lake where the sampling was done.

(Plate 2.1)

2.4.1 Physical characteristics :

The physical parameters noted were the nature of the substrate or sediments, the water depth and the temperature. The water depth was measured using a tape measure, the measurements were taken at three points within each quadrat (4 x 2)m marked out from the banks of the lake . Measurements were taken from the banks along a perpendicular transect ie a) close to the banks of the lake about 0.6 m in the quadrat, b) about 1.3m in the middle of the quadrat and c) further away from the banks of the lake, about 1.8m towards the furthest end of the quadrat.

Sediments were collected from the bottom of the water in each of the quadrats separately, these were brought to the laboratory for observation. The surface and bottom temperatures of the water were measured at three different points per quadrat as in the case of the water depth measurements. Three different surface and bottom temperatures were taken for each point respectively using a temperature meter (AQUA LYTIC : OXI921).

2.4.2 Biological characteristics :

The biological factors noted were the aquatic macrophytes present at the sites and the molluscan species available. The macrophytes found in each quadrat were noted. The molluscan species collected in each quadrat were counted. The dead snails, represented by empty shells were also counted.

2.4.3 Chemical characteristics :

The chemical parameters noted were pH , oxygen concentration of the water, and also the conductivity of the water. Six readings were taken at each water depth for each of the above parameters, three at the surface and three at the bottom respectively. Oxygen concentration was measured using an oxygen meter (AQUA LYTIC : OXI 921). pH and conductivity were measured using a pH / Redox Digimeter (AQUA LYTIC : pH 21) and a conductivity meter (AQUA LYTIC : L21 / L21C) respectively.

2.5 Snail cultures :

Due to the large number of snails required for the main experiments, cultures were set up to raise snails of possibly the same size, weight and age. The two snails used were *Melanoides tuberculata* and *Bulinus truncatus*. Adult snails collected from Weija Lake as a result of the preliminary studies were used as brood stock for initiation of the cultures.

2.5.1 *Bulinus truncatus* cultures :

Snails were cultured in the laboratory in ten glass aquaria measuring 50cm x 35cm x 20cm (length x width x height) each containing about 20 litres of tap water. The cultures were maintained as described by Madsen (1984). They were kept at ambient temperature of 29 ± 1 ^{o}C and under artificial light of photoperiod of 12hrs light and 12hrs darkness. Fifteen adult snails were introduced into each glass aquaria and were kept in the aquaria for 5 days to allow enough time for these snails to lay eggs .

After the 5 day period the adult snails were removed leaving the eggs laid on the walls of the aquaria and on the leave of *Launea taraxacifolia* (Plate 2.2) and *Latuca sativa* (which was added as food) to hatch and grow to the size required for the experiment. The snails were fed on dried and fresh leaves of wild and edible lettuce (*Launea taraxacifolia* and *Latuca sativa*). Feeding was done once every other day to ensure adequate supply of food. The pH and the conductivity of the water were monitored and maintained at 7.02 ± 0.1 and 107 ± 2µs/m respectively. Once every week the water in the aquaria was changed to prevent it from becoming toxic. Air pumps were used to aerate the water.

2.5.2 *Melanoides tuberculata* cultures :

Adult snails obtained from the preliminary studies were used in setting up these cultures. The cultures were set up as in the case of the *Bulinus truncatus* cultures with the exception of the following modifications. About 4cm layer of sediment collected from the same site as the snails was introduced into each aquaria. This was necessary because of the ovoviparous nature of *Melanoides tuberculata* (Hicklin (1988). The aquaria for the *Bulinus truncatus* cultures were without any sediments.

The leaves of *Launaea taraxacifolia* which was added to the culture tanks to serve as food.

(Plate 2.2)

2.6 RESULTS:

2.6.1 Water Depth:

From Table 2.1, it could be observed that water depth for all the quadrats ranged between 19.8 and 31.7 cm. Quadrats 1 and 4 were the deepest with mean depth of 31.7cm and 31.1 cm respectively. The water was found to be at its shallowest in quadrat 5 with a mean depth of 19.8cm.

2.6.2 pH:

Table 2.1 shows that the pH of the water on the surface and the bottom was acidic. Generally the pH was less acidic in quadrats 1, 4 and 5 and more acidic in quadrats 2 and 3. Differences between the surface and the bottom pHs for each of the quadrats ranged between 0.1 and 0.4 and the average pH recorded on the surface was 6.1 and for the bottom it was 5.9.

2.6.3 Conductivity:

From Table 2.2 the highest conductivity of 430.8μs/m was recorded in quadrat 3 and the lowest 324.3μs/m was recorded in quadrat 4. The conductivity in the remaining quadrats ranged between 328.7 and 331.5μs/m.

Table 2.1 Table showing averages of some of the parameters measured during the preliminary survey.

	WATER	PH		CONDUCTIVITYus/m		TEMPERATURE (OC)		O 2 CONC (mg/l)	
QUADRAT	DEPTH (CM)	SURFACE	BOTTOM	SURFACE	BOTTOM	SURFACE	BOTTOM	SURFACE	BOTTOM
1	31.7	6.1	6.0	328.3	330.0	28.3	28.6	2.4	1.4
2	28.7	6.1	5.7	330.0	333.0	29.6	29.3	4.9	1.9
3	21.2	5.8	5.7	432.3	438.3	29.0	29.0	4.7	4.1
4	31.1	6.2	6.1	323.3	325.3	29.9	29.7	4.4	3.9
5	19.8	6.4	6.1	328.0	329.3	30.2	30.0	5.0	4.1
Average	26.5	6.1	5.9	348.4	351.2	29.4	29.3	4.3	3.1

Table 2.2 Table showing some of the parameters measured during the preliminary survey (Quadrats 2 and 5 are water contact sites).

QUADRAT	AVER WATER DEPTH/cm	PH	ME AN CONDUCTIVITY (us/m)	AVERAGE TEMPERATURE (oC)	AVERAGE OXYGEN CONC. (mg/l)	TOTAL NO OF SNAILS	NATURE OF SEDIMENT
1	31.7	6.2	329.2	28.5	1.9	112	SANDY
2	28.7	5.9	331.5	29.5	3.4	315	SANDY
3	21.2	5.8	430.8	29	4.4	125	SANDY GRAVEL
4	31.1	6.2	324.3	29.8	4.2	124	SANDY CLAY LOAM
5	19.8	6.2	328.7	30.1	4.6	102	SANDY CLAY LOAM

Sand (particle size : 0.05 - 2.0mm)
Gravel (particle size : > 2.0mm)
Clay (particle size : < 0.002mm)
Silt (particle size : 0.002 - 0.05mm)

Sandy gravel = 70% gravel and 30% sand
Sandy clay loam = 68.2% sand, 26.9% clay and 5% silt

2.6.4 Temperature:

The temperature differences as shown in Table 2.1 between the surface and the bottom are not very sharp as the range was between 0.0 and 0.3 °C with an average surface temperature of 29.4 °C and an average bottom temperature of 29.3 °C. The highest surface temperature of 30.3 °C was recorded for quadrat 1. For the bottom temperature the highest was 30.0 °C and the lowest was 28.6 °C. These were recorded for quadrats 5 and 1 respectively.

2.6.5 Oxygen Concentration:

Table 2.1 shows that the surface oxygen concentrations are higher that the bottom concentrations. The dissolved oxygen concentrations at the surface ranged between 2.4 and 5.0mg/l while those of the bottom were between 1.4 and 4.1 mg/l. The highest surface concentration of 5.0 mg/l was recorded for quadrat 4 while the lowest which was 2.4 mg/l was recorded for quadrat 1. The highest and lowest bottom concentrations of 4.1 and 1.4 mg/l were recorded for quadrats 3 and 5 respectively. The differences in the surface and bottom concentrations for the different quadrats ranged between 0.7 and 3.0. Averages of 4.3 and 3.1 were recorded for the surface and bottom oxygen concentrations respectively.

2.6.6 Aquatic Macrophytes:

From Table 2.3 six aquatic plants namely *Ceratophyllum demersum, Echinochloea pyramidalis, Ipomoea aquatica, Lemna purpusilla, Pistia stratiotes* and *Polygonum senegalense* were identified at the sampling sites. Of all these *Echinochloea pyramidalis* was the most abundant and *Pistia stratiotes* the least abundant. The observed order of abundance for the remaining plants was as follows : *Ceratophyllum demersum, Ipomoea aquatica, Lemna purpusilla and Polygonum senegalense.* Quadrat 1 had the richest macroflora while quadrat 4 had the least.

2.6.7 Snail Species:

Table 2.4 shows that the total number of snails found in all the quadrats was 778. Out of these 85% (677) were *Melanoides tuberculata*, 8% (61) were *Bulinus truncatus* and 5% (40) were *Biomphalaria pfeifferi.* These are depicted in Figure 2.2. The highest number of snails (315) was found in quadrat 2 while the least number (102) recorded in quadrat 5. For the individual snails, *Melanoides tuberculata* was most common in quadrat 2 (295) while it had the lowest occurrence (39) in quadrat 5. *Bulinus truncatus* snails on the other hand were most common in quadrat 5 and least common in quadrats 4 and 1 in which only one snail was found. For *Biomphalaria pfeifferi* the highest number of snails ie. 19 was recorded for quadrat 5 followed by quadrat 2 which recorded 7 snails. The least number of *Biomphalaria pfeifferi* snails (4) were recorded for both quadrats 1 and 4.

Table 2.3 AQUATIC PLANTS IDENTIFIED AT THE VARIOUS SITES DURING THE PRELIMINARY SURVEY.

NAME OF PLANTS	QUADRATS				
	1	2	3	4	5
Ceratophyllum demersum	1+	2+	0	0	3+
Echinochloea pyramidalis	3+	3+	3+	3+	3+
Ipomoea aquatica	2+	0	2+	1+	0
Lemna purpusilla	1+	1+	1+	0	0
Pistia stratiotes	0	0	0	0	1+
Polygonum senegalense	1+	1+	0	0	0

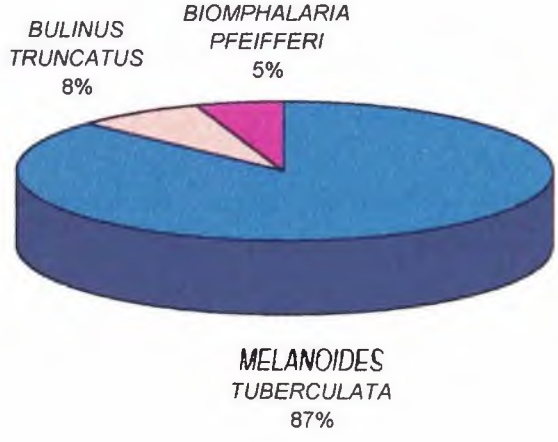
The figures 1+, 2+, 3+, indicate the relative abundance of the plant species found at the site where the survey was conducted. (3+ > 2+ >1+)

Table 2.4 SNAIL SPECIES FOUND IN THE VARIOUS QUADRATS DURING THE PRELIMINARY SURVEY.

QUADRAT	*MELANOIDES TUBERCULATA*	*BULINUS TRUNCATUS*	*BIOMPHALARIA PFEIFFERI*	TOTAL
1	107	1	4	112
2	295	13	7	315
3	117	2	6	125
4	119	1	4	124
5	39	44	19	102
Total	677	61	40	778

Figure 2.2

A CHART SHOWING THE PERCENTAGES OF THE DIFFERENT SNAIL SPECIES FOUND DURING THE SURVEY.



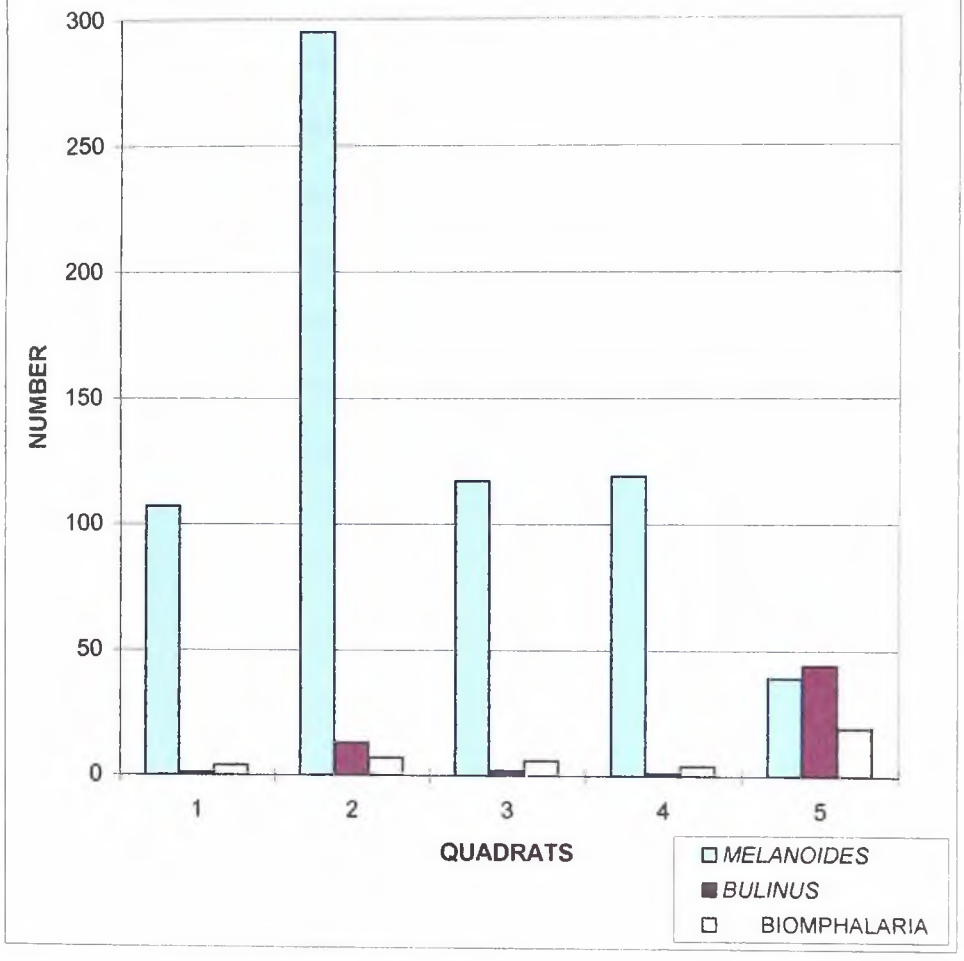
2.7 DISCUSSION:

The molluscan fauna is clearly dominated by *Melanoides tuberculata*. This is clearly seen from Figure 2.3 where with the exception of quadrat 5, *Melanoides tuberculata* outnumbered the rest of the snails. For the first four quadrats *Melanoides tuberculata* seem to exert a strong negative effect on *Bulinus truncatus*. In such areas the whole of the sediments were covered with a layer of shells and both dead and living *Melanoides tuberculata*. These shells were closely packed together.

It is interesting to note that the host snails ie *Bulinus truncatus* and *Biomphalaria pfeifferi* seem to be more common at the water contact sites than the non-water contact sites. Thus in quadrats 2 and 5, the host snails were found to relatively more abundant. Quadrat 5 which appeared to be more intensively used (judging from the level of human effects and the number of people present there) had the highest number of host snails. *Melanoides tuberculata* numbers were at the same time much lower. It is possible that the pollution and damage to the habitat negatively affected the *Melanoides tuberculata* and thus leading to the ascendancy of the host snails. On the other hand, the low levels of pollution could enhance the growth rate of *Bulinus truncatus* which would then be able to outcompete *Melanoides tuberculata*.

Figure 2.3

GRAPH SHOWING THE TYPES AND NUMBER OF SNAIL SPECIES FOUND AT THE SAMPLING SITES .



Other factors which may account for the differences observed in the population distribution pattern in quadrat 5 may be the nature of the sediment (sandy clay loam) and also the type of plants (*Ceratophyllum demersum and Echinochloea pyramidalis*) found in the quadrat. This is the only quadrat with sandy clay loam sediments, it more likely that *Bulinus truncatus*prefers the condition created by this type of sediment and plants . Whatever the case may be, these findings suggest the importance of other environmental factors 'tipping' the competitive interactions either in favour of the host snails or the non-host snail.

The present study reported in the subsequent chapters of this thesis investigates one of these factors which may have an effect on the outcome of the competitive interactions between *Melanoides tuberculata* a non-host snail and *Bulinus truncatus* a host snail of schistosomiasis.

CHAPTER 3

COMPETITION BETWEEN *MELANOIDES TUBERCULATA* AND *BULINUS TRUNCATUS* USING NORMAL AND HEAT TREATED SANDY GRAVEL AS SEDIMENT.

3.1 INTRODUCTION :

Melanoides tuberculata (Melaniidae), a natural component of the snail fauna in freshwater habitats in sub-Saharan Africa (Mkoji et al, 1992), has demonstrated the capacity to colonise many types of habitats. It has been found to limit and exclude certain pulmonate species, particularly snail hosts of schistosomiasis without any adverse effect on the environment, (Thomas and Tait, 1984; Pointier, 1989). In an investigation in Martinique, it caused a decline in the population of *B. glabrata* and *B. straminea*, (Pointier et al, 1989) and proved to be a successful competitor for *Biomphalaria glabrata,* (Prentice, 1983). In sub-Saharan Africa, it has been found to compete with *B. pfeifferi*, (Mkoji et al, 1992). However the exact nature of this competition is not known. *Melanoides tuberculata* is known to be successful on organically rich, eroding types of substrate while the host snails on the contrary require depositing substrate and macrophytes for survival (Thomas and Tait, 1984). These observations suggest that the nature of the substrate may be involved in the competitive interactions between *Melanoides tuberculata* and the host snails.

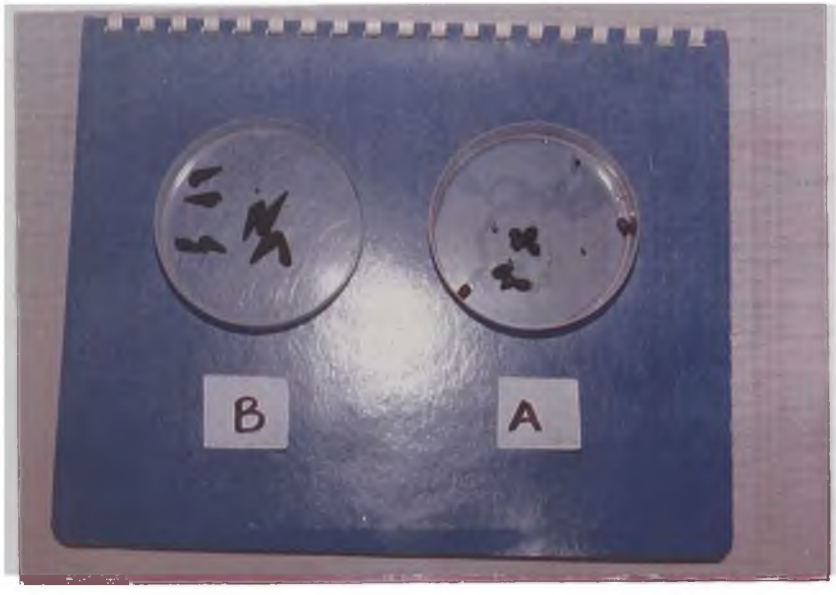
In this present work, the effects of a) varying the relative density of *Melanoides tuberculata* and *Bulinus truncatus* and b) sediment nature and condition on the competitive interaction between the two snail species under laboratory conditions were investigated using normal and heat treated sandy gravel (69.8% gravel and 30.2% sand) sediment.

3.2 MATERIALS AND METHODS

3.2.1 The Snails :

The two snail species (Plate 3.1) used for the experiments originated from the Weija Lake which is 17km west of Accra. *Bulinus truncatus* snails were from the cultures which had been established in the laboratory for four months . *Melanoides tuberculata* snails, however, were obtained directly from the lake and acclimatized for seven days prior to the experimentation. For the experiment using normal sediment the *B. truncatus* snails used were about four weeks old and had an average shell length ± SD between 0.388 ± 0.031cm and 0.685 ± 0.013 cm and an average weight ± SD between 0.040 ± 0.006g and 0.087 ± 0.007g. The *M. tuberculata* snails were about 7 - 8 weeks old according to the demographic studies done by Hicklin (1988). Their average shell length ± SD ranged between 1.555 ± 0.127cm and 2.033 ± 0.248cm their average weight ± SD between 0.200 ± 0.034 and 0.27 ± 0.045g.

For the experiment using the heat treated sediments the *B. truncatus* snails used were also about four weeks old and had an average shell length ± SD of between 0.525 ± 0.033cm and 0.718 ± 0.033 cm and an average weight ± SD between 0.052 ± 0.008g and 0.085 ± 0.011 g. The *M. tuberculata* snails were about 7 - 8 weeks old. Their average shell length ± SD ranged between 0.128 ± 0.049cm and 1.38 ± 0.038cm and their average weight ± SD between 0.091 ± 0.010 and 0.148 ± 0.006g.



Scale I----------I
9cm

Samples of the two snail species used (specimens in Dish A are *Bulinus truncatus*, while specimens in Dish B are *Melanoides tuberculata* snails)

Plate 3.1

3.2.2 Building of experimental cages :

Cages as shown in Plate 3.2, were made from transparent plastic spring water bottles (Astek INSU bottles). The top portions of these bottles were carefully cut off leaving a cylindrical container (open at one end only) with a height of 20cm and a diameter of 8.6cm. Two holes (7x8cm) were cut into two opposite sides of the container , one being 3cm above the base of the container and the other 4cm from the top cut edge. These holes were then covered with a nylon net (mesh size 1.5mm^2) using carpenters glue. The holes were to ensure proper streaming of the water from one cage to another to establish a uniform physico - chemical environment in all the cages.

The net was to prevent the snails from escaping from their respective cages. The cages were then allowed to dry for a period of two days to get rid of the strong smell of the glue and also to allow proper adherence of the net to the plastic container. The snails were then introduced into their respective labelled cages. Pieces of the net used in building the cages were fastened to the top of the cages by means of a rubber band to prevent the snails from escaping.

3.2.3 Preparation of sediments :

The sediment (sandy gravel) used was collected from the same site as the snails used for the experiment. This was used in two conditions ie Normal condition and Heat treated condition.

A sample of the experimental cages used

Plate 3.2

3.2.3.1 *Normal Sediments :*

This sediment was not heat treated. It was washed to remove some of the debris, and stored in clean bottles in the laboratory from where it was used for the experiment. This sediment had a composition of 30.2% sand with a particle size between 0.05 - 2mm and 69.8% of gravel with particle size > 2mm. (United States Department of Agriculture (USDA) Classification; Marshall and Holmes, 1992).

3.2.3.2 *Heat treated Sediments :*

The sediment used was collected from the same site as the snails. This was washed to remove debris and was then put in an oven at a temperature of 60 °C for 24 hrs to kill all the living micro- organisms such as fungi etc. which may be growing on the sediments. The sediment was then cooled and stored in clean bottles in the laboratory from where it was used for the experiment. The sediment used had the same composition as the Normal sediment. The normal sediment was used in section A while the heat treated sediment was used in section B of this chapter

3.2.4 Experimental setup :

The method used by Meyer-Larsen and Madsen (1989) was followed with minor modifications. To prevent changes in the growth patterns of the different groups of snails due to soluble substances produced by the snail species involved and also to minimize the depletion of some calcium compounds in the water and experimental cages, the aquatic medium used

was as homogenous as possible for all groups and combinations. Two glass aquaria measuring 50 x 35 x 20 cm (ie length x width x height) with a volume of 35,000cm^3 and twenty cages which measured 20 x 8.6 cm (ie height x diameter) with a volume of 1161.17cm^3 were used for this experiment. Each of the glass aquaria contained about 20 litres of dechlorinated tap water.

To each cage was added 50cm^3 of the prepared sediment which covered the base of the cage to the height of about 1cm . Ten cages were placed in each aquarium as shown in Plates 3.3 and 3.4. The snails were fed on 5g of dried and crushed wild lettuce (*Latuca taraxacifolia*) and also 5 discs of fresh *L. taraxacifolia* leaves with diameter 1.1cm. This same amount of food was added every other day to each cage to ensure an excess supply of food and also to avoid the possibility of food shortage acting as an experimental parameter interfering with the set experimental conditions. This also gave each snail in each cage access to the same amount of food. Usually at the next feeding about one third of the previous food was uneaten which meant that food was in excess of the snails requirements. The sails were first weighed to the nearest 0.0001g by using an electronic balance (AND Model : ER - 180A). Each species was weighed separately according to their densities and the shell length of each snail was measured by the use of a laminated graph sheet.

In all twenty snails were introduced into each cage and four cages were use for each of the following combinations of the two snail species (number of *M .tuberculata* / number of *B. truncatus* - M/B) : 0/20, 5/15, 10/10, 15/5, 20/0. These are referred to in the rest of the text, tables and graphs as CB (ie control for *Bulinus truncatus*), M5B15, M10B10, M10B5 and CM (ie control for *Melanoides tuberculata*) respectively. The cages were also labelled accordingly. At the centre of each aquarium was placed an air pump to aerate the water.

Side view of the experimental setup.

Plate 3.3

Top view of the experimental setup (note the arrangement of the various cages and the position of the air pump

Plate 3.4

Once every other day the cages were lifted and returned into the water almost immediately to promote good circulation of the water. The water was changed once every week after snail measurements had been taken. The experiment was maintained at a constant room temperature of 29 ± 1 °C and was followed for seven weeks. This duration was considered because of the limited time available and also the various experiments to be run.

At the end of each week , three indices were used to assess the competitive interactions, these were growth, mortality and reproduction. Growth was monitored using weight and shell length measurements. Mortality was measured by counting the number of snails dead at the end of each week, the dead snails were removed and replaced with snails of approximately the same size and weight. Reproduction was measured in the case of *B. truncatus* by the removal of all the egg masses found in each cage and the counting of all the individual eggs per cage. *M. tuberculata* being viviparous gives birth to juveniles which are released from their brood pouches into the sediments. Reproduction in the case of *M. tuberculata* was therefore measured by counting all juveniles found taking into account the fact that *M. tuberculata* is known to be parthenogenetic and also the fct that males are rare if not absent. The pH and conductivity of the water were measured twice every week at the start and termination of the week.

3.2.5 Definition of the various indices :

The rate of weight increase was calculated by the formula (Wp1 - Wp2) X 100, where (Wp1) is the average weight of snails for the previous week and (Wp2) is the average weight of snails for the present week. Increase in rate of shell length was calculated by the formula (Slp1 - SLp2) X 100, where (SLp1) is the average shell length of snails for the previous week and (SLp2) is the average shell length of snails for the present week. The rate

of mortality increase was calculated by the formula (Mp1 - Mp2) X 100, where (Mp1) is the average mortality of snails for the previous week and (Mp2) is the average mortality of snails for the present week. The rate of reproduction increase was calculated by the formula (Rp1 - Rp2) X 100, where (Rp1) is the average reproduction of snails for the previous week and (Rp2) is the average reproduction of snails for the present week. The error bars inserted onto the various graphs were based on standard error calculations.

3.3 RESULTS

The results are in two sections, the results obtained using the normal sediments are in section A and the results obtained using the heat treated sediments are in section B.

3.3.1 SECTION A : NORMAL SEDIMENTS

3.3.1.1 Experimental Group M5B15 :

3.3.1.1.1 *Weight :*

Table 3.2 and Figure 3.1 show a lowered rate of weight increase of *Bulinus truncatus* in the experimental combination below that of the control group for 5 (ie weeks 1, 2, 3, 4 and 5) out of the 7 weeks of the experiment (P(T<=t) value of 0.00518). The rate of weight increase rose gradually from the first week reaching a peak in week 3 and then decreasing to a fairly stable rate to the end of the experiment in week 7. In week 6, the rate (10.73%) went up above that of the control group (2.53%). However when the rates of weight changes were compared over the entire period using ANOVA, no statistically significant differences were found.

The rate of weight increase of *Melanoides tuberculata* also seems to have been strongly influenced by that of *Bulinus truncatus*. Thus during the first three weeks and also weeks 5 to 7, the rate of weight increase of *Melanoides tuberculata* in this experimental

TABLE 3.1 RATE OF INCREASE IN WEIGHT OF *M. TUBERCULATA*

USING NORMAL SANDY GRAVEL AS SEDIMENTS

GROUP	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7
CM	0	11.25	27.4	23.875	2.35	15.35	28.25	25.6
M5B15 - M	0	7.775	0.05	6.125	5.725	7.525	0.55	19.4
M10B10 - M	0	11.125	0.55	6.525	5.675	0.775	1.525	11.75
M15B5 - M	0	1.85	10.975	6.925	6.1	24.575	0.9	2.725

TABLE 3.2 RATE OF INCREASE IN WEIGHT OF *B. TRUNCATUS*

USING NORMAL SANDY GRAVEL AS SEDIMENTS

GROUP	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7
CB	0	14.425	13.825	24.35	14.375	14.45	2.525	11.525
M5B15 - B	0	3.8	2.225	10.425	7.7	8.425	10.725	10.225
M10B10 - B	0	3.925	4.85	5.25	2.2	8.575	5.05	5.55
M15B5 - B	0	-7.8	4.025	11	-1.7	0.775	1.375	5.275

GRAPHS FOR COMBINATION M5B15 (*BULINUS TRUNCATUS*)

USING NORMAL SANDY GRAVEL AS SEDIMENTS

FIG. 3.1

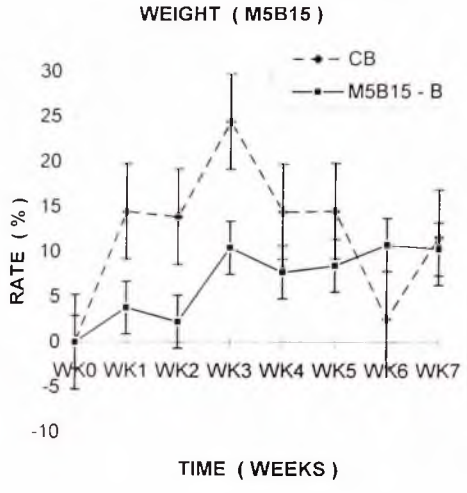
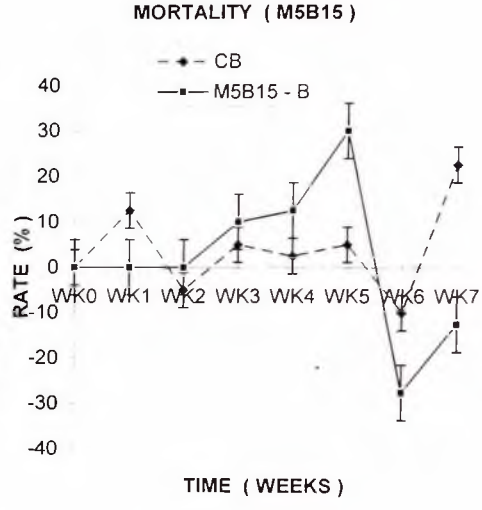


FIG. 3.2



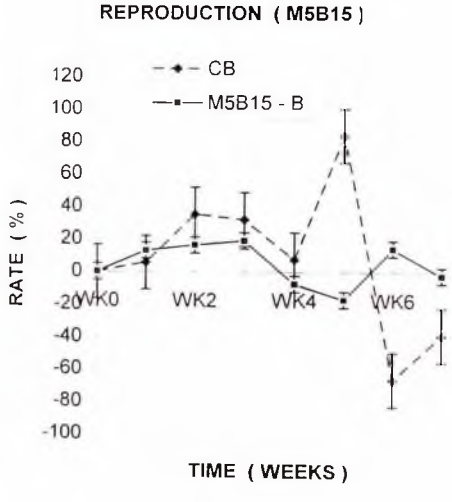


FIG. 3.3

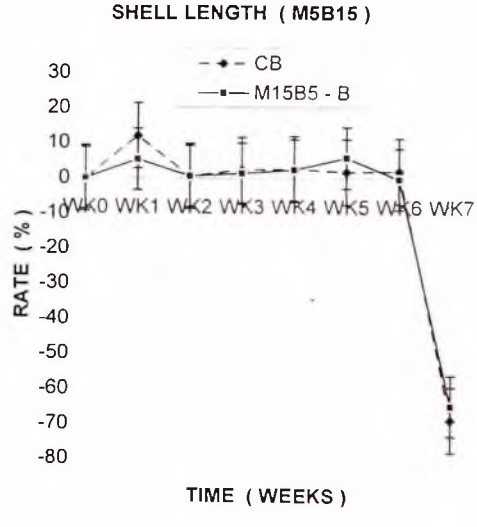


FIG. 3.4

GRAPHS FOR COMBINATION M5B15 (*M. TUBERCULATA*)

USING NORMAL SANDY GRAVEL AS SEDIMENTS

FIG. 3.5

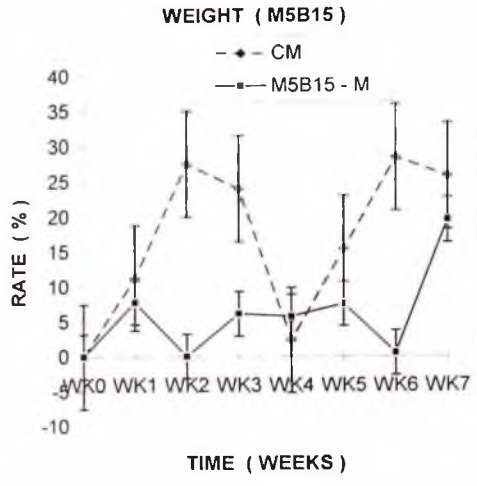
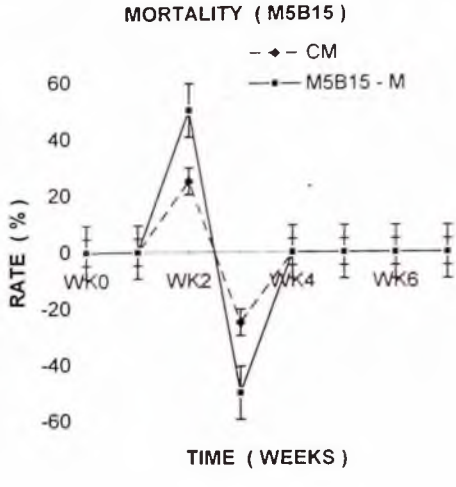


FIG. 3.6



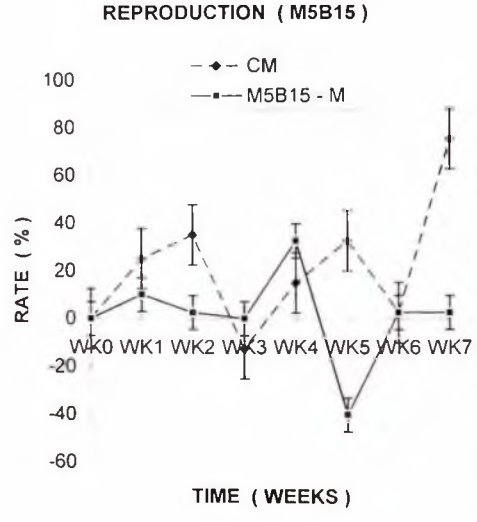


FIG. 3.7

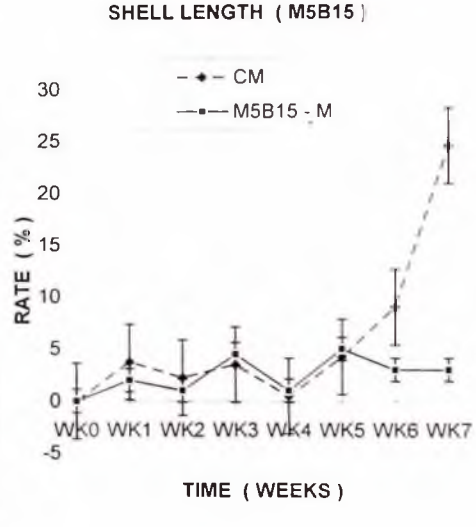


FIG. 3.8

combination decreased below that of the controls as shown in Figure 3.5. Analysis of the results for the entire seven week period showed that the differences are statistically significant (P(T<=t) value of 0.01878) .

3.3.1.1.2 *Mortality :*

From Table 3. 4 and also Figure 3.2, it was observed that for 4 (ie week 2,3,4 and 5) out of the 7 weeks of the experiment, the rate of mortality of *Bulinus truncatus* in the experimental cages increased above that of the control group. The increase in rate started gradually from week 2 (0%) to a peak in week 5 (27.5 %). The highest decrease was in week 6 (-27.5%). Analysis of the results of weeks 2 to 5 during which some clear differences occurred between the rate of mortality of *Bulinus truncatus* and the control group showed that the differences are statistically significant (P(T<=t) value of 0.04893). However the overall differences between *Bulinus truncatus* and the control group was not significant (p<0.71458).

3.3.1.1.3 *Reproduction :*

From Table 3.6 and also Figure 3.3, it could be seen that for 4 (ie weeks 2,3, 4 and 5) out of the 7 weeks of the experiment, the rate of reproduction of *Bulinus truncatus* decreased below that of the control group. However these differences were not statistically significant (P(T<=t) value of 0.09016). The rate of increase in reproduction of *Melanoides tuberculata* (Fig. 3.7) was not influenced to a statistically significant extent by the presence of *Bulinus truncatus* (P(T<=t) value of 0.11692).

TABLE 3.3 RATE OF INCREASE IN MORTALITY OF *M. TUBERCULATA*

USING NORMAL SANDY GRAVEL AS SEDIMENTS

GROUP	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7
CM	0	0	25	-25	0	0	0	0
M5B15 - M	0	0	50	-50	0	0	0	0
M10B10 - M	0	25	0	25	-50	0	0	0
M15B5 - M	0	50	-25	-25	0	0	0	0

TABLE 3.4 RATE OF INCREASE IN MORTALITY OF *B. TRUNCATUS*

USING NORMAL SANDY GRAVEL AS SEDIMENTS

GROUP	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7
CB	0	12.5	-5	5	2.5	5	-10	22.5
M5B15 - B	0	0	0	10	12.5	27.5	-27.5	-12.5
M10B10 - B	0	2.5	0	5	2.5	25	-12.5	2.5
M15B5 - B	0	5	-5	7.5	2.5	0	0	0

TABLE 3.5 RATE OF INCREASE IN REPRODUCTION OF *M. TUBERCULATA*

USING NORMAL SANDY GRAVEL AS SEDIMENTS

GROUP	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7
CM	0	25	35	-12.5	15	32.5	2.5	75
M10B10 - M	0	17.5	2.5	0	15	20	-32.5	25
M15B5 - M	0	30	5	15	75	-35	-40	25
M5B15 - M	0	10	2.5	0	32.5	-40	2.5	2.5

TABLE 3.6 RATE OF INCREASE IN REPRODUCTION OF *B. TRUNCATUS*

USING NORMAL SANDY GRAVEL AS SEDIMENTS

GROUP	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7
CB	0	5.6	35.4	32.325	7.75	83.625	-65.9	-38.75
M10B10 - B	0	10.95	7.975	17.575	-6.275	2.25	-21.8	7
M15B5 - B	0	3.775	9.775	15.85	-20.925	-3.1	8.8	-1.05
M5B15 - B	0	12.95	16.375	19.2	-7.3	-16.875	14.25	-2.3

3.3.1.1.4 *Shell Length :*

Table 3.8 and Figure 3.4 show a fairly constant rate of shell length increase in *Bulinus truncatus* throughout the period of the experiment with the exception of week 7 where the rate decreased to a value of (-66.25%). Analysis of the results of the first six weeks of the experiment during which some differences occurred between the rate of increase of shell length of *Bulinus truncatus* in this experimental combination and the control group showed that these differences were not statistically significant (P(T<=t) value of 0.24027)

3.3.1.2 Experimental Combination M10B10 :

3.3.1.2.1 *Weight :*

Table 3.2 and Figure 3.9 show that for 6 (ie weeks 1 to 5 and week 7) out of the 7 weeks of the experiment, the rate of weight increase of *Bulinus truncatus* in this experimental combination fell below that of the control group. These differences were found to be highly significant (P(T<=t) value of 0.00761) over the first five weeks of the experiment. Further analysis of the differences between the rate for *Bulinus truncatus* and the control group over the entire 7 week period using ANOVA was also found to be statistically significant (p< 0.01740).

The rate of weight increase of *Melanoides tuberculata* in this experimental combination seems to have been (with the exception of week 6) influenced by that of *Bulinus truncatus* during the period of the experiment . This could be seen from Figure 3.13. Analysing these results the differences were found to be statistically significant (P(T<=t) value of 0.01234).

