

**SNAIL FAUNA AND *Schistosoma haematobium*
TRANSMISSION PATTERNS IN FRESHWATER SYSTEMS OF
ISHIELU LOCAL GOVERNMENT AREA, EBONYI STATE**

BY

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AWARD OF MASTER OF SCIENCE (MSc) DEGREE IN
PUBLIC HEALTH PARASITOLOGY**

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CERTIFICATION

I, Ubaka, Uchenna Athanasius, hereby, declare that this work on “Snail Fauna and *Schistosoma haematobium* Transmission Patterns in Freshwater Systems of Ishielu Local Government Area, Ebonyi State” is the product of my own research efforts, undertaken under the supervision of Prof. C.A. Ekwunife and has not been presented elsewhere for the award of a degree or certificate. All sources of information collected and materials used for the research have been duly distinguished and appropriately acknowledged.

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APPROVAL

The research work on “Snail Fauna and *Schistosoma haematobium* Transmission Patterns in the Freshwater Systems of Ishielu Local Government Area of Ebonyi State” was carried out by Ubaka, Uchenna Athanasius, NAU/PG/MSc/2011586002P, and has been approved for the award of Master of Science (MSc) degree in Public Health Parasitology, Department of Parasitology and Entomology, Faculty of Biosciences, Nnamdi Azikiwe University, Awka.

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DEDICATION

This research work is dedicated to the Glory of God Almighty.

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ABSTRACT

Freshwater snails are widespread in both tropical and temperate regions of the world where they serve as intermediate hosts for larval stages of parasitic trematodes which cause schistosomiasis that is endemic in Nigeria. A study of freshwater snail fauna and patterns of *Schistosoma haematobium* transmission was carried out around freshwater bodies in Ishielu Local Government Area, Ebonyi State between June 2014 and May 2015. Using scoop and hand-picking techniques, snail collection was done within each 10m² of site area sampled at 100m intervals against the water current in flowing waters habitats (FWH) and between 20m to 50m in stagnant water habitats (SWH). *Bulinus* species of snails collected were light-induced for cercarial shedding in the laboratory. A total of 923 snails were collected among which 625(67.7%) were *Lymnaea natalensis*, 213(23.1%) *B. globosus* and 85(9.2%) *B. truncatus*. The numbers of these snail species were significantly different ($P<0.05$). The number of *Bulinus* snails was a total of 82.4% in dry season months and 17.6% during rainy months in FWH whereas, in SWH, the number of *Bulinus* snails was a total of 5.6% in dry season and 94.4% in rainy season. The monthly collections of snails were significantly different ($P<0.05$) for FWH and SWH. Out of the 136 *Bulinus* snails collected from FWH, 43 (31.6%) were infected and out of 162 collected from SWH, 30 (18.5%) were infected. The monthly collection of *Bulinus* spp infected from either flowing waters or stagnant waters were not significantly different, FWH ($P>0.05$); SWH ($P>0.05$). In the FWH, the highest infection rates were observed to coincide with the dry season with a peak in February (45.8%). For SWH, the highest infection rates coincided with the rainy season with a peak in July (26.8%). The transmission potential (T.Ps) of the two types of habitats were shown to be very high during the dry season months (a total of 93.0%) with a peak in February (34.9%) in FWH and very low (a total of 0%) for SWH in the same season. During the rainy season months, the T.P was very high (a total of 100%) with a peak in July (50%) in SWH and very low (a total of 7.0%) for FWH in the same season. These findings indicate that season and habitat type influence the patterns of transmission of *Schistosoma haematobium* in Ishielu L.G.A. and demonstrate the periods of the year when the human populations are at the greatest risk of infections in the study habitats and can help in establishing programmes for proper health awareness and snail control.

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CHAPTER ONE

INTRODUCTION

1.1 Background

Snails are invertebrate animals, belonging to the Phylum: Mollusca; class: Gastropoda; subclass: Pulmonata. Bassommatophora and Stylomatophora are two orders belonging to the above subclass. Bassomatophores are the fresh water snails while the Stylomatophores include the terrestrial snails and slugs (Okafor and Obiezue, 2015). A freshwater snail is a gastropod that lives in a watery non-marine (freshwater) habitat. The majority of freshwater gastropods have a shell, with very few exceptions. Some snails that live in freshwater respire using gills, whereas other groups need to reach the surface to breathe air. According to the present classification efforts, there are about 4,000 species of freshwater gastropods (3,795 - 3,972) (Strong *et al.*, 2008). At least 33 - 38 independent lineages of gastropods have successfully colonized freshwater environments (Strong *et al.*, 2011). It is not possible to quantify the exact number of these lineages yet. Freshwater snails are widespread; they are found in both tropical and temperate regions of the world (Brown, 1965), in habitats ranging from ephemeral pools to the largest lakes, and from small seeps and springs to major rivers. The snails serve as intermediate hosts for major digenean trematodes. They could be ovoviviparous or viviparous. Eggs are laid in batches or masses and a snail could lay up to 1000 eggs before the end of its life, which may last up to 1 year (McCullough, 1992). The eggs are deposited on plants or solid objects in water. Most feed on algae, but many are detritivores and some are filter feeders. A single snail, especially planorbid snails, could invade and establish within a new environment over a short period of time, due to its multiplication advantage

(Brown, 1965). Freshwater snails serve as intermediate host(s) for some major parasitic trematode diseases. Such diseases are schistosomiasis, paragonimiasis, fascioliasis and angiostrongyliasis.

Schistosomiasis is water-borne pathological condition resulting from infection by parasitic worms (blood flukes) of the genus *Schistosoma*. It affects about 250 million people worldwide, mainly in Africa, the Middle East, South America, some Caribbean Islands and the orient, which results in about one million deaths annually (Rollinson, 1999). It is one of the six tropical infections which World Health Organization (WHO) has singled out for priority owing to its public health significance (Ukpai and Ezeike, 2002). It is second to malaria in endemicity (Adetokunbo and Herbert, 2003).

Three genera of freshwater snails serve as intermediate hosts for the three major schistosomes (Ekwunife and Okafor, 2004; Roberts and Janovy, 2010). They are *Bulinus*, *Biomphalaria* and *Oncomelania*. *Bulinus* is the genus of freshwater snails that are hosts to *Schistosoma haematobium* that causes urinary schistosomiasis. *Biomphalaria* is the genus containing the various species that are intermediate hosts to *S. mansoni* that causes large intestinal schistosomiasis. The third snail hosts are of the genus *Oncomelania* which are vectors of *S. japonicum* that causes small intestinal schistosomiasis mainly in some Asian countries. In addition to these three are other schistosomiasis that can infect humans of which snail intermediate hosts are sparsely distributed. They include *Schistosoma intercalatum* and *Schistosoma mekongi*. Snail hosts of *S. intercalatum* are also of the genus *Bulinus* while *Neotricula aperta* serves as intermediate hosts to *S. mekongi* (Roberts and Janovy, 2010).

Fasciolosis is a disease caused by the liver trematode flukes, *Fasciola hepatica* and *Fasciola gigantica*. *Fasciola hepatica* which was first reported by de Brie in 1379 in sheep with 'Liver rot' has been known as important parasite of sheep and cattle for hundreds of years (Arora and Arora, 2010). It is one of the largest flukes of the world reaching a length of 30mm and a width of 13mm. *F. gigantica* is longer and more slender than but otherwise very similar to *F. hepatica*. It is the largest of the human liver and lung flukes. It measures up to 75mm in length and 12mm in width (Arora and Arora, 2010). They pass their life cycle in one definitive host and two intermediate hosts.

Various species of the molluscan family, lymnaeidae, serve as the first intermediate hosts. These snails include *Lymnaea natalensis*, *L. cousini*, and *L. diaphana*. The second intermediate host is aquatic vegetation e.g. water cress.