TABLE 3.7 RATE OF INCREASE IN SHELL LENGTH (CM) OF *M. TUBERCULATA* USING NORMAL SANDY GRAVEL AS SEDIMENTS

GROUP	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7
CM	0	3.75	2.25	3.5	0.5	4.25	9	24.5
M15B5 - M	0	5.5	2.75	0	3	0	3.25	4.25
M10B10 - M	0	4.5	2.5	4	1	0.75	-0.25	4.75
M5B15 - M	0	2	1	4.5	1	5	3	3

TABLE 3.8 RATE OF INCREASE IN SHELL LENGTH (CM) OF *B. TRUNCATUS* USING NORMAL SANDY GRAVEL AS SEDIMENTS

GROUP	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7
CB	0	12	0.5	2.25	2.5	1.75	2	-68.5
M15B5 - B	0	5.25	0.5	1.25	2.25	5.75	-0.25	-64.5
M10B10 - B	0	6	3.5	0.5	4	2.5	5	-68
M5B15 - B	0	7.5	3.75	2.5	1.25	1.25	3.75	-66.25

GRAPHS FOR COMBINATION M10B10 (*BULINUS TRUNCATUS*)

USING NORMAL SANDY GRAVEL AS SEDIMENTS

FIG. 3.9

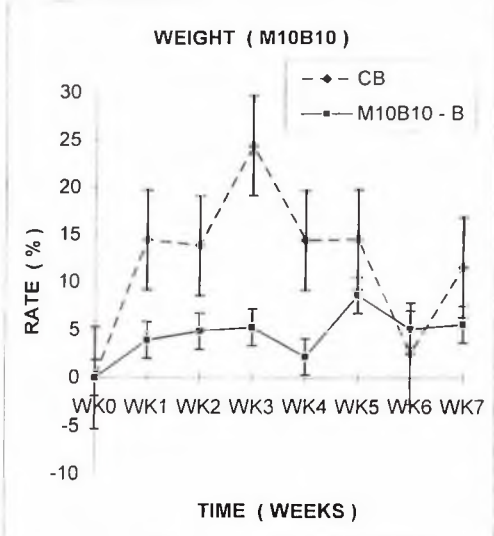
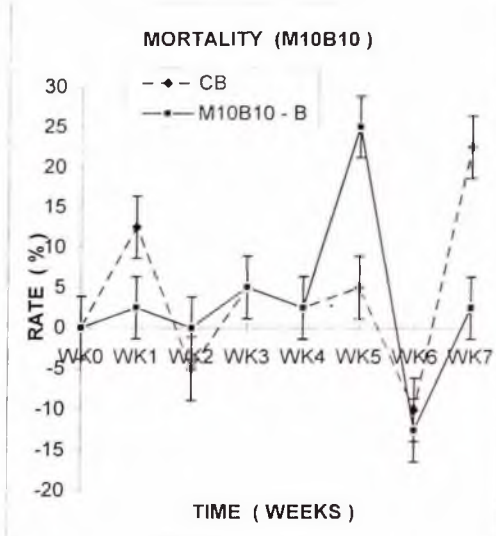


FIG. 3.10



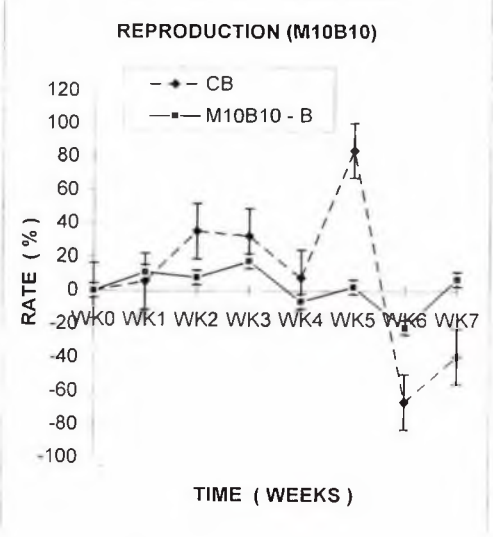


FIG. 3.11

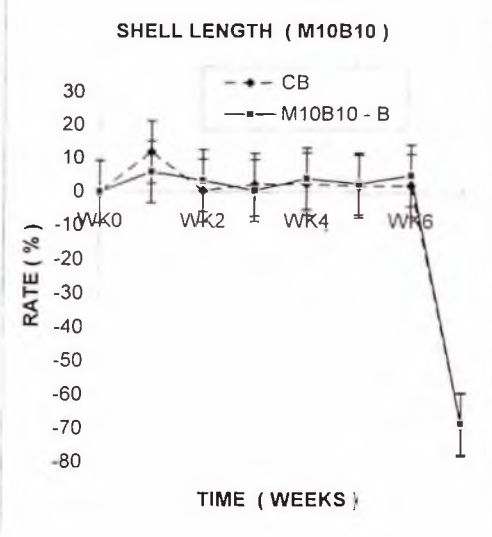


FIG. 3.12

GRAPHS FOR COMBINATION M10B10 (*M. TUBERCULATA*)

USING NORMAL SANDY GRAVEL AS SEDIMENTS

FIG. 3.13

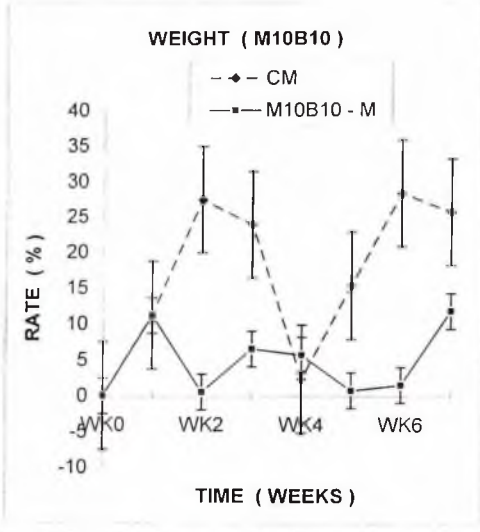
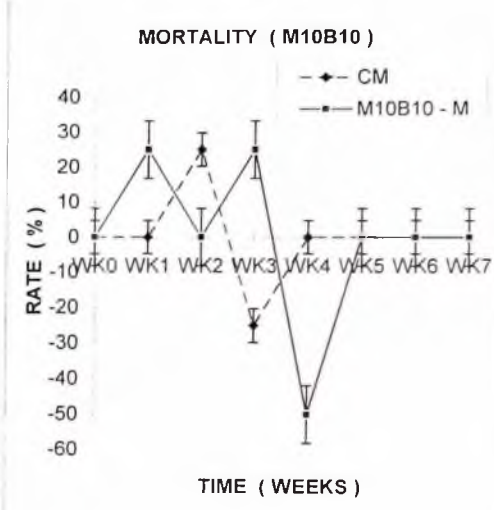


FIG. 3.14



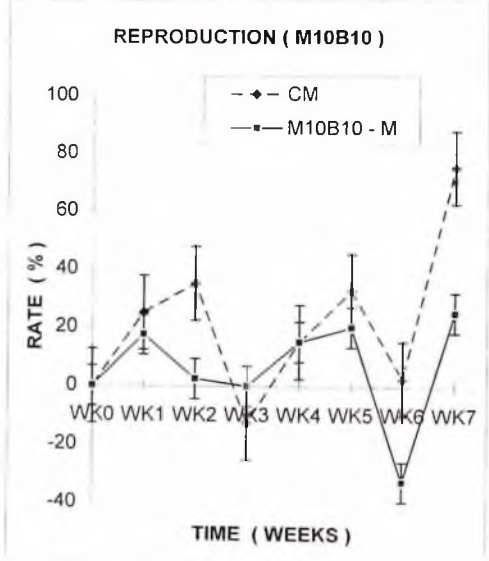


FIG. 3.15

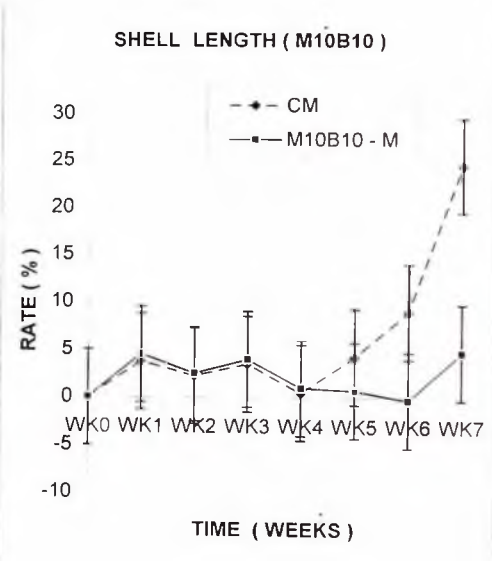


FIG. 3.16

3.3.1.2.2 ***Mortality :***

Table 3.4 and Figure 3.10 show fluctuations in the rate of mortality of *Bulinus truncatus* in this experimental combination. In 2 (weeks 2 and 5) out of the 7 weeks of the experiment the rate of mortality of *Bulinus truncatus* increased above that of the control group. The highest increase was recorded in week 5 (25%). In weeks 3 and 4 the mortality rate (5 and 2.5% respectively) of *Bulinus truncatus* coincided with that of the control group, and for the rest of the weeks the mortality rate of *Bulinus truncatus* in the experimental combination decreased below that of the control group. Statistical analyses of the results of the first five weeks where most of the differences occurred between the rate for *Bulinus truncatus* and the control group showed that these differences were not statistically significant (P(T<=t) value of 0.28116).

3.3.1.2.3 ***Reproduction :***

Table 3.6 and Figure 3.11 show that for 3 (ie weeks 1, 6 and 7)out of the 7 weeks of the experiment the rate of reproduction of *Bulinus truncatus* in this combination increased above that of the control group. For the other 4 weeks the control group had a higher rate of reproduction than the *Bulinus truncatus* in this experimental combination. Analyses of the results of weeks 1 to 5 during which period most of the differences occurred between the rate of reproduction for *Bulinus truncatus* and the control group as shown in Figure 3.11, showed that these differences were not statistically significant (P(T<=t) value of 0.07318).

The rate of increase in reproduction of *Melanoides tuberculata* in this experimental combination seems to have been influenced by that of *Bulinus truncatus* during the first two weeks and the last two weeks of the experiment (Figure 3.15). Analysing the result for these

weeks, P(T<=t) values of 0.15381 and 0.04822 respectively were obtained showing that the differences observed for the first two weeks are not statistically significant while for weeks 5 to 7, the differences were statistically significant.

3.3.1.2.4 *Shell Length :*

Table 3.8 and Figure 3.12 show a fairly constant rate of shell length increase in *Bulinus truncatus* throughout the period of the experiment with the exception of week 7 where the rate decreased to a value of (-68%). Throughout this period the differences between the rate of shell length increase of *Bulinus truncatus* in the experimental combination and that of the control group were not statistically significant (p< 0.90727).

3.3.1.3 Experimental Combination M15B5 :

3.3.1.3.1 *Weight :*

Table 3.2 and Figure 3.17 show that throughout the period of the experiment, the rate of weight increase of *Bulinus truncatus* in this experimental combination was below that of the control group. A sharp decrease (-7.8%) was recorded in week 1 and a high rate (10.43%) was observed in week 3. The differences in weight changes observed between *Bulinus truncatus* and the control group were found to be highly significant over the first 6 weeks (P(T<=t) value of 0.00565). A similar high level of significance was obtained when the weight changes were compared over the entire period of the experiment (7weeks) using ANOVA (p<0.00632).

GRAPHS FOR COMBINATION M15B5 (*BULINUS TRUNCATUS*)

USING NORMAL SANDY GRAVEL AS SEDIMENTS

FIG. 3.17

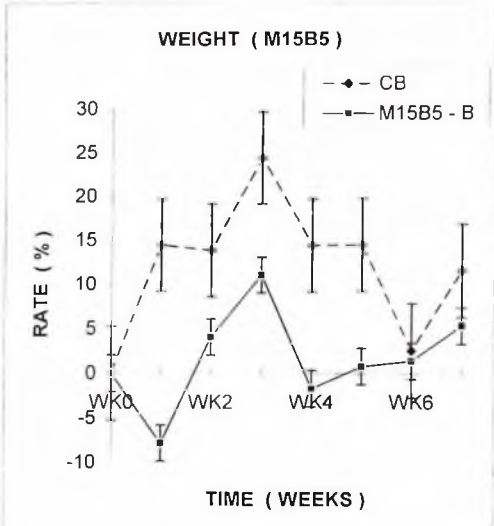
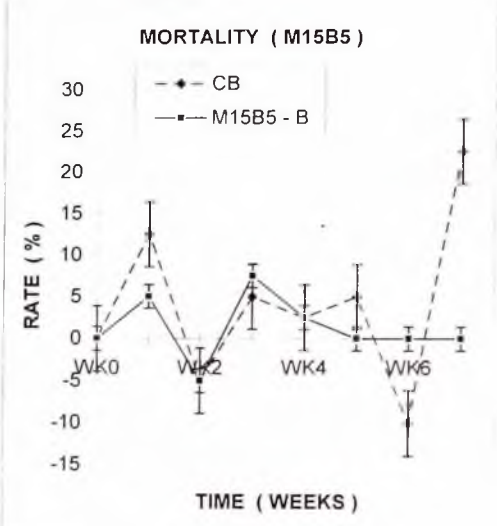


FIG. 3.18



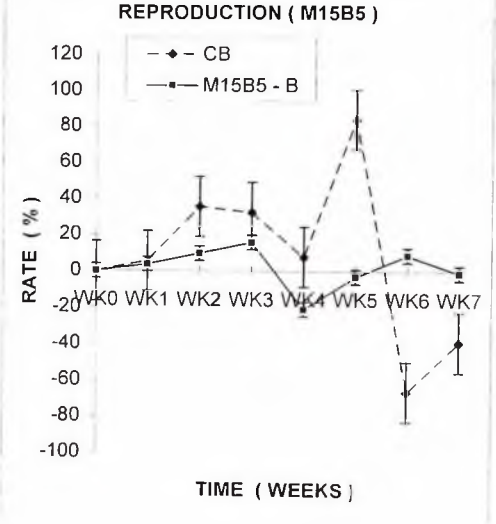


FIG. 3.19

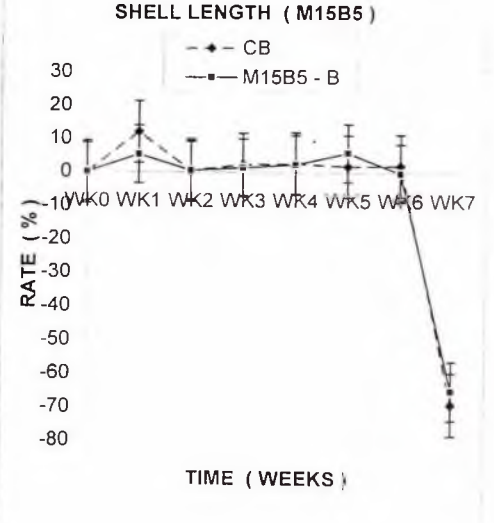


FIG. 3.20

The rate of weight increase of *Melanoides tuberculata* in this experimental combination seems to have been repressed to some extent by *Bulinus truncatus* snails (Figure 3.21) P(T<=t) values of 0.03684.

3.3.1.3.2 *Mortality :*

Table 3.6 and Figure 3.18 show that the rate of mortality of *Bulinus truncatus* in this experimental combination increased above that of the control group only in 3 out of the 7 weeks of the experiment. For the rest of the time the rate was below that of the control group. No significant differences ($p<0.42959$) were observed between the mortality rate of *Bulinus truncatus* and the control group.

3.3.1.3.3 *Reproduction :*

Table 3.6 and Figure 3.19 show that, for 4 weeks (ie weeks 2, 3, 4 and 5) the rate of reproduction of *Bulinus truncatus* in this experimental combination was below that of the control group (P(T<=t) value of 0.0463). For the rest of the weeks (ie weeks 1, 6 and 7) the rate of reproduction of *Bulinus truncatus* increased above that of the control group. Analysing the overall results for *Bulinus truncatus* and the control group shown using ANOVA, no significant differences were observed ($p< 0.73879$).

The rate of reproduction increase of *Melanoides tuberculata* in this experimental combination was influenced adversely by that of *Bulinus truncatus* during weeks 5 to 7 of the experiment (Figure 3.23). Analysing the result for these 3 weeks, the differences as shown in the Figure, were found to be statistically significant (P(T<=t) values of 0.00937).

GRAPHS FOR COMBINATION M15B5 (*M. TUBERCULATA*)

USING NORMAL SANDY GRAVEL AS SEDIMENTS

FIG. 3.21

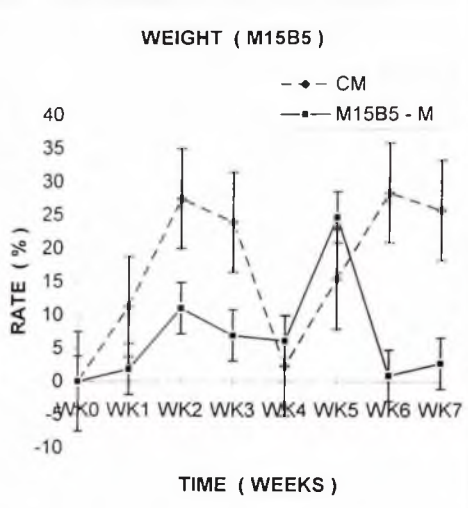


FIG. 3.22

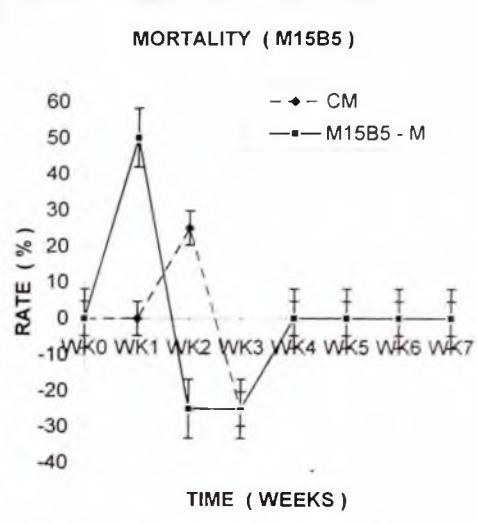


FIG. 3.23

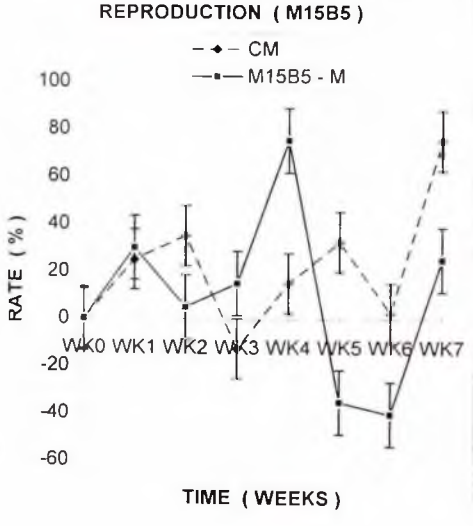
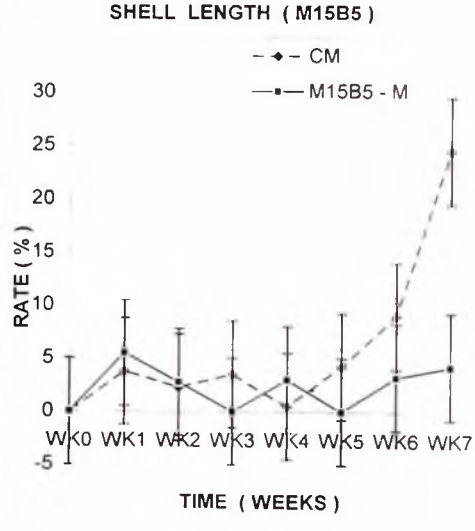


FIG. 3.24



3.3.1.3.4 *Shell length :*

Table 3.8 and Figure 3.20 show a fairly constant rate of shell length increase in *Bulinus truncatus* throughout the period of the experiment with the exception of week 7 where the rate decreased to a value of (-64.5%). Throughout this period the differences between the rate of shell length increase of *Bulinus truncatus* in the experimental combination and that of the control group were insignificant (P(T<=t) value of 0.24525).

The rate of shell length increase of *Melanoides tuberculata* in this experimental combination (Figure 3.24) was not influenced significantly by the presence of *Bulinus truncatus* snails (P(T<=t) values of 0.09337).

3.3.1.4 Density dependent effects :

3.3.1.4.1 *Weight :*

From Table 3.9 and Figures 3.26 and 3.33, it was observed that the significance of the results increased with an increase in the density of the *Melanoides tuberculata* snails. Thus *Melanoides tuberculata* in the combination M15B5 ($p<0.00632$) had a greater influence on the rate of weight increase of *Bulinus truncatus* than M10B10 ($p< 0.01740$) and M5B15 ($p< 0.08109$) respectively.

3.3.1.4.2 *Mortality :*

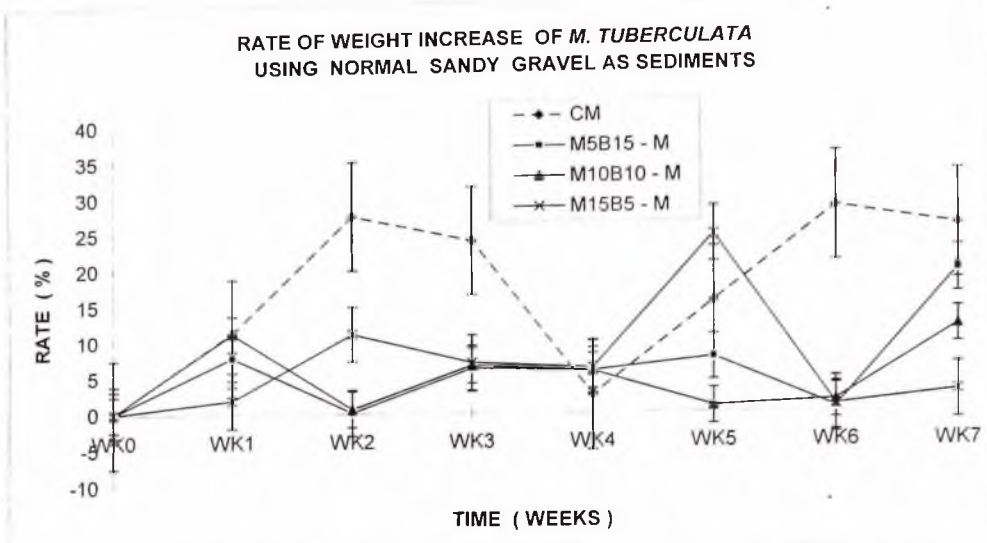
From Table 3.9 and Figures 3.28 and 3.33, it was observed that, the density of *Melanoides tuberculata* did not have any significant effect on the mortality of *Bulinus truncatus* in the various combinations. The results of all the various combinations were insignificant, M5B15 ($p< 0.71458$) M10B10 ($p< 0.82409$) and M15B5 ($p< 0.42960$).

Table. 3.9 ANOVA : Two-Factor Without Replication for *Bulinus truncatus*

USING NORMAL SANDY GRAVEL AS SEDIMENTS

Combinations	Weight	Mortality	reproduction	Shell Length
M5B15B	0.08109	0.71458	0.87519	0.55591
M10B10B	0.01740	0.82409	0.53821	0.90727
M15B5B	0.00632	0.42960	0.73879	0.82396

FIG. 3.25



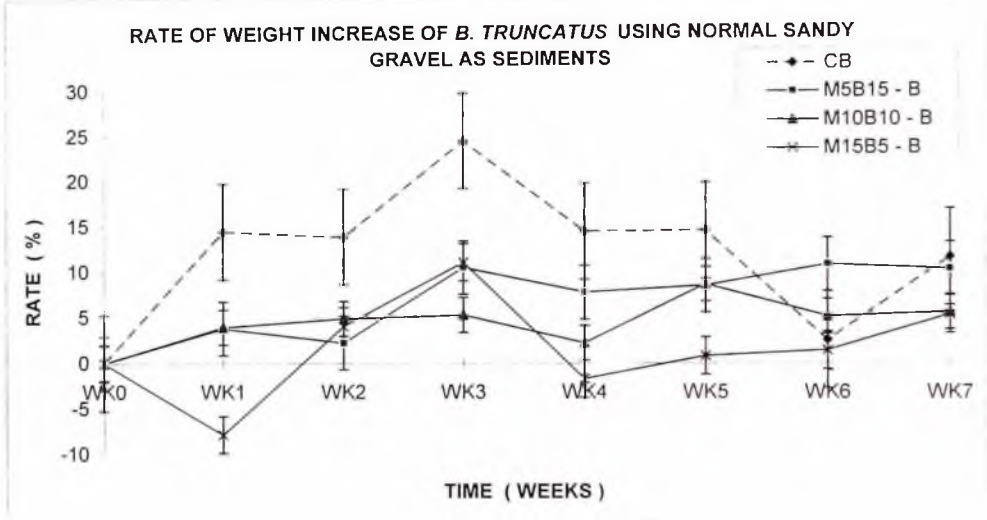
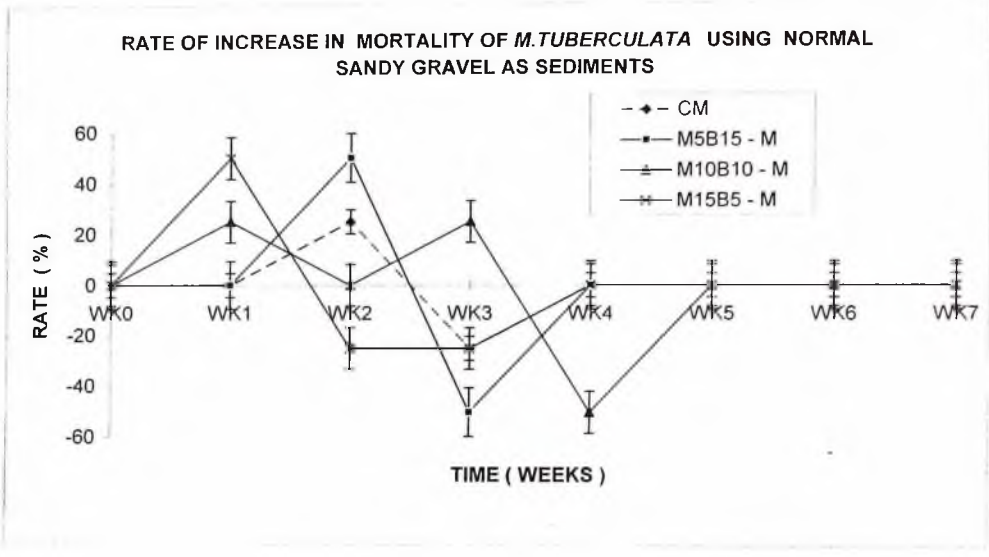


FIG. 3.26

FIG. 3.27



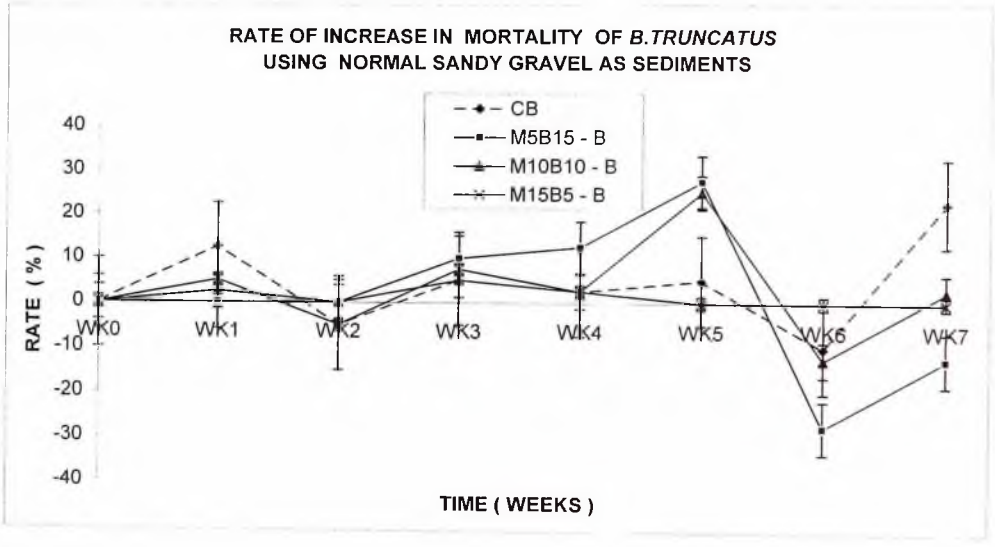


FIG. 3.28

3.3.1.4.3 *Reproduction :*

From Table 3.9 and Figures 3.30 and 3.33, no significant effect was exerted by the increase in the density of *Melanoides tuberculata* on the rate of increase of reproduction of *Bulinus truncatus* in the various combinations. The results of all the various combinations were insignificant, M5B15 ($p < 0.87519$) M10B10 ($p < 0.53821$) and M15B5 ($p < 0.73879$).

3.3.1.4.4 *Shell Length :*

From Table 3.9 and Figures 3.32 and 3.33, the observations made were similar to those for mortality and reproduction. The results of the differences between all the various combinations were insignificant, M5B15 ($p < 0.55591$) M10B10 ($p < 0.90727$) and M15B5 ($p < 0.82396$). Thus the increase in the density of *Melanoides tuberculata* has no effect on the rate of increase of shell length of *Bulinus truncatus* in the various combinations.

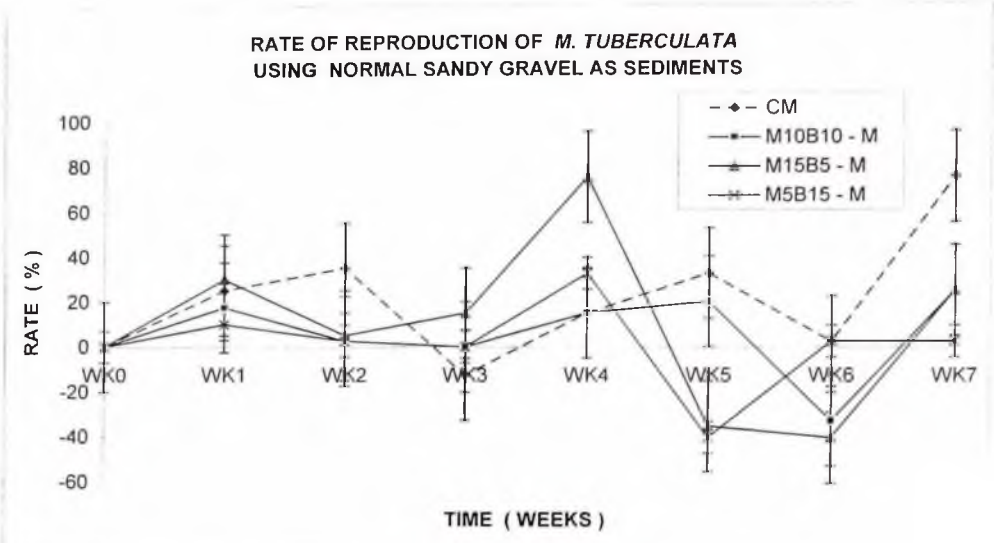
3.3.2 SECTION B : HEAT TREATED SEDIMENTS

3.3.2.1 Experimental Combination M5B15 :

3.3.2.1.1 *Weight :*

Table 3.11 and Figure 3.34 show a rise (26.45%) in the rate of weight increase of *Bulinus truncatus* for this experimental combination in week 1 above that of the control group (12.53%). This was followed by a sharp decrease in the rate in week 2. The rate increased from week 2 to week 3 and thereafter remained fairly constant but was above that of the

FIG. 3.29



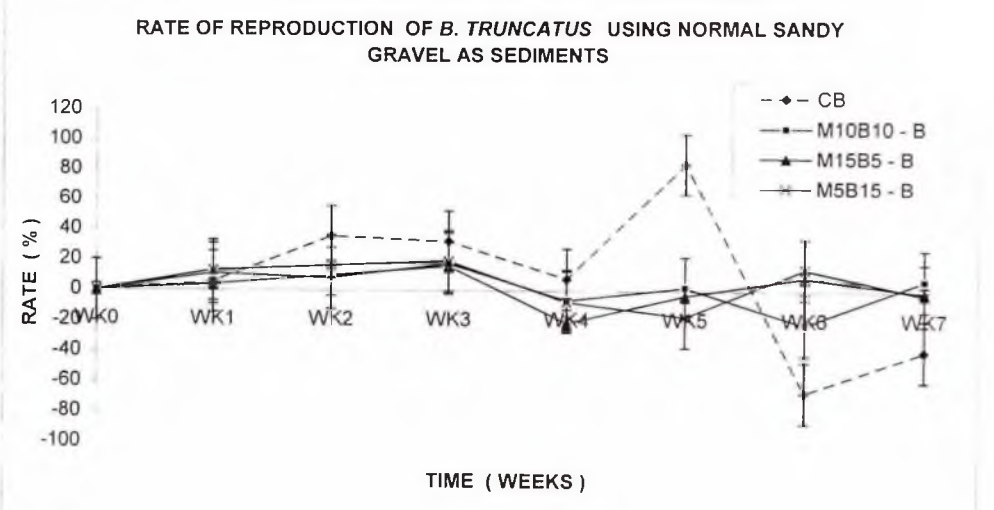
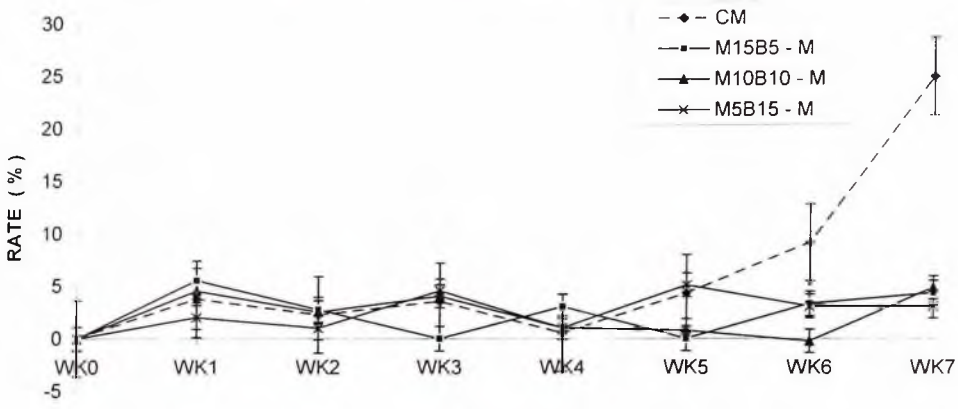


FIG. 3.30

FIG. 3.31

RATE OF INCREASE IN SHELL LENGTH OF *M. TUBERCULATA* USING NORMAL SANDY GRAVEL AS SEDIMENTS



RATE OF INCREASE IN SHELL LENGTH OF *B. TRUNCATUS* USING NORMAL SANDY GRAVEL AS SEDIMENTS

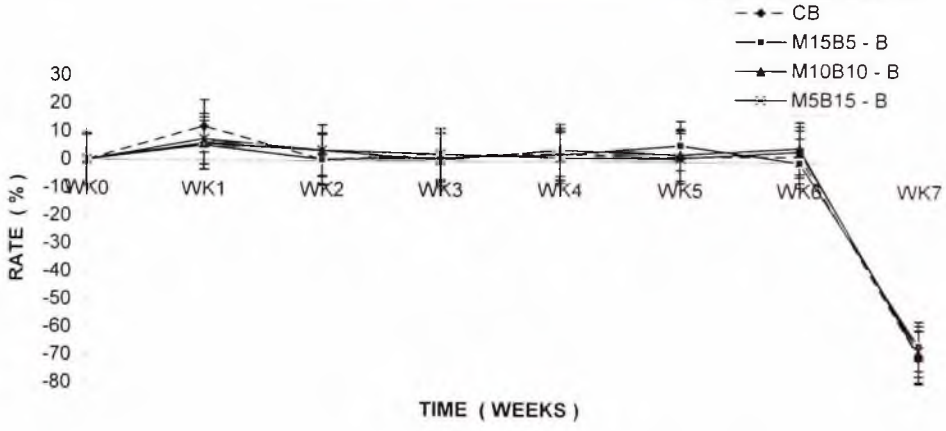


FIG. 3.32

FIG. 3.33

GRAPHICAL PRESENTATION OF THE STATISTICAL ANALYSIS OF THE RESULTS OF *B. TRUNCATUS* (USING NORMAL SANDY GRAVEL)

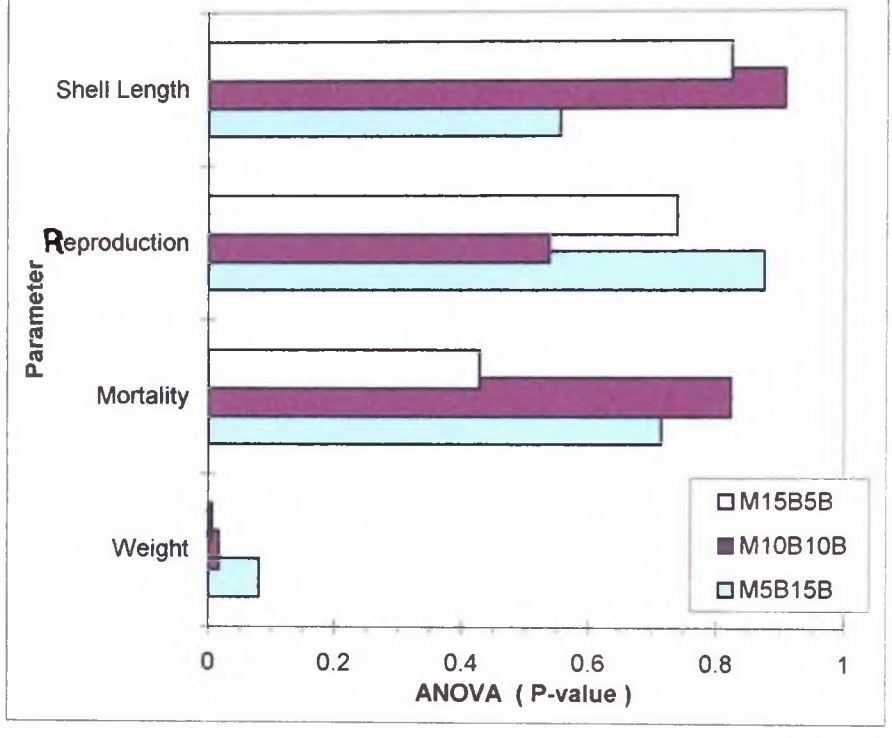


TABLE 3.10 RATE OF INCREASE IN WEIGHT OF *M. TUBERCULATA* USING HEAT TREATED SANDY GRAVEL AS SEDIMENTS

GROUP	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7
CM	0	11.4575	10.0425	13.325	13.425	18.25	9.725	37.45
M5B15 - M	0	22.2433	-19.19	3.35	4.34333	4.74667	6.8	6.20667
M10B10 - M	0	24.1925	-15.238	6.6525	4.95	7.8	5.8075	9.25
M15B5 - M	0	23.13	-12.12	8.6025	11.84	15.18	13.165	7.82

TABLE 3.11 RATE OF INCREASE IN WEIGHT OF *B. TRUNCATUS* USING HEAT TREATED SANDY GRAVEL AS SEDIMENTS

GROUP	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7
CB	0	12.5225	9.6625	2.7225	2.865	7.1975	6.7325	14.4775
M5B15 - B	0	26.4525	-3.5875	6.045	7.5825	3.77	13.1625	5.1825
M10B10 - B	0	31.52	-18.928	3.4175	11.6625	5.33	36.2	7.2
M15B5 - B	0	24.2058	-12.321	-2.44	2.785	-0.7075	3.57	-1.235

GRAPHS FOR COMBINATION M5B15 (*B. TRUNCATUS*)

USING HEAT TREATED SANDY GRAVEL AS SEDIMENTS.

FIG. 3.34

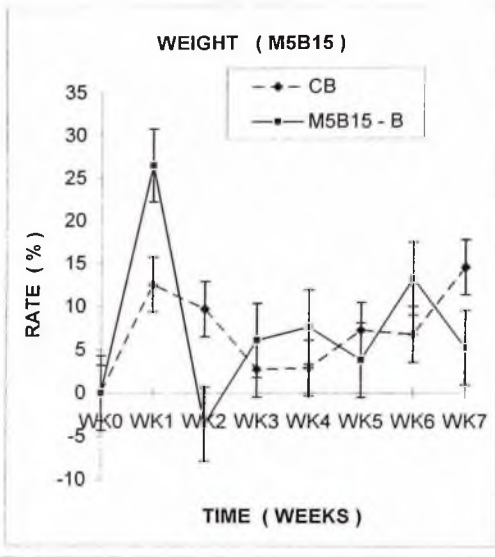
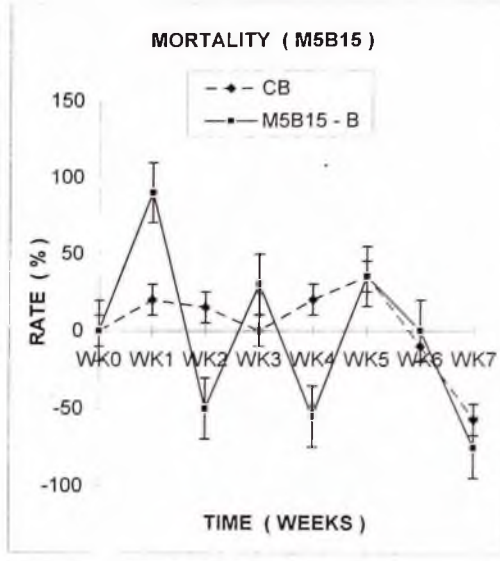
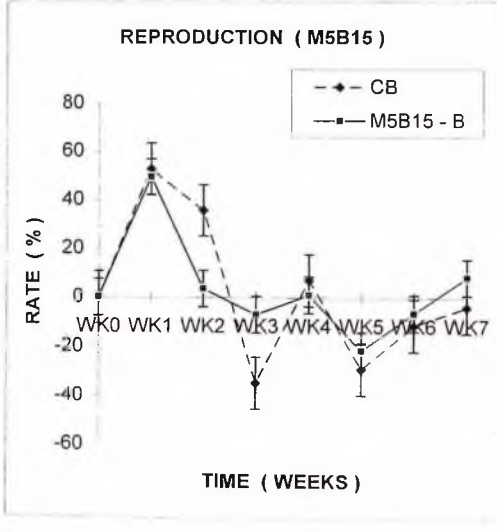


FIG 3.35





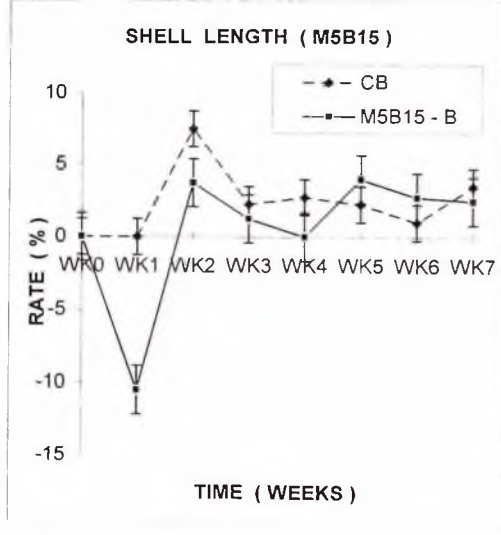


FIG. 3.36

FIG. 3.37

control group till after week 4 when it decreased again to 13.16%. The rate then increased from week 5 to week 6. Analysis of the differences between the rate of weight increase of *Bulinus truncatus* and the control showed that the observed differences were not significant ($p < 0.92501$)

The rate of weight increase of *Melanoides tuberculata* seems to have been influenced by that of *Bulinus truncatus*. This is because from Figure 3.38 , during weeks 2 and 6, the rate of weight increase of *Melanoides tuberculata* in this experimental combination decreased below that of the control group. These differences were found to be statistically significant ($P(T<=t) = 0.02136$) .

3.3.2.1.2 ***Mortality :***

Table 3.13 and Figure 3.35 show that there were fluctuations in the rate of mortality in this experimental combination for *Bulinus truncatus* . The highest increase (90%) in the rate was recorded in week 1 and the largest decrease (-80%) was recorded in week 7. In all the weeks except in week 5 where the rate (35%) coincided with that of the control group, the rate alternatively fluctuated above (in weeks 1, 3 and 6) and below (in weeks 2, 4, and 7) that recorded for the control group over the weeks. Analysis of the overall results for *Bulinus truncatus* under experimental conditions and the control group using ANOVA, did not yield any significant difference between the two groups ($p < 0.73135$).

GRAPHS FOR COMBINATION M5B15 (*M. TUBERCULATA*)

USING HEAT TREATED SANDY GRAVEL AS SEDIMENTS.