Fasciolopsis buski – The large or giant intestinal fluke is the largest fluke that infects man. It is a common parasite of humans and pigs in the orient. Stoll estimated 10 million human infections in 1947 (Roberts and Janovy, 2010). The number is greater today with prevalence ranging up to near 60% in India and Mainland, China (Graczyk *et al.*, 2001). Although *F. buski* is a typical fasciolid, it is peculiar because it lives in the small intestine of its definite host rather than in the liver precisely in duodenum and jejunum. Pigs serve as reservoir of infection for man. It also has two intermediate hosts. The first is the snails of the genera *Segmentina* and *Hippentis* (Planorbidae) while the second intermediate hosts are aquatic vegetations especially the seed pods of the water caltrop, the bulb of the water chestnut, and the roots of lotus and water bamboo (Arora and Arora, 2010).

Paragonimiasis is a disease caused by trematodes of the genus *Paragonimus* (the lung fluke), family Troglotrematidae. It is an excellent example of a zoonosis. *Paragonimus westermani* – the Oriental lung fluke was discovered by Kerbert in 1878 in the lungs of two Bengaltigero that died in two different zoological gardens in Hamburg and Amsterdam, Europe. Two years later eggs were found in rusty-brown sputum of a Chinese patient residing in Formosa (Taiwan) (Roberts and Janovy, 2010; Bunnag *et al.*, 2000). Many species especially *P. westermani* have been described in Western, sub-saharan Africa, Asia, Oceania and South and Central America.

Life cycle is passed in three hosts, one definitive and two intermediate hosts. Definitive hosts are man, wolf, fox, tiger, leopard, cat, dog and monkey. First intermediate host is a freshwater snail of the genera *Semisulcospira* and *Brotia* or *Oncomelania* (in Asia) while the second intermediate host is a freshwater crayfish usually *Autopotamobius pallipes* or a crab usually *Cherax quadricarinatus* (crustacea). Some evidence suggests that crustaceans may become infected by eating infected snails (Markell and Voge, 2006).

Angiostrongyliasis is caused by the nematode flukes, *Angiostrongylus cantonensis* and *A. costaricensis* which cause human eosinophilic meningitis or eosinophilic meningoencephalitis and abdominal angiostrongyliasis respectively. Eosinophilic meningitis or eosinophilic meningoencephalitis of which means that patients have high eosinophil counts in peripheral blood and spinal fluid in about 75% of cases and increased lymphocytes in cerebrospinal fluid (Roberts and Janovy, 2010).

1.2 Justification

A number of types of molluscs serve as intermediate hosts, including slugs and aquatic and terrestrial snails. Malex (1981) pointed out that terrestrial snails and slugs serve as the intermediate hosts, although aquatic snails could be infected experimentally. *Amphicyclotus olssoni*, *Potertia gigantean* and *Potertia confuse* are examples of terrestrial snails, while *Bradybraena similis*, *Cepolis varians* and *Vaginalus bovellianus* are examples of terrestrial slugs. Rats remain the definitive hosts. *Bulinus (Physopsis) globosus* (Morelet) is a major snail host of *Schistosoma haematobium* in Anambra State (Okafor, 1984; Ikpeze *et al.*, 2012). Field observations in the old Anambra State, Nigeria, also show that humans are in frequent contact with the habitats from which *Bulinus (Ph.) globosus* that shed schistosome cercariae were isolated (Okafor and Anya, 1991; Anya and Okafor, 1986). The frequency of such contact was also shown to be correlated with seasons for various activities such as collection of water for domestic uses, washing of clothes and utensils, bathing after work and swimming during recreation in schools. It is reasonable to expect that opportunities for transmission to human hosts would vary according to some environmental factors. Extreme changes in suitability of the snails' habitats are bound to have profound effect on the snail populations.