FIG. 3.38

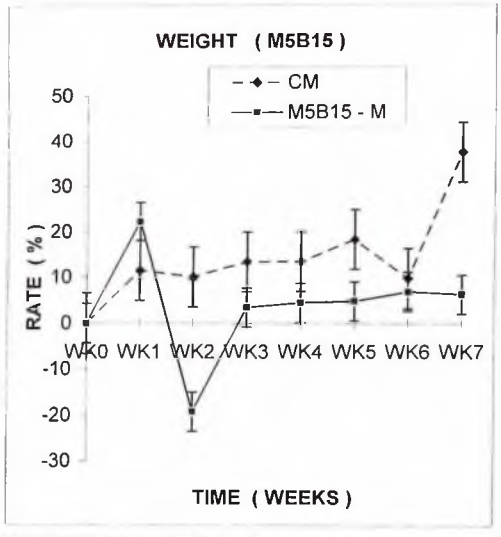
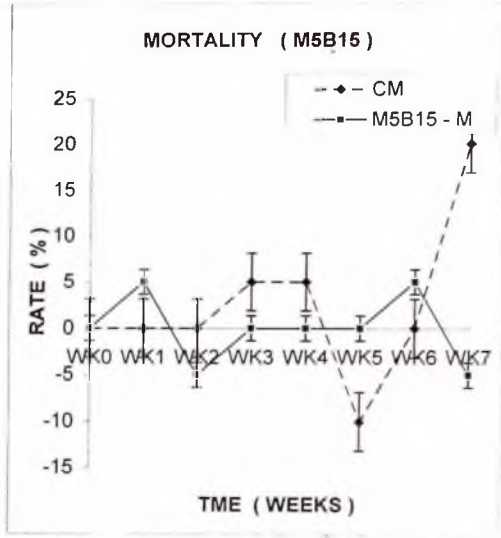


FIG. 3.39



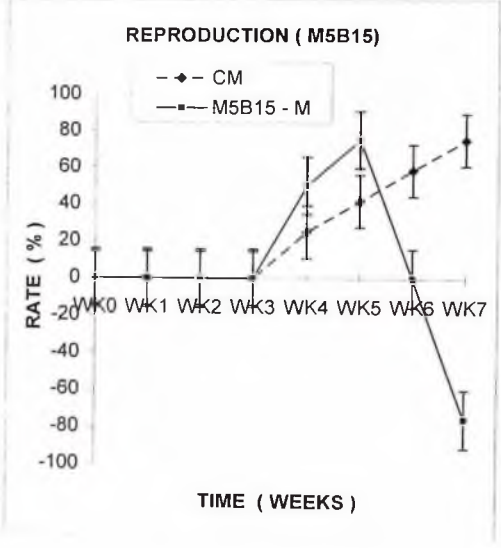


FIG. 3.40

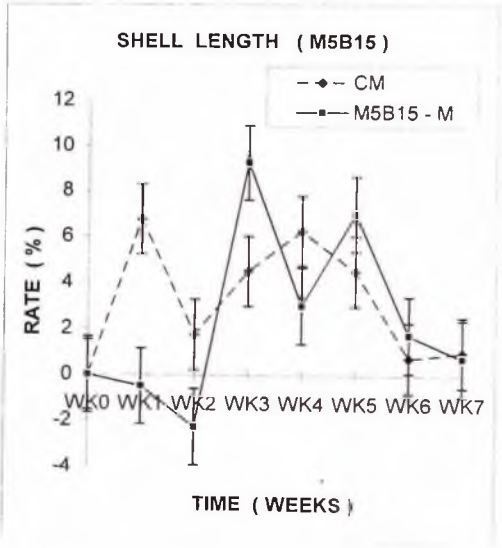


FIG. 3.41

TABLE 3.12 RATE OF INCREASE IN MORTALITY OF *M. TUBERCULATA* USING HEAT TREATED SANDY GRAVEL AS SEDIMENTS

GROUP	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7
CM	0	0	0	5	5	-10	0	20
M5B15 - M	0	5	-5	0	0	0	5	-5
M10B10 - M	0	5	0	0	-5	0	10	-10
M15B5 - M	0	5	-5	0	0	0	20	-20

TABLE 3.13 RATE OF INCREASE IN MORTALITY OF *B. TRUNCATUS* USING HEAT TREATED SANDY GRAVEL AS SEDIMENTS

GROUP	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7
CB	0	20	15	0	20	35	-10	-57
M5B15 - B	0	90	-50	30	-55	35	0	-75
M10B10 - B	0	30	5	-25	10	5	25	-80
M15B5 - B	0	40	-35	25	0	0	-5	-25

3.3.2.1.3 *Reproduction :*

Table 3.15 and Figure 3.36 show the results of the rate of reproduction increase in *Bulinus truncatus* in this experimental combination. In week 1, the rate increased considerably (49.28%) but was below that of the control (52.26 %) and also in weeks 2 and 4 the rate was below that of the control . For the rest of the weeks, the rate of reproduction of *Bulinus truncatus* increased but above that of the control group to the end of the experiment. ANOVA based on the overall results of *Bulinus truncatus* under experimental conditions and the control showed that the differences were insignificant ($p< 0.82292$).

The rate of reproduction of *Melanoides tuberculata* seem to have been influenced by the presence of *Bulinus truncatus* from Figure 3.40, however these differences were found to be statistically insignificant (P(T<=t) value of 0.09585).

3.3.2.1.4 *Shell length :*

Table 3.17 and Figure 3.37 show the results of the rate of increase in shell length of *Bulinus truncatus*. In weeks 5 and 6 the rate increased above that of the control with values 4 and 2.75% respectively. The rate however decreased and remained below that of the control group for the other weeks with the largest decrease of -10.5% recorded in week 1. T-test calculated based on the results of the first three weeks where most of the differences occurred between the rate of increase of shell length of *Bulinus truncatus* and the control showed that these differences are not statistically significant (P(T<=t) value of 0.10274). Considering the overall results for *Bulinus truncatus* and the control group using ANOVA, the difference were found to be statistically insignificant ($p< 0.73135$).

TABLE 3.14 RATE OF INCREASE IN REPRODUCTION OF *M. TUBERCULATA* USING HEAT TREATED SANDY GRAVEL AS SEDIMENTS

GROUP	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7
CM	0	0	0	0	25	41.7	58.3	75
M5B15 - M	0	0	0	0	50	75	0	-75
M10B10 - M	0	0	25	0	0	50	-75	85
M15B5 - M	0	0	0	0	50	0	-25	75

TABLE 3.15 RATE OF INCREASE IN REPRODUCTION OF *B. TRUNCATUS* USING HEAT TREATED SANDY GRAVEL AS SEDIMENTS

GROUP	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7
CB	0	52.65	35.55	-34.65	6.975	-29.025	-11.3	-3.775
M5B15 - B	0	49.275	3.6	-6.9	1.05	-21.375	-6.3	8.325
M10B10 - B	0	28.35	15.75	-15.975	8.55	-12.375	-5.725	-0.8
M15B5 - B	0	22.825	9.125	-4.5	2.025	-13.725	-2.325	-4.425

TABLE 3.16 RATE OF INCREASE IN SHELL LENGTH (CM) OF *M. TUBERCULATA* USING HEAT TREATED SANDY GRAVEL AS SEDIMENTS

GROUP	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7
CM	0	6.75	1.75	4.5	6.25	4.5	0.75	1
M5B15 - M	0	-0.5	-2.25	9.25	3	7	1.75	0.75
M10B10 - M	0	5	7.75	1.25	4.25	5.5	0.5	1.5
M15B5 - M	0	2	1.75	3.75	10.25	2.25	4.25	3

TABLE 3.17 RATE OF INCREASE IN SHELL LENGTH (CM) OF *B. TRUNCATUS* USING HEAT TREAED SANDY GRAVEL AS SEDIMENTS

GROUP	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7
CB	0	0	7.5	2.25	2.75	2.25	1	3.5
M5B15 - B	0	-10.5	3.75	1.25	0	4	2.75	2.5
M10B10 - B	0	-1	8	4.5	1	1.5	1.75	0.25
M15B5 - B	0	4.75	4	4	3	3.25	1.5	1.75

3.3.2.2 Experimental Combination M10B10 :

3.3.2.2.1 *Weight :*

Table 3.11 and Figure 3.42 show the results of the rate of increase in weight of *Bulinus truncatus* in this experimental combination. The results show a considerable rise in the rate of weight increase of *Bulinus truncatus* in weeks 1, 4 and 6 above the rate recorded for the control group. This increase was high in weeks 1 and 6 (31.52% and 36.2% respectively). For the rest of the weeks (2, 3, 5 and 7) the rate of weight increase of *Bulinus truncatus* in the experimental cages were below that of the control group with the lowest drop (-18.93%) recorded in week 2. These observed differences however were not statistically significant ($p< 0.69414$).

The rate of increase in weight of *Melanoides tuberculata* was influenced by that of *Bulinus truncatus* as shown in Figure 3.46. From weeks 2 to 7, the rate for *Melanoides tuberculata* in this experimental group decreased below that of the control group. This difference was found to be statistically significant (P(T<=t) value of 0.01074).

3.3.2.2.2 *Mortality :*

Table 3.13 and Figure 3.43 show the results of the rate of mortality increase of *Bulinus truncatus* in this experimental combination. The mortality rate increased during weeks 1 and week 6 (30 and 25% respectively) above the control while in week 2, 3, 4 and 5, the rates dropped below that of the control group, the highest decrease was recorded in week 7 (-80%).

GRAPHS FOR COMBINATION M10B10 (*B. TRUNCATUS*)

USING HEAT TREATED SANDY GRAVEL AS SEDIMENTS.

FIG. 3.42

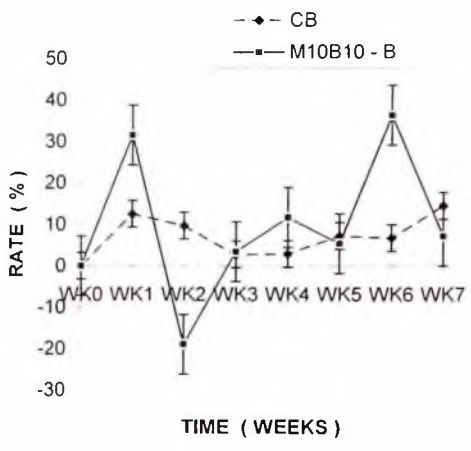
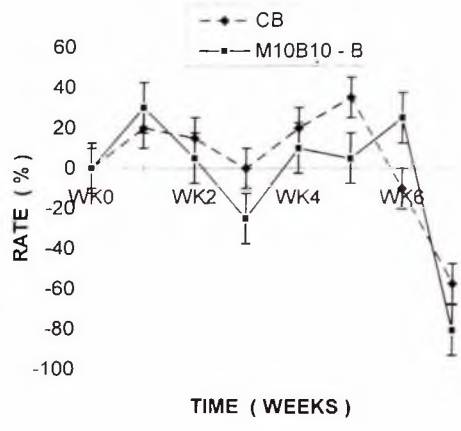


FIG. 3.43



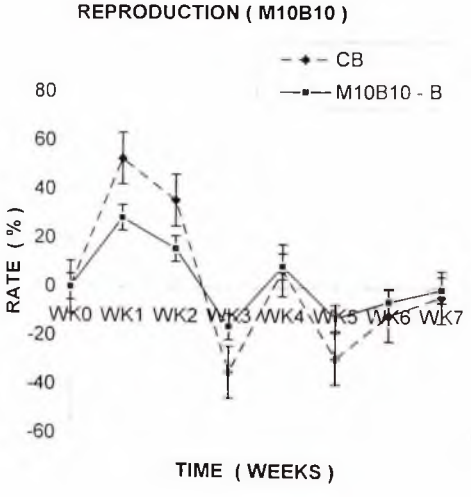


FIG. 3.44

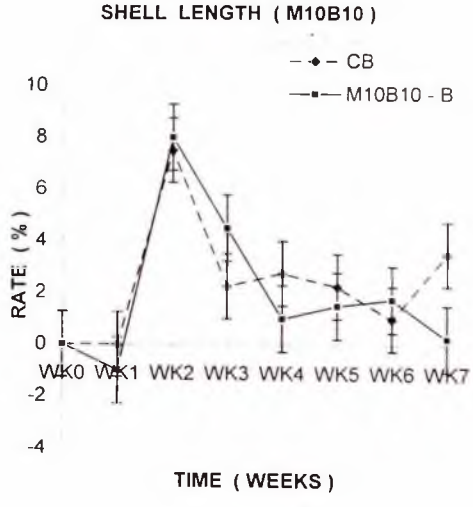


FIG. 3.45

GRAPHS FOR COMBINATION M10B10 (*M. TUBERCULATA*)

USING HEAT TREATED SANDY GRAVEL AS SEDIMENTS.

FIG. 3.46

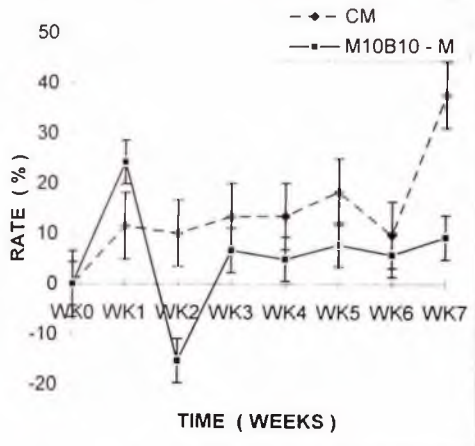
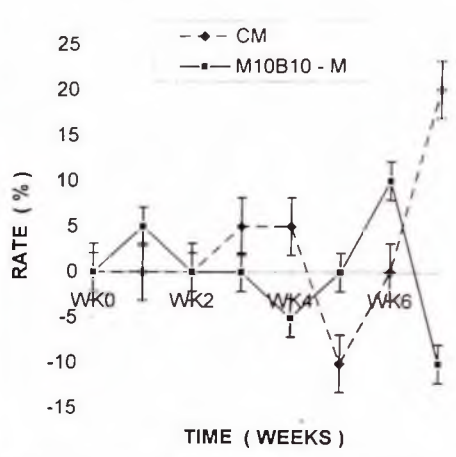


FIG. 3.47



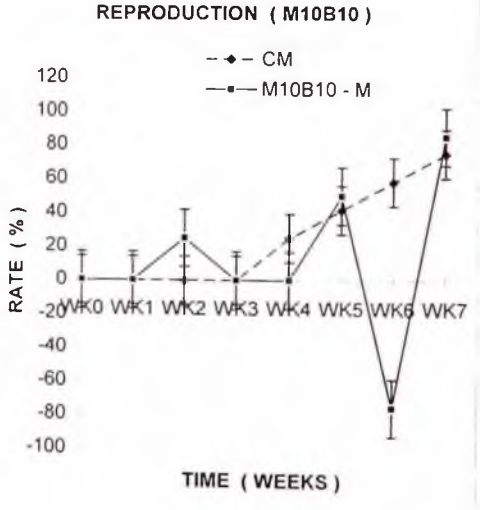


FIG. 3.48

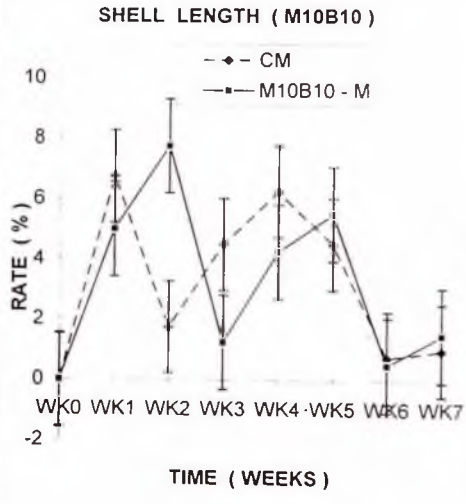


FIG. 3.49

T-test analysis of the results of weeks 2 to 5 where most of the differences occurred between the mortality rate increase of *Bulinus truncatus* and the control group, gave a significant difference (P(T<=t) value of 0.01790). However the differences over the entire period of the experiment were found to be statistically insignificant (P-value of 0.41180).

3.3.2.2.3 *Reproduction :*

Table 3.16 and Figure 3.44 show the results of the rate of reproduction of *Bulinus truncatus* in this experimental combination. These results show that apart from weeks 1 and 2 where the rate (28.35 and 15.75% respectively) was lower than the rate for the control (52.65 and 35.55% respectively), the rate of reproduction of *Bulinus truncatus* in this experimental combination increased above that of the control group during the duration of the experiment. Comparing the overall results for *Bulinus truncatus* and the control group for the entire period of the experiment using ANOVA, no statistically significant differences were found ($p < 0.97607$).

3.3.2.2.4 *Shell length :*

Table 3.17 and Figure 3.45 show the results of the rate of increase in shell length of *Bulinus truncatus* in this experimental combination. The results show that in weeks 1, 4, 5 and 7 the rate of increase in shell length of *Bulinus truncatus* decreased below that of the control group. The lowest decrease of -1% was recorded in week 1. For the remaining weeks 2, 3 and 6 the rate increased above that of the control group. The highest increase (4.5%) above that of the control was recorded in week 3. The rates of shell length increase of *Bulinus truncatus* and

that of the control group as shown in the graph (Figure 3.45) did not show any clear differences which needed to be analysed in segments. When the overall results for *Bulinus truncatus* and the control group were compared using ANOVA, no statistically significant differences were found ($p< 0.51585$).

3.3.2.3 Experimental Combination M15B5 :

3.3.2.3.1 *Weight :*

Table 3.11 and Figure 3.50 show the results of the rate of increase in weight of *Bulinus truncatus* in this experimental combination. From the results it was noticed that with the exception of week 1 where the rate of weight of *Bulinus truncatus* for this experimental combination (24.21%) increased above the rate of the control group (12.52%), the rate remained below that of the control group for the rest of the period of the experiment. These differences were however not found to be statistically significant (ANOVA : $p< 0.06741$).

The rate of increase in weight of *Melanoides tuberculata* (Figure 3.54) did not seem to have been influenced to any statistically significant extent by that of *Bulinus truncatus* (P(T<=t) value of 0.09957).

3.3.2.3.2 *Mortality :*

Table 3.13 and Figure 3.51 show the results of the rate of mortality of *Bulinus truncatus* in this experimental combination. The results show some fluctuations in the rate of mortality of *Bulinus truncatus*. Thus during four out of the seven weeks ie weeks (1, 3, 6, and 7) the rate of mortality of this experimental combination went above that of the control group, the

GRAPHS FOR COMBINATION M15B5 (*B. TRUNCATUS*)

USING HEAT TREATED SANDY GRAVEL AS SEDIMENTS.

FIG. 3.50

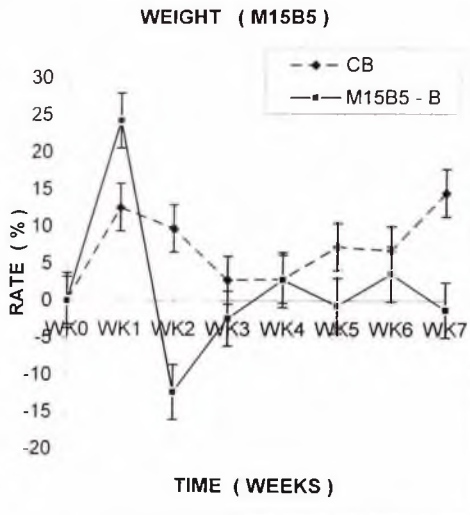
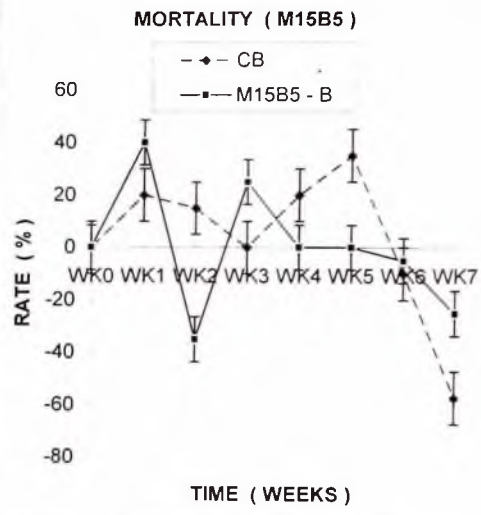


FIG. 3.51



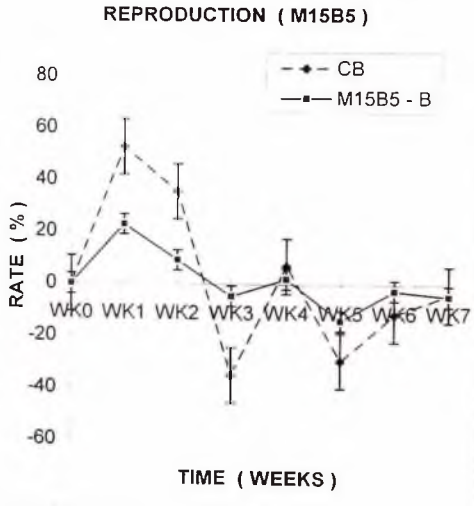


FIG. 3.52

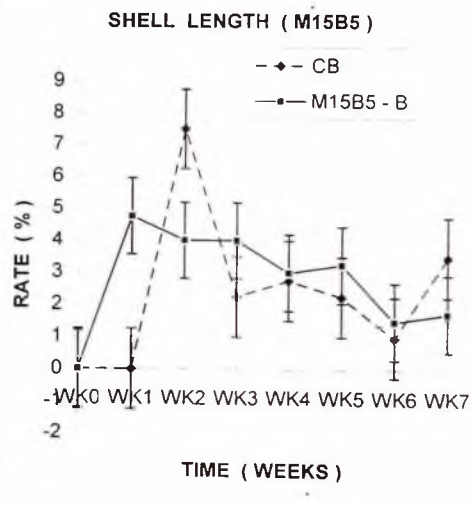


FIG. 3.53

GRAPHS FOR COMBINATION M15B5 (*M. TUBERCULATA*)

USING HEAT TREATED SANDY GRAVEL AS SEDIMENTS.

FIG. 3.54

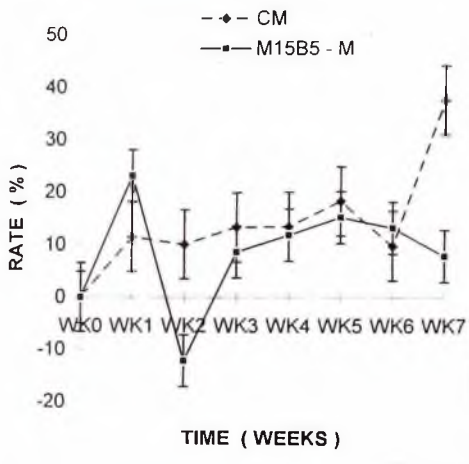
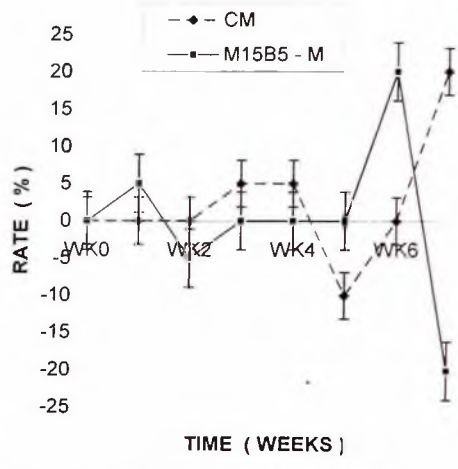


FIG. 3.55



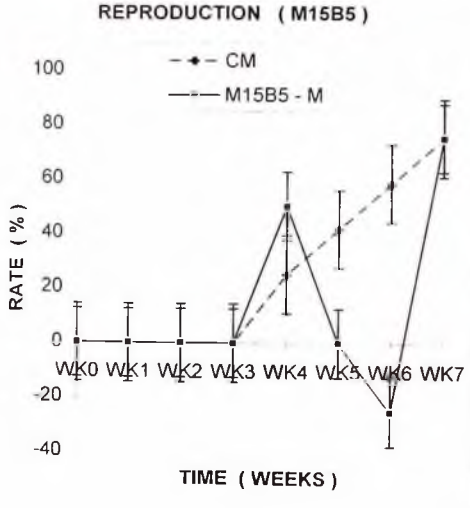


FIG. 3.56

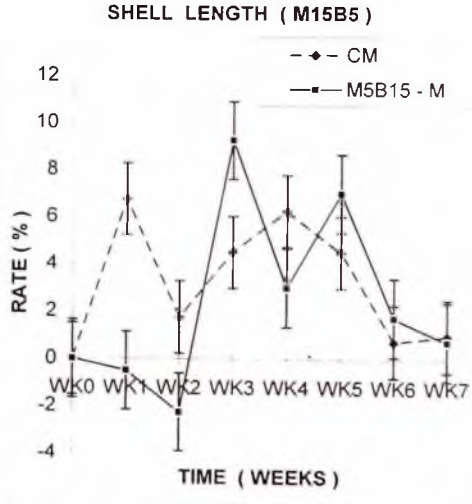


FIG. 3.57

highest increase was found in week 3 (40%), while the reverse was found in weeks 2, 4 and 5. Considering the overall results for *Bulinus truncatus* and the control group using ANOVA, the differences observed were found to be insignificant ($p < 0.79146$).

3.3.2.3.3 *Reproduction :*

Table 3.15 and Figure 3.52 show the results of the rate of reproduction of *Bulinus truncatus* in this experimental combination. From the results it was noticed that in weeks 1, 2 and 4, the rate of reproduction (22.83, 9.13 and 2.03% respectively) of *Bulinus truncatus* in this experimental combination decreased below that of the control group (52.65, 35.55, and 6.97% respectively). During the rest of the experimental period, the rate of reproduction of *Bulinus truncatus* increased above that of the control. However the results of the first two weeks and also weeks 4 to 7 where most of the differences occurred between the rate of *Bulinus truncatus* and the control group as shown in the graph were found to be statistically insignificant (P(T<=t) value of 0.09250 and 0.19180 respectively).

The rate of increase in reproduction of *Melanoides tuberculata* (Figure 3.56) did not seem to have been influenced by that of *Bulinus truncatus* (P(T<=t) value of 0.11271).

3.3.2.3.4 *Shell length :*

Table 3.17 and Figure 3.53 show the results of the rate of increase in shell length of *Bulinus truncatus* in this experimental combination . The results show that for five out of the seven weeks (1, 3, 4, 5 and 6) of the experiment, the rate of increase of shell length of *Bulinus truncatus* in this experimental combination went above that of the control group.

Calculated t-test based on the results of weeks 3 to 6 where most of the changes occurred between the rate *Bulinus truncatus* and the control group gave a one-tail P(T<=t) value of 0.03864. This means the differences observed between the rates as shown in the graph, during this period are statistically significant. Considering the overall results for *Bulinus truncatus* and the control group as shown in Figure 3.65 using ANOVA, a P-value of 0.67486 was obtained meaning the differences observed between the rate are insignificant.

The rate of increase in shell length of *Melanoides tuberculata* (Figure 3.57) was not influenced by that of *Bulinus truncatus* to any statistically significant extent (P(T<=t) value of 0.10779).

3.3.2.4 Density dependent effects :

3.3.2.4.1 ***Weight :***

From Table 3.18 and Figures 3.59 and 3.66, no statistically significant difference was observed in the results of the various combinations. Thus *Melanoides tuberculata* in all the combinations M15B5 (p<0.92501), M10B10 (p< 0.69414) and M5B15 (p< 0.73135) did not influence the rate of increase in weight of *Bulinus truncatus* differently. However it was observed (Figures 3.58) that between weeks 2 and 5 the rate of *Melanoides tuberculata* was influenced significantly by the increase in the density of *Bulinus truncatus* in combinations M5B15 (P (T<=t) value of 0.02136) and M10B10 (P (T<=t) value of 0.01074).

3.3.2.4.2 ***Mortality :***

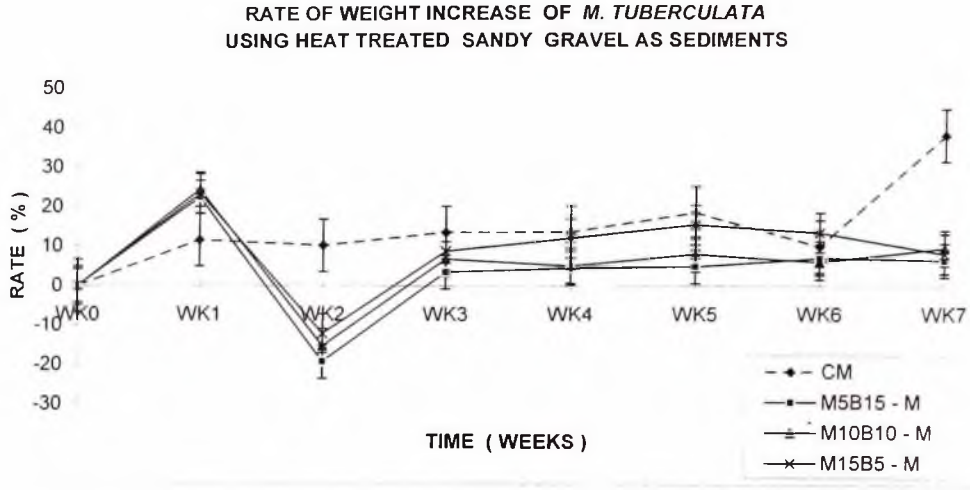
From Table 3.18 and Figures 3.61 and 3.66, it could be observed that, the density of *Melanoides tuberculata* did not have any significant effect on the mortality of *Bulinus truncatus* in the various combinations (M5B15 (p< 0.73135), M10B10 (p< 0.41180) and M15B5 (p< 0.79145)

Table. 3.18 ANOVA : TWO - FACTOR WITHOUT REPLICATION FOR .

B. TRUNCATUS USING HEAT TREATED SANDY GRAVEL AS SEDIMENTS

Combinations	Weight	Mortality	reproduction	Shell Length
M5B15B	0.92501	0.73135	0.82292	0.20928
M10B10B	0.69414	0.41180	0.97607	0.51585
M15B5B	0.73135	0.79145	0.89978	0.67486

FIG. 3.58



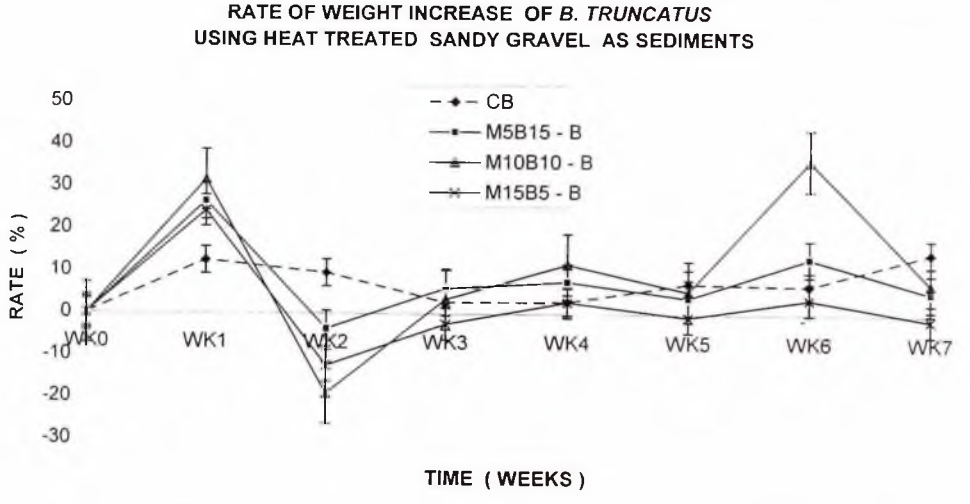
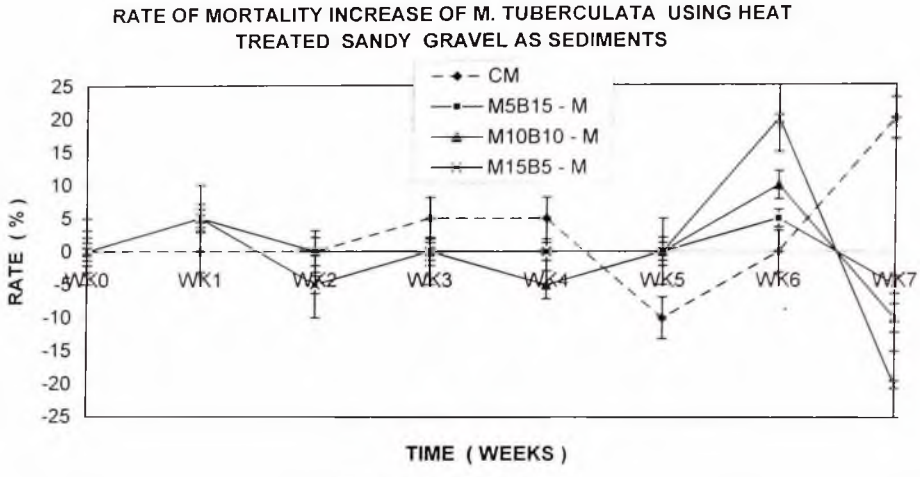


FIG. 3.59

FIG. 3.60



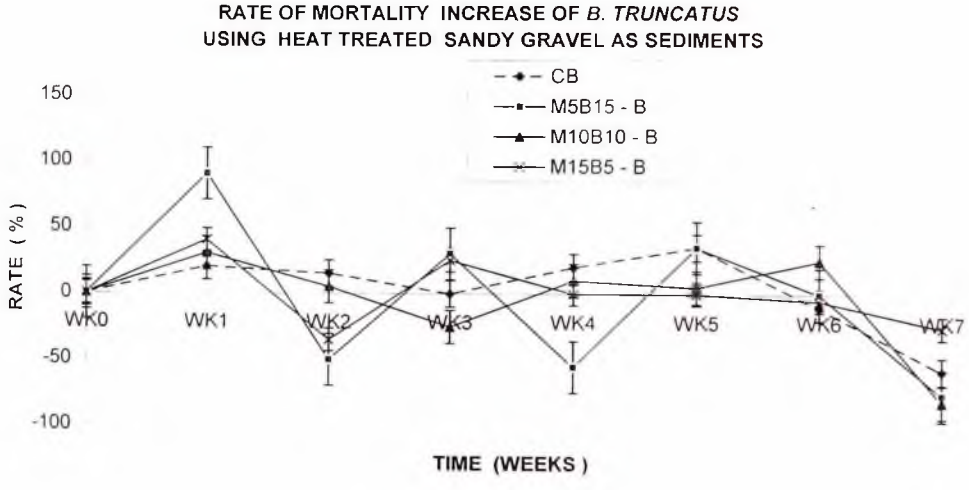


FIG. 3.61

3.3.2.4.3 *Reproduction :*

From Table 3.18 and Figures 3.63 and 3.66, no significant effect was exerted by the increase in the density of *Melanoides tuberculata* on the rate of increase of reproduction of *Bulinus truncatus* in the various combinations (M5B15 ($p< 0.82292$), M10B10 ($p< 0.97607$) and M15B5 ($p< 0.89978$).

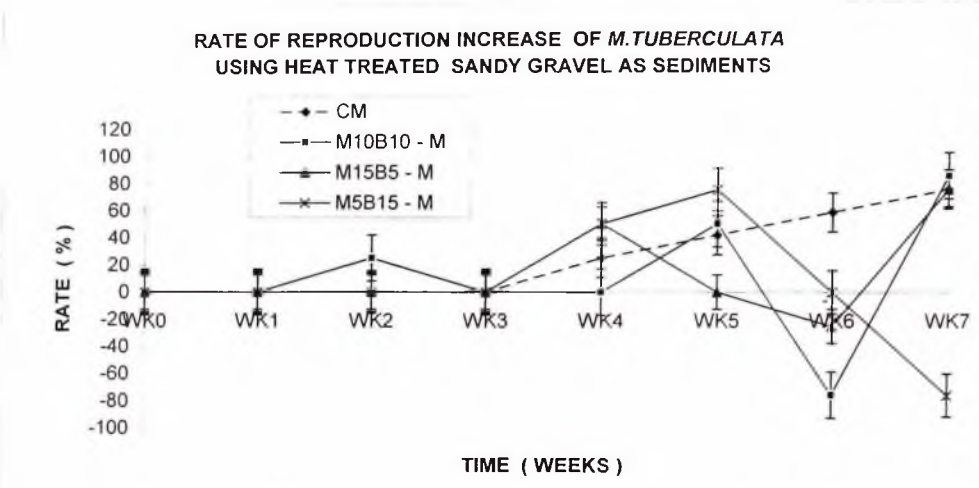
3.3.2.4.4 *Shell Length :*

From Table 3.18 and Figures 3.65 and 3.66, the observations made were similar to that made for mortality and reproduction. Thus the increase in the density of *Melanoides tuberculata* has no effect on the rate of increase of shell length of *Bulinus truncatus* in the various combinations (M5B15 ($p< 0.20928$), M10B10 ($p< 0.51585$) and M15B5 ($p< 0.67486$

3.4 DISCUSSION :

The present investigations were undertaken to test two hypotheses, firstly that *Melanoides tuberculata* would be at a competitive advantage over *Bulinus truncatus* and replace them in freshwater systems. Secondly, that sediment *type* and *condition* can affect the ecological interactions of snails and thus influence the ability of *Melanoides tuberculata* to act as a biological agent. The results of the experiment are therefore discussed in relation to these two hypotheses.

FIG. 3.62



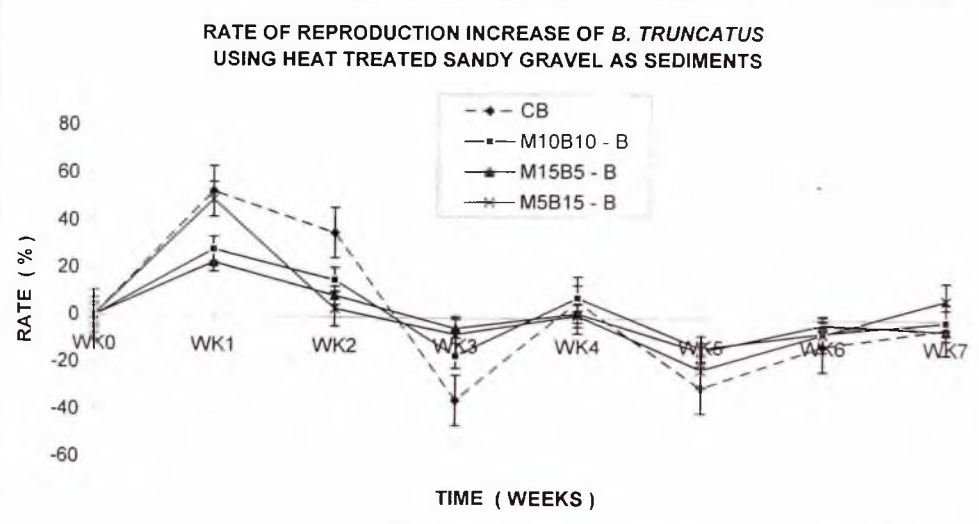
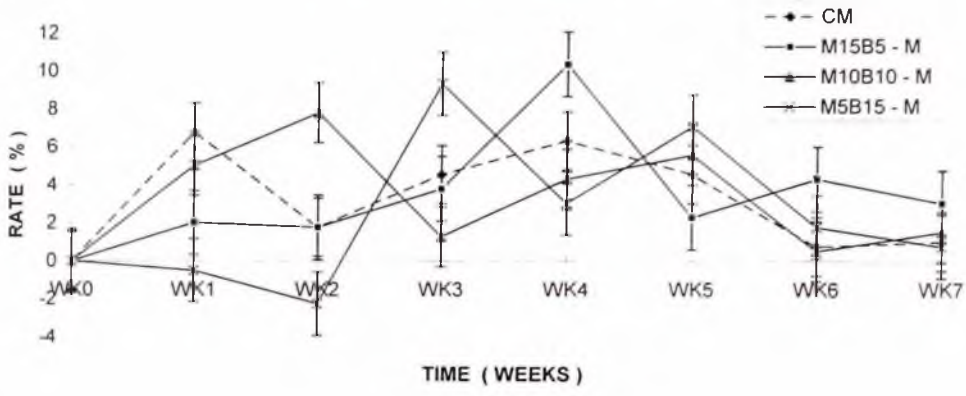


FIG. 3.64

RATE OF SHELL LENGTH INCREASE OF *M. TUBERCULATA*
USING HEAT TREATED SANDY GRAVEL AS SEDIMENTS



RATE OF SHELL LENGTH INCREASE OF *B. TRUNCATUS*
USING HEAT TREATED SANDY GRAVEL AS SEDIMENTS

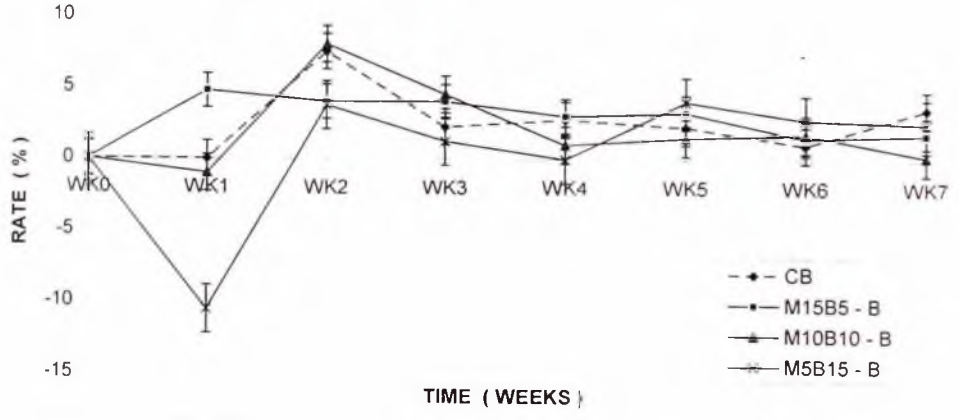
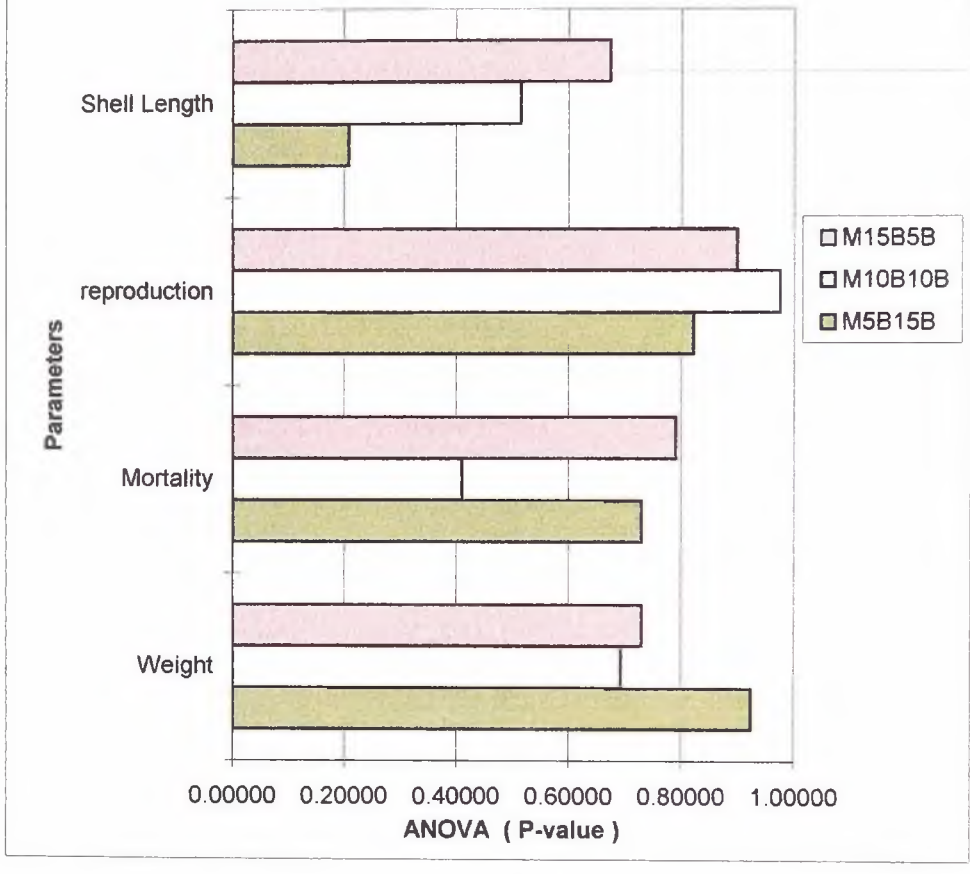


FIG. 3.65

Fig. 3.66

Graphical presentation of the statistical analysis of the results for *Bulinus truncatus* using heat treated sandy gravel as sediment



3.4.1 The effect of *Melanoides tuberculata* on *Bulinus truncatus* :

The results show that for all the various combinations, mortality rate, reproduction rate, and the rate of shell length increase of *Bulinus truncatus* were not affected to any significant extent by the presence or densities of *Melanoides tuberculata*. However when changes in weight was used as the indicator of growth, it was discovered that the growth rate of *Bulinus truncatus* in the presence of *Melanoides tuberculata* was significantly lower than under control conditions. Furthermore the severity of the effect of *Melanoides tuberculata* on *Bulinus truncatus* increased as the density of *Melanoides tuberculata* increased. This density dependent effect can be arranged as follows : M5B15 (0.081.9) < M10B10 (0.01740) < M15B5 (0.00632). These findings contrast with the observations made in section B, where heat treated gravel was used as the sediment. With heat treated gravel *Melanoides tuberculata* was not found to exert any significant effect on the growth rate of *Bulinus truncatus*.

It has been shown that the competitive performance of *Melanoides tuberculata* changes that under different ecological conditions. Thus Pointier (1993), found different impacts of *Melanoides tuberculata* on *Biomphalaria glabrata* in different water bodies which had different ecological conditions. They found out that in water- cress beds more than in cattle ponds, *Melanoides tuberculata* was able to cause the disappearance of the schistosomiasis host snails. Although two factors, ie physico-chemical stability and homogeneity of habitat, were suggested as enhancing the competitive ability of *Melanoides tuberculata* in water-cress beds, the exact nature of the underlying factor (s) has not been identified.

The results of the present work suggest that the condition of the sediment may be critical in determining the outcome of the competitive interaction. The process of heat treating the sediments would have had at least two major effects on the sediments. Firstly, the organic component must have been destroyed. Secondly, the chemical condition of the particles might have been changed. These two factors could probably account for the drastic effects of this process on the competitive advantage of the *Melanoides tuberculata* snails over the *Bulinus truncatus* snails.

Under normal field conditions sediments are known to be covered with epilithic algae (Round, 1981), micro algae, bacteria, fungi, organic matter of vegetable or animal origin (Pointier and McCullough, 1989), which act as important food items for aquatic snails. *Melanoides tuberculata* snails are mainly bottom dwellers and are therefore more likely to be dependent on this food source than *Bulinus truncatus* snails which tend to feed more on epiphytic algae and decaying macrophytes. (Thomas and Tait, 1984; Kpikpi, 1990). Consequently damage to the food source in the sediments (through heat treatment) had a much more adverse effect on the competitive ability of *Melanoides tuberculata* than on *Bulinus truncatus*.

It is also possible that the chemical condition of the particles of the gravel could have been altered through the heat treatment. If this is the case then the uptake of essential cations eg Ca^{2+} and traces of copper which are known to be assisted by ingestion of sand grains would also be impaired. This can reduce the growth rate and consequent competitive ability of *Melanoides tuberculata*. It would be of considerable interest to examine the nature of the

epilithic community on the surface of both the heat treated and the normal sediment to unearth the main differences which can account for the observed differences in the competitive performance of *Melanoides tuberculata* under the two conditions.

Based on the investigations conducted by Mkoji et al., (1992) in Kenya where *Melanoides tuberculata* was found to co-exist with *Biomphalaria pfeifferi* in some water bodies, these workers concluded that *Melanoides tuberculata* was not capable of totally displacing populations of *Biomphalaria pfeifferi* or other pulmonate snails. Pointier et al., (1989) on the other hand showed that *Melanoides tuberculata* successfully eliminated *Biomphalaria glabrata* in several locations through competitive displacement. Mkoji et al., (1992) indicated that one possible explanation for their observations in Africa was that *Melanoides* and the indigenous pulmonates have lived together for a very long period of time and have evolved towards co-existence. Secondly, they also suggested that the habitat investigated by them were not ideal for total displacement of pulmonates by *Melanoides tuberculata.*

Unfortunately, they did not study the sediment characteristics to find out what impact any differences observed might have had on the interactions between the two snail species studied. It is possible that differences in the nature and condition of the sediment in these habitats might have greatly influenced the results of their investigations. These present observation indicates that, at least in closed systems, *Melanoides tuberculata* given the right nature and condition of sediments can repress the growth rate of *Bulinus truncatus*. Therefore the co-evolutionary theory proposed by Mkoji et al., (1992) may not be the reason for the co-existence of the two snail species.

The results of the present experiment seem to suggest that under such conditions (ie in cases where the sediments are more gravel-like and rich in epilithic micro-organisms) *Melanoides tuberculata* can depress the growth rate of *Bulinus truncatus* although the mortality and reproduction rates of *Bulinus truncatus* are not equally affected. It is possible that, if the experiment had been run for a much longer period, a wider range of effects of *Melanoides tuberculata* on *Bulinus truncatus* would have been discovered.

3.4.2 The effect of *Bulinus truncatus* on *Melanoides tuberculata* :

The weight gains and reproductive rates of *Melanoides tuberculata* were found to have been repressed in the various experimental combinations. For example, compared with controls, the rate of weight increase of *Melanoides tuberculata* was lowered significantly for the entire 7 week period of the experiment in experimental combination M5B15 ($P(T<=t)=0.01878$). In the case of the experimental combination M10B10, the reduction in the rate of growth was noticeable in 6 out of 7 weeks while similar effects were observed over only a two week period in the combination with the least number of *Bulinus truncatus* competitor snails ie M15B5. It is of interest to find that *Melanoides tuberculata* snails not only fail to repress growth, mortality, and reproduction of *Bulinus truncatus* snails under unfavourable sediment conditions, these non-host are actually adversely affected by the *Bulinus truncatus* snails.

This finding shows that the competitive interactions work both ways and observed effects must be the end results of complex interactions between the two species. In this case the condition of the sediment used has been shown to be significant in determining which species 'wins' the interaction. It would be of interest to investigate the possible factors such as the presence or absence of aquatic macrophytes and also the effect of water current on the competitive interaction since flow rate is known to influence host snail distribution (Appleton,1978).

CHAPTER 4

COMPETITION BETWEEN *MELANOIDES TUBERCULATA* AND *BULINUS TRUNCATUS* USING NORMAL AND HEAT TREATED SANDY CLAY LOAM AS SEDIMENT.

4.1 INTRODUCTION :

The first part of this study which is concerned with investigating the competitive potential of *Melanoides tuberculata* has revealed two things. First of all, that *Melanoides tuberculata* can exert a negative effect on the growth rate of *Bulinus truncatus*, a snail intermediate host of *Schistosoma haematobium*. This finding has also been reported by Thomas and Tait (1984) and Pointier et al. (1989). Secondly, it has been shown that the competitive ability of *Melanoides tuberculata* depends to a considerable extent on the condition of the sediment used. Thus not only was *Melanoides tuberculata* unable to repress the growth rate of *Bulinus truncatus* when heat treated sediments were used, its growth rate was adversely affected by *Bulinus truncatus*.

This observation that the nature of the competitive interactions between freshwater snails can be influenced by the nature of the environment has also been recorded by Thomas and Tait (1984), although the precise elements of importance in the environment have not been identified up till now. Having identified the importance of the condition of a particular type of sediment ie sandy gravel, it was considered useful to investigate a different kind of sediment, ie sandy clay loam. In this chapter, as in the previous one the sediment is investigated in two forms ie as Normal sediment and as Heat treated sediment. The findings of this experiment are reported in this chapter.

4.2 MATERIALS AND METHODS

4.2.1 The snails :

The two snail species used for this experiment also originated from the Weija Lake as those used for the experiments in chapters 3. *Bulinus truncatus* snails were from the cultures established in the laboratory for four months, the *Melanoides tuberculata* snails however, were obtained from the snails acclimatized in the laboratory for seven days prior to the experimentation. For the experiment using normal sediments the *Bulinus truncatus* snails used were about four weeks old and had an average shell length ± SD between 0.513 ± 0.045cm and 0.665 ± 0.0480cm and an average weight ± SD between 0.048 ± 0.007g and 0.088 ± 0.012g. The *M. tuberculata* snails were about 7 - 8 weeks old according to the demographic studies done by Hicklin (1988). Their average shell length ± SD ranged between 1.660 ± 0.059cm and 1.730 ± 0.013cm their average weight ± SD between 0.234 ± 0.002 and 0.261 ± 0.013g.

For the experiment using the heat treated sediments the *B. truncatus* snails used were also about four weeks old and had an average shell length between 0.535 ± 0.131 cm and 0.615 ± 0.013cm and an average weight between 0.043 ± 0.007g and 0.091 ± 0.005g. The *M. tuberculata* snails were about 7 - 8 weeks old and had an average shell length ranging between 1.570 ± 0.063cm and 1.610 ± 0.081cm and average weight ranging between 0.119 ± 0.018 and 0.22 ± 0.007g.

4.2.2 Experimental cages :

The cages used were similar and had the same dimensions as those used for experiments one and two in chapters 3. These cages were also made from the same material [transparent plastic spring water bottles (Astek INSU bottles)] and had the same shape and size as those used in experiments one and two. The design and treatment were as the cages used in experiment one to ensure a uniform physico - chemical environment and free streaming of water in each of the cages. The snails were introduced into their respective labelled cages and pieces of the net used in building the cages were fastened to the cages by means of a rubber band to prevent the snails from escaping as was done in chapter 3.

4.2.3 Preparation of sediments :

The sediment used was collected from Weija Lake as in the previous experiments (in chapter 3) but from a different site in the lake. The sediment ie sandy clay loam was used in two conditions ie Normal condition and Heat treated condition.

4.2.3.1 *Normal sediments* :

The sediment used was collected from the same site as the snails and was washed to remove some of the debris. This was then stored in clean bottles in the laboratory from where it was used for the experiment. This sediment had a composition of 68.2% sand with a particle size between 0.05 - 2mm, 26.9% clay with particle size <0.002mm and 5.0% silt with particle size of 0.002 - 0.05mm according to the United States Department of Agriculture (USDA) Classification (Marshall and Holmes, 1992).

4.2.3.2 *Heat treated sediments :*

This sediment was heat treated as in the case of the sediment used in the previous experiment using the same procedure. The sediment was then cooled and stored in clean bottles in the laboratory from where it was used for the experiment. This sediment had the same composition as the normal sediments used in this chapter.

4.2.4 Experimental setup :

The materials and method used for the previous work using sandy gravel sediments was followed with no modifications except for the difference in sediments. Growth measurements of the snails were taken as was done in experiments one and two (in chapter 3) using the same methods. As in experiments one and two, twenty snails were introduced into each cage and four cages were used for each of the following combinations of the two snail species (ie number of *M .tuberculata* / number of *B. truncatus*) : 0/20, 5/15, 10/10, 15/5, 20/0 which were labelled accordingly. Aeration of the water was done by the use of an aspirator. The water was changed once every week after snail measurements have been taken and the experiment was maintained at a constant room temperature of 29 ± 1 °C. This was followed for seven weeks, at the end of each week however, the competitive interactions of the snails were assessed by the three indices (growth, mortality, and reproduction) used in the previous experiments. The pH and conductivity of the water were also measured twice every week at the start and termination of the week.

4.2.5 Definition of the various indices :

The different rates were calculated by using the same formulae as was done in the case of the experiments in chapter 3. The rate of weight increase was calculated by the formula (Wp1 - Wp2) X 100. Increase in rate of shell length was calculated by the formula (SLp1 - SLp2) X 100. The rate of mortality increase was calculated by the formula (Mp1 - Mp2) X 100. The rate of reproduction increase was calculated by the formula { (Rp1 - Rp2) X 100 }) 1000. The symbols used in these formulae are the same as indicated in experiment one in chapter 3. The error bars inserted onto the various graphs were based on standard error calculations.

4.3 RESULTS

The results are in two sections, the results obtained using the Normal sediments are in Section A and the results obtained using the Heat treated sediments are in Section B.

4.3.1 SECTION A : NORMAL SEDIMENTS

4.3.1.1 Experimental Group M5B15 :

4.3.1.1.1 *Weight* :

Table 4.2 and Figure 4.1 show that the rate of weight increase of *Bulinus truncatus* in this experimental combination decreased below that of the control group for 4 (ie weeks 3, 4, 6 and 7) out of the 7 weeks of the experiment. These differences were found to be statistically insignificant ($p < 0.56204$). The rate of weight increase of *Melanoides tuberculata* did not seem to have been influenced by the presence of *Bulinus truncatus* snails ($p < 0.09025$) (Figure 4.5).

TABLE 4.1 RATE OF INCREASE IN WEIGHT OF *M. TUBERCULATA* USING NORMAL SANDY CLAY LOAM AS SEDIMENTS

GROUP	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7
CM	0	7.225	17.325	5.125	3.925	3.625	11.5	6.4
M5B15 - M	0	7.175	6.025	4.05	2.675	1.6	1.45	6.275
M10B10 - M	0	0.425	8.075	3.875	2.425	12	14.1	8.6
M15B5 - M	0	-5.075	8.3	16.875	13.15	-11.525	5.7	13.85

TABLE 4.2 RATE OF INCREASE IN WEIGHT OF *B. TRUNCATUS* USING NORMAL SANDY CLAY LOAM AS SEDIMENTS

GROUP	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7
CB	0	2.75	9.8	21	7.025	10.975	17.875	9.725
M5B15 - B	0	4.975	16.125	18.725	5.4	13.5	7.85	3.7
M10B10 - B	0	5.2	4.325	14.425	-1.025	11.625	7.65	6.85
M15B5 - B	0	6.625	3.65	8.15	2.975	7.475	2.35	2.125

GRAPHS FOR COMBINATION M5B15 (*BULINUS TRUNCATUS*)

USING NORMAL SANDY CLAY LOAM AS SEDIMENTS

FIG. 4.1

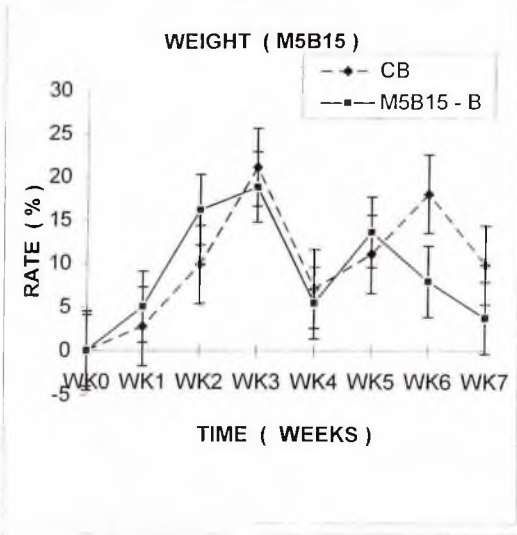
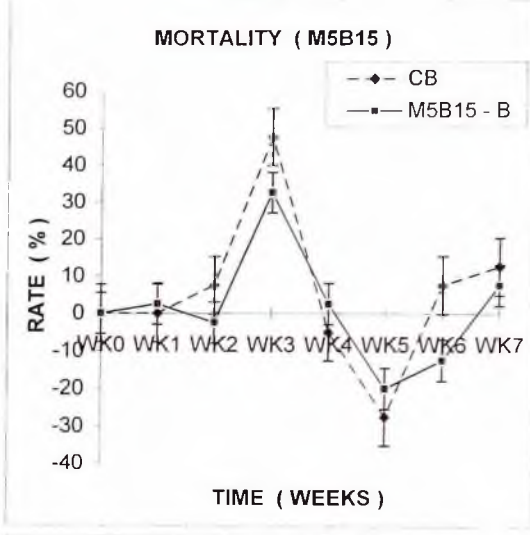


FIG. 4.2



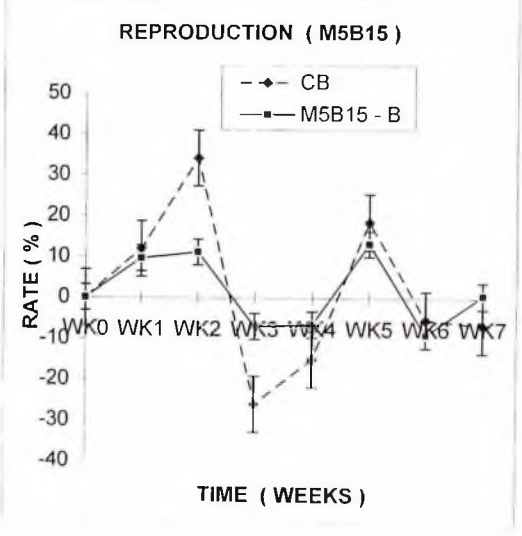


FIG. 4.3

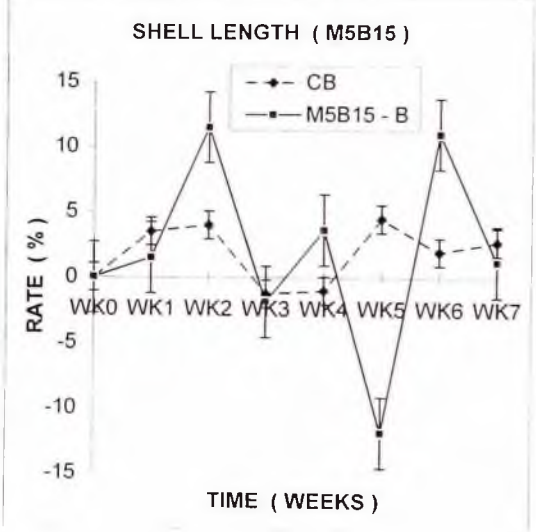


FIG. 4.4

4.3.1.1.2 *Mortality :*

From Table 4.4 and also Figure 4.2, it was observed that for 3 (ie week 1, 4 and 5) out of the 7 weeks of the experiment, the rate of mortality of *Bulinus truncatus* in the experimental cages increased above that of the control group and decreased for the remaining period. Analysis of the overall results of the 7 week period of the experiment showed that the differences are statistically insignificant ($p< 0.29964$).

4.3.1.1.3 *Reproduction :*

From Table 4.6 and also Figure 4.3, it was observed that for 4 (ie weeks 1,2, 5 and 6) out of the 7 weeks of the experiment, the rate of reproduction of *Bulinus truncatus* decreased below that of the control group. However these differences were not statistically significant ($p< 0.99164$). The rate of increase in reproduction of *Melanoides tuberculata* (Fig. 4.7) was not influenced to a statistically significant extent by the presence of *Bulinus truncatus* (P(T<=t) value of 0.49582).

4.3.1.1.4 *Shell Length :*

Table 4.8 and Figure 4.4 show fluctuations in the rate of shell length increase in *Bulinus truncatus* throughout the period of the experiment. For 3 (ie weeks 2, 4 and 6) out of the 7 weeks, the rate of increase in shell length of *Bulinus truncatus* in this experimental combination increased above that of the control group. Thus for most of the period of the experiment the rate of shell length of *Bulinus truncatus* was suppressed. Analysis showed that these differences were not statistically insignificant ($p< 0.97398$)

TABLE 4.3 RATE OF INCREASE IN MORTALITY OF *M. TUBERCULATA* USING NIORMAL SANDY CLAY LOAM AS SEDIMENTS

GROUP	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7
CM	0	2.5	5	0	0	-2.5	2.5	0
M5B15 - M	0	5	-2.5	2.5	0	-2.5	2.5	-2.5
M10B10 - M	0	0	2.5	-2.5	0	0	2.5	5
M15B5 - M	0	5	-5	7.5	5	-7.5	2.5	0

TABLE 4.4 RATE OF INCREASE IN MORTALITY OF *B. TRUNCATUS* USING NORMAL SANDY CLAY LOAM AS SEDIMENTS

GROUP	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7
CB	0	0	7.5	47.5	-5	-27.5	7.5	12.5
M5B15 - B	0	2.5	-2.5	32.5	2.5	-20	-12.5	7.5
M10B10 - B	0	0	0	15	22.5	-20	-2.5	10
M15B5 - B	0	0	0	2.5	12.5	-5	-10	12.5

TABLE 4.5 RATE OF INCREASE IN REPRODUCTION OF *M. TUBERCULATA* USING NORMAL SANDY CLAY LOAM AS SEDIMENTS

GROUP	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7
CM	0	22.5	55	-25	5	12.5	22.5	30
M5B15 - M	0	5	40	-2.5	-32.5	15	0	10
M10B10 - M	0	12.5	32.5	27.5	37.5	-15	-7.5	-2.5
M15B5 - M	0	15	32.5	50	-17.5	-10	-7.5	-2.5

TABLE 4.6 RATE OF INCREASE IN REPRODUCTION OF *B. TRUNCATUS* USING NORMAL SANDY CLAY LOAM AS SEDIMENTS

GROUP	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7
CB	0	11.925	34.05	-25.75	-14.8	18.45	-5.25	-6.525
M5B15 - B	0	9.65	11.175	-6.875	-6.4	13.2	-8.85	0.575
M10B10 - B	0	9.15	31.55	-19.325	-9.175	7.9	-5.675	2.25
M15B5 - B	0	2.5	8.15	4.35	-10.675	9.475	-4.45	-1.875

GRAPHS FOR COMBINATION M5B15 (*MELANOIDES TUBERCULATA*)

USING NORMAL SANDY CLAY LOAM AS SEDIMENTS

FIG. 4.5

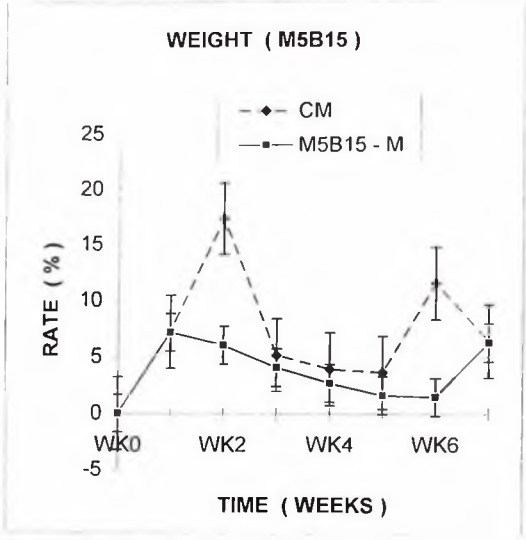


FIG. 4.6

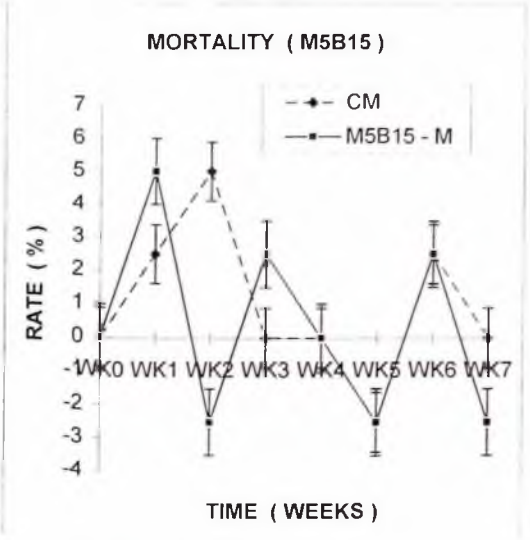


FIG. 4.7

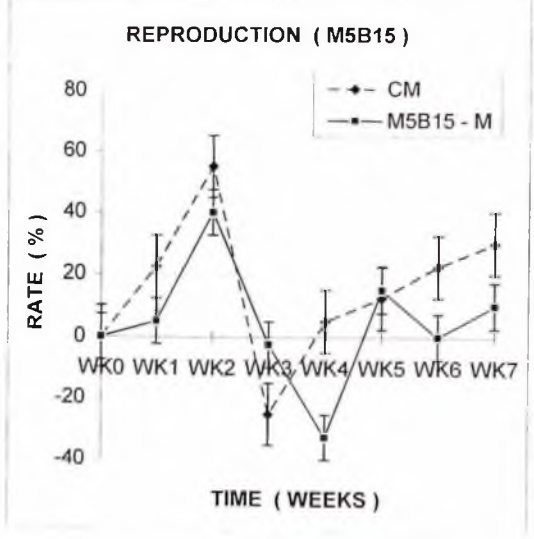


FIG. 4.8

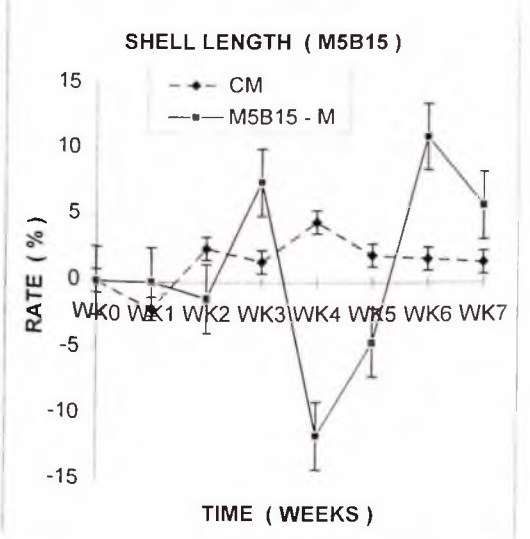


TABLE 4.7 RATE OF INCREASE IN SHELL LENGTH (CM) OF *M. TUBERCULATA* USING NORMAL SANDY CLAY LOAM AS SEDIMENTS

GROUP	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7
CM	0	-2	2.5	1.5	4.5	2	1.75	1.5
M5B15 - M	0	0	-1.25	7.5	-11.5	-4.5	11	5.75
M10B10 - M	0	-1.75	3	1.25	3.75	-3.75	5	2.25
M15B5 - M	0	1.75	0.0325	0.08	2	1.5	1.5	2.75

TABLE 4.8 RATE OF INCREASE IN SHELL LENGTH (CM) OF *B. TRUNCATUS* USING NORMAL SANDY CLAY LOAM AS SEDIMENTS

GROUP	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7
CB	0	3.5	4	-1.25	-1	4.5	2	2.75
M5B15 - B	0	1.5	11.5	-1.85	3.65	-11.8	11	1.25
M10B10 - B	0	5	6	-0.5	-2.5	-4.25	5.25	3.25
M15B5 - B	0	0	14	0	-1.25	3.5	4.25	2.5

4.3.1.2 Experimental Combination M10B10 :

4.3.1.2.1 *Weight* :

Table 4.2 and Figure 4.9 show that for 5 (ie weeks 2 to 4, 6 and 7) out of the 7 weeks of the experiment, the rate of weight gain of *Bulinus truncatus* in this experimental combination decreased below that of the control group. These differences were found to be statistically significant (P(T<=t) value of 0.00609) for weeks 2 to 4 of the experiment. Further analysis of the differences between the rate for *Bulinus truncatus* and the control group over the entire 7 week period (Figure 4.26) using ANOVA, was also found to be statistically significant ($p< 0.05136$).

The rate of weight increase of *Melanoides tuberculata* in this experimental combination seems to have been influenced by that of *Bulinus truncatus* during weeks 0 (ie the start of the experiment) to week 2 and also weeks 5 to 7 as shown in Figure 4.13. These differences however were found to be statistically insignificant (P(T<=t) value of 0.09642 and 0.07933 respectively).

4.3.1.2.2 *Mortality :*

Table 4.4 and Figure 4.10 show fluctuations in the rate of mortality of *Bulinus truncatus* in this experimental combination. In 2 (weeks 4 and 5) out of the 7 weeks of the experiment the rate of mortality of *Bulinus truncatus* increased above that of the control group. The highest increase was recorded in week 5 with a value of 25% . Thus for 5 of the weeks the rate of mortality of *Bulinus truncatus* was suppressed by that of the control group. Statistical analysis of the overall results showed that these differences were not statistically significant ($p< 0.72468$).

GRAPHS FOR COMBINATION M10B10 (*BULINUS TRUNCATUS*)

USING NORMAL SANDY CLAY LOAM AS SEDIMENTS

FIG. 4.9

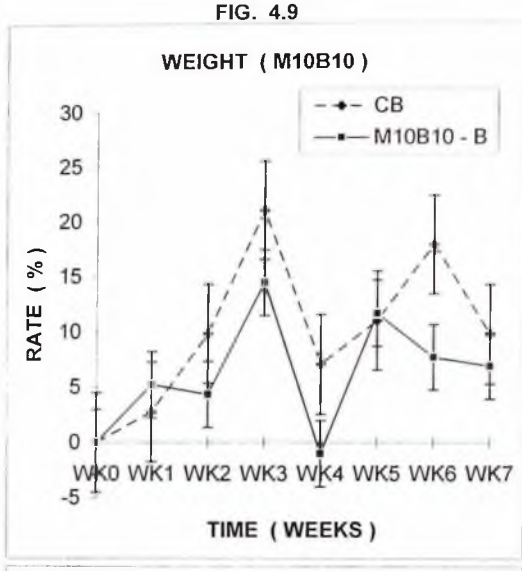


FIG. 4.10

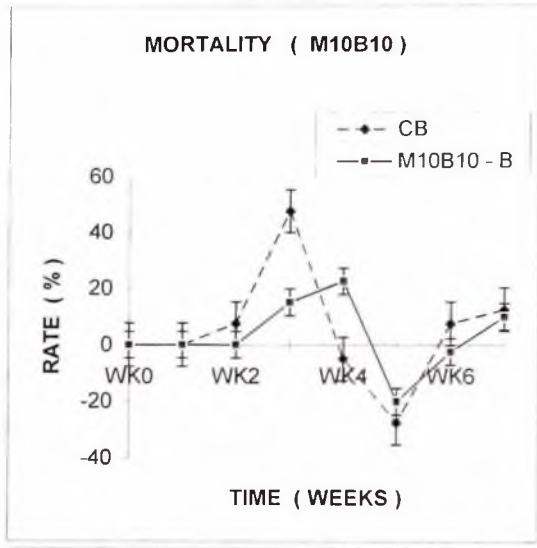


FIG. 4.11

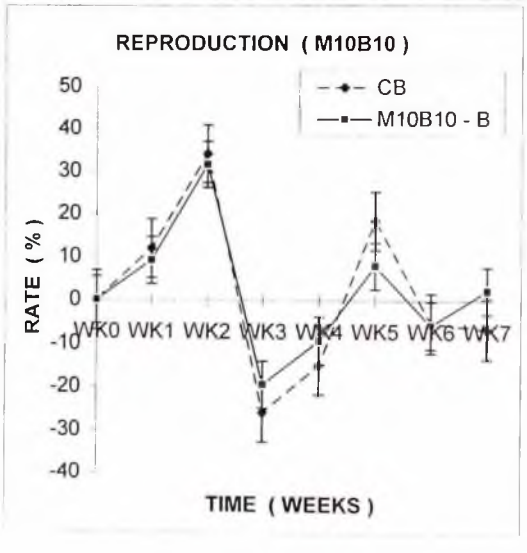
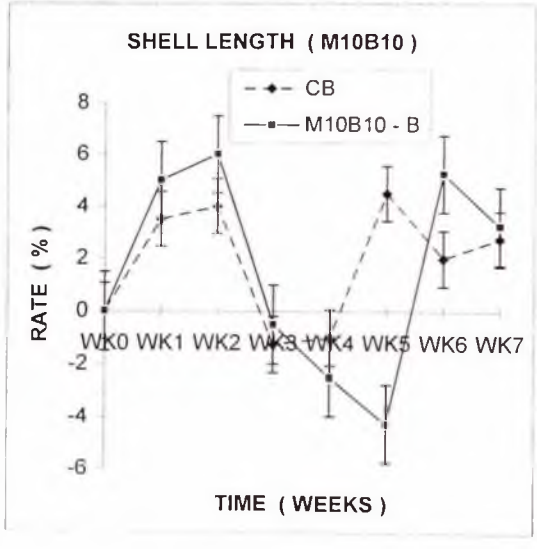


FIG. 4.12



GRAPHS FOR COMBINATION M10B10 (*MELANOIDES TUBERCULATA*)

USING NORMAL SANDY CLAY LOAM AS SEDIMENTS

FIG. 4.13

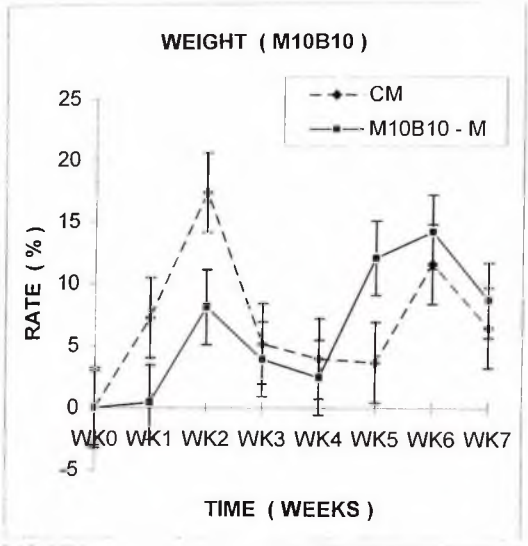


FIG. 4.14

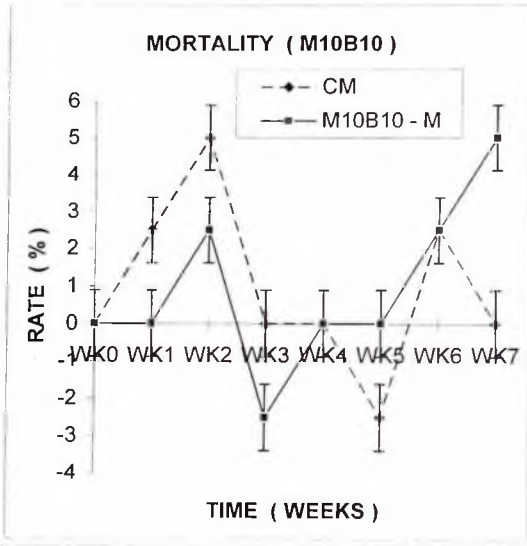


FIG. 4.15

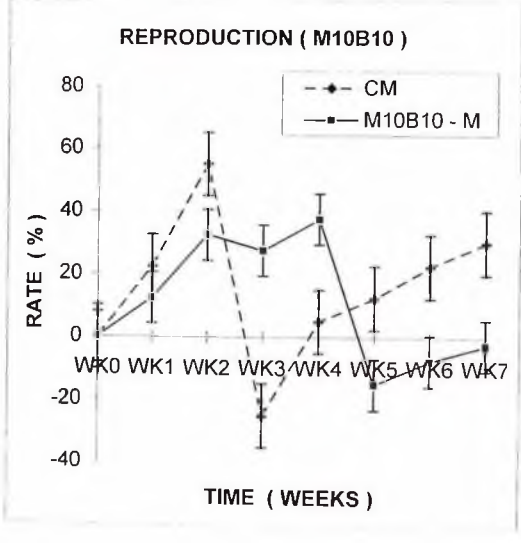
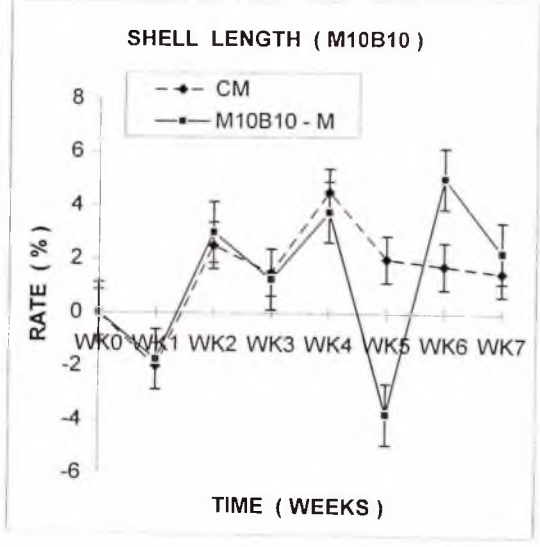


FIG. 4.16



The rate of mortality increase of *Melanoides tuberculata* in this experimental combination was influenced by the presence of *Bulinus truncatus* snails during the first three weeks as shown in Figure 4.14. These differences were found to be statistically significant (P(T<=t) value of 0.02883).

4.3.1.2.3 *Reproduction :*

Table 4.6 and Figure 4.11 show that for 3 (ie weeks 3, 4 and 7)out of the 7 weeks of the experiment the rate of reproduction of *Bulinus truncatus* in this combination increased above that of the control group. For the other 4 weeks the control group had a higher rate of reproduction than the *Bulinus truncatus*. Analysis of the overall showed that these differences were not statistically significant (p< 0.80299).

The rate of increase in reproduction of *Melanoides tuberculata* in this experimental combination seems to have been influenced by the presence of *Bulinus truncatus* during the last two weeks of the experiment (Figure 4.15). These differences were statistically significant (P(T<=t) value of 0.00115).

4.3.1.2.4 *Shell Length :*

Table 4.8 and Figure 4.12 show that for 5 (ie weeks 1, 2, 3, 6 and 7) out of the 7 weeks of the experiment, the rate of shell length increase of *Bulinus truncatus* increased above that of the control group. The differences observed during the first three weeks were found to be statistically significant (P (T<= t) value of 0.04472. For the entire period of the experiment, the differences were found to be statistically insignificant (p< 0.97398).

GRAPHS FOR COMBINATION M15B5 (*BULINUS TRUNCATUS*)

USING NORMAL SANDY CLAY LOAM AS SEDIMENTS

FIG. 4.17

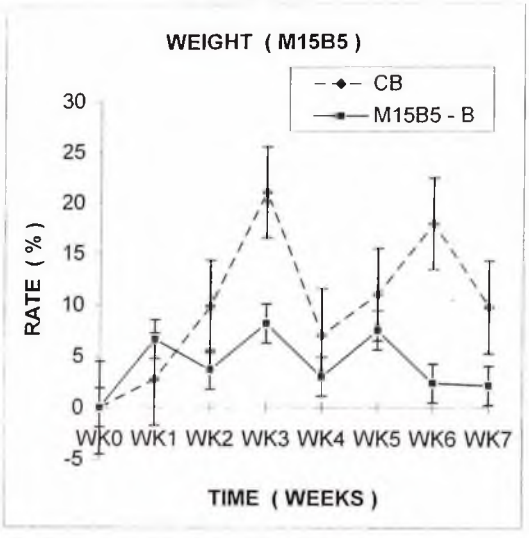
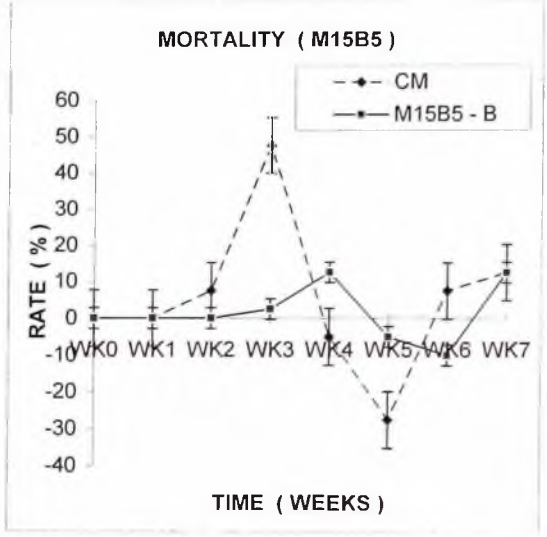


FIG. 4.18



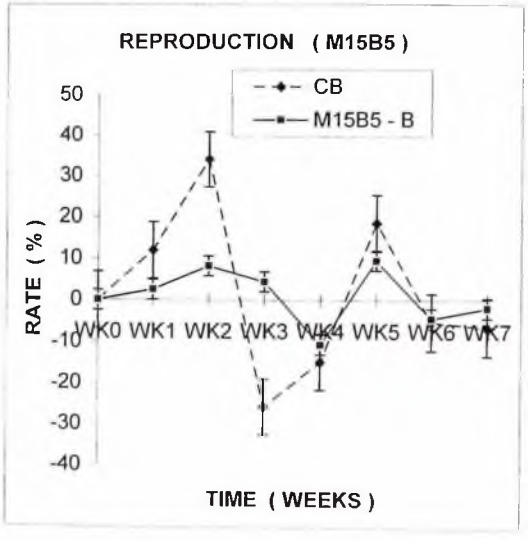


FIG. 4.19

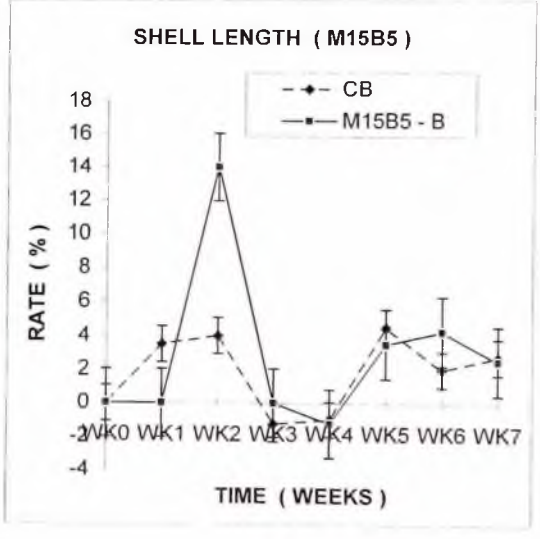


FIG. 4.20

4.3.1.3 Experimental Combination M15B5 :

4.3.1.3.1 *Weight :*

Table 4.2 and Figure 4.17 show that throughout the period of the experiment (with the exception of start of the experiment), the rate of weight increase of *Bulinus truncatus* in this experimental combination was below that of the control group. The differences in weight changes observed between *Bulinus truncatus* in the experimental cages and the control group were found to be highly significant over the first 6 weeks ($P(T<=t)$ value of 0.01767). A similar high level of significance was obtained when the weight changes were compared over the entire period of the experiment (7 weeks) using ANOVA ($p<0.0383$).

4.3.1.3.2 *Mortality :*

Table 4.6 and Figure 4.18 show that the rate of mortality of *Bulinus truncatus* in this experimental combination increased above that of the control group only in weeks 4 and 5 out of the 7 weeks of the experiment. For the rest of the time, the rate was below that of the control group. No significant differences ($p<0.62904$) were observed between the mortality rate of *Bulinus truncatus* and the control group.

4.3.1.3.3 *Reproduction :*

Table 4.6 and Figure 4.19 show that, for 3 weeks (ie weeks 1, 2 and 5) the rate of reproduction of *Bulinus truncatus* in this experimental combination was below that of the control group. For the rest of the weeks (ie weeks 3, 4, 6 and 7) the rate of reproduction of *Bulinus truncatus* increased above that of the control group. Analysing the overall results for *Bulinus truncatus* and the control group shown in Figure 4.19 using ANOVA, no significant differences were observed ($p< 0.92126$).

The rate of reproduction increase of *Melanoides tuberculata* in this experimental combination was influenced by the presence of *Bulinus truncatus* during the first two weeks of the experiment (Figure 4.23). These differences were found to be highly significant (P(T<=t) values of 0.0009).

4.3.1.3.4 *Shell length :*

Table 4.8 and Figure 4.20 show that for 3 out of the 7 weeks of the experiment the rate of shell length increase in *Bulinus truncatus* in this experimental combination increased above that of the control group. The highest increase (14%) was recorded in week 2. These differences between the rates were found to be statistically insignificant ($p < 0.47500$).

4.3.1.4 Density dependent effects :

4.3.1.4.1 *Weight :*

From Table 4.9 and Figures 4.26 and 4.33, it can be observed that the P-values (which indicate the level of significance of the results) decreased with an increase in the density of the *Melanoides tuberculata* snails (ie M15B5 > M10B10 > M5B15). Thus *Melanoides tuberculata* in the combination M15B5 ($p<0.03833$) had a greater influence on the rate of weight increase of *Bulinus truncatus* than M10B10 ($p< 0.05136$) and M5B15 ($p< 0.56204$) respectively.

GRAPHS FOR COMBINATION M15B5 (*MELANOIDES TUBERCULATA*)

USING NORMAL SANDY CLAY LOAM AS SEDIMENTS

FIG. 4.21

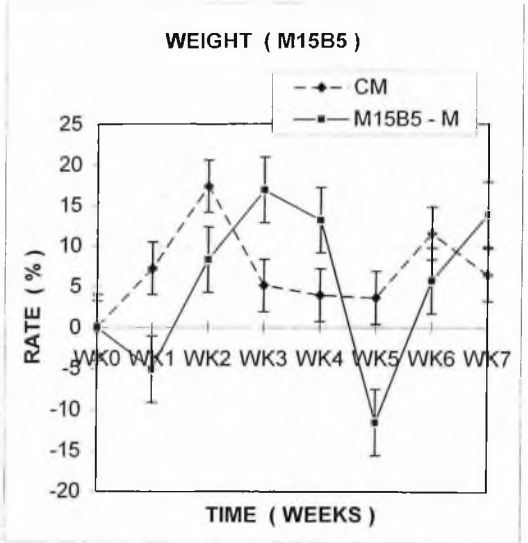
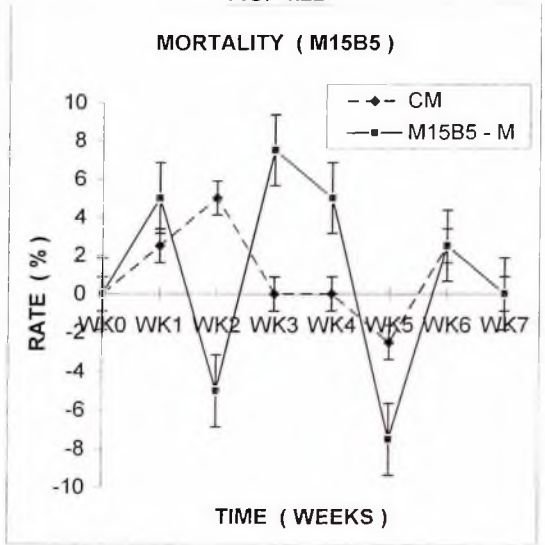


FIG. 4.22



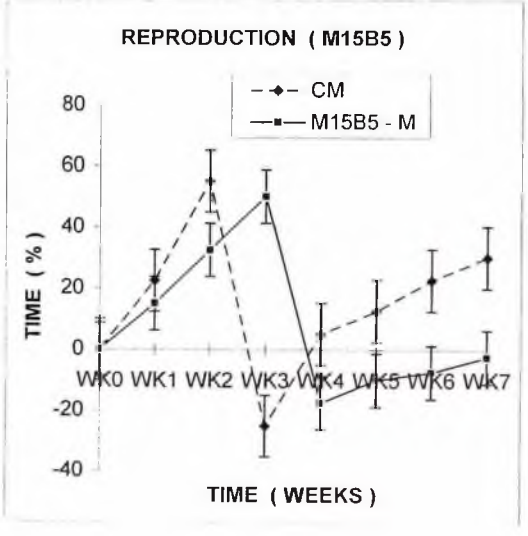


FIG. 4.23

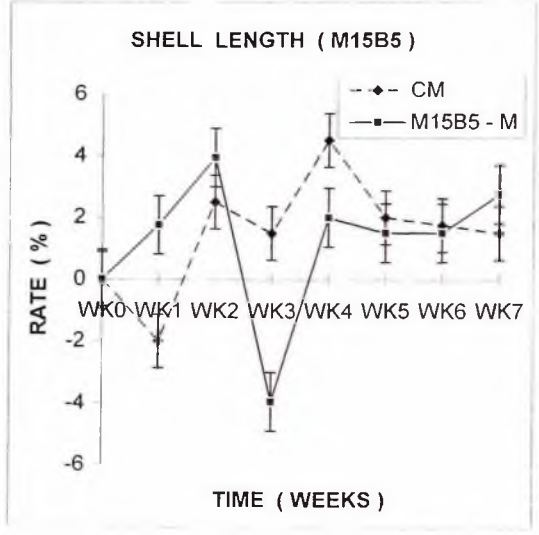
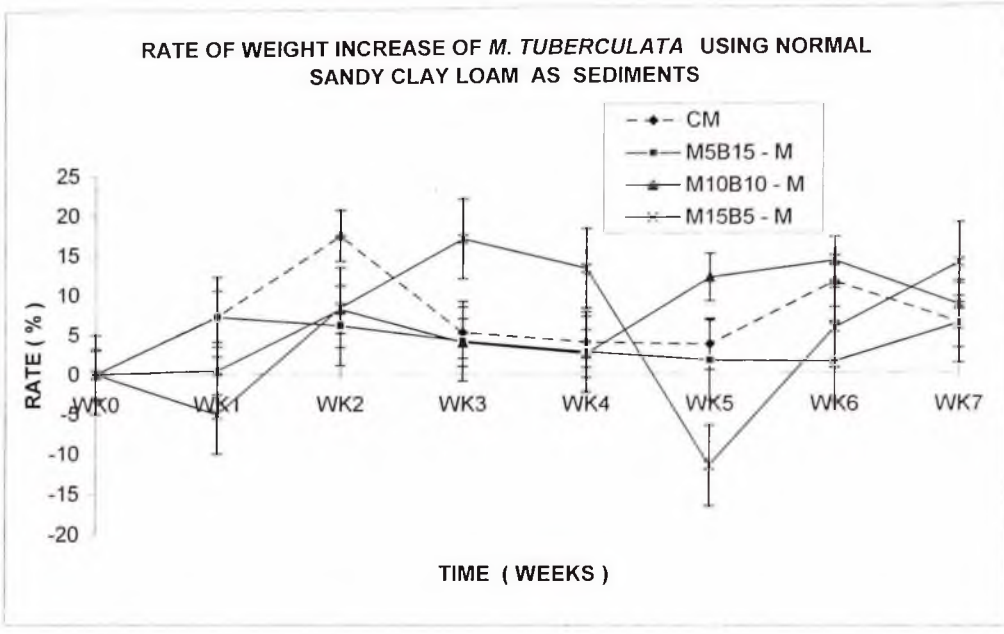


FIG. 4.24

Table. 4. 9 ANOVA : Two-Factor Without Replication for *Bulinus truncatus* using normal sandy clay loam as sediments

Combinations	Weight	Mortality	Reproduction	Shell Length
M5B15B	0.56204	0.29964	0.99164	0.97399
M10B10B	0.05136	0.72468	0.80298	0.83587
M15B5B	0.03833	0.62904	0.92126	0.4750

FIG. 4.25



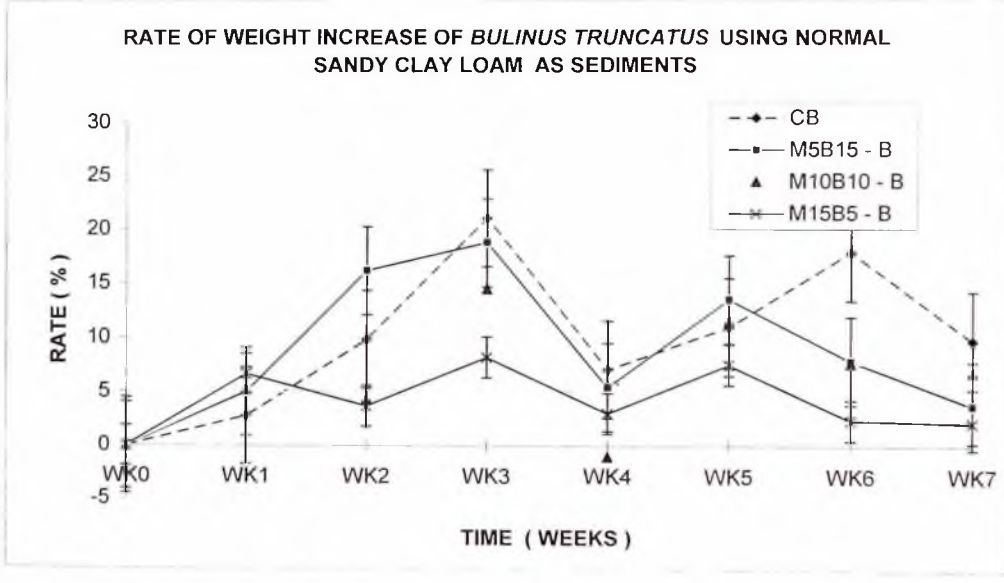


FIG. 4.26

4.3.1.4.2 *Mortality :*

From Table 4.9 and Figures 4.28 and 4.33, it was observed that, the density of *Melanoides tuberculata* did not have any significant effect on the mortality of *Bulinus truncatus* in the various combinations [M5B15 ($p< 0.29964$) M10B10 ($p< 0.72468$) and M15B5 ($p< 0.62904$)].

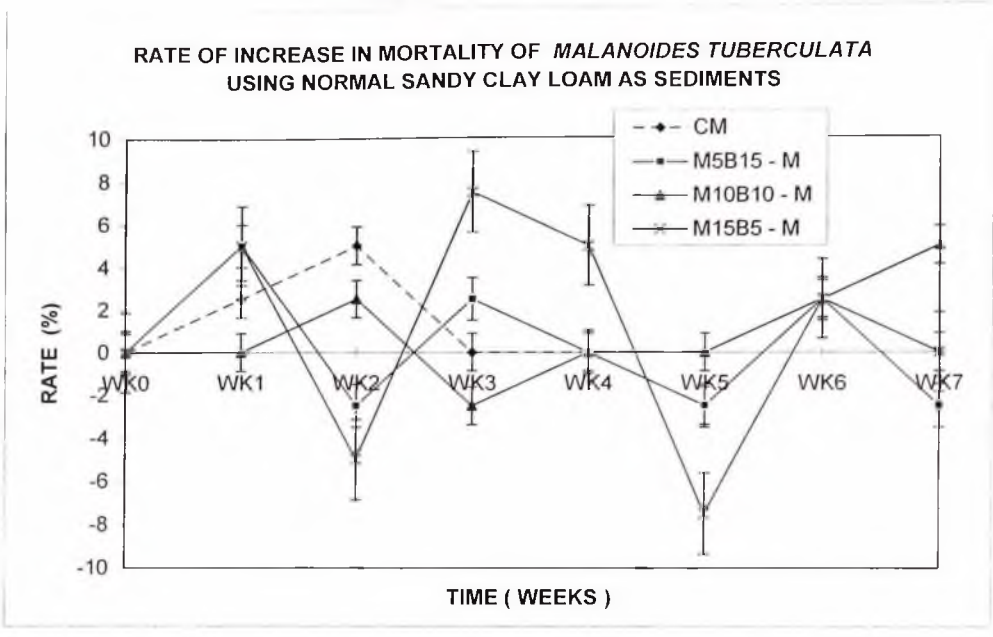
4.3.1.4.3 *Reproduction :*

From Table 4.9 and Figures 4.30 and 4.33, no significant effect was exerted by the increase in the density of *Melanoides tuberculata* on the rate of increase of reproduction of *Bulinus truncatus* in the various combinations. None of the comparisons between the reproductive rate of *Bulinus truncatus* in the experimental and control groups yielded any significant results (M5B15 ($p< 0.99164$) M10B10 ($p<0.80298$) and M15B5 ($p<0.92126$).

4.3.1.4.4 *Shell Length :*

From Table 4.9 and Figures 4.32 and 4.33, the observations made were similar to that made for mortality and reproduction. The differences between the growth rate of the shell under experiment and control conditions under all the various combinations were not significant (M5B15 ($p<0.97399$) M10B10 ($p<0.83587$) and M15B5 ($p<0.47500$).

FIG. 4.27



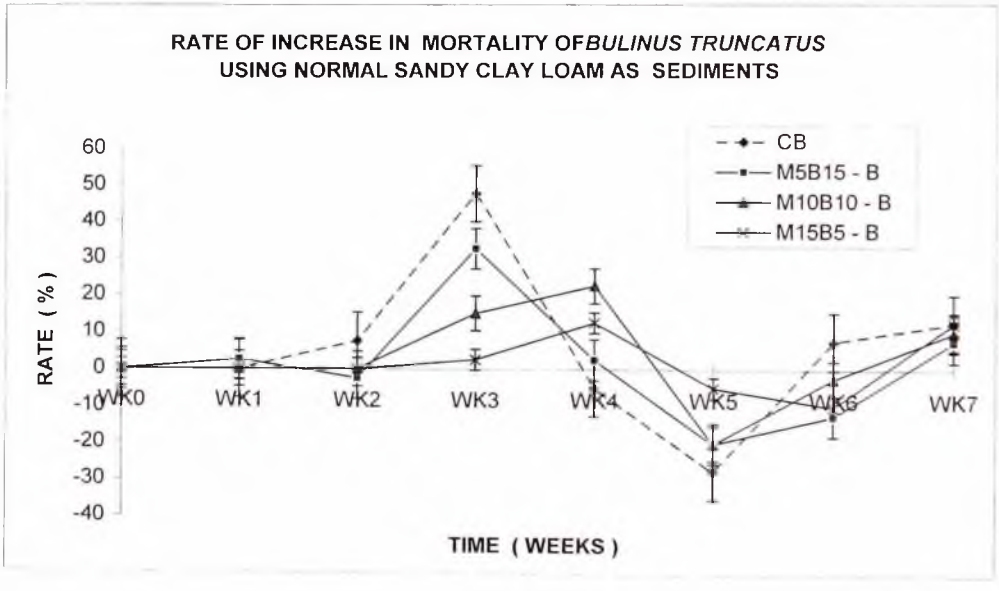
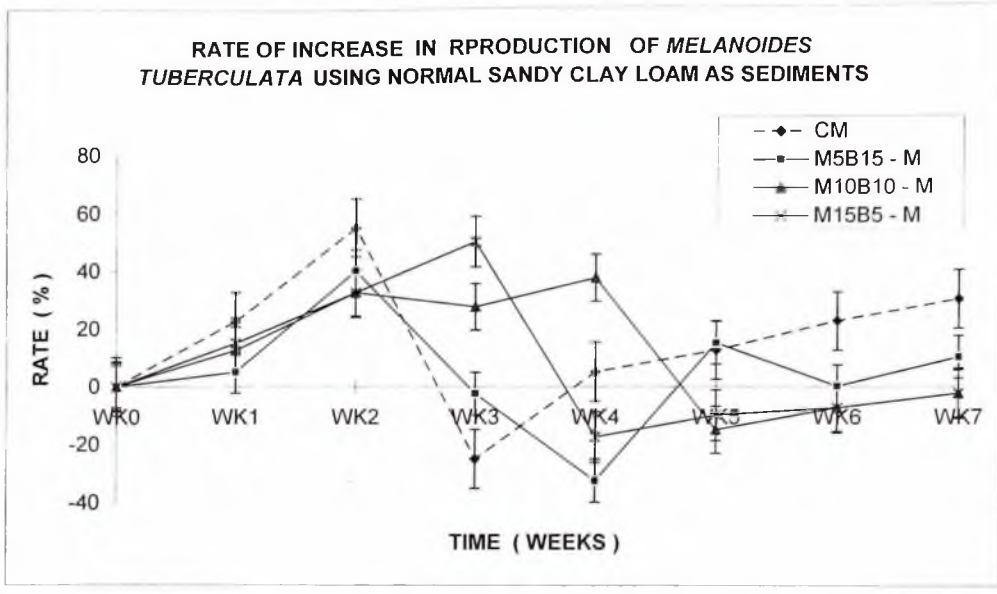


FIG. 4.28

FIG. 4.29



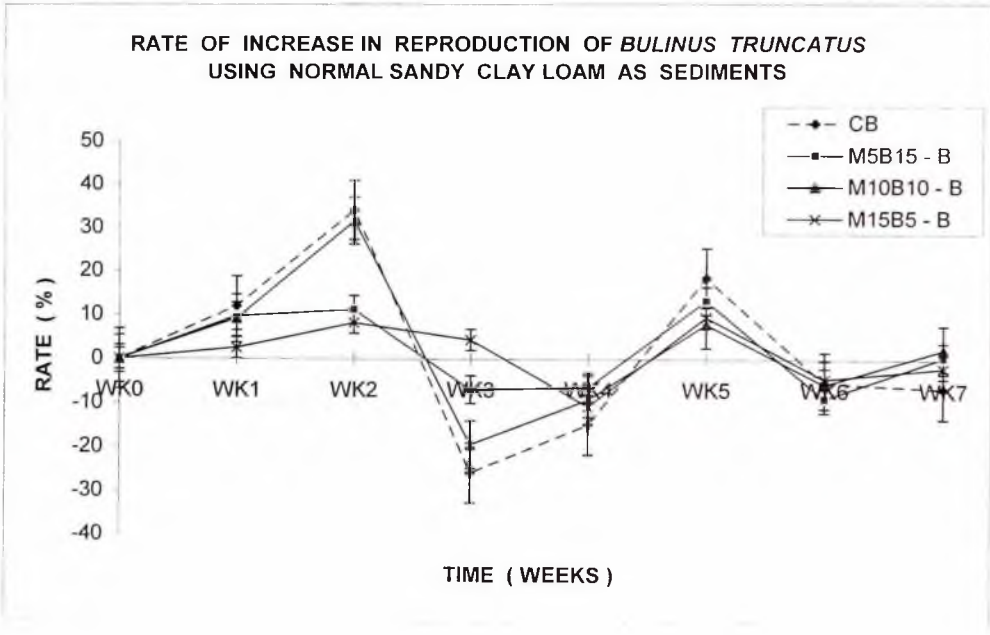
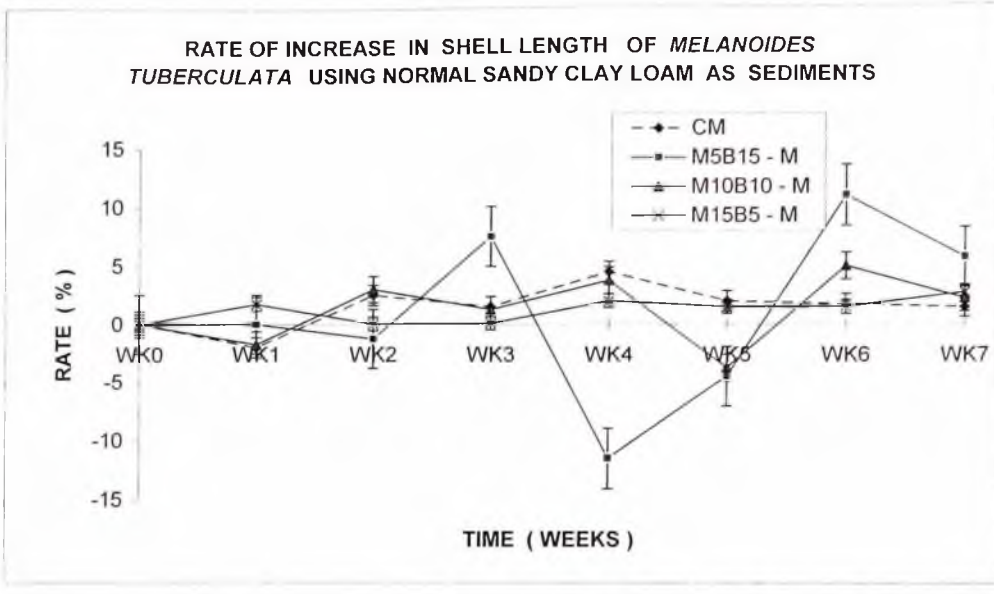


FIG. 4.30

FIG. 4.31



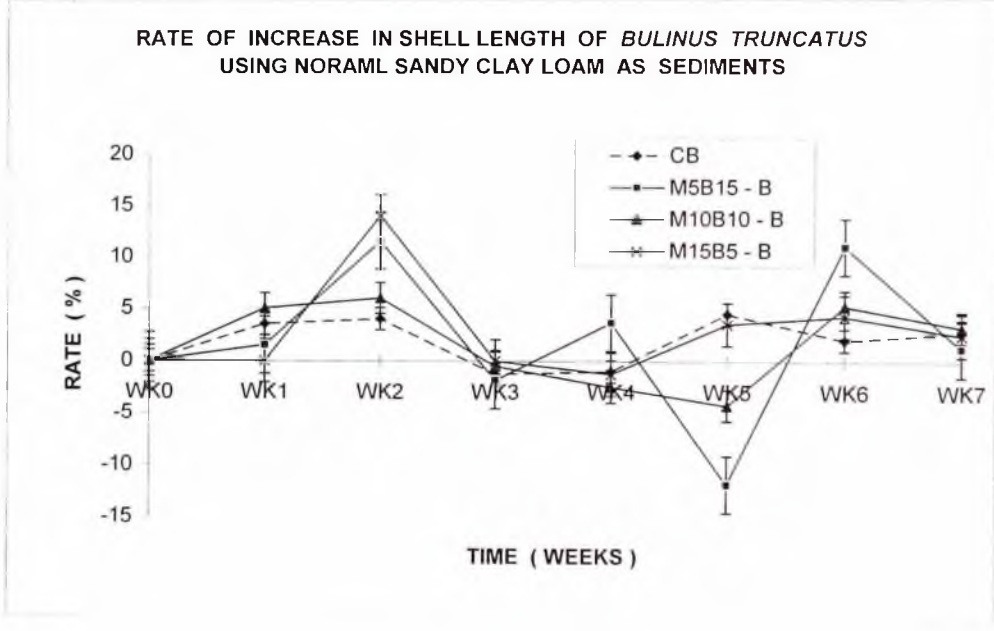
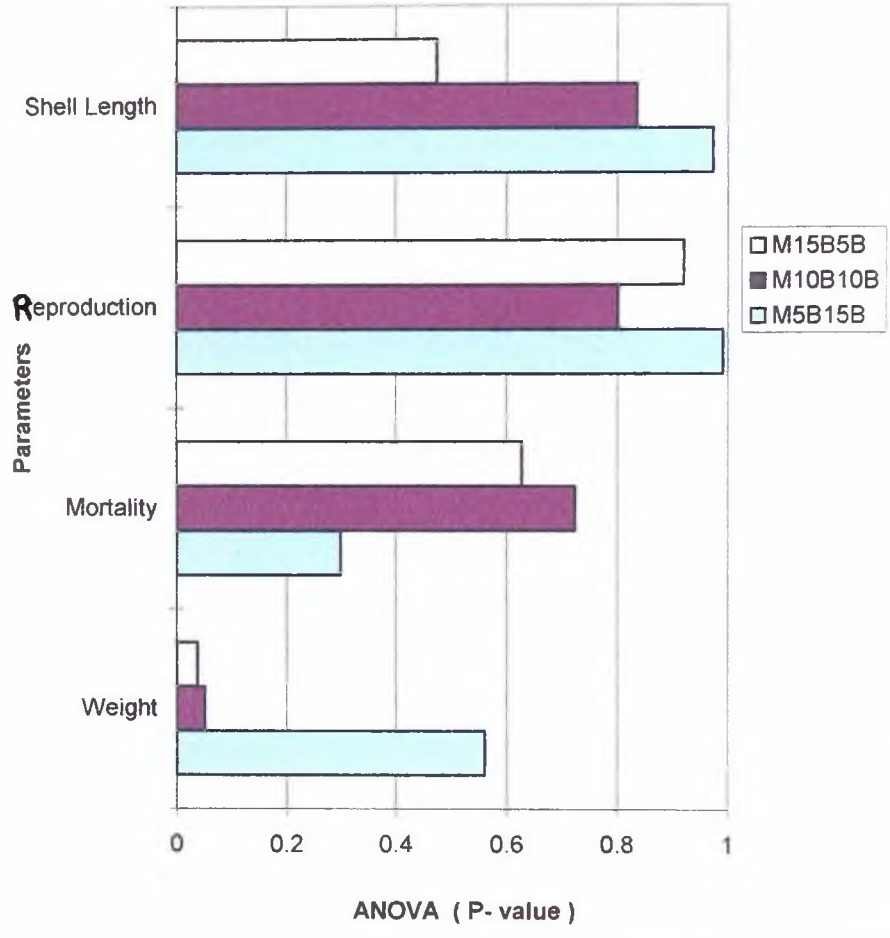


FIG. 4.32

Fig. 4.33

Graphical presentation of the statistical analysis of the results for *Bulinus truncatus* using normal sandy clay loam as sediments.



4.3.2 SECTION B : HEAT TREATED SEDIMENTS

4.3.2.1 Experimental Group M5B15 :

4.3.2.1.1 *Weight :*

Table 4.11 and Figure 4.34 show a decrease in the rate of weight gain of *Bulinus truncatus* in this experimental combination below that of the control group for 4 (ie weeks 1, 2, 3, and 5) out of the 7 weeks of the experiment. The highest decrease in the rate was recorded in week 3 with a value of -0.3525. From week 6 to week 7, the rate (1.008 and 1.67% respectively) increased above that of the control group (-0.2075 and 0.575% respectively). Statistical analysis of the results for weeks 5 and 7 showed that the differences were statistically insignificant (P(T<=t) value of 0.14633). Analysing the overall results using ANOVA, the differences observed between the growth rates of *Bulinus truncatus* in experimental and control conditions for the seven week period were found to be insignificant ($p<0.9497$).

The rate of weight increase of *Melanoides tuberculata* was found to have been influenced by the presence and density of *Bulinus truncatus*. From Figure 4.38 , between weeks 1 and 6, the rate of weight increase of *Melanoides tuberculata* in this experimental combination was found to have decreased. Analysing these results, the differences observed during these weeks was found to be statistically significant (P(T<=t) value of 0.00783).

4.3.2.1.2 *Mortality :*

From Table 4.13 and also Figure 4.35, it can be seen that for 4 (ie week 3,4,6 and 7) out of the 7 weeks of the experiment, the rate of mortality of *Bulinus truncatus* decreased below that of the control group. The highest decrease was in week 5 (-35%).

TABLE 4.10 RATE OF INCREASE IN WEIGHT OF *M. TUBERCULATA* USING HEAT TREATED SANDY CLAY LOAM AS SEDIMENTS

GROUP	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7
CM	0	15.1575	16.625	5.95	1.5	10.875	9.75	4.55
M5B15 - M	0	-3.625	2.525	-0.225	-1.975	5.9	-0.175	9.725
M10B10 - M	0	-1.825	2.225	-1.25	-8.5	3.2	-1.575	13.45
M15B5 - M	0	5.775	-11.4	1.825	-16	-15.925	7.125	19.425

TABLE 4.11 RATE OF INCREASE IN WEIGHT OF *B. TRUNCATUS* USING HEAT TREATED SANDY CLAY LOAM AS SEDIMENTS

GROUP	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7
CB	0	3.2675	1.8175	1.305	1.9875	0.8575	-0.2075	0.575
M5B15 - B	0	2.85	1.735	-0.305	2.23	0.58	1.0075	1.67
M10B10 - B	0	2.6475	1.775	0.3525	0.38	0.5725	0.1425	2.415
M15B5 - B	0	1.575	0.7825	0.5375	0.4025	0.525	0.41	1.105

GRAPHS FOR COMBINATION M5B15 (*BULINUS TRUNCATUS*)

USING HEAT TREATED SANDY CLAY LOAM AS SEDIMENTS

FIG. 4.34

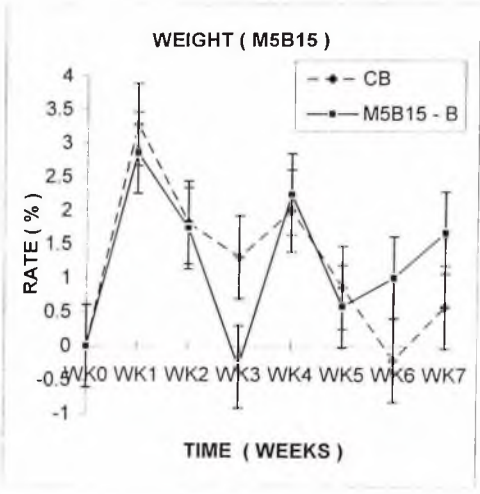
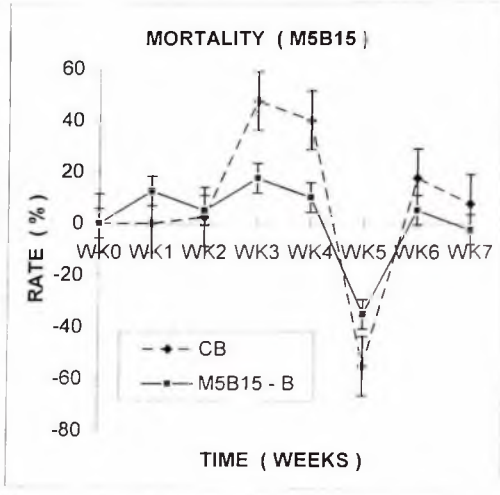


FIG. 4.35



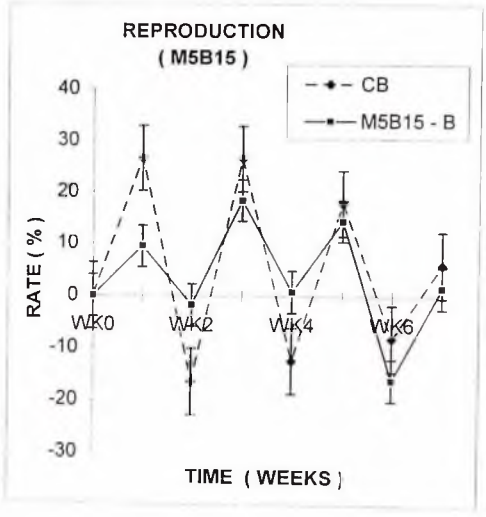


FIG. 4.36

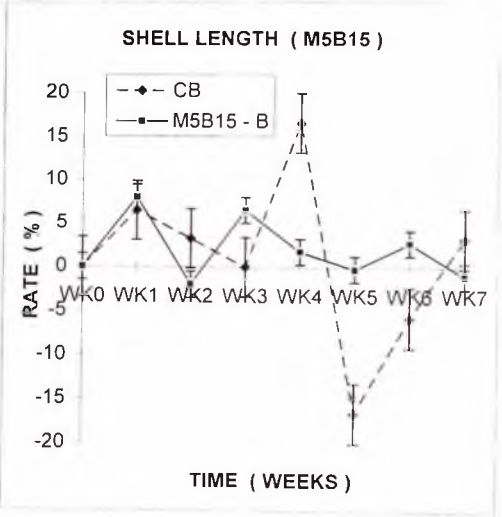


FIG. 4.37

GRAPHS FOR COMBINATION M5B15 (*MELANOIDES TUBERCULATA*)

USING HEAT TREATED SANDY CLAY LOAM AS SEDIMENTS .

FIG. 4.38

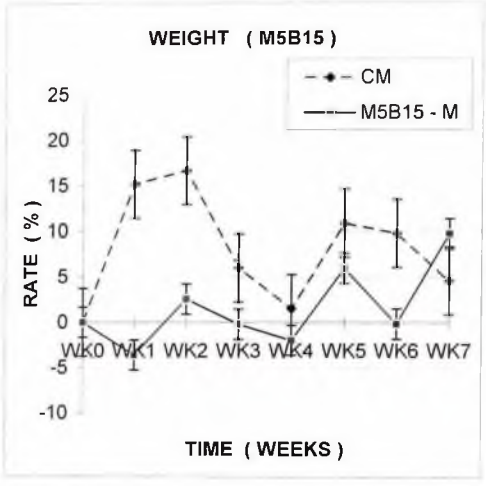
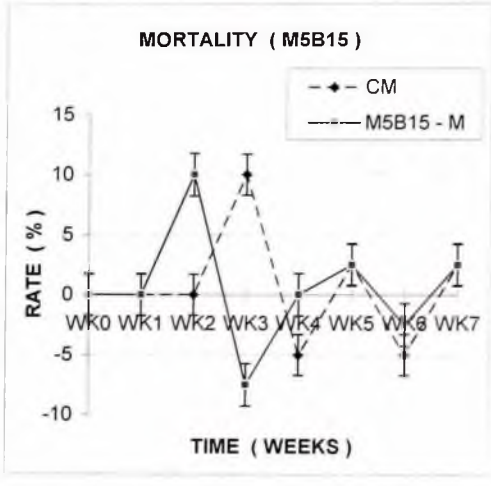


FIG. 4.39



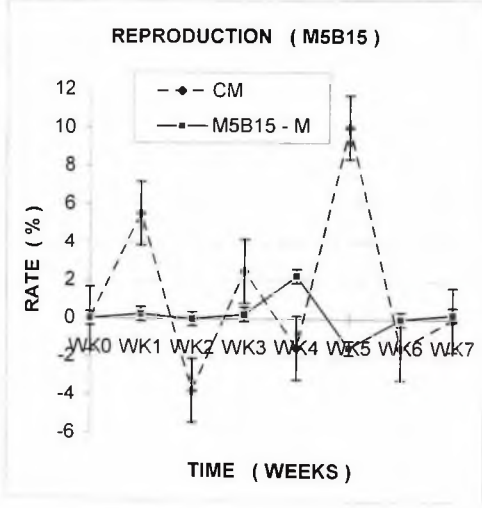


FIG 4.40

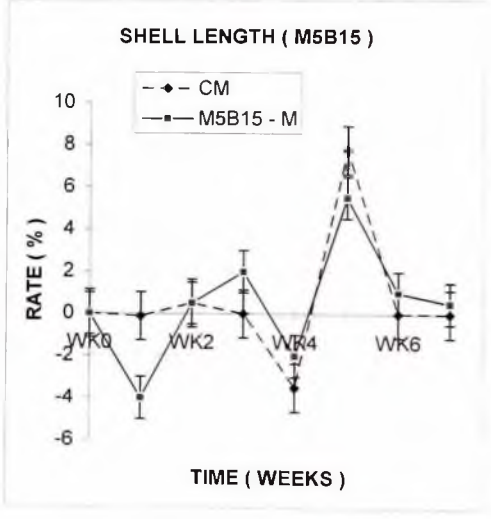


FIG. 4.41

TABLE 4.12 RATE OF INCREASE IN MORTALITY OF *M. TUBERCULATA* USING HEAT TREATED SANDY CLAY LOAM AS SEDIMENTS

GROUP	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7
CM	0	0	0	10	-5	2.5	-5	2.5
M5B15 - M	0	0	10	-7.5	0	2.5	-2.5	2.5
M10B10 - M	0	0	2.5	0	-2.5	5	0	0
M10B10 - M	0	5	-5	5	-2.5	5	-2.5	0

TABLE 4.13 RATE OF INCREASE IN MORTALITY OF *B. TRUNCATUS* USING HEAT TREATED SANDY CLAY LOAM AS SEDIMENTS

GROUP	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7
CB	0	0	2.5	47.5	40	-55	17.5	7.5
M5B15 - B	0	12.5	5	17.5	10	-35	5	-2.5
M10B10 - B	0	10	2.5	20	-5	-2.5	-10	5
M15B5 - B	0	0	7.5	5	-5	-2.5	0	7.5

These differences were found to be statistically insignificant (P(T<=t) value of 0.1094). The analysis of the overall results using ANOVA, also failed to yield any statistically insignificant ($p<0.38386$) difference between the mortality rate of *Bulinus truncatus* in the experimental and control cages.

4.3.2.1.3 *Reproduction :*

From Table 4.15 and also Figure 4.36, it can be seen that for 5 (ie weeks 1,3,5,6 and 7) out of the 7 weeks of the experiment, the rate of reproduction of *Bulinus truncatus* decreased below that of the control group. The two rates showed some fluctuations during the period of the experiment. These differences however were found to be statistically insignificant (P(T<=t) value of 0.33865).

4.3.2.1.4 *Shell Length :*

Table 4.17 and Figure 4.37 show that for 4 weeks out of the 7 weeks of the experiment the rate of shell length increased for *Bulinus truncatus* in this experimental combination over that of the control group. Analysing the results, the differences observed were found to be statistically insignificant (P(T<=t) value of 0.38302).

4.3.2.2 Experimental Combination M10B10 :

4.3.2.2.1 *Weight :*

Table 4.11 and Figure 4.42 show that for the first 5 weeks out of the 7 weeks of the experiment, the rate of weight increase of *Bulinus truncatus* in this experimental combination fell below that of the control group. The differences were more marked between weeks 3

TABLE 4.14 RATE OF INCREASE IN REPRODUCTION OF *M. TUBERCULATA* USING HEAT TREATED SANDY CLAY LOAM AS SEDIMENTS

GROUP	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7
CM	0	5.5	-3.75	2.5	-1.5	10	-1.5	0
M5B15 - M	0	0.25	0	0.25	2.25	-1.5	0	0.25
M10B10 - M	0	0.5	-0.25	1.5	-0.5	2	1.25	0.5
M15B5 - M	0	1.5	-0.75	3	-2.5	6.75	-0.5	1

TABLE 4.15 RATE OF INCREASE IN REPRODUCTION OF *B. TRUNCATUS* USING HEAT TREATED SANDY CLAY LOAM AS SEDIMENTS

GROUP	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7
CB	0	26.6	-16.35	26.5	-12.375	17.825	-8.1	5.95
M5B15 - B	0	9.475	-1.725	18.4	0.775	14.375	-16.05	1.525
M10B10 - B	0	18.1	-5.925	6.175	2.4	21.75	-16.25	3.2
M15B5 - B	0	14.65	-5.4	10	-17.225	31.05	-16.3	78.75

TABLE 4.16 RATE OF INCREASE IN SHELL LENGTH (CM) OF *M. TUBERCULATA* USING HEAT TREATED SANDY CLAY LOAM

GROUP	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7
CM	0	-0.125	0.5	0	-3.5	7.75	0	0
M5B15 - M	0	-4	0.5	2	-2	5.5	1	0.5
M10B10 - M	0	-4.25	-2.25	6	-7.25	5	3.75	0.25
M15B5 - M	0	0	1.325	-1.575	-4.5	4	-2.25	5.5

TABLE 4.17 RATE OF INCREASE IN SHELL LENGTH (CM) OF *B. TRUNCATUS* USING HEAT TREATED SANDY CLAY LOAM

GROUP	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7
CB	0	6.5	3.25	0	16.59	-16.58	-5.75	3.25
M5B15 - B	0	8	-2	6.5	1.75	-0.25	2.75	-1
M10B10 - B	0	2.25	8.25	-4.5	7	-9.25	7	1.75
M15B5 - B	0	6.5	6	4.75	-2.75	-3.5	-2.5	5

GRAPHS FOR COMBINATION M10B10 (*BULINUS TRUNCATUS*)

USING HEAT TREATED SANDY CLAY LOAM AS SEDIMENTS

FIG. 4.42

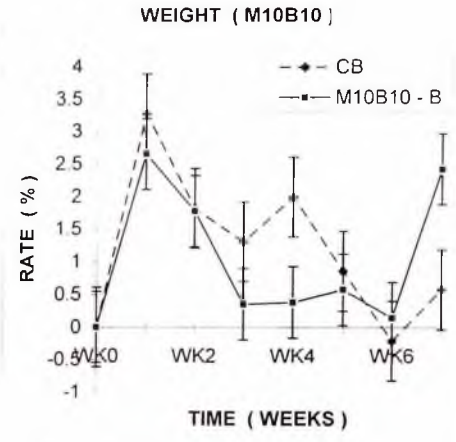
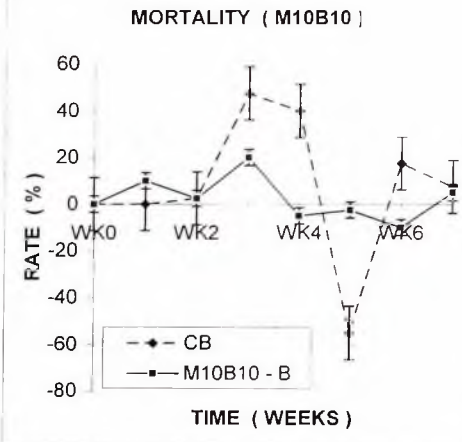


FIG. 4.43



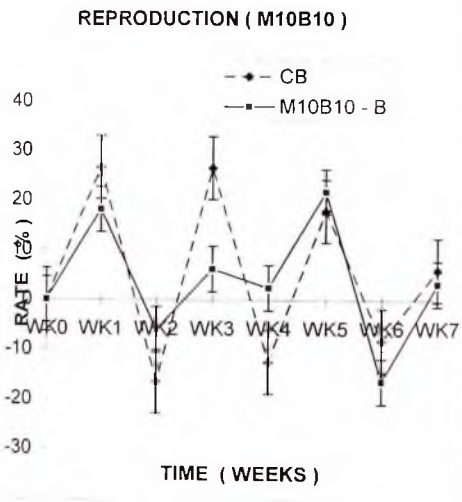


FIG. 4.44

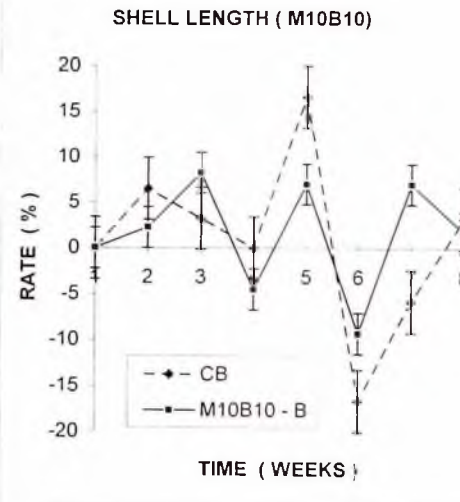


FIG. 4.45

and 5. Statistical analysis of the results obtained between weeks 3 and 5 showed that the differences were statistically insignificant (P(T<=t) value of 0.06548). Also analysis of the overall results showed that the differences observed for the entire 7 week period of the experiment were statistically insignificant (p< 0.66103).

The rate of weight increase of *Melanoides tuberculata* was found to have been influenced by *Bulinus truncatus*. From Figure 4.46, it was observed that during the first six weeks, the rate of weight increase of *Melanoides tuberculata* in this experimental combination decreased below that of the control group. The observed difference was found to be statistically significant (P(T<=t) value of 0.00178).

4.3.2.2.2 *Mortality :*

Table 4.13 and Figure 4.43 show fluctuations in the rate of mortality of *Bulinus truncatus* in this experimental combination. In four (ie weeks 3, 4, 6 and 7) out of the 7 weeks of the experiment, the rate of mortality of *Bulinus truncatus* decreased below that of the control group. These differences were however found to be statistically insignificant (P(T<=t) value of 0.10315).

4.3.2.2.3 *Reproduction :*

Table 4.15 and Figure 4.44 show fluctuations in the results. In 4 (ie weeks 1, 3, 6 and 7)out of the 7 weeks of the experiment the rate of reproduction of *Bulinus truncatus* in this combination decreased below that of the control group. For the other 3 weeks the control group had a lower rate of reproduction than the *Bulinus truncatus* in the experimental combination. Analysing the overall results, the differences observed were found to be statistically insignificant (P(T<=t) value of 0.37640).

GRAPHS FOR COMBINATION M10B10 (*MELANOIDES TUBERCULATA*)

USING HEAT TREATED SANDY CLAY LOAM AS SEDIMENTS

FIG. 4.46

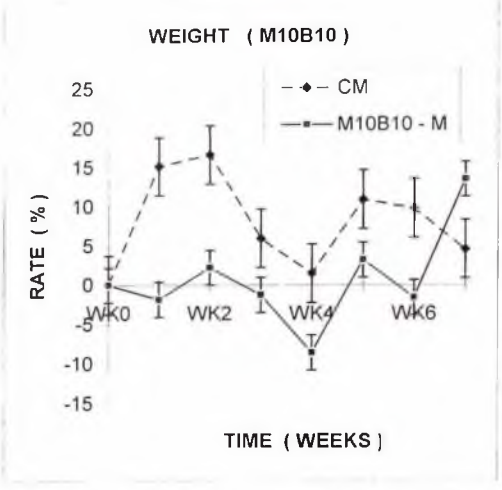
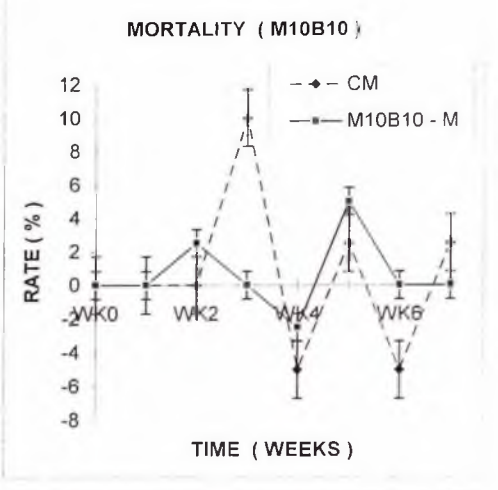


FIG. 4.47



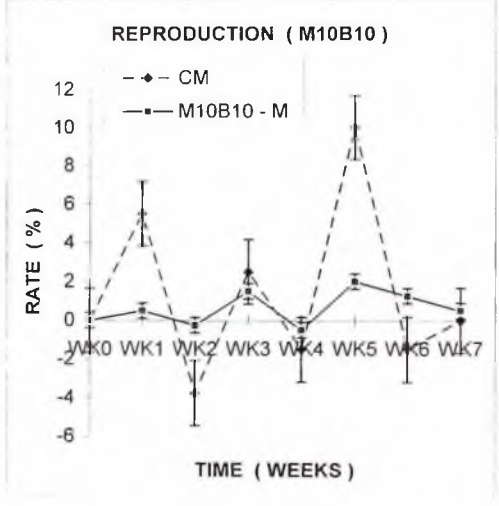


FIG. 4.48

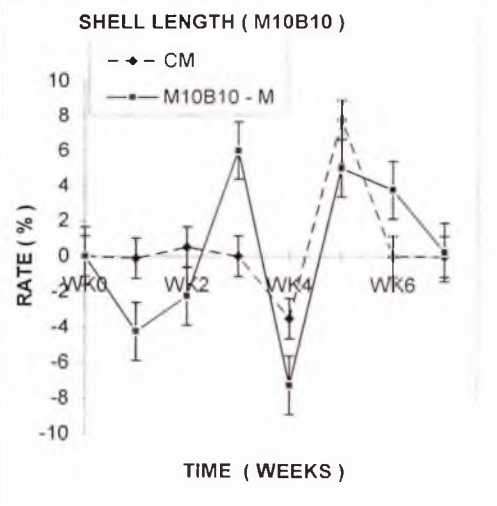


FIG. 4.49

4.3.2.2.4 *Shell Length :*

Table 4.17 and Figure 4.45 show fluctuations in the rate of shell length increase in *Bulinus truncatus* and the control group throughout the period of the experiment Analysing the overall results, the observed differences were found to be statistically insignificant ($p<0.80587$). The rate of shell length increase of *Melanoides tuberculata* was not found to have been influenced by the presence and density of *Bulinus truncatus* to any significant extent (P(T<=t) value of 0.0997).

4.3.2.3 Experimental Combination M15B5 :

4.3.2.3.1 *Weight :*

Table 4.11 and Figure 4.50 show that throughout the period of the experiment except for weeks 6 and 7, the rate of weight increase of *Bulinus truncatus* in this experimental combination fell below that of the control group. The results of the first five weeks where most of these differences were observed between the rate of weight increase of *Bulinus truncatus* and the control group was found to be statistically significant (P(T<=t) value of 0.01094). The overall results however, showed that the differences observed for the stretch of the experiment was statistically insignificant ($p< 0.13378$).

The rate of weight increase of *Melanoides tuberculata* was found to have been influenced by the presence of *Bulinus truncatus*. This is because from Figure 4.54 , during the first six weeks of the experiment, the rate of weight increase of *Melanoides tuberculata* in this experimental combination decreased. Analysing these results showed that the observed differences were statistically significant (P(T<=t) value of 0.01388).

GRAPHS FOR COMBINATION M15B5 (*BULINUS TRUNCATUS*)

USING HEAT TREATED SANDY CLAY LOAM AS SEDIMENTS

FIG. 4.50

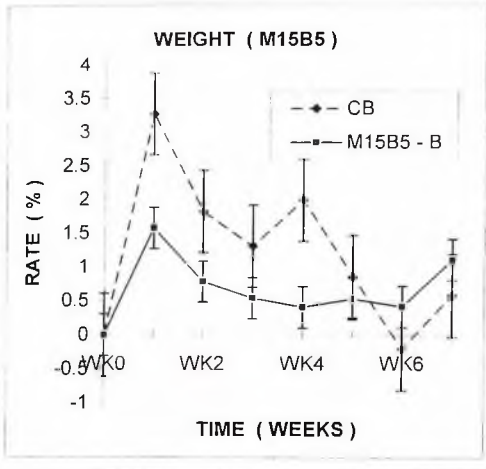
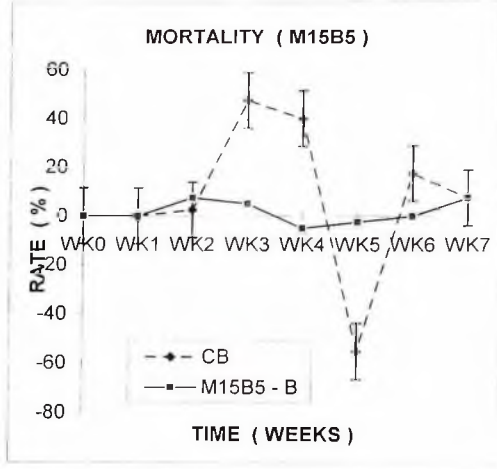


FIG. 4.51



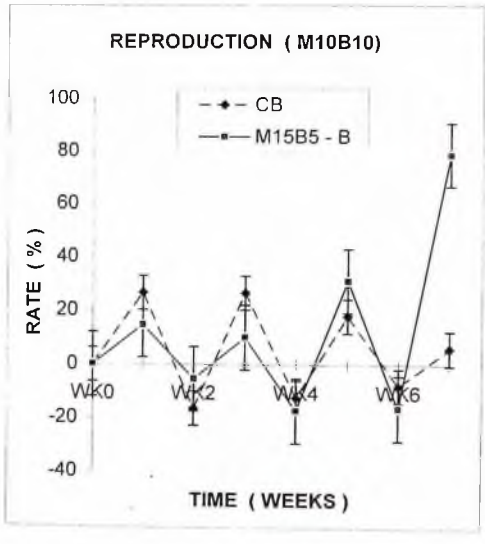


FIG. 4.52

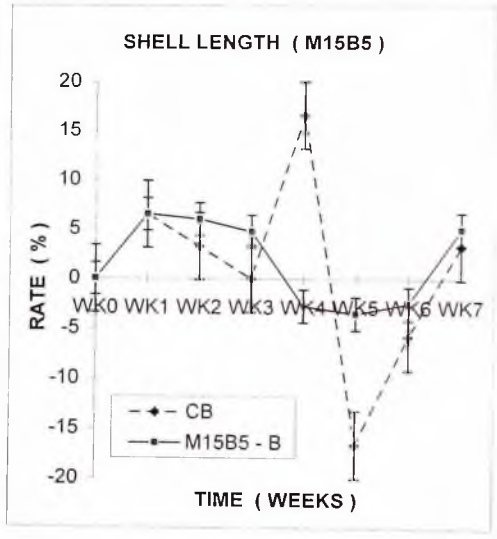


FIG. 4.53

GRAPHS FOR COMBINATION M15B5 (*MELANOIDES TUBERCULATA*)

USING HEAT TREATED SANDY CLAY LOAM AS SEDIMENTS

FIG. 4.54

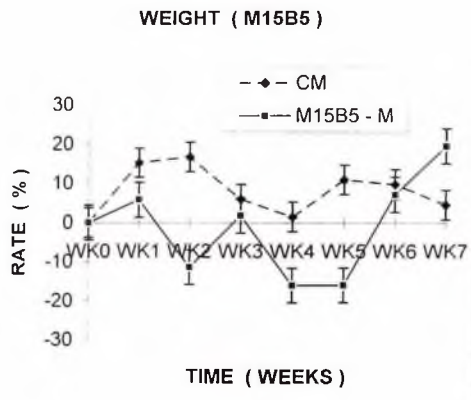
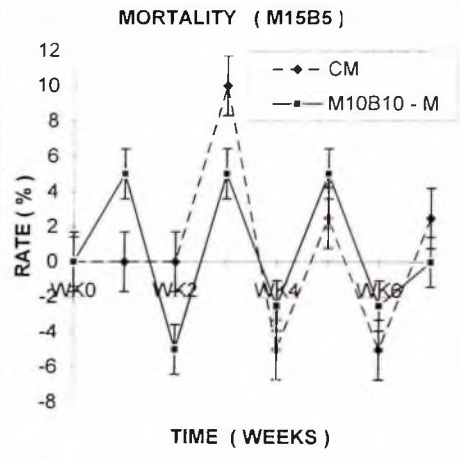


FIG. 4.55



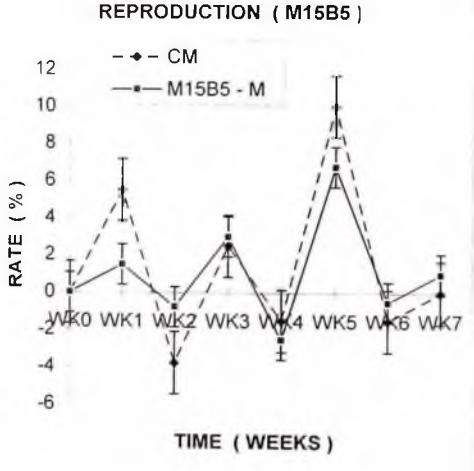


FIG. 4.56

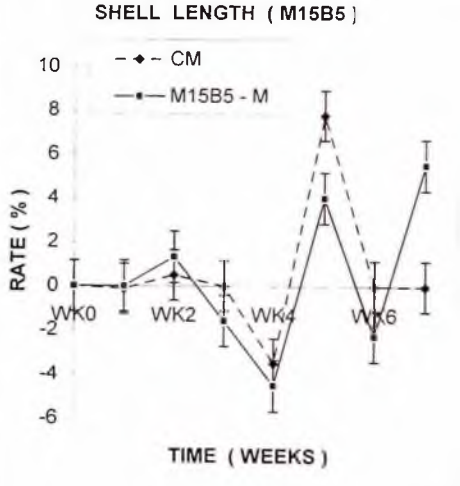


FIG. 4.57

4.3.2.3.2 *Mortality :*

Table 4.13 and Figure 4.51 show that the rate of mortality of *Bulinus truncatus* in this experimental combination increased above that of the control group only in weeks 2 and 5. For the rest of the time the rate was below that of the control group. The overall differences observed were found to be statistically insignificant ($p<0.60241$).

4.3.2.3.3 *Reproduction :*

Table 4.15 and Figure 4.52 show fluctuations in the results of the reproduction rates of *Bulinus truncatus* in this experimental combination and that of the control group. In four (ie weeks 1, 3, 4, and 6) out of the 7 weeks of the experiment, the rate of reproduction of *Bulinus truncatus* decreased below that of the control group. These differences were however found to be statistically insignificant (P(T<=t) value of 0.29066). The overall differences in the results were found to be statistically insignificant ($p< 0.51456$).

4.3.2.3.4 *Shell length :*

Table 4.17 and Figure 4.53 show that in five (ie weeks 2, 3,5,6 and 7) out of the seven weeks of the experiment the rate of shell length increase in *Bulinus truncatus* increased above that of the control group. Statistical analysis of the results of the first three weeks and also the last two weeks (during which periods clear differences occurred between the rate of *Bulinus truncatus* and the control group) showed that the observed differences were statistically insignificant (P(T<=t) value of 0.10554 and 0.11597 respectively). The overall results also showed that the differences were statistically insignificant ($p< 0.81571$).

4.3.2.4 Density dependent effects :

4.3.2.4.1 *Weight :*

From Table 4.18 and Figures 4.59 and 4.66, no statistically significant differences were observed in the result of the various combinations. Thus *Melanoides tuberculata* in all the combinations M15B5 ($p<0.94974$), M10B10 ($p< 0.66103$) and M5B15 ($p< 0.13378$) did not influence the rate of increase in weight of *Bulinus truncatus* differently. However it was observed (Table 4.19 and Figures 4.58 and 4.67) that for the first six weeks, the rate of increase in weight of *Melanoides tuberculata* was influenced significantly by the presence and density of *Bulinus truncatus* in all the combinations M5B15 (P (T<=t)=0.00783) and M10B10 (P (T<=t)= 0.00178) and M15B5 (P (T<=t)= 0.01388) was statistically insignificant.

4.3.2.4.2 *Mortality :*

From Table 4.18 and Figures 4.61 and 4.66, it could be observed that, the density of *Melanoides tuberculata* did not have any significant effect on the mortality of *Bulinus truncatus* in the various combinations. The results of all the various combinations were insignificant [M5B15 ($p< 0.38858$) M10B10 ($p< 0.64912$) and M15B5 ($p< 0.60241$)].

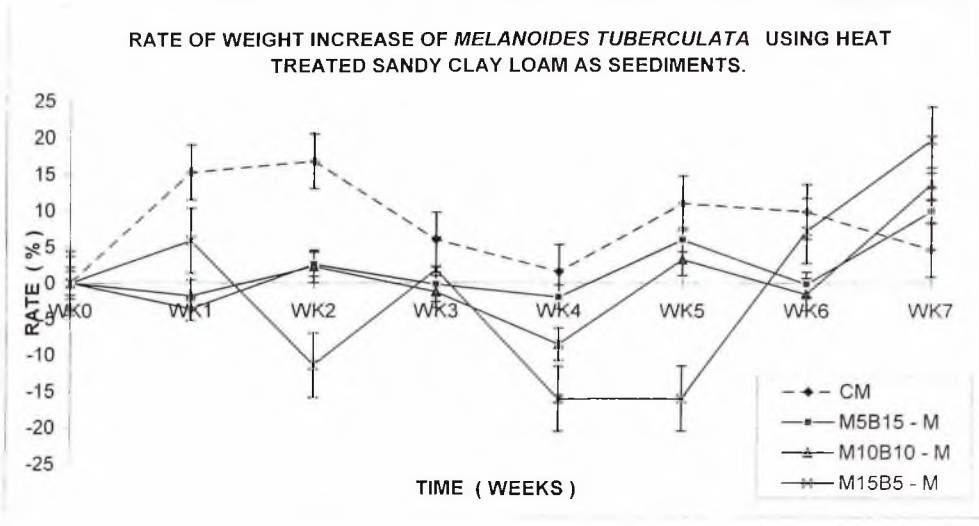
4.3.2.4.3 *Reproduction :*

From Table 4.18 and Figures 4.63 and 4.66, no significant effect was exerted by the increase in the density of *Melanoides tuberculata* on the rate of increase of reproduction of *Bulinus truncatus* in the various combinations [M5B15 ($p< 0.67729$) M10B10 ($p< 0.74903$) and M15B5 ($p< 0.51455$)].

Table. 4.18 ANOVA : Two-Factor Without Replication for *Bulinus truncatus* using heat treated sandy clay loam as sediments

Combinations	Weight	Mortality	Reproduction	Shell Length
M5B15B	0.94974	0.38858	0.67729	0.76244
M10B10B	0.661029	0.64912	0.74903	0.80587
M15B5B	0.13378	0.60241	0.51455	0.81571

FIG. 4.58



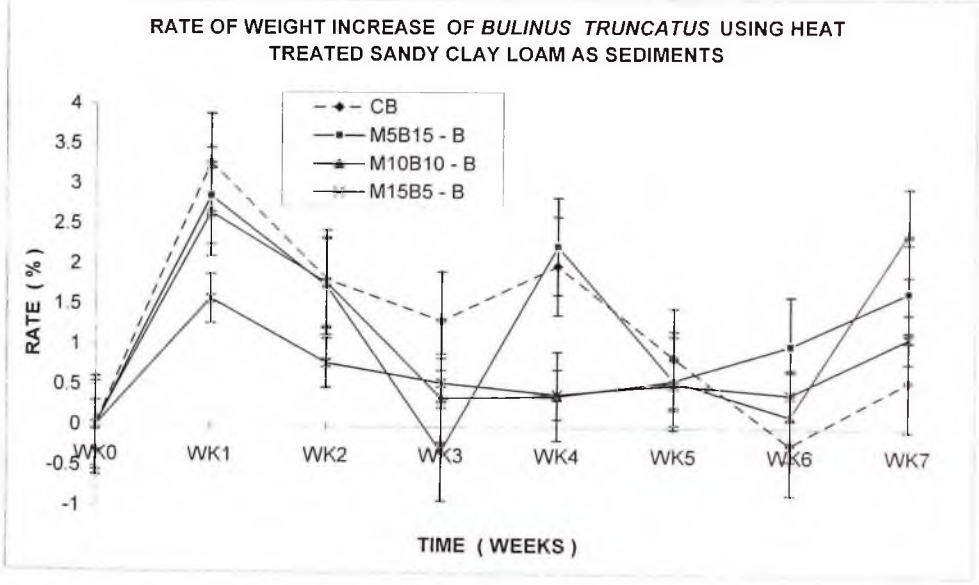
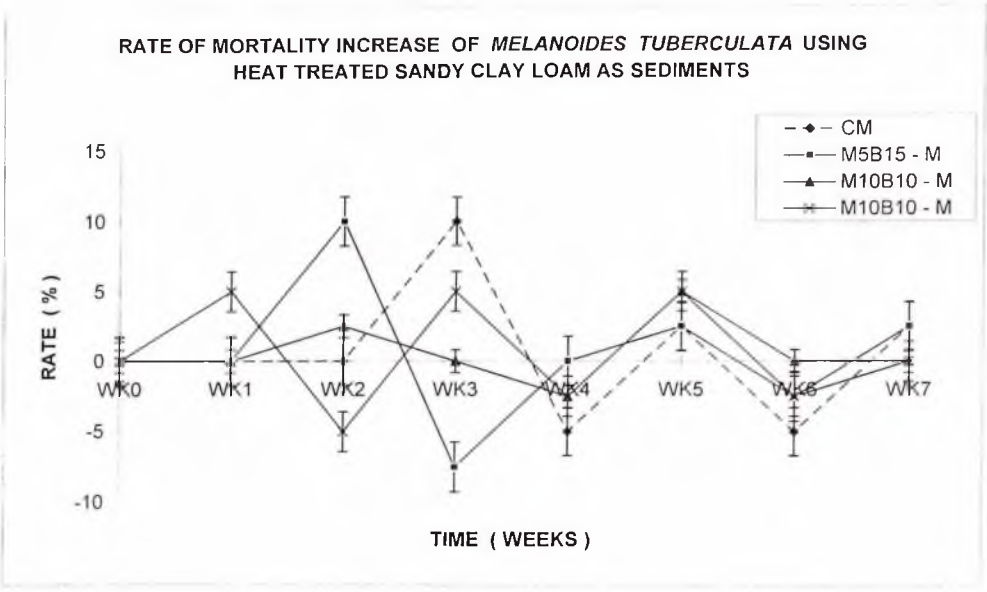


FIG. 4.59

FIG. 4.60



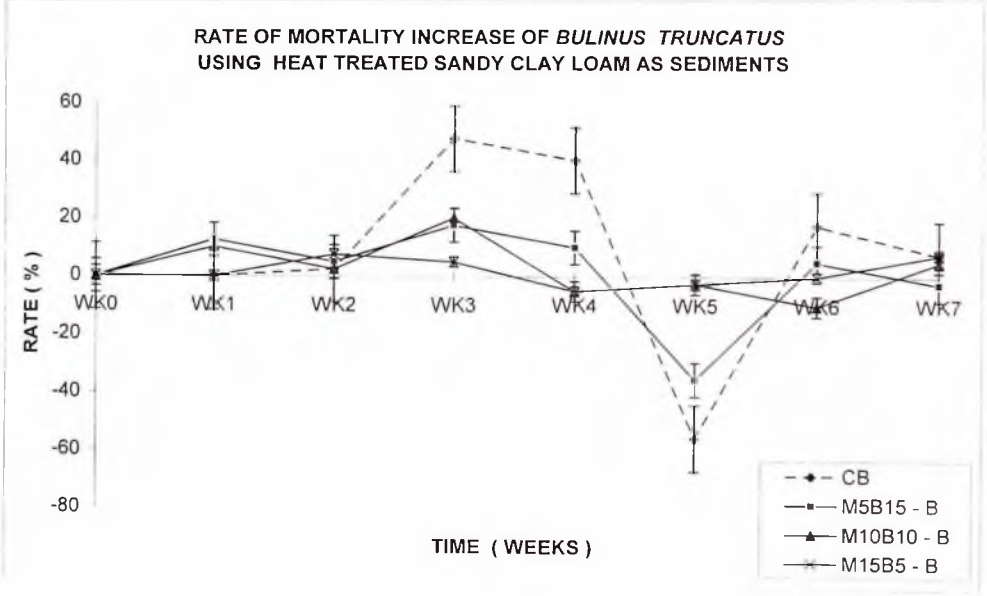
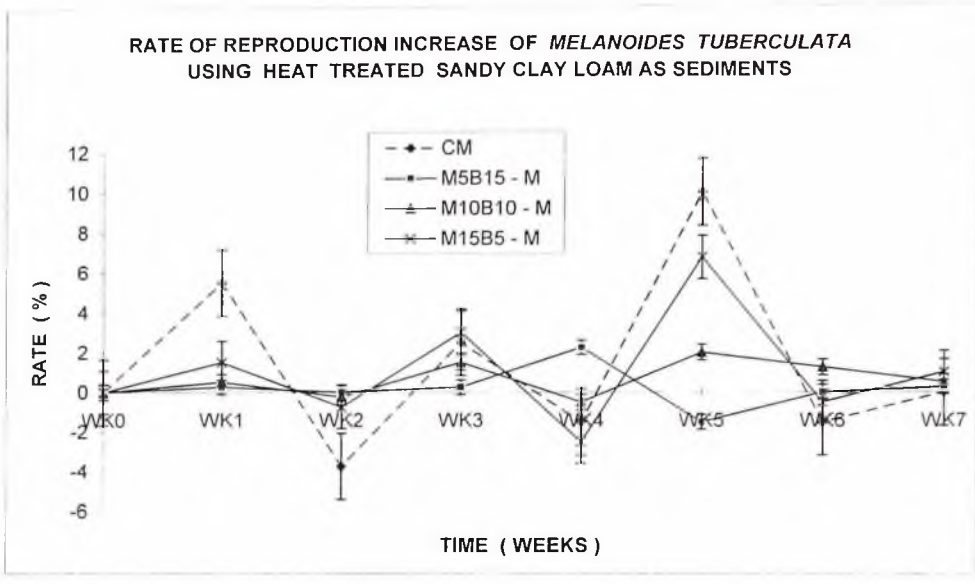


FIG. 4.61

FIG. 4.62



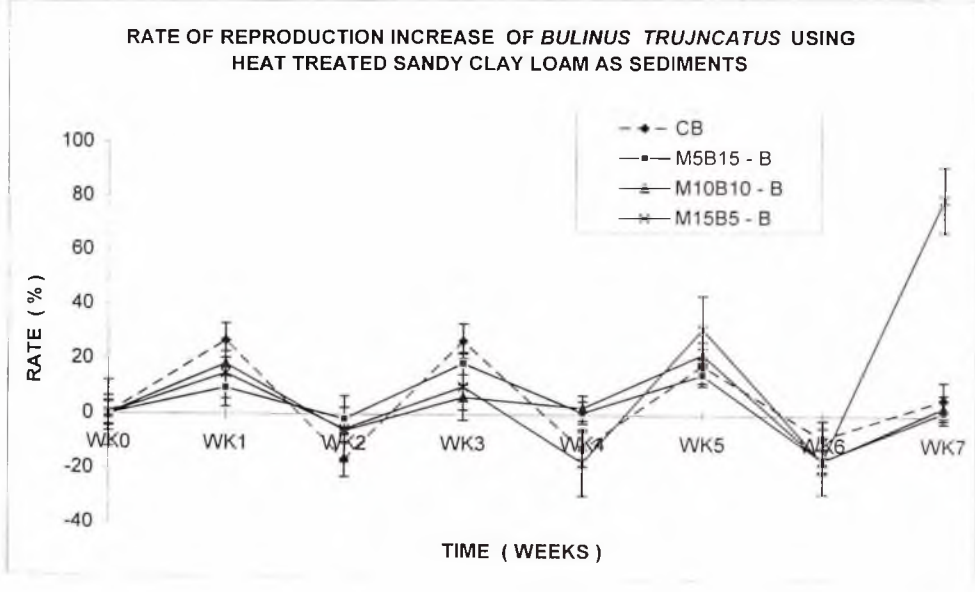


FIG. 4.63

4.3.2.4.4 *Shell Length :*

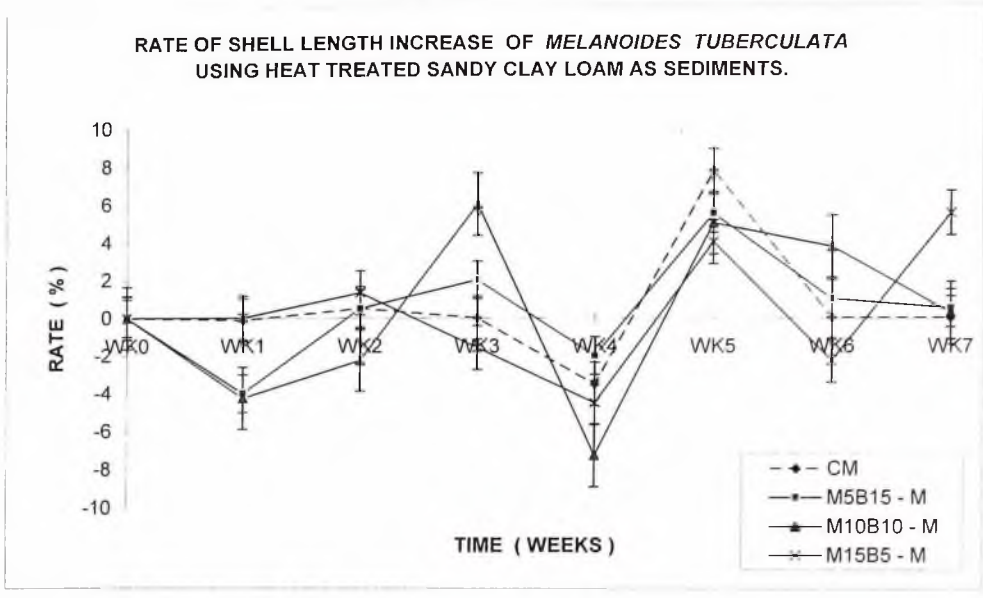
From Table 4.18 and Figures 4.64 and 4.66, the observations made were similar to that made for mortality and reproduction. No significant differences were observed between the increase in the shell length with the different densities of *Melanoides tuberculata* (M5B15 ($p< 0.76244$) M10B10 ($p< 0.80587$) and M15B5 ($p< 0.81571$). Thus the increase in the density of *Melanoides tuberculata* had no effect on the rate of increase of shell length of *Bulinus truncatus* in the various combinations.

4.4 DISCUSSION :

The findings reported in this chapter lend further support to the conclusions of the previous chapter that the nature and condition of the sediments may be of critical importance in determining the outcome of the competitive interactions between *Melanoides tuberculata* and *Bulinus truncatus*. As in the previous study (chapter 3), the adverse effects of the presence of *Melanoides tuberculata* on *Bulinus truncatus* were demonstrated clearly in the area of growth (ie total body weight gain). The other parameters which were investigated ie reproduction, mortality, and increase in shell length did not show any clear competitive interactions.

The present results show that *Melanoides tuberculata* is able to suppress the growth rate of *Bulinus truncatus* when sandy clay loam is used (in the normal condition) as the sediment. Thus two sediment types have now been shown as suitable substrates for the possible competitive exclusion of *Bulinus truncatus* by *Melanoides tuberculata*.

FIG. 4.64



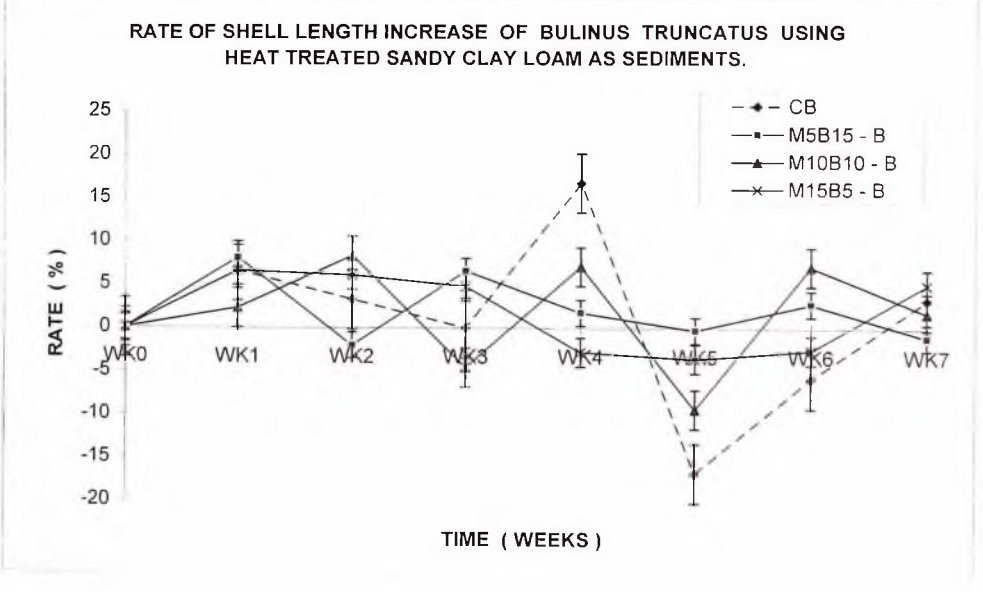
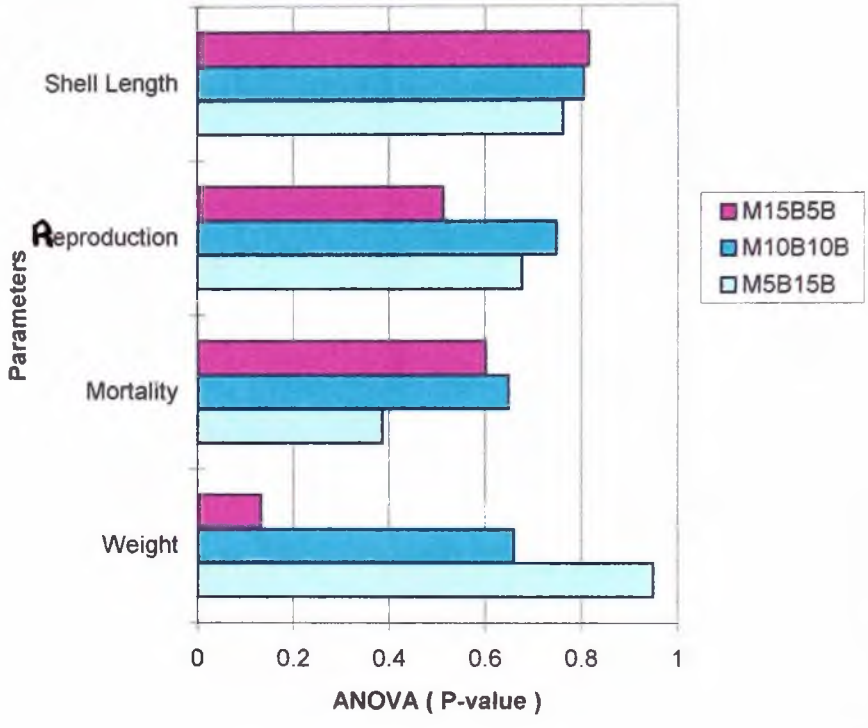


FIG. 4.65

Fig. 4.66

Graphical presentation of the statistical analysis of the results for *Bulinus truncatus* using sandy clay loam as sediments



The first substrate which was identified was sandy gravel. It is significant to note that with both kinds of sediments, the heat treatment prior to the experiments reduced the competitive ability of *Melanoides tuberculata* rather drastically.

In the present studies *Melanoides tuberculata* did not exert any significant influence on *Bulinus truncatus* snails in all the experiments using heat treated sandy clay loam. On the contrary, its growth rate in the three experimental combinations was significantly lowered below that of the control. This was found to be the case whether as few as five *Bulinus truncatus* snails were used or as many as ten or fifteen.

The observed changes in competitive advantage of *Melanoides tuberculata* over *Bulinus truncatus* when the condition of the sediment is changed can be explained by postulating that

i) the sandy clay loam particles play an important role in the nutrition of *Melanoides tuberculata* snails. This role may be direct or indirect.

ii) heat treatment has a deteriorating effect on the nutritive value of the particles of the sediment.

In the direct nutritive role, the particles which are usually covered with epilithic algae and diatoms (Round, 1981) may be a source of food or diatoms for the *Melanoides tuberculata* snails. Indirectly the sand particles may facilitate the mechanical digestion or trituration of larger food particles in the gut (Storey, 1970).

Heat treatment is likely to destroy the epilithic communities thus eliminating a possible food source for the snails. The fact that *Melanoides tuberculata* is adversely affected by this treatment of the sediment suggests that this species is more dependent on this food source than *Bulinus truncatus*. This can be inferred from earlier observations (Hicklin,1988) that

Melanoides tuberculata is usually found inhabiting the sediments while *Bulinus truncatus* is usually more closely associated with aquatic macrophytes. Thus the elimination of this food source through heat treatment of the sediments introduces some amount of food stress on *Melanoides tuberculata*.

Since the physical nature of the sand particles remained unchanged at the end of the heat treatment, it is possible to conclude that the direct nutritive function of the epilithic communities may be of more value to the growth of the *Melanoides tuberculata* snails than the indirect one. It is also known that the ingestion of sand grains may assist in the uptake of essential cations such as Ca^{2+} and traces of copper (Hicklin, 1988). It is possible that heat treatment could have altered the surface chemistry of the sand particles thus interfering with the uptake of Ca^{2+}. If this is the case, it could partly explain the reduction in growth rate of *Melanoides tuberculata* when the experiments were done using heat treated sediments.

4.4.1 Density dependent effects :

The results indicate that as the density of *Melanoides tuberculata* increased the adverse effects on *Bulinus truncatus* became more severe. This observation is not surprising since competitive interactions in nature are often dependent on the density of the competing species (Pointier et al., 1989; Pointier, 1993; Meyer-Larsen & Madsen, 1989). If the present findings, are a good indicator, any attempt to introduce *Melanoides tuberculata* snails for the control of *Bulinus truncatus* snails should aim for a rather high *Melanoides tuberculata* / *Bulinus truncatus* ratio at the start. The determination of the exact ratios to be employed would warrant further investigations.

CHAPTER 5

FOOD PREFERENCE AND FEEDING BEHAVIOUR OF *BULINUS TRUNCATUS* AND *MELANOIDES TUBERCULATA*

5.1 INTRODUCTION :

In biological control methods using intramolluscan competition, a knowledge of the diet of the molluscs can be a very useful tool for the selective elimination of target species. This is because the population growth rate of pulmonate snails is known to be regulated by their food quality and quantity (Eisenberg, 1966, 1970). Unfortunately little information about the natural diet of freshwater snails and their feeding methods (Graham, 1955; Calow, 1970), is contradictory. Thus while Boycott (1936) ; WHO (1957) are of the view that freshwater snails feed on algae (including those that form part of the periphyton and detritus) rather than living macrophytes, Frömming, (1953, 1956) stressed that macrophytes are more important in the diet of pulmonates.

Due to the equivocal nature of the evidence, it was decided to investigate the feeding habits of the two snail species whose competitive interactions have been reported in chapters 3 and 4. First of all, the feeding apparatus of each species ie the radula was studied using scanning electron microscope (SEM) in Section A of this chapter.

Secondly, the gut contents of both snails species were examined in detail (Section B). The rationale for doing this was to obtain information about the feeding process. It is hoped that this information might throw some light on the mechanisms underlying the competitive interactions observed between *Melanoides tuberculata* and *Bulinus truncatus*.

5.2 SECTION A : THE RADULA

5.2.1 MATERIALS AND METHODS :

5.2.1.1 Preparation of the radula.

Snails were obtained from the Weija lake. They were fixed in 70% alcohol and examined using S E M at the Robert Ogg Electron Microscopy Laboratory (26 Gianinni Hall) of the University of California at Berkeley. The snails were first removed from the 70% alcohol and rehydrated through alcohol series from 60 % to 10% alcohol and then in distilled water as described by Hickman (1983). The snails were then dissected to remove their buccal mass. These were then treated in a 10% sodium hydroxide solution at 70^0C. This dissolved most of the buccal tissue leaving the radula intact. The radulae were removed before all the buccal tissues were dissolved completely to avoid contact of the sodium hydroxide with the chitin of the teeth and the radula membrane.

Radulae were picked up with a brush and washed thoroughly in distilled water and dehydrated through ethanol series from 60% to 100%. These were then cleaned by brief immersion in an ultrasonic cleaner. The clean radulae were critical point dried and manipulated to expose maximum dentition. Each dry radula was transferred to S E M stubs and mounted on a thin cellophane paper . The radula was then coated with gold (200 - 400^{0}A). This was then transferred to an ISI (International Scientific Instrument , DS - 130 with an accelerating default of 10KV) S E M which was then viewed at accelerating high tension

voltages of 10 kv or less to minimize charging and specimen damage. The radula was rotated and oriented selectively to allow diagonal presentation that maximizes areal coverage and resolution. Photographs were then taken of these radulae for illustrations, selection being more in favour of morphology than systematics and topology.

5.2.2 RADULA MORPHOLOGY :

5.2.2.1 *Melanoides tuberculata* :

The radula ribbon of a *Melanoides* species is believed to be of the taenioglossan type with formula 3 + R + 3 or 2 + 1 + R + 1 + 2 (Fretter and Graham, 1962 ; Hicklin, 1988). This means there is a central tooth R (the rachidian), one lateral tooth and two (outer and inner) marginals making the number of teeth per row 7 (Figs. 5.1 and 5.2). However the number of teeth per row in the *Melanoides* under study is five (Plate 5.1) which means there is a rachidian R with one lateral and one marginal on both sides. Thus the possible formula will be 1 + 1 + R + 1 + 1 or 2 + R + 2. An average of 104 to 106 ± 2 rows of teeth were counted thus about 520 to 530 individual teeth were found on the radula ribbon. The central tooth which is short, stout and multi-cuspid has one lateral tooth and one marginal tooth on either side of it .

The central tooth (the rachidian) has a cuspid or denticular formula of 4 - 1 - 4 / 0 + 0 instead of 4 - 1 - 4 / 1 + 1 as observed by Abbott, 1952 and also Hicklin, 1988. This means that the leading edge bears one large denticle or cusp (mesocone) in the centre with four smaller endocones and ectocones (Baker, 1945) on each side (Plate 5.1). There were no basals found at corners of each tooth hence the 0 + 0 in the formula instead of 1 + 1 as reported out by Abbott, 1952; and Hicklin, 1988. Most of the central teeth were symmetrical.

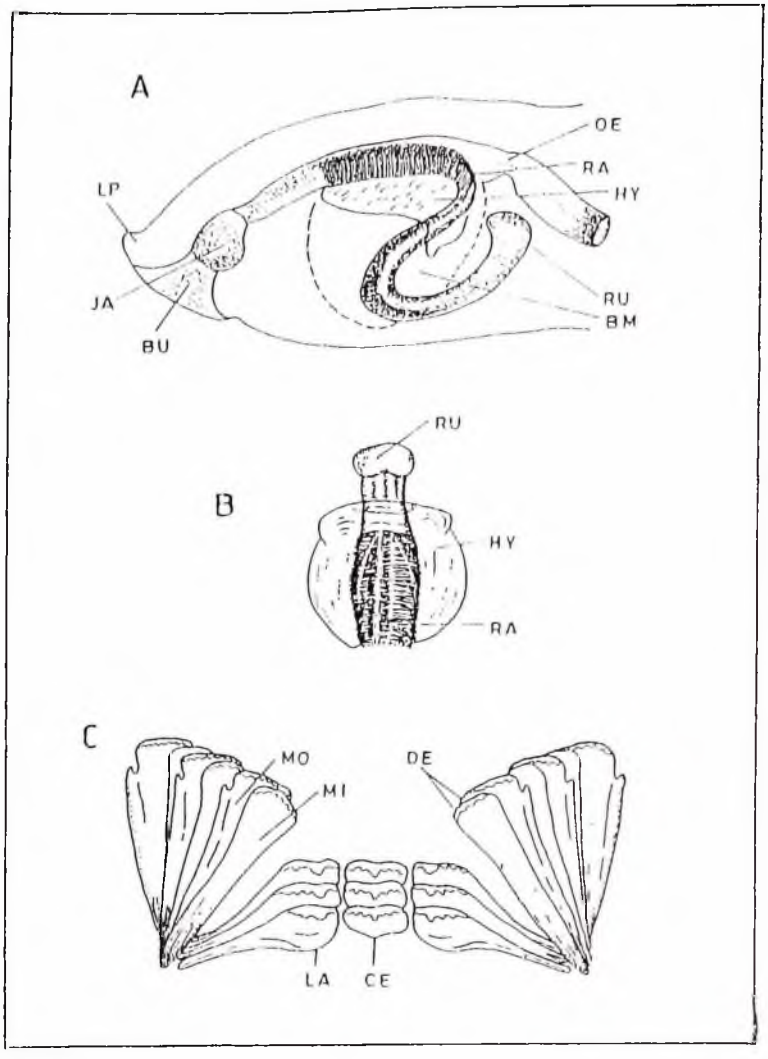


Fig. 5.1

PROBOSIS AND ITS PARTS : **A**, Sagittal section (semi-grammatic) of probosis (BM, dotted line showing limits of buccal muscle and cartilage, BU, oral cavity; HY, hyaline sheath or buccal membrane of radula; JA, left jaw, LP, labial pads; OE, oesophagus; RA, radula or odontophore; RU, rudiment of radula). **B**, Dorsal view of the exposed radula. **C**, Sample rows of radula teeth in their respective positions. (CE, central or rachidian tooth; DE, denticles found on leading edge of all teeth; LA, lateral tooth; MI, inner marginal; MO, outer marginal) [**Adopted from Abbott, 1952**]

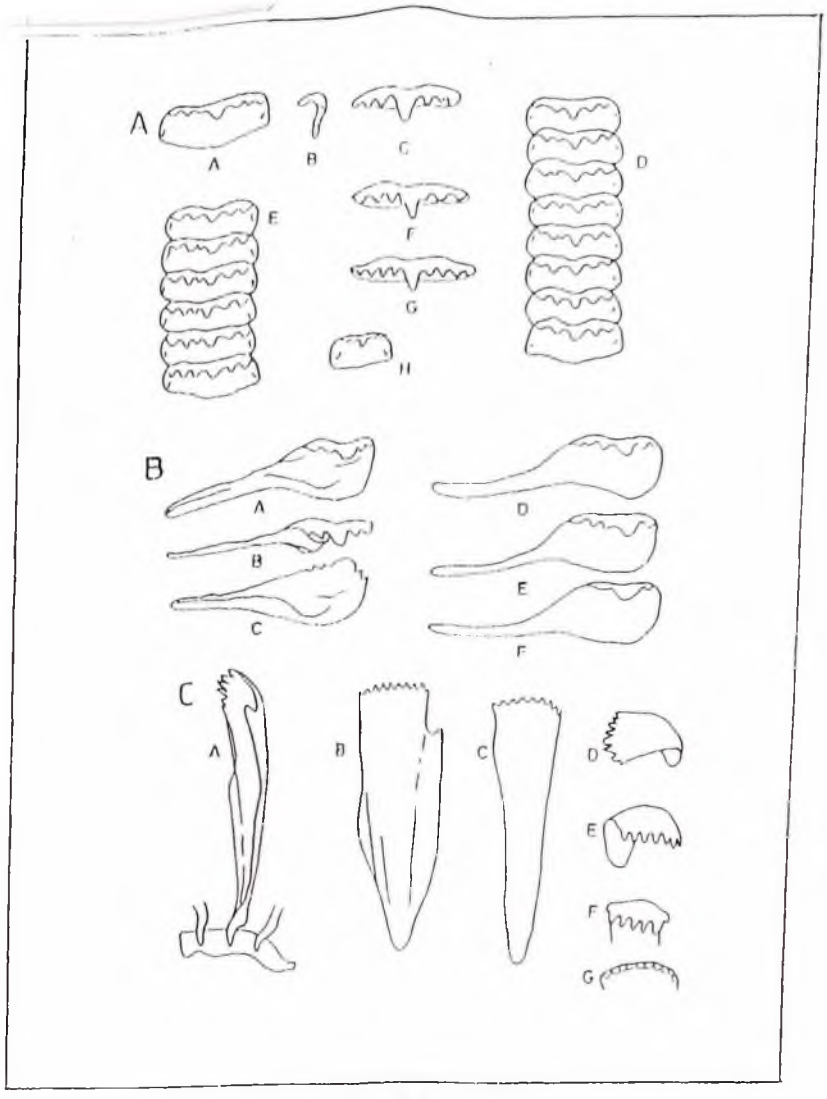


Fig. 5.2

DETAILS OF RADULA : A. ***Central tooth*** (A, anterior view; B, lateral view; C, dorsal view; D, row of 8 centrals showing variation in number of denticles; E, rows of 6 centrals showing similar variation; F,G. dorsal view showing denticle variation; H. central of young snail removed from parent's brood pouch, showing prominence of the two basal denticles in each lower corner). B. *Lateral tooth* (A, C to F, anterior view of 5 different laterals; B, dorsal view of the lateral). C. *Marginal tooth* (A, side view of outer marginal showing attachment to basal membrane; B, outer marginal; C, inner marginal; D to G, dorsal view of inner marginals showing variations in the number of denticles). **[Adopted from Abbott, 1952]**

Plate : 5.1 A transverse section of radula of *Melanoides tuberculata* showing the five teeth in a row: (**r** = rachidian tooth, **c** = lateral tooth and **a** = marginal tooth).

Plate : 5.2 Marginal teeth of *Melanoides tuberculata* :
Note the long shaft (**q**) *and the denticles or cusps eg.* (**s**)

The laterals are slightly longer than the centrals, however they are arm-like and seem to arise from the same source as the marginals. The cusps counted on the lat-

PLATE 5.1

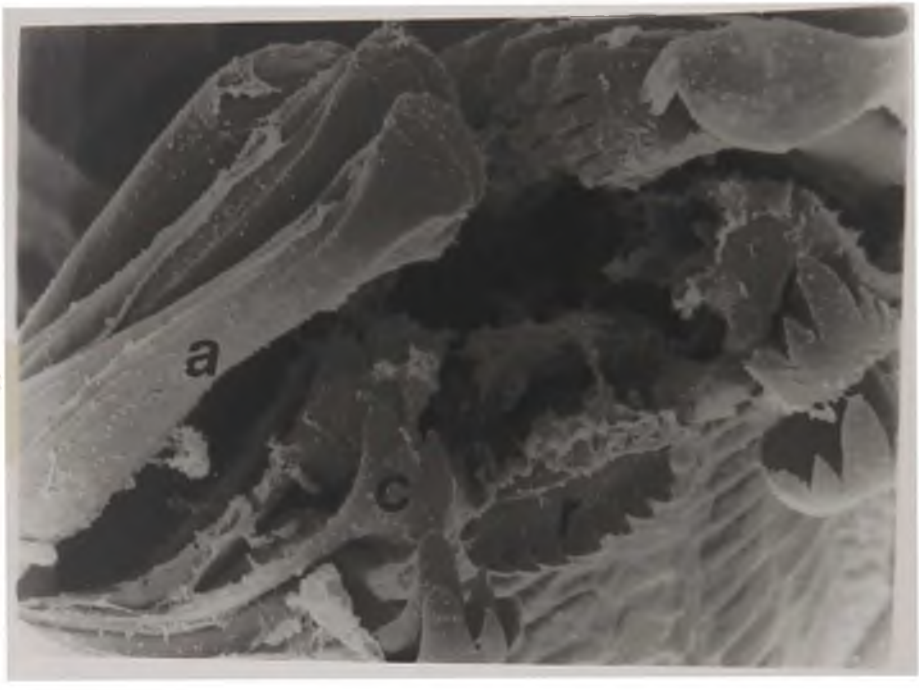
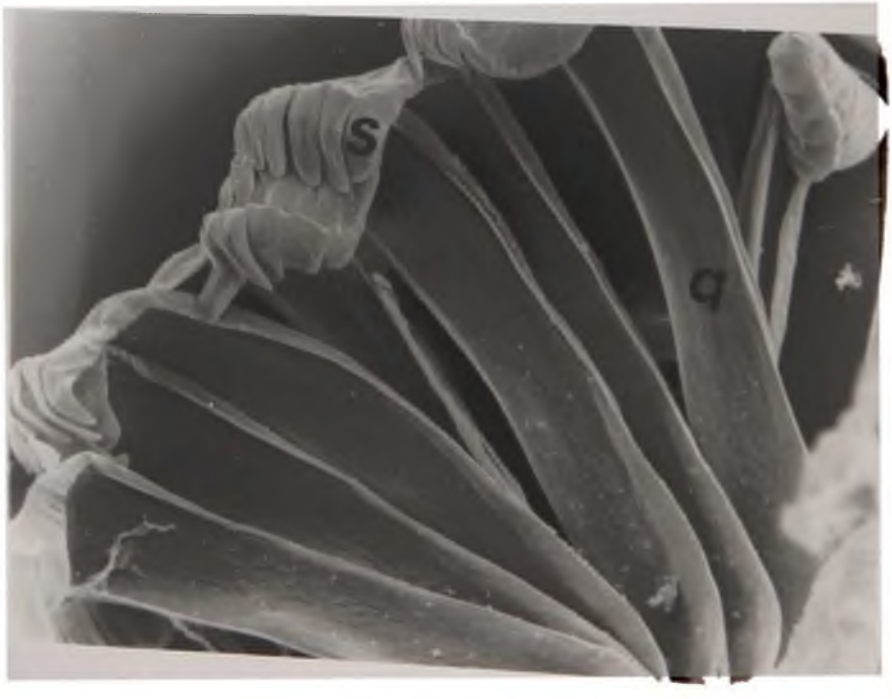


PLATE 5.2



The laterals are slightly longer than the centrals, however they are arm-like and seem to arise from the same source as the marginals. The cusps counted on the laterals were most frequently 1 - 1 - 4 and occasionally 0 - 1 -2, showing asymmetry.

The marginals are slightly club-like or spatulate in appearance, often very long and slender with lots of cusps (Plate 5.2). It must be noted that most of the denticles found on the teeth of this *Melanoides* under study were not sharp but blunt at their tips and were similar in size. Usually six flappy denticles were found on each marginal tooth, however, occasionally a few were found to be forked close to the tips and thus raising the number between 7 and 9 (Plate 5.3).

5.2.2.2 *Bulinus truncatus* :

The radula of *Bulinus truncatus*, unlike that of *Melanoides tuberculata* is sheet-like. It is shaped like a long ribbon with not less than 120 transverse rows of teeth. Each transverse row comprised of not less than 40 individual teeth. Thus roughly as many as 4,800 teeth could be found on just one radula (Plates 5.4 & 5.9). There are series of duplicating teeth of different shapes and forms on either side of the rachidian tooth. These are laterals, intermediates and marginals.

The central teeth are bicuspid as is common in most Planorbidae (Baker, 1945; Kpikpi, 1990). The two cusps, which are of equal size, are sharp and pointed. The most striking feature about these central teeth is that a) they seem to be two monocuspid teeth lying close to one another so close that they look like one tooth (Plates 5.5) and b) the mesocone has no characteristic angular shape as was the case of *B. truncatus* investigated by Oberholzer et al, 1970 cited in Kpikpi, 1990.

Plate : 5.3 Cusps or denticles on the flappy marginal teeth of *Melanoides tuberculata*. *Note the blunt ends and the forked cusps.*

Plate : 5.4 Part of the transverse rows of teeth on the rdula of *Bulinus truncatus*. *Note the modifications of the teeth as one moves from the central (rachidian) tooth.*

PLATE
5.3

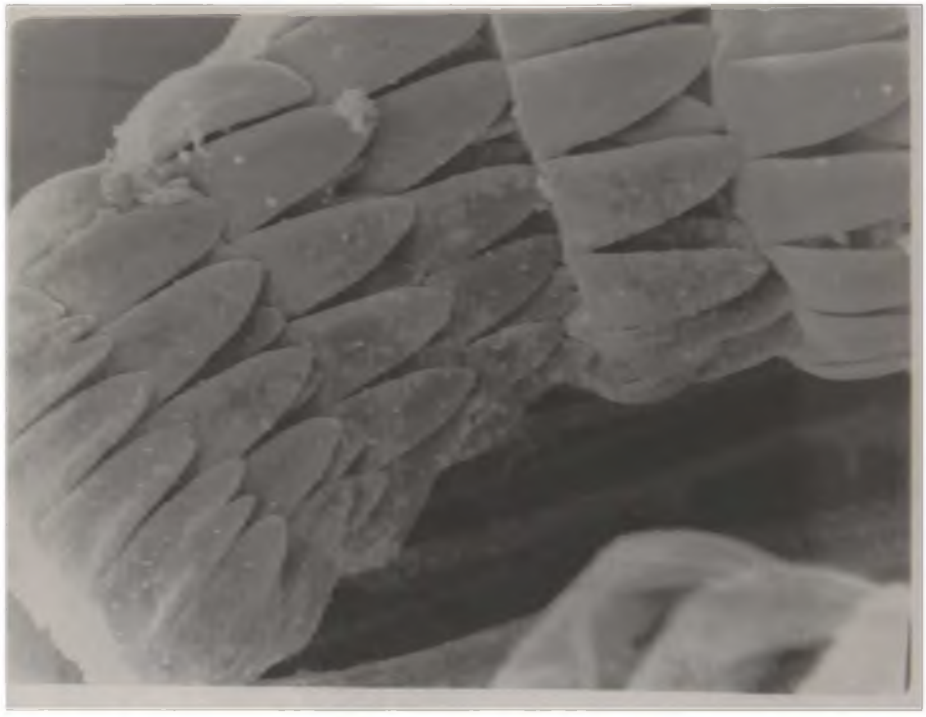


PLATE
5.4

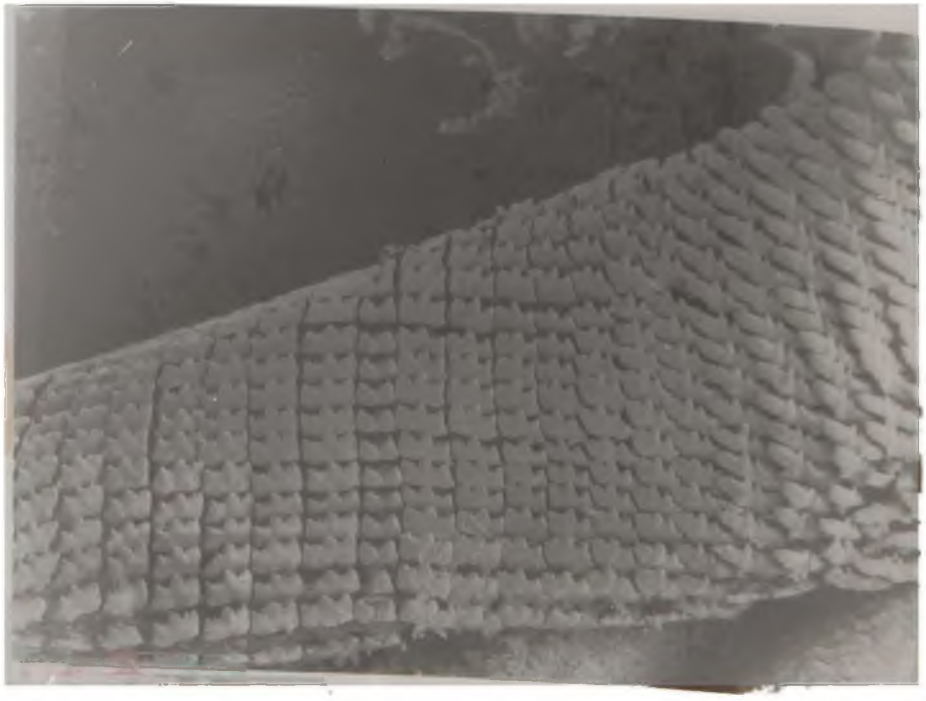


Plate : 5.5 Rachidian tooth (**n**) and lateral tooth (**o**) of *Bulinus truncatus*.

Plate : 5.6 Some lateral or intermediate teeth of *Bulinus truncatus* eg. (**k**) : *Note the endocone* (**z**), *the ectocone* (**x**) *and the mesocone* (**y**).

The lateral teeth are most commonly tricuspid and lie on both sides of the central teeth. Those teeth between the first (typical) laterals and the first marginals are a varying number

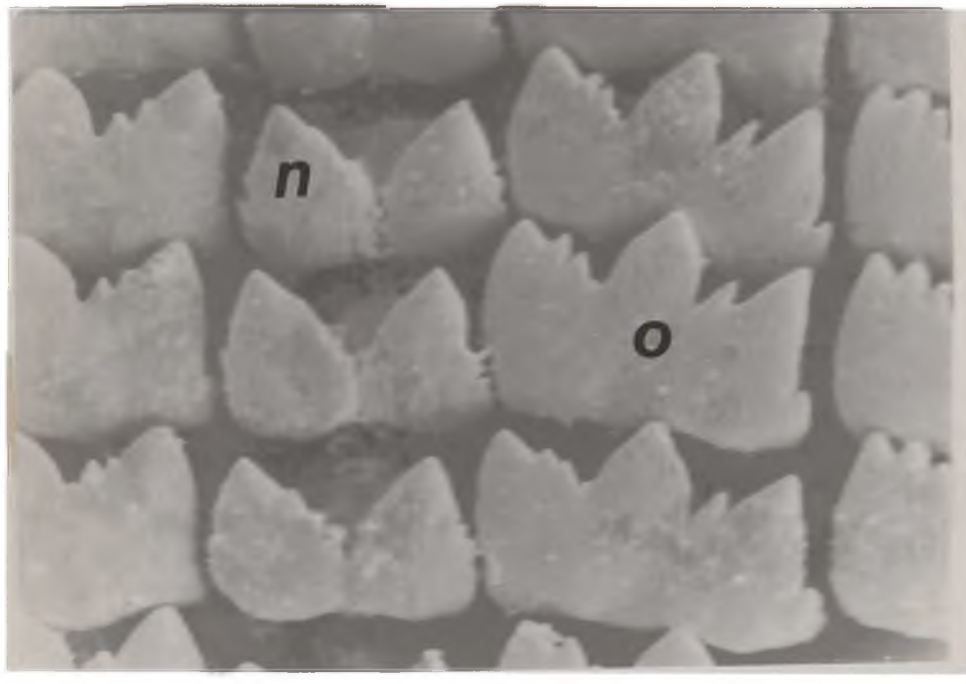


PLATE 5.5.

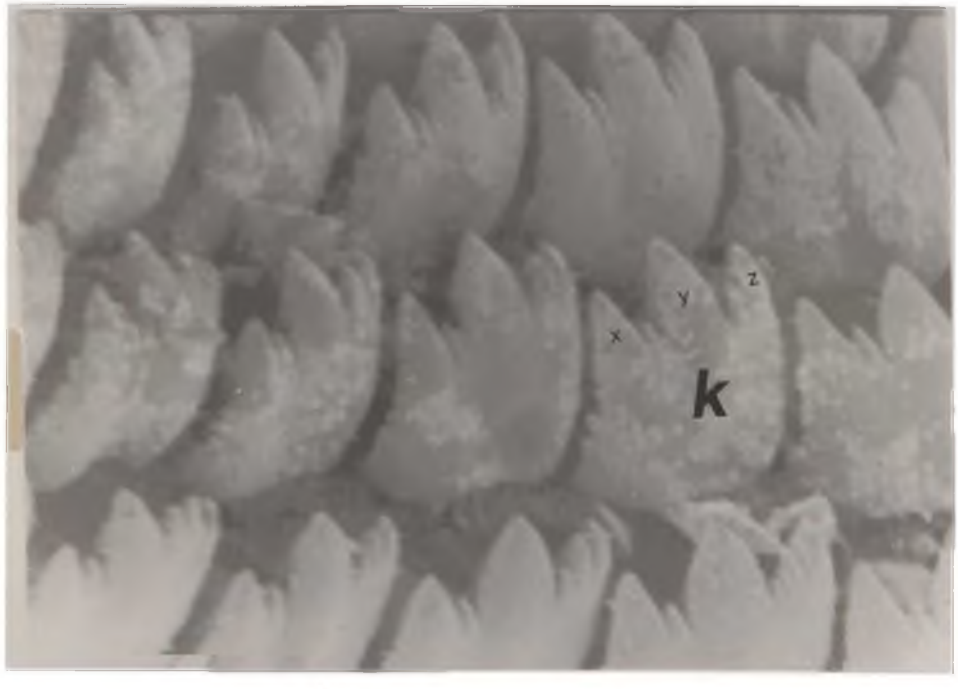


PLATE 5.6.

The lateral teeth are most commonly tricuspid and lie on both sides of the central teeth. Those teeth between the first (typical) laterals and the first marginals are a varying number of teeth referred to as the intermediates. These usually show certain modifications as splitting of the ecto and endocones into smaller cusps. There are as many as one to five intermediate teeth on either side of the typical (the first tooth after the central tooth) lateral tooth in a transverse row (Plate 5.4). Beyond these are a vast array of comb-like marginals.

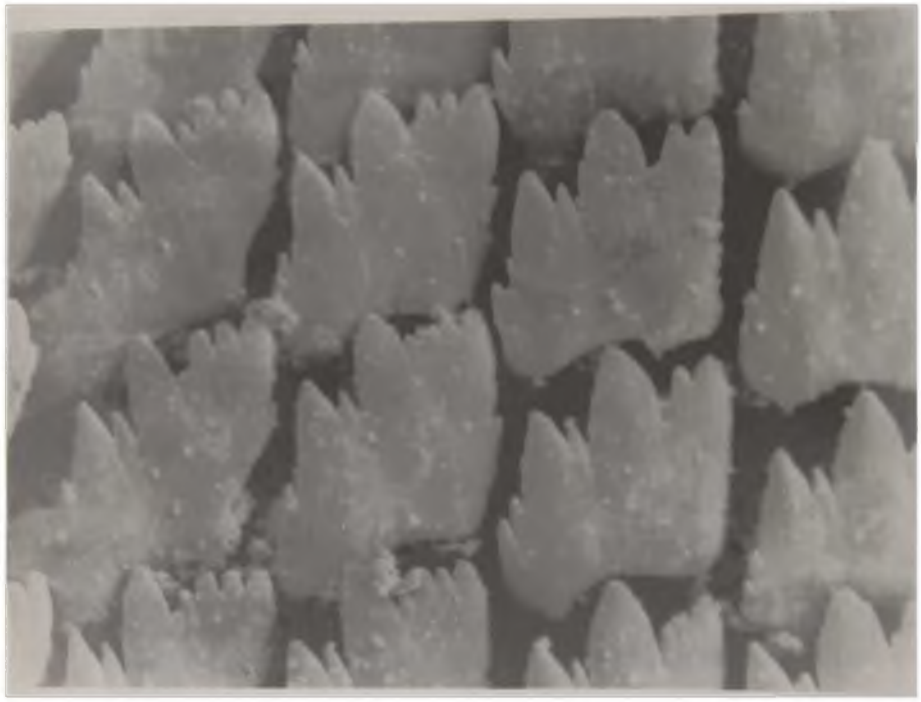
The tricuspid laterals have an intact central or median mesocone and show varying degrees of subdivisions of inner endocones and outer ectocones as these laterals are converted to intermediates. The mesocone is usually large and prominent (Plate 5.6, 5.7 & 5.8) . The subdivisions of the endocone vary between 1 and 4 and those of the ectocones vary between 1 and 2. Basal cusps are found at the bases of the ectocones and endocones, with those found on the latter being more prominent. The number of basal cusps vary between 1 and 3. These subdivisions tend to increase along a transverse row towards the marginals.

The marginals are numerous. Not less than 16 teeth lie on either side of the central teeth just after the laterals and intermediates on the transverse row. The exact number of marginal teeth could not be ascertained because some had degenerated. These are comb-like and claw-like teeth with varying numbers of cusps and elaborate divisions of the endo and ectocones (Plate 5.9). The mesocone however usually persists and may be recognized by its large size and central position. The number of subdivisions of the endocones varies between 7 and 9, usually they are of the same size and are pointed. The ectocones however have only one subdivision but have basals which vary between 1 and 3 in number. These basals are prominent and sharp.

Plate : 5.7 Lateral and intermediate teeth of *Bulinus truncatus* :
Note the divisions of the endocones and ectocones.

Plate : 5.8 Marginal teeth of *Bulinus truncatus* showing pronounced subdivisions of the endocones and ectocones : *Note the prominent median mesocone.*

ATE
.7



ATE
.8

Plate : 5.9 Radula ribbon of *Bulinus truncatus* :
Note the numerous teeth and the folding of the ribbon.

Plate : 5.10 Radula of *Melanoides tuberculata* showing transverse rows of the teeth

5.3 DISCUSSION :

PLATE 5.9

PLATE 5.10

5.3 DISCUSSION :

Melanoides tuberculata was found to have a more robust and boat-like radula ribbon with 500 and 530 teeth (Plate 5.10). This is in contrast with the observations made by Hicklin, (1988) who found a total of between 742 and 840 teeth. *Bulinus truncatus* on the other hand has a sheet-like radula ribbon which bears about 40 teeth in the transverse row and over 120 teeth in a longitudinal row (Plates 5.4 & 5.5) . Total number of individual teeth per ribbon therefore is not less than 4,800, this supports the observation of Thomas et al, (1985) who found a total of 4,425 teeth. The radula teeth of *Bulinus truncatus* are therefore finer that those of *Melanoides tuberculata*. It is believed that there is a direct correlation between the shape of the teeth and the feeding habits of the animal while the length of the teeth has been linked to the amount of work done by the teeth during feeding (Fretter and Graham; 1962).

Thus the differences suggest that *Melanoides tuberculata* has a different feeding strategy to *Bulinus truncatus*. The robust structure (Plate 5.10) of *Melanoides* with large teeth, long shaft, blunt cusps and also the relatively wide interteeth gaps seem to be better adapted for feeding on larger food items (like decayed macrophytes, algae, diatoms and sand grains) rasped from the substrate. The rachidian being the shortest with slightly sharper and finer cusps suggesting their ability to rasp from the substrate. The marginals being slender and longer may be involved in selective gathering of food from the surrounding substrate (Fretter & Graham, 1962). The blunt cusps of these marginals also suggest that they are not used for scraping. It is therefore likely that their food is composed of soft and decaying material of both animal and plant origin. The teeth of *Melanoides tuberculata* did not show any wear and tear.

The sheet-like structure of the radula teeth of *Bulinus truncatus* (Plate 5.4 and 5.9) with stronger and sharper cusps, short and stout shafts and the relatively small interteeth gaps and the duplications of the various teeth types suggests that it is better adapted for scraping food as the last 10 to 13 rows of teeth on either side of the central teeth show increased wear and tear. This is as a result of their overuse and possibly a state of high efficiency as pointed out by Hickman (1980). The rather small size of these teeth in *Bulinus truncatus* may be related to the size of the epiphytic algae and diatoms which form its diet. It is likely that the narrow interteeth gaps prevent losses of the food scraped from the substrate. From these findings it is clear that if competition for food was the main interaction between these two snail species competitive exclusion was not to be expected .

The most striking features of the radula of *Melanoides tuberculata* which differ from that of previous observations and which might be of taxonomic importance are a) the presence of 5 teeth in a transverse row instead of 7 as found by Hicklin (1988). This shows the absence of outer marginals. This means that instead of a true taenioglossan teeth type with a formula of 3 + R + 3 or 2 + 1 + R + 1 + 2 (Fretter and Graham,1962), one with a formula of 2 + R + 2 or 1 + 1 + R + 1 + 1 was observed. b) the absence of basal cusps at the two corners of the central teeth (Hicklin, 1988; Brown, 1980) hence instead of 4 - 1 - 4 / 1 + 1 as the formula for the central teeth (Abbott, 1952 and Hicklin, 1988) 4 - 1 - 4 / 0 + 0 or 4 - 1 - 4 was observed and c) the absence of the small, sharp, thumb-like protrusion on the outer edge of the marginal teeth. It is possible that the loss of the two outer marginal teeth and the differences observed for the *Melanoides* under study might be of adaptive significance

(Hickman,1980) which might have been handed down the generations (Figure. 5.3) or simply that, this *Melanoides* may be a different species altogether. However, one cannot be conclusive on this, since radula structure is just one of the numerous features used in the classification of species.

Further investigations into the other features of taxonomic importance eg shell structure may be required to confirm this. It is possible that some authors have used the species *T. granifera* and *M. tuberculata* interchangeably and as such what has been described as the radula of *M. tuberculata* might actually be for *T. granifera* or that the two species are not the same as some investigators claim (Pointier et al., 1989; Mkoji et al., 1992).

The most striking feature of the radula teeth of *Bulinus truncatus* is the monocuspid appearance of some of the central teeth (Plate 5.5) lying close to one another suggesting that the whole ribbon is probably bilaterally symmetrical. This may be a useful taxonomic tool, but since it did not occur in all the radulae studied, it needs to be looked at more critically before final conclusions could be made.

It could be concluded that a) the radula of the two snails are different morphologically and also function differently. b) although the *Melanoides* species under study bear some resemblances to those studied by Abbott (1952) and Hicklin (1988), the differences seem significant enough to warrant the suggestion that they might be different species.

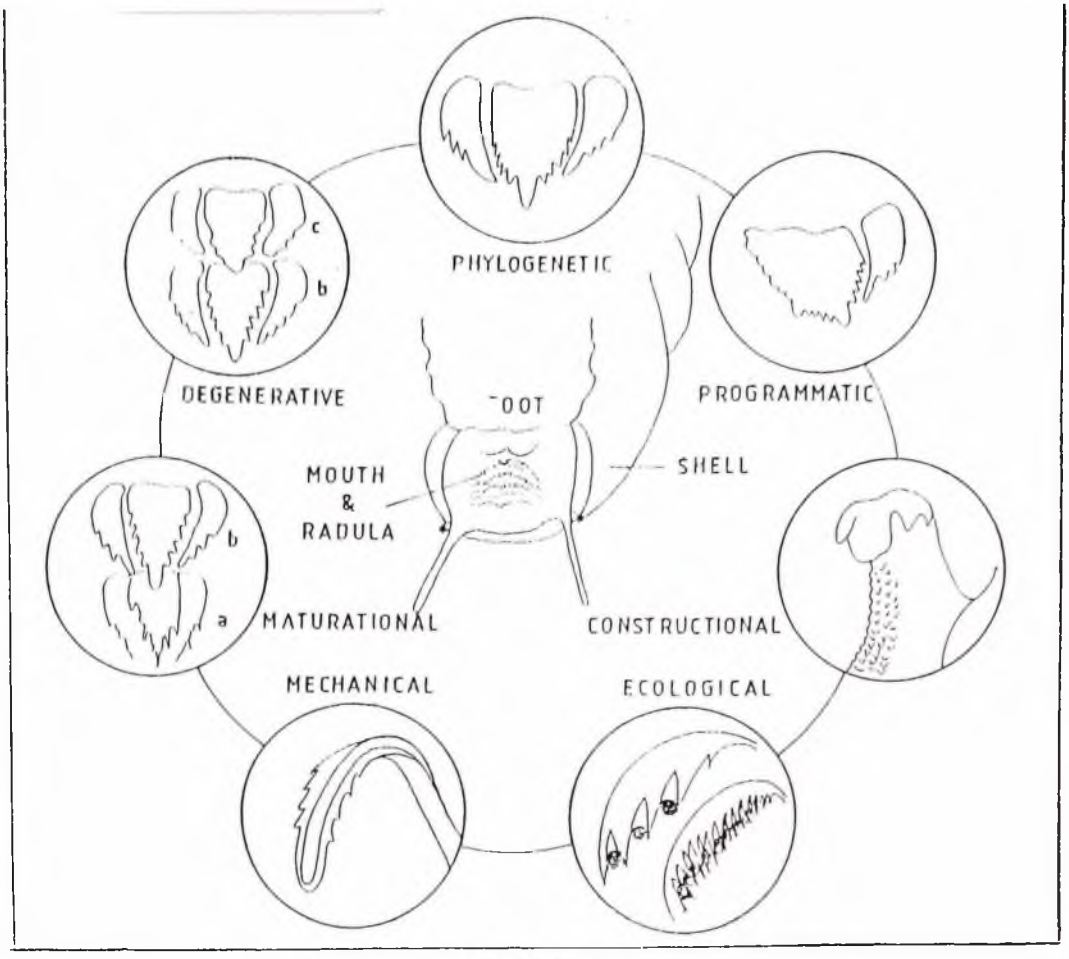


Fig. 5.3.

DIAGRAMMATIC MODEL OF SEVEN MAJOR FACTORS CONTRIBUTING TO FORM AND PATTERN IN GASTROPOD RADULAE.

Clockwise from the top, **Phylogenetic factors** comprise the genetically coded basic ground planof inherited form or pattern. **Pro-grammatic errors** produce repeated deviation from basic ground plan and may be genetic or somatic. **Constructional factors** related to the properties of chitin and the way it is secreted produce form and patten of little or no direct functional significance but serve as a source of morphogenetic information. **Ecological factors** such as the interactions between tooth cuspsand substrates produce adaptive modification of morphological detailand are superimposed on the basic ground plan. **Mechanical factors** such as forces acting on teeth during feeding produce adaptive solutions to stresssuch as development of compressional ridges. **Maturational factors** relating to the continuous secretion, hardening, and anterior migrationof new teeth produce a sequence of form and pattern that serve as a source of ontogenetic rather than functional information. **Degenmerative factors** related to the wear of the teeth provide information about patterns of use and behaviour. a - a newly formed tooth; c - worn tooth. [**Adopted from Hickman, 1980**]

5.4 SECTION B : GUT CONTENT ANALYSIS

5.4.1 MATERIALS AND METHODS :

Adults and juveniles of *Melanoides tuberculata* (0.048 ± 0.012 and 0.099 ± 0.002g) and *Bulinus truncatus* (0.096 ± 0.001 and 0.177 ± 0.010g) were collected from Weija lake. The snails were immediately transferred into 70% alcohol to kill them. This treatment was meant to arrest the ongoing digestion process and also to avoid the egestion of the gut contents. Twenty snails of each species were then removed from their shells and dissected under water to remove the gut. The method used by Thomas et al (1985) was then followed with slight modifications. Each portion of the gut was transferred separately into a labelled Sedgwick - Rafter counting cell containing 200µl of water. The contents of these portions of the gut were thoroughly mixed with the water in the cell.

Using a micropipette (Socorex Swiss - Heigar model), 50µl of the mixture was taken from the cell and placed on a clean slide. This was then examined under the microscope (Olympus CH - 2) to identify the gut as well as the relative sizes of the food items as this moves from the crop to the rectum. This was repeated. The number of particles belonging to a particular plant and animal species were counted within each of 10 randomly selected fields. The number obtained was then multiplied by 100 to give a rough estimate of the total number of that particular food item present in that portion of the gut. The identification of the algae and the diatoms was done with the aid of Palmer (1962).

Tissues of living macrophytes were identified by means of the unique appearance of their epidermal cell patterns. To quantify the food items the following indices were used.

Percentage occurrence $_{(1)} = (T_1/T_2)\ 100$

Mean number of particles $_{(2)} = (M_1 / T_1)$

Relative abundance $_{(3)} = (M_1 / T_1) / T_3$

where T_1 represents the number of portions in which item 1 was found, T_2 represents the total number of portions of gut investigated, M_1 represents the number of item 1 present in a portion and T_3 represents the total of all the food items in that portion of the gut. By the use of an eyepiece grid graticule calibrated by means of a standard millimetre of a stage micrometer, the sizes of the various food items were measured at X40 magnification.

5.5 RESULTS

5.5.1 Items ingested by the two snail species :

The common food items found in the guts of the snails include, algae, diatoms, living macrophytes, decayed macrophytes and also sand grains. The percentage occurrence of the various food items and the relative sizes of the sand grains and the decayed macrophytes differed markedly amongst the two snail species (Tables 5.1 - 5.4 and Figures 5.4 & 5.5). *Bulinus truncatus* was found to have ingested a greater variety of food items than *Melanoides*

Table 5.1. The area (mm2) of some of the items found in the gut of *Melanoides tuberculata*

Items	Minimum area	Maximum area	Mean
Sand grain	0.009	0.09	0.047 +/- 0.028
Living macrophytes	0.163	0.642	0.331 +/- 0.170
Decayed macrophytes	0.088	2.425	1.039 +/- 0.547

Table 5.2. The area (mm2) of some of the items found in the gut of *Bulinus truncatus*

Items	Minimum area	Maximum area	Mean
Sand grain	0.006	0.019	0.0125 +/- 0.0079
Living macrophytes	0.125	0.224	0.133 +/- 0.027
Decayed macrophytes	0.354	0.724	0.598 +/- 0.324

Table 5.3. Relative abundance of food items found in the gut contents of *Bulinus truncatus*.

	Crop	Intestines	Rectum
Diatoms	35.94	27.96	27.14
Algae	9.60	9.54	8.93
Living macrophytes	13.84	13.16	7.14
Decayed macrophytes	24.55	23.03	17.86
Sand grains	16.07	26.32	39.28

Table 5.4. Relative abundance of food items found in the gut contents of *Melanoides tuberculata*.

	Crop	Intestines	Rectum
Diatoms	10.78	10.27	8.71
Algae	0.94	0.83	2.07
Living macrophytes	7.17	4.59	4.14
Decayed macropytes	64.29	50.08	39.45
Sand grains	16.82	33.39	45.64

FIGURE 5.4
GUT CONTENTS OF *BULINUS TRUNCATUS.*

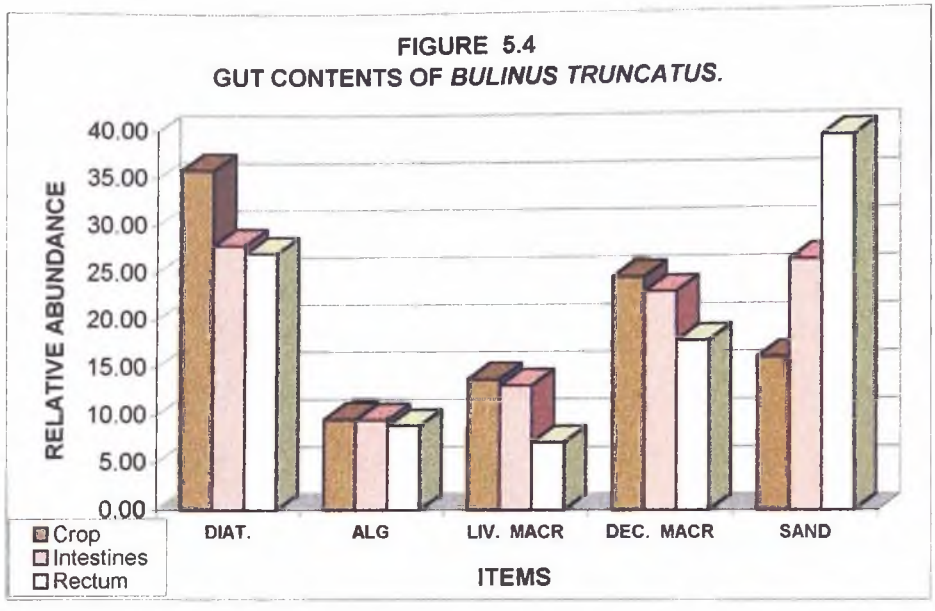
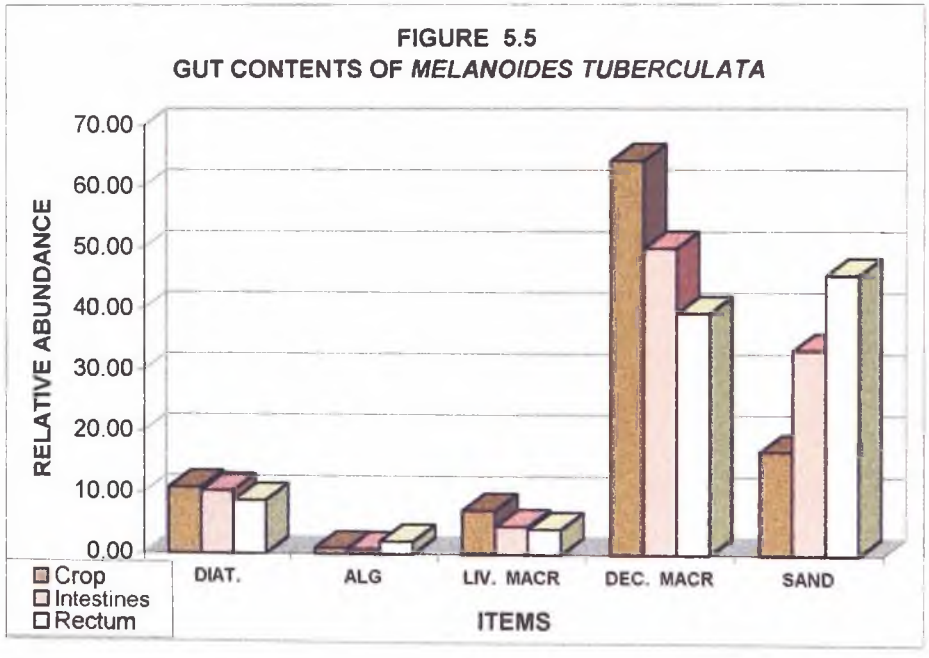


FIGURE 5.5
GUT CONTENTS OF *MELANOIDES TUBERCULATA*



DIAT.= Diatoms, ALG.= Algae, LIV MACR. = living macrophytes and DEC. MACR. = Decayed macrophytes.

tuberculata. Thus more than 25 different food items made up of 14 species of diatoms, 8 species of algae, 2 species of living macrophytes and several kinds of unidentified decayed macrophytes were found in the gut contents of *Bulinus truncatus*. *Melanoides tuberculata* on the other hand was found to have ingested 19 different items made up of 11 species of diatoms, 4 species of algae, 2 species of living macrophytes and also lots of unidentified macrophytes.

5.5.1.1 ***Diatoms :***

On average (Table 5.5) in the crop of *Bulinus truncatus,* 14 genera of diatoms were identified. The most common ones were, *Navicula* > *Cocconeis* > *Fragilaria* > *Rhoicosphenia* > *Synedra* and the less abundant ones include, *Nitzschia* > *Amphora* > *Achnanthes* and *Actirella* > *Cyclotella* and *Diatoma* > *Licmophora, Melosira* > *Auricula.* In contrast to the above observations, Table 5.6 shows that *Melanoides tuberculata* had only 11 species of diatoms, the most common ones being *Cocconeis* > *Rhoicosphenia* > *Fragilaria* and the less abundant ones were *Navicula* > *Cyclotella* > *Nitzschia* > *Amphora* > *Achnanthes* and *Synedra* > *Actirella* > *Diatoma.*

5.5.1.2 ***Algae :***

Table 5.5 shows that 8 genera of algae were found in the gut contents of *Bulinus truncatus* with *Scenedesmus* > *Pediastrum* > *Rhizochlonium* being the most common. The less common ones were *Anacystis* > *Chlorococum* > *Agmenellun* > *Anabaena* >

Table 5.5 Percentage occurence, mean number of cells (particles) and relative abundance of food items found in the crop of *Bulinus truncatus*.

Food Items	Percentage occurrence	Mean number	Relative abundance
Achnanthes	30	200	0.89
Actirella	30	250	1.12
Amphora	40	100	0.45
Auricula	5	50	0.22
Cocconeis	85	1200	5.36
Cyclotella	20	100	0.45
Diatoma	20	500	2.23
Fragilaria	80	400	1.79
Licmophora	10	150	0.67
Melosira	10	100	0.45
Navicula	100	2000	8.93
Nitzschia	50	500	2.23
Synedra	60	1200	5.36
Rhoicosphenia	70	1300	5.80
Agmenellum	30	200	0.89
Anabaena	25	300	1.34
Anacystis	50	500	2.23
Batracospermum	10	100	0.45
Chlorococum	40	150	0.67
Pediastrum	70	100	0.45
Scenedesmus	80	700	3.13
Rhizochlonium	60	100	0.45
Ceratophyllum	60	600	2.68
Lemna	70	2500	11.16
Sand grain.	100	3600	16.07
Decayed macrophytes	100	5500	24.55

Batracospermum. Table 5.6 however shows that the gut contents of *Melanoides tuberculata* had only 4 types of algae, again *Scenedesmus* and *Rhizochlonium* being the most common and *Anacystis* and *Ankistrodesmus* being the least. Thus *Bulinus truncatus* had 5 more species of algae which were not identified in the gut of *Melanoides tuberculata* while *Ankistrodesmus* was only identified in the gut of *Melanoides tuberculata*.

5.5.1.3 *Living Macrophytes :*

Ceratophyllum and *Lemna* as recorded in Tables 5.5 and 5.6 were the two most common living macrophytes identified in the gut of the two snail species . The *Lemna* appeared as fronds or sheets of tissues of cells but the *Ceratophyllum* showed up as long strands. These were found in each of the snails, however, the relative sizes and quantities of these varied considerably amongst the two snail species. Tables 5.1 and 5.2 show that whilst an average size *Melanoides tuberculata* ingested living macrophyte tissue of size ranging between 0.163 to 0.64 mm^2 with an average ± SD of 0.331 ± 0.170mm^2 , an average size *Bulinus truncatus* ingested smaller tissues with size ranging between 0.125 and 0.224 mm^2 with an average ± SD of 0.133 ± 0.027mm^2. Between 2500 to 600 cells of living tissues were found to have been consumed by one *Bulinus truncatus* snail of average size. However an average size *Melanoides truncatus* was found to have consumed between 100 to 50 cells.

Table 5.6 Percentage occurence, mean number of cells (particles) and relative abundance of food items found in the crop of *M. tuberculata.*

Food Items	Percentage occurrence	Mean number	Relative abundance
Achnanthes	20	20	0.99
Actirella	10	20	0.99
Amphora	30	10	0.49
Cocconeis	100	35	1.73
Cyclotella	40	8	0.40
Diatoma	2	5	0.25
Fragilaria	60	50	2.47
Navicula	45	20	0.99
Nitzschia	30	15	0.74
Synedra	20	10	0.49
Rhoicosphenia	70	25	1.24
Ankistrodesmus	5	2	0.10
Anacystis	5	2	0.10
Scenedesmus	20	5	0.25
Rhizochlonium	20	10	0.49
Ceratophyllum	50	55	2.72
Lemna	100	90	4.45
Sand grain.	100	340	16.82
Decayed macrophytes	100	1300	64.29

5.5.1.4 *Decayed macrophytes :*

By far the largest and most common single item found in the gut of all the snails was decayed macrophytes. Again the relative sizes and the quantities differ for the two snail species. More decayed unidentified macrophytes were found in *Bulinus truncatus* than in *Melanoides tuberculata*. From Table 5.5, as many as 5500 cells were found in an adult *Bulinus truncatus* but in an adult *Melanoides tuberculata*, only 1300 cells were found as is shown in Table 5.6. For *Bulinus truncatus* as shown in Table 5.2, the relative sizes ranged between 0.354 to 0.724 mm^2 with an average size of 0.598 ± 0324 mm^2 and for *Melanoides tuberculata* as shown in Table 5.1, the relative sizes ranged between 0.088 to 2.425 mm^2 with an average size of 1.039 ± 0.547 mm^2

5.5.1.5 *Sand grains :*

All the snails were found to have ingested sand grains. Size differences in the grains were observed in the gut of the different snail species. Larger grains with sizes ranging between 0.009 to 0.090 mm^2 with a mean size of 0.047 ± 0.028 mm^2 were however found in *Melanoides tuberculata*, while smaller grains with sizes ranging between 0.006 to 0.019 mm^2 with a mean size of 0.0125 ± 0.0079 mm^2 were however found in *Bulinus truncatus*.

5.5.2 The contents of the crop :

5.5.2.1 *Bulinus truncatus :*

From Table 5.3, it could be seen that on average, the crop of *Bulinus truncatus* contains 35.94% of diatoms, 9.60% of algae, 13.84% of living macrophytes, 24.55% of decayed macrophytes and 16.07% of sand grains. Thus the most abundant food items were diatoms and decayed macrophytes. From Table 5.5, all the snails were found to have ingested *Navicula* (of all the 14 species of diatoms) decayed macrophytes and sand grains . Over half of the snails investigated had *Cocconeis, Fragilaria, Synedra* and *Rhoicosphenia.* Half of the snails had ingested *Nitzschia* and less than half of the snails had were found to have ingested the rest of the diatoms.

For the algae, more than half of the snails were found to have ingested *Pediastrum, Scenedesmus* and *Rhizochlonium* and also the macrophytes *Ceratophyllum* and *Lemna.* Half of the snails were found to have ingested *Anacystis* and less than half of the snails had ingested the rest of the algae.

5.5.2.2 *Melanoides tuberculata :*

From Table 5. 4, it could be observed that the gut content of an adult *Melanoides tuberculata* is made up of 10.78% diatoms, 0.94% algae, 7.17% living macrophytes, 62.29% decayed macrophytes and also 16.82% of sand grains. It is important to note that while there is almost the same amount of sand grains in the gut of an average size *Bulinus truncatus* and

Melanoides tuberculata, the single most abundant food item in *Melanoides tuberculata* was the macrophytes. *Melanoides tuberculata* had ingested 39.74% more of this food item than *Bulinus truncatus*, while *Bulinus truncatus* had ingested 25.16% more of diatoms, 8.66% more of algae and 6.67% more of living macrophytes than *Melanoides tuberculata*.

Table 5.6 shows that all the snails investigated had ingested the single most abundant diatom *Cocconeis*, the macrophyte *Lemna*, decayed macrophytes and also sand grains. Over half of the snails had ingested *Fragilaria* and *Rhoicosphenia* and half of the snails had ingested the macrophyte *Ceratophyllum*.

5.5.3 The contents of the Intestines :

5.5.3.1 *Bulinus truncatus :*

The intestinal contents of *Bulinus truncatus* from Table 5.3, contained 27.95% diatoms (7.98% less than the crop contents), 9.54% algae (0.06% less the crop contents), 13.16% living macrophytes (0.68% less the crop contents), 23.03% unidentified decayed macrophytes (1.52% less the crop contents) and 26.32% sand grains (10.25% less the crop contents). This shows a considerable reduction in the percentage occurrence of the various food items in the intestines as compared to that in the crop. This difference is more pronounced in the case of diatoms which showed a difference of 7.98%.

Table 5. 7 Percentage occurence, mean number of cells (particles) and relative abundance of food items found in the intestines of *Bulinus truncatus*.

Food Items	Percentage occurrence	Mean number	Relative abundance
Achnanthes	10	60	0.99
Actirella	5	40	0.66
Amphora	15	60	0.99
Auricula	2	20	0.33
Cocconeis	35	100	1.64
Cyclotella	10	40	0.66
Diatoma	15	40	0.66
Fragilaria	50	200	3.29
Licmophora	5	20	0.33
Melosira	2	40	0.66
Navicula	70	600	9.87
Nitzschia	20	80	1.32
Synedra	45	120	1.97
Rhoicosphenia	50	280	4.61
Agmenellum	20	20	0.33
Anabaena	10	100	1.64
Anacystis	10	120	1.97
Batracospermum	8	20	0.33
Chlorococcum	30	60	0.99
Pediastrum	40	40	0.66
Scenedesmus	70	200	3.29
Rhizochlonium	10	20	0.33
Ceratophyllum	60	200	3.29
Lemna	50	600	9.87
Sand grain.	100	1600	26.32
Decayed macrophytes	100	1400	23.03

Table 5. 8 Percentage occurence, mean number of cells (particles) and relative abundance of food items found in the intestines of *M. tuberculata*.

Food Items	Percentage occurrence	Mean number	Relative abundance
Achnanthes	5	20	1.67
Actirella	5	5	0.42
Amphora	2	10	0.83
Cocconeis	50	30	2.50
Cyclotella	10	5	0.42
Diatoma	0	0	0.00
Fragilaria	10	5	0.42
Navicula	10	15	1.25
Nitzschia	5	3	0.25
Synedra	10	20	1.67
Rhoicosphenia	20	10	0.83
Ankistrodesmus	0	0	0.00
Anacystis	2	0	0.00
Scenedesmus	10	5	0.42
Rhizochlonium	15	5	0.42
Ceratophyllum	50	30	2.50
Lemna	20	25	2.09
Sand grain.	100	400	33.39
Decayed macrophytes	100	600	50.08

From Table 5.7 one can see that in spite of the reduction in the percentage occurrence of the various food items, all the snails still had decayed macrophytes and sand grains in their respective guts. Over half of the snails had ingested the diatom *Navicula*, *Scenedesmus* and the macrophyte *Ceratophyllum*, half of the snails were found to have ingested *Fragilaria* and *Rhoicosphenia* and also the macrophyte *Lemna* while below half of the snails had ingested the rest of the other food items.

5.5.3.2 ***Melanoides tuberculata :***

From Table 5.4 it could be observed that the intestinal contents of *Melanoides tuberculata* consists of 10.27% diatoms (0.51% less the crop contents), 0.83% algae (0.11% less the crop contents), 4.59% living macrophytes (2.58% less the crop contents), 50.08% decayed macrophytes 9 14.12% less the crop contents) and 33.39% sand grains (an increase of 16.57% on the crop contents). Also it could be noticed from Tables 5.3 and 5.4 that the intestinal contents of *Bulinus truncatus* was more than that of *Melanoides tuberculata* by 17.69% diatoms, 8.71% algae, 8.57% living macrophytes and less by 27.05% decayed macrophytes and 7.07% of sand grains.

A look at Table 5.8 showed a clear reduction in the percentage occurrences. All the snails still had decayed macrophytes and sand grains. Half of the snails had *Cocconeis* and the macrophyte *Ceratophyllum* and below half of the snails had the rest of the food items. The diatom *Diatoma* and the alga *Ankistrodesmus* were not found in the intestinal contents of *Melanoides tuberculata.*

Table 5.9 Percentage occurence, mean number of cells (particles) and relative abundance of food items found in the rectum of *Bulinus truncatus*.

Food Items	Percentage occurrence	Mean number	Relative abundance
Achnanthes	5	10	0.71
Actirella	0	5	0.36
Amphora	2	10	0.71
Auricula	2	5	0.36
Cocconeis	15	30	2.14
Cyclotella	2	0	0.00
Diatoma	1	0	0.00
Fragilaria	20	20	1.43
Licmophora	0	0	0.00
Melosira	0	0	0.00
Navicula	15	95	6.79
Nitzschia	5	45	3.21
Synedra	20	35	2.50
Rhoicosphenia	10	125	8.93
Agmenellum	0	0	0.00
Anabaena	2	5	0.36
Anacystis	0	5	0.36
Batracospermum	0	0	0.00
Chlorococcum	5	15	1.07
Pediastrum	20	40	2.86
Scenedesmus	25	35	2.50
Rhizochlonium	10	25	1.79
Ceratophyllum	20	40	2.86
Lemna	15	60	4.29
Sand grain.	100	550	39.29
Decayed macrophytes	100	250	17.86

Table 5.10 Percentage occurence, mean number of cells (particles) and relative abundance of food items found in the rectum of *M. tuberculata*.

Food Items	Percentage occurrence	Mean number	Relative abundance
Achnanthes	0	0	0.00
Actirella	1	1	0.41
Amphora	0	0	0.00
Cocconeis	2	5	2.07
Cyclotella	1	0	0.00
Diatoma	0	0	0.00
Fragilaria	2	0	0.00
Navicula	5	5	2.07
Nitzschia	2	0	0.00
Synedra	10	5	2.07
Rhoicosphenia	10	5	2.07
Ankistrodesmus	0	0	0.00
Anacystis	2	0	0.00
Scenedesmus	5	0	0.00
Rhizochlonium	10	5	2.07
Ceratophyllum	15	5	2.07
Lemna	10	5	2.07
Sand grain.	100	110	45.64
Decayed macrophytes	100	95	39.42

5.5.4 The contents of the Rectum :

5.5.4.1 *Bulinus truncatus :*

Table 5.3 shows that the rectal contents of an average *Bulinus truncatus* still contained 27014% diatoms (8.8% less the crop contents), 8.93% algae (0.67% less the crop contents), 7.14% living macrophytes (6.7% less the crop contents) 17.86% of decayed macrophytes (6.69% less the crop contents) and 39.28% sand grains, an increase of 23.21% over the crop content.

From Table 5.9, it could be noticed that most of the items are greatly reduced. Only the sand grains and the decayed macrophytes were common to all the snails . All the other food items were below 35% some items like *Actirella, Licmophora, Melosira, Agmenellum, Anacystis,* and *Batracospermum* had disappeared completely.

5.5.4.2 *Melanoides tuberculata :*

Considerable reductions in the percentage occurrences of the various food items in the rectum of *Melanoides tuberculata* is noticed in Tables 5.4 & 5.10. From Table 5.4, 8.71% of diatoms were still found in the food contents, a reduction of 2.07% of what was observed in the crop. 2.07% of algae were found in the rectum showing an increase of 1.135 over that of the crop. 4.14% of living macrophytes were also found (3.03% less the crop contents), 39.45% decayed macrophytes were noted indicating a significant reduction from the crop contents by 24.84%. Sand grains increased in percentage from 16.82% to 45.64% showing a difference of 28.82% over that of the crop.

Table 5.11 **Percentage of food items digested by the two snail species.**

	Bulinus truncatus.	*Melanoides tuberculata.*
Diatoms	38.5	5.6
Algae	2.9	1.1
Living Macrophytes	29.3	8.3
Decayed Macrophytes	29.3	85.1

FIGURE 5.6

PERCENTAGE OF FOOD ITEMS DIGESTED BY *BULINUS TRUNCATUS*

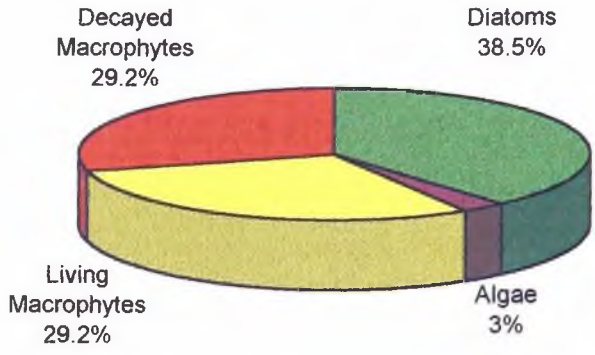
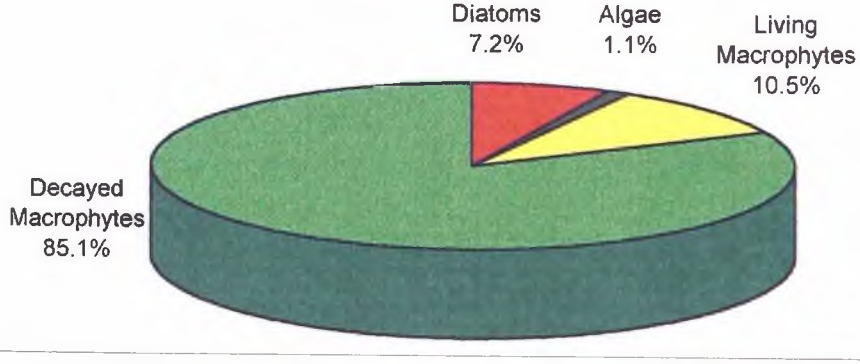


FIGURE 5.7

PERCENTAGE OF FOOD ITEMS DIGESTED BY *MELANOIDES TUBERCULATA*



5.5.5 DISCUSSION :

5.5.5.1 Food ingested by the snails :

Food items ingested by *Melanoides tuberculata* and *Bulinus truncatus* were found to differ in quantity and size. In the gut of *Melanoides tuberculata* as shown in Table 5.11 and also Figures 5.6 & 5.7, decayed macrophytes (the most abundant food item ingested) formed 85.1% of the total food items ingested, living macrophytes formed 8.3%, diatoms formed 5.6% and algae formed 1.1% . Similarly Madsen (1992) ; Dudgeon and Yipp (1983) and Hicklin (1988) in their investigations found detritus to be the most abundant food item among the stomach contents of *Melanoides tuberculata.* For example in the investigations conducted by Dudgeon and Yipp, (1983) decayed macrophytes formed 70% of the gut contents, inorganic material formed 24.7%, algae formed 4.2%, diatoms formed 0.6% and terrestrial macrophytes formed 0.4%.

Considering the gut of *Bulinus truncatus,* from Table 5.11, diatoms (the most abundant food item ingested) formed 38.5% of the total food items ingested, living macrophytes formed 29.3%, unidentified decayed macropytes formed 29.2% and algae formed 2.9%. This was found to be in contrast to the observations made by Thomas et al, (1985) who recorded decaying but recognizable macrophyte as the most abundant item in the gut of *B. glabrata.* It could be observed from the above that food selection or preference among the two snail species though similar is not exactly the same as there are differences in the quantity of the ingested items. When this was tested, the difference was found to be statistically significant ($P(T=t)= 0.001$).

Two possible reasons can be advanced to account for the observed differences in food preference . Firstly, the nature of the feeding apparatus. Graham, (1985) has pointed out that taenioglossan radulae (ie that of *Melanoides*) are incapable of digging deep enough into the substrate to cause the removal of any tightly attached material. In *Melanoides tuberculata* the teeth have long shafts with blunt denticles and large inter-teeth gaps which militate against selective feeding on particular fractions such as diatoms, algae, and the cutting and scraping of living macrophytes. This may be one reason why they have been found to prefer coarse (gravel-like) sediments (Thomas and Tait, 1984). In *Bulinus truncatus* the teeth are very strong with short shafts and sharp denticles (modified for cutting and scraping). Their inter-teeth gaps also appear to be small suggesting their efficacy in selective feeding.

The ingestion of varieties of food items is known to be advantageous to snails (Frömming, 1956) and this might explain why most snails were found to have ingested a mixture of detritus, epiphytic, algae, decaying macrophytes and also some animal remains (Madsen, 1992). Sometimes some of these snails become opportunistic or inadvertent scavengers and ingest small organisms such as rotifers, gastrotrichs, acarina, crustacea, ostracods, insects, molluscan eggs and juvenile snails.

Secondly, the spatial separation of *Melanoides tuberculata* and *Bulinus truncatus*. It has been suggested that the abundance or predominance of a particular food item eg detritus in the gut content is a reflection of its abundance in the environment (Bovbjerg, 1968). It is therefore possible that *Melanoides tuberculata* which is benthic and largely related to the sediments finds itself in the abundance of decayed unidentified macrophytes.

Observation on *Melanoides tuberculata* while feeding showed that they are not able to float in the water column as *Bulinus truncatus*. Thus they cannot feed on floating macrophytes which probably might be a good source of the particular species of algae and diatoms (eg *Auricula, Licmophora, Melosira, Agamenellum, Anabaena, Batracospermum, Chlorococum and Pediastrum*) which were found to be absent from their diet. These probably were not present on the sand grains at the bottom nor on the decayed macrophytes which formed the most common food item ingested *Melanoides tuberculata*.

5.5.5.2 Food selection and relative importance :

The extent to which different food items are selected by different species may be determined by their food value and the ability of the organism to digest a particular food item. For example, in a study conducted by Gratham et al, (1993), it was observed that *M. cornuarientis* preferred *Ludwigia repens* to *Vallisneria americana* because *L. repens* contained a higher amount of holocellulose which appeared to be readily digested than *V. americana*. In another investigation *B. glabrata* was found to prefer or select senescing or decayed fronds of *Lemna* in preference to the green ones (Thomas et al, 1985a).

Feeding on decayed macrophytes may have two major advantages, firstly, it may require less cost (work) and less expenditure of energy to feed on this item since decayed macrophytes are more flaccid and thus can be manoeuvred readily into the groove of the odontophore. The turgid mature leave may have to be cut first and this when done continuously leads to the wearing away of the teeth (Runham and Hunter, 1970). This was

commonly found in *Bulinus truncatus* but only scarcely in *Melanoides tuberculata*. This may explain why *Bulinus truncatus* has more living macrophytes in its gut than *Melanoides tuberculata*. The long marginals of *Melanoides tuberculata* which do not wear off are possibly adapted for pushing long fronds of decayed food into the gut and this may explain the greater amount of decayed macrophytes found in the gut of *Melanoides tuberculata*.

Secondly it is more biochemically advantageous for the snail to ingest plant materials in the early stages of decay more that the green living plant tissue because the decayed materials contain a considerable amount of C_2 - C_5 carboxylic acid (Patience et al, 1983) which are easily taken up through the body wall and metabolised (Thomas et al, 1984). This is a useful source of energy since C_2 - C_5 compounds can easily be adsorbed into the Krebs Cycle to produce ATP and NADPH and can be aminated to produce amino acids which are useful in protein synthesis (Thomas et al, 1985b). It is possible that *Melanoides tuberculata* has less energy demanding enzymes to digest the highly resistant polymeric plant carbohydrates which might also contain some antifeedants. This may be one reason why *Melanoides tuberculata* did not have large quantities of living macrophytes in its gut. It is possible that the little which was found to have been ingested came in accidentally mixed up with decayed macrophytes.

It is also likely that *Melanoides tuberculata* is able to get a considerable amount of the needed proteins from the decayed macrophytes thus the absence of algae and diatoms leave nothing to be desired. The selective ingestion of algae and diatoms by *Melanoides tuberculata* and *Bulinus truncatus* might be due to mechanical inabilities of the snails (Bowker et al, 1983), or the presence of antifeedants like repellants or arrestants (Carmichael, 1981).

The relative importance of the various food categories can be evaluated by comparing their relative abundance in the crop with that of the rectum. The absence of a particular item indicates that it has been ingested as food. In the case of *Bulinus truncatus*, the diatoms, *Cyclotella, Diatoma, Licmophora* and *Melosira* appeared to have been completely used up. *Auriculla, Fragilaria and Navicula* were found to have been underutilised as high proportions were still found in the rectum. With the algae, *Agamenellum* and *Batracospermum* were completely utilized while *Chlorococcum, Pediastrum, Scenedesmus*, and *Rhizochlonium* were not effectively utilized probably because they are less easily digestible. In the case of *Melanoides tuberculata,* the diatoms found to have been used up completely are *Achnanthes, Amphora,, Cyclotella, Diatoma, Fragilaria* and *Nitzschia.* For the algae, *Ankistrodesmus, Anacystis* and *Scenedesmus* appeared to have been completely digested.

Willoughby and Earnshaw, (1982) found in their investigations that the delicate external membrane of diatoms make them vulnerable to digestive enzymes. Thus the presence of recognizable siliceous frustules of diatoms in the rectum of the two species of snails studied does not therefore signify that they have not been used as a food source by the snails. It is possible that the dissolved organic matter has been absorbed.

5.5.5.3 Sand grains and their possible relevance in the gut :

Sand grains are known to be common in the crop of most gastropods (Reavell, 1980). Thus it is not surprising the gut of the two snail species studied contained a high proportion of sand grains. In the present study, *Melanoides tuberculata* was found to have

ingested more sand grains than *Bulinus truncatus*. Aside of this, the sizes of the grains differed, an average size ± SD of 0.047 ± 0.028 mm^2 was found in *Melanoides tuberculata*, while an average size ± SD of 0.0125 ± 0.0079mm^2 was found in *Bulinus truncatus*. The presence of sand grains in the guts of the two snails studied show that they are being taken in continuously and passed through the gut . It is not very clear whether the snails intentionally ingest the grains or because they are covered by epilithic algae (Round,1981).

However according to Storey, (1970) pulmonates select mineral particles and use them to triturate tough food in the crop or gizzard. Other possible reasons for the ingestion of sand grains include : the possibility of the surface of the grains being covered with epilithic organisms which might be utilised by the snails (Lopez and Levilin, 1978; Round, 1981). This might be one of the reasons for the quantity and also the large sizes of the sand grains found in the gut of *Melanoides tuberculata*. Since the radula of *Melanoides tuberculata* is not well adapted for scraping off epiphytic algae it probably picks up the grains as another way of ingesting the algae. It is also known that the ingestion of sand grains may assist in the uptake of essential cations eg Ca^{2+} and traces of copper (Hicklin, 1988).

5.5.5.4 Relevance to biological control :

It could be observed that although the contents of the guts of *Melanoides tuberculata* and *Bulinus truncatus* show a lot of similarities, the food preference of these snails could be said to be different (P(T=t)=0.001). This is because the food items found in the respective guts

differed in quantity, while *Melanoides tuberculata* was found to have digested 85.1% of decayed macrophytes, 10.5% of living macrophytes, 7.2% of diatoms and 1.1% of algae. *Bulinus truncatus* on the other hand was found to have digested 29.3% of decayed macrophytes, 29.3% of living macrophytes, 38.5% of diatoms and 2.9% of algae.

These observed differences might be due to the differences in their microhabitats. *Melanoides tuberculata* is predominantly a bottom dweller (benthic organism) usually found on the sediments feeding largely on decayed macrophytes. In contrast *Bulinus truncatus* is usually found on the surface of the water body in association with submerged and floating macrophytes where they feed mainly on diatoms, living macrophytes, the decayed portions of these floating or submerged plants (Hicklin, 1988). This shows that if these two species are present in the same water body they will possibly occupy different niches and possibly coexist. Competition for food may only arise in the absence of living macrophytes or water bodies dominated by watercress macrophytes which are known to be a poor resource for host snails (Thomas, 1987).

Thus food competition may not be the main underlying mechanism for the competitive interactions observed between *Melanoides tuberculata* and *Bulinus truncatus*. This conclusion has also been arrived at by Madsen, (1986) who studied exhaustively the interactions between *Helisoma duryi* and *Bulinus truncatus*. He argued that interference competition might be of greater importance than food in explaining the observed competitive interactions between the two species. It would be of considerable interest to conduct further experiments on the competitive effects of *Melanoides tuberculata* on *Bulinus truncatus* in the presence or absence of macrophytes as wells as the different kinds of sediments.

It seems from the above that the removal of macrophytes from water bodies as suggested by Ferguson, (1977) may help to eliminate the host snails as this will not only cut down on their food supplies, it will also intensify any competitive interactions between these snails and the benthic community. Furthermore, their oviposition sites and shelter will be removed thus they will be exposed to ultra-violet radiation and water currents. This approach appears to be very promising, however according to McMullen (1973) removal of macrophytes alone may not cause sufficient changes in the microhabitats to reduce the number of the snail hosts below the threshold value. This is because snail hosts are known to adapt to feeding on a variety of food substance in such macrophyte free environments, (Thomas, 1987).

The investigations carried out by Thomas and Tait, (1984), tend to support the observations of McMullen, (1973) in that the mean population density of *B. pfeifferi* in habitats containing macrophytes was found to be 51 snails per m^2 and 16 per m^2 in habitats devoid of macrophytes respectively. Although 16 snails per m^2 is by far lower than 51 snails per m^2, it is still sufficienty high to cause serious public health threats because according to Duke and Moore, (1976a-c) as in Hicklin, (1988) snail population need to be reduced to zero to achieve the breakpoint in transmission in endemic areas.

There is therefore the need to investigate and manipulate more than one factor eg sediments and macrophytes to identify the real mechanisms which underly the observed competitive interactions between *Melanoides tuberculata* and *Bulinus truncatus*.

CHAPTER 6

GENERAL DISCUSSION

Thomas and Tait, (1984) in their field observations carried out in Southern Nigeria, concluded that *Melanoides tuberculata* would be at an advantage over host snails such as *Biomphalaria pfeifferi* in both lentic and lotic systems under specific environmental factors since *Melanoides* snails are benthic and the host snails are usually associated with macrophyte. They postulated that, competition between the host snails and *Melanoides* is unlikely to occur under normal circumstance but only in the absence of macrophytes. Thus *Melanoides* could ultimately replace the host snails by competitive exclusion in areas devoid of aquatic macrophytes. This hypothesis was supported by the investigations of Hicklin, (1988) who made similar observations.

6.1 The preliminary field observations :

During the preliminary investigations carried out at the beginning of the present work in chapter 2, it was observed that *Melanoides tuberculata* outnumbered the two host snails of schistosomiasis ie *Bulinus truncatus* and *Bulinus pfeifferi*, the effects being more pronounced on *Bulinus truncatus*. A possible negative association between *Melanoides tuberculata* and the host snails thus seem to exist in the Weija Lake in Ghana. This observation is in support of the observations of Thomas and Tait, (1984) and Hicklin, (1988) but differed slightly in that, it was found from the field observations that competition is still

evident even in situations where aquatic macrophytes were present. Thus other factors may be involved in influencing the outcome of the interactions, for example physical interferences among snails as demonstrated by Madsen,(1986) using *Helisoma duryi* and *Bulinus truncatus*. The nature of interferences operating in the present study might be different from that investigated by Madsen,(1986) since unlike his studies the two snails under the present study seem to prefer different niches.

Of the possible factors which may account for the differences observed in snail distribution pattern at the sampling sites in Weija Lake, the type of sediment seems to be most plausible. It was observed that a mixture of sand and clay does not seem to favour the growth and reproduction of *Melanoides tuberculata*. This is in support of the observations of Thomas and Tait; (1984) who pointed out in their investigations that *Melanoides tuberculata* seem to prefer coarse (gravel-like) sediments. It therefore became necessary to investigate further the significance of the sediments in the competitive interactions between *Melanoides tuberculata* and the host snails (*Bulinus truncatus* and *Biomphalaria pfefferi)*.

6.2 Sediment replacement series experiments :

In chapter 3, competition between *Melanoides tuberculata* and *Bulinus truncatus* using normal and heat treated sandy gravel as sediment was investigated to test two hypotheses, firstly that *Melanoides tuberculata* would be at a competitive advantage over *Bulinus truncatus* and replace them in freshwater systems. Secondly, that sediment type and condition affect the ecological interactions of snails and influence the ability of *Melanoides tuberculata* to act as a biological control agent.

6.3 The effect of *Melanoides tuberculata* on *Bulinus truncatus* :

The results of the investigations carried out in chapter three which were discussed in relation to the above hypotheses show in Section A where normal gravel and sand were used as the sediment that, for all the various combinations, mortality rate, reproduction rate, and the rate of shell length of *Bulinus truncatus* were not affected to any significant extent by the presence or densities of *Melanoides tuberculata*. However when changes in weight was used as the indicator of growth, it was discovered that the growth rate of *Bulinus truncatus* in the presence of *Melanoides tuberculata* was significantly lower than under control conditions.

Furthermore the severity of the effect of *Melanoides tuberculata* on *Bulinus truncatus* increased as the density of *Melanoides tuberculata* increased. This density dependent effect can be arranged as follows : M5B15 (0.081.9) < M10B10 (0.01740) < M15B5 (0.00632). These findings contrast with the observations made in section B where heat treated sandy gravel was used as the sediments. With heat treated sandy gravel, *Melanoides tuberculata* was not found to exert any significant effect on the growth rate of *Bulinus truncatus*.

Thus the competitive performance of *Melanoides tuberculata* changes under different ecological conditions. The results of the present work suggest that the condition of the sediment may be critical in determining the outcome of the competitive interaction. The process of heat treating the sediments would have had at least two major effects on the sediments. Firstly, the organic component must have been destroyed. Secondly, the chemical condition of the particles might have been changed. These two factors could probably account for the drastic effects of this process on the competitive advantage of the *Melanoides tuberculata* snails over the *Bulinus truncatus* snails.

Under normal field conditions, sediments are known to be covered with epilithic algae (Round, 1981) micro algae, bacteria, fungi, organic matter of vegetable or animal origin (Pointier and McCullough,1989) which act as important food items for aquatic snails. *Melanoides tuberculata* snails are mainly bottom dwellers and are therefore more likely to be dependent on this food source than *Bulinus truncatus* snails which tend to feed more on epiphytic algae and decaying macrophytes (Thomas and Tait, 1984; Kpikpi, 1990). Consequently damage to the food source in the sediments (through heat treatment) had much more adverse effect on the competitive ability of *Melanoides tuberculata* than *Bulinus truncatus*.

It is also possible that the chemical condition of the particles of the gravel and sand could have been altered through the heat treatment thus impairing the uptake of essential cations eg Ca^{2+} and traces of copper which are known to be assisted by ingestion of sand grains. This can reduce the growth rate and consequent competitive ability of *Melanoides tuberculata*. It would therefore be of considerable interest to examine the nature of the epilithic community on the surface of both the heat treated and the normal sediment to unearth the main differences which can account for the observed differences in the competitive performance of *Melanoides tuberculata* under the two conditions.

The results of the present experiment seem to suggest that under such conditions (ie in cases where the sediments are more gravel-like and rich in epilithic micro-organisms) *Melanoides tuberculata* can depress the growth rate of *Bulinus truncatus* although the

mortality and reproduction rates of *Bulinus truncatus* are not equally affected. It is possible that, if the experiment had been run for a much longer period, a wider range of effects of *Melanoides tuberculata* on *Bulinus truncatus* would have been discovered.

6.4 The effect of *Bulinus truncatus* on *Melanoides tuberculata* :

The weight gains and reproductive rates of *Melanoides tuberculata* were found to have been depressed in the various experimental combinations. It is of interest to find that *Melanoides tuberculata* snails not only fail to repress growth, mortality, and reproduction of *Bulinus truncatus* snails under unfavourable sediment conditions, these non-host snails are actually adversely affected by the *Bulinus truncatus* snails.

These findings show that the competitive interactions work both ways and observed effects must be the end results of complex interactions between the two species. In this case the condition of the sediment used has been shown to be significant in determining which species wins the interaction. It would be of interest to investigate the possible factors such as the presence or absence of aquatic macrophytes and also the effect of water current on the competitive interaction.

6.5 The effect of sediment type and condition on the competitive interactions between *Melanoides tuberculata* and *Bulinus truncatus*.

In chapter 4, competition between *Melanoides tuberculata* and *Bulinus truncatus* using normal and heat treated sandy clay loam as sediments was investigated. The findings reported in this chapter lend further support to the conclusions of the previous chapter that the

nature and condition of the sediments may be of critical importance in determining the outcome of the competitive interactions between *Melanoides tuberculata* and *Bulinus truncatus*. As in the previous study (chapter 3), the adverse effects of the presence of *Melanoides tuberculata* on *Bulinus truncatus* were demonstrated clearly in the area of growth (ie total body weight gain). The other parameters which were investigated ie reproduction, mortality, and increase in shell length did not show any clear competitive interactions.

The present results show that *Melanoides tuberculata* is able to suppress the growth rate of *Bulinus truncatus* when sandy clay loam is used (in the normal condition) as the sediment. Thus two sediment types have now been shown as suitable substrates for the possible competitive exclusion of *Bulinus truncatus* by *Melanoides tuberculata*. The first substrate which was identified was sandy gravel. It is significant to note that with both kinds of sediments, the heat treated sediment prior to the experiments reduced the competitive ability of *Melanoides tuberculata* rather drastically.

In the present studies *Melanoides tuberculata* did not exert any significant influence on *Bulinus truncatus* snails in all the experiments using heat treated sandy clay loam. On the contrary, its growth rate in the three experimental combinations was significantly lowered below that of the control. This was found to be the case whether as few as five *Bulinus truncatus* snails were used or as many as ten or fifteen.

The observed changes in competitive advantage of *Melanoides tuberculata* over *Bulinus truncatus* when the condition of the sediment is changed can be explained along the same line as was done in chapter 3 by postulating that

i) the sandy clay loam particles play an important role in the nutrition of *Melanoides tuberculata* snails. This role may be direct or indirect.

ii) heat treatment has a deteriorating effect on the nutritive value and chemical composition of the particles of the sediment.

In the direct nutritive role, the particles which are usually covered with epilithic algae and diatoms (Round, 1981) may be a source of food or vitamins for the *Melanoides tuberculata* snails. Indirectly the sand particles may facilitate the mechanical digestion or trituration of larger food particles in the gut (Thomas et al., 1985b; Storrey, 1970).

Heat treatment is likely to destroy the epilithic communities thus eliminating a possible food source for the snails and introducing food stress on *M.tuberculata*. The fact that *Melanoides tuberculata* is adversely affected by this treatment of the sediment suggests that this species is more dependent on this food source than *Bulinus truncatus* as can be inferred from earlier observations that *Melanoides tuberculata* is usually found inhabiting the sediments while *Bulinus truncatus* is usually more closely associated with aquatic macrophytes (Hicklin, 1988). It is possible that heat treatment could have altered the surface chemistry of the sand particles thus interfering with the uptake of Ca^{2+}. This could partly explain the reduction in growth rate of *Melanoides tuberculata* when the experiments were done using heat treated sediments.

6.6 Density dependent effects :

The results have indicated that as the density of *Melanoides tuberculata* increased the adverse effects on *Bulinus truncatus* became more severe. This observation is not surprising since competitive interactions in nature are often dependent on the density of the competing species (Pointier et al., 1989; Pointier, 1993; Meyer-Lassen & Madsen, 1989). If the present findings, are a good indicator, any attempt to introduce *Melanoides tuberculata* snails for the control of *Bulinus truncatus* snails should aim for a rather high *Melanoides tuberculata / Bulinus truncatus* ratio at the start. The determination of the exact ratios to be employed would warrant further investigations.

Now in the sediment replacement series experiments it was established that sediment type as well as its condition play a major role in the competitive interaction between *Melanoides tuberculata* and *Bulinus truncatus*. Secondly this role is suspected to be either direct or indirect. In the case of the direct nutritive role, the particles which are known to be covered with epilithic algae and diatoms (Round, 1981) may be a source of food or diatoms for the *Melanoides tuberculata* snails. Indirectly the sand particles may facilitate the mechanical digestion of the or trituration of larger food particles in the gut (Thomas et al., 1985b). This brought about the need to ascertain the nature of food eaten by the two snails investigated and the importance of sediment particles in the digestion of these snails.

6.7 Food preference and feeding behaviour of *B. truncatus* and *M. tuberculata* :

In chapter 5, the feeding apparatus, the radulae of *Melanoides* and *Bulinus* were examined to note any morphological differences and also to ascertain their relevance in feeding. The radulae of the snails were found to differ. *Melanoides tuberculata* was found to have a more robust and boat-like radula ribbon with a few teeth between 500 and 530, this was found to be in contrast to the observations made by Hicklin (1988) who found a total of between 742 and 840 teeth. *Bulinus truncatus* on the other hand has a sheet-like radula ribbon which bears about 40 teeth in the transverse row and over 120 teeth in a longitudinal row . The total number of individual teeth per ribbon therefore is not less than 4,800, this supports the observation of Thomas et al, (1985) who found a total of 4,425 teeth.

This means that the radula teeth of *Bulinus truncatus* are much finer than those of *Melanoides tuberculata*. Since it is believed that there is a direct correlation between the shape of the teeth and the feeding habits of the animal, while the length of the teeth is known to be linked to the amount of work done by the teeth during feeding (Fretter and Graham, 1962), we can infer from the observations made that the two different forms of radulae are specialised for different ways of feeding. Thus the differences show that *Melanoides tuberculata* and *Bulinus truncatus* have different feeding strategies.

These investigations did not only point out some differences in the radula morphology of the two snails species with respect to their habitats but also led to the discovery of some striking features of the radula of the two snail species which differ from those of previous observations and which might be of taxonomic importance. For example in the case of the

radula of *Melanoides tuberculata*, the following new observations were made. a) the presence of 5 teeth in a transverse row instead of 7 as found by Hicklin, (1988) indicating the absence of outer marginals and a deviation from a true taenoglossan teeth type. b) the absence of basal cusps at the two corners of the central teeth as was found by Hicklin; (1988) and Brown; (1980) and c) the absence of the small, sharp, thumb-like protrusion on the outer edge of the marginal teeth.

These observations which might be of adaptive significance might be due to the effect of some of the determinants of radula form (Figure 5.3) as described by Hickman, (1980) or simply that, the *Melanoides* used in the present study is a different species altogether. However one cannot be conclusive on this, since radula structure is just one of the numerous features used in the classification of species. Thus further investigations into the other features is required to confirm this. It is possible that some authors have used the species *T. granifera* and *M. tuberculata* interchangeably and as such what has been described as the radula of *M. tuberculata* might actually be for *T. granifera* or that, the two species are not the same as some investigators claim.

In the case of *Bulinus truncatus*, the most striking feature of the radula was the monocuspid appearance of some of the the central teeth lying close to one another suggesting that the whole ribbon is probably bilaterally symmetrical. This may be a useful taxonomic tool, but since it did not occur in all the radulae studied, it needs to be looked at more critically before final conclusions could be made. Throughout these investigation it was established

that, the radula of the two snails are different morphologically and also function differently with respect to feeding. To confirm this, the gut contents of the two snail species were examined.

6.8 Food ingested by the snails :

During this investigation, the food items ingested by *Melanoides tuberculata and Bulinus truncatus* were found to differ in quantity and size. The food items digested by *Melanoides tuberculata* was found to consist of 85.1% decayed macrophytes, 8.3% living macrophytes, 5.6% diatoms and 1.1% algae . This was found to be in support of the observations of Madsen, (1992b); Dudgeon and Yipp, (1983) and Hicklin, (1988). The food digested by *Bulinus truncatus* on the other hand was found to consist of 38.5% diatoms, 29.3% living macrophytes, 29.2% decayed macropytes and 2.9% algae.

It could be observed from the above, that food selection or preference among the two snail species though similar is not exactly the same as there are differences in the quantity of the ingested items. When this was statistically tested, the difference was found to be statistically significant (P(T=t)=0.001). The observed differences might possibly be due to the differences in the respective radula morphology of the two snail specie, the value of the food item to the snail, the ability of the organism to digest a particular food item and also the differences in their microhabitats.

Melanoides tuberculata is a known bottom dweller (benthic organism) usually found on the sediments feeding largely on decayed macrophytes. In contrast *Bulinus truncatus* is usually found on the surface of the water body in association with submerged and floating macrophytes where they feed mainly on diatoms, living macrophytes and the decayed portions of these floating or submerged plants. This shows that considering food as a limiting factor, if these two species are present in the same water body they will possibly occupy different niches and coexist and competition should not occur.

It is possible that competition for food may only arise in the absence of living macrophytes as suggested by Ferguson, (1977) since this will not only cut down on their food supplies of the host snails but also remove their oviposition sites and shelter thereby exposing them to ultra-violet radiation and swift water currents. Thus in water bodies with lots of plants of malacological importance as was found at the sites of investigations, there should not under normal conditions be any competition. This was however not the case in the present work as competition was still evident in the presence of these macrophytes.

Thus from the above findings, the removal of macrophytes from water bodies alone, though very promising may not cause enough change in the microhabitats to the extent that will reduce the the number of the snail hosts below the threshold value as pointed out by McMullen, (1973). This is because snail hosts are known to adapt to feeding on a variety of food substances in such macrophyte free environments (Thomas,1987).

The presence of sand grains in the guts of the two snails studied show that they are being taken in continuously and passed through the gut. This means that sand grains do play a major role in the ecology of freshwater snails. In the present study, *Melanoides tuberculata* was found to have ingested more sand grains than *Bulinus truncatus*. Furthermore, the sizes of the grains differed. Grains of an average size of 0.047 ± 0.028 mm^2 was found in *Melanoides tuberculata*, while those of an average size of 0.0125 ± 0.0079mm^2 was found in *Bulinus truncatus*. It is not very clear whether the snails intentionally ingested the grains or because they are covered by epiphytic algae (Round, 1981).

There are differences of opinion on this issue, while Storey, (1970) and Franzel, (1979) believe that pulmonates select mineral particles and use them to triturate tough food in the crop or gizzard, the possibility of the surface of the grains being covered with epilithic organisms which might be utilised by the snails is believed to be the main reason for the uptake of the grains by Lopez and Levilin, (1978).

6.9 CONCLUSIONS :

Now, of the two normal sediments, it could be noted that *Melanoides tuberculata* competes more effectively on a sandy gravel than on a sandy clay loam mixture because the results obtained on the adverse effects of the rate of weight increase of *Bulinus truncatus*

using a sandy gravel sediment was found to be more promising M15B5 (0.00632) < M10B10 (0.01740) < (M5B15 (0.0819) than the results obtained when the sandy clay loam was used M15B5 (0.03833) < M10B10 (0.05136) < (M5B15 (0.56204) .

From the present study, the following were observed from the results.

1. It has been established that, the radula of the two snails are different morphologically and also function differently.
2. Food selection or preference among the two snail species was found to be different in percentage composition (P(T=t)=0.001)., though similar in type.
3. *Melanoides tuberculata* has been found to be at a competitive advantage over *Bulinus truncatus* and is suspected that they actually could replace them if the ratio of *Melanoides* to *Bulinus truncatus* is 3:1 or higher in closed freshwater systems.

This observation is in support of the first hypothesis. The effect was however found to be more directed towards the reduction in the rate of weight increase and not mortality or reproduction. The rates of mortality and reproduction of *Bulinus truncatus* might probably be affected if the study was prolonged over several weeks. This is because in a study conducted by Pointier, (1986), it took between 20 and 32 weeks for *Melanoides* to control *Biomphalaria glabrata*.

4. Sediment type and condition has been found to a) affect the ecological interactions of snails and b) influence the ability of *Melanoides tuberculata* to act as a biological control agent. Thus the living organic components of sediments have a remarkable effect on snail ecology. This is in support of the second hypothesis.
5 Although *Melanoides tuberculata* competes with *Bulinus truncatus* the competitive ability of *Melanoides tuberculata* reduces when there is food stress.

6.10 RECOMMENDATIONS :

1. It is vital to confirm the taxonomy of this *Melanoides* to distinguish between this *Melanoides* and that used by others because of the differences in the radula morphology since the success of one species in eliminating host snails may not necessarily imply the success of other related species.
2. There is the need to conduct carefully controlled field experiments to further test these hypotheses. This should be allowed to last for at least 30 weeks.
3. Before *Melanoides* could be used to control the host snail in freshwater bodies in which both snails occur, there is the need to sample to ascertain the ratio of one species to another. A minimum ratio of 3 *Melanoides* to 1 *Bulinus* is recommended.
4. Before the third recommendation is followed, the investigator should try to examine the nature of the sediments at the site of investigation. The control method using *Melanoides* will be more effective if the sediment is coarse and gravel-like. For clay type of sediments, an integrated control method such as the removal of macrophytes may be effective.
5. A possible consideration for enhancing biological control using *Melanoides tuberculata* could involve altering the sediment at water contact sites either through adding of a particular kind of sediment or removal of what is already present. By manipulating the sediments in the appropriate manner, the competitive ability of *Melanoides tuberculata* could be increased considerably.

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