1.3 Statement of the Problem

The control of aquatic vector snails is an important aspect of public health in the tropics. This is because it has been found to reduce the level of transmission of associated diseases. But it is both impossible and unnecessary to estimate the total number of snails in an area, in the preliminary studies of population

dynamics of snails, usually required for effective planning and execution of the control programmes. Rather, useful information can be obtained by calculating some indices especially those that help to determine the ecological and temporal aspects of the transmission patterns. Availability of cercariae is also an important factor and must be investigated to identify the periods in the year when human population in endemic areas is at the greatest risk of acquiring schistosomiasis infection.

1.4 Aim and Objectives

The Aim of this research work was to study snail fauna and *Schistosoma haematobium* transmission patterns in freshwater systems of Ishielu Local Government Area of Ebonyi State, Nigeria.

The specific objectives were to:

1. identify the freshwater snail species in Ishielu LGA
2. determine the monthly snail species abundance.
3. determine the relative abundance of snail species.
4. determine the habitat and seasonal changes in the population of snails infected with *Schistosoma haematobium*
5. determine the Prevalence of *Schistosoma haematobium* in the infected snails collected from the habitats
6. determine the cercarial transmission potentials in the water bodies.

CHAPTER TWO

LITERATURE REVIEW

2.1 Identification and Brief Morphology of Freshwater Snails

Snails, especially freshwater snails, possess external shell that is composed of calcium carbonate. The shell plays an important role in the identification of snail and its colour is determined by the colour of the cuticle (Okafor and Obiezue, 2015).

Species of the genus *Bulinus* has shells that are sinistral (Fig. 1). It has a very large body whorl and a small spire. *Bulinus globosus* is 22.5 x 14mm in size. It belongs to the *Bulinus africanus* group. The shell shows no readily definable differences from *B. africanus* but is comparatively smaller. The largest individuals found in some localities are much smaller and large races with different biological characteristics exist in Nigeria. *Bulinus truncatus* is smaller than *B. globosus*. The size is usually 14.5 x 10mm (reaching 20mm high). Highly variable, though common with the whorls somewhat flattened at the sides and even shouldered near the suture (Okafor and Obiezue, 2015).



Figure 1: *Bulinus globosus*



Figure 2: *Bulinus truncatus*



Figure 3: *Lymnaea natalensis*

Source: Okafor and Obiezue (2015)

Oberholzer and Van Eeden (1967)

Many nominal species are synonyms (Mandahl-Barth, 1958) and some names have been applied incorrectly to snails collected in Southern Africa.

For example, the name *B. contortus* has been commonly used but if it is maintained should be applied only to a local form in the Liberian Peninsula and North-West Africa. *B. sericinus* is one of the most senior nominal species of this group and was employed for snails from Ethiopia (Mandahl-Barth, 1958; Wright and Brown, 1962) and South-West Arabia (Wright, 1963). There is present in Lake Mutanda, Uganda, a distinctive form of *B. truncatus* with an obtuse apex (Brown and Wright, 1972; Mandahl-Barth, 1960, 1965).

Lymnaea natalensis are 25 x 14.5mm in size (Brown, 1965). The spire is generally much less high than the aperture. The surface may have spiral rows of small transverse grooves, but always lacks strong spiral ridges of peristria which are characteristics of *L. columella* (Umechukwu, 2009). Considerable variation is seen both between and within populations. Two forms with exceptionally low spire were described from Ethiopia, *L. exerta* and *L. gravieri*.

The name *L. cailladi* has frequently been used for snails from Eastern Africa with the belief that they belong to a narrower form than typical *L. natalensis*. However, intermediate specimens connect these and also many other nominal species and there does not appear to be any distinct geographical pattern in the distribution of variation. *L. natalensis* belongs to the *L. auricularia* super species, with representatives also in Europe and Asia. Brown (1965) indicated overlap between the ranges of the African species and *L. auricularia* in Lower Egypt and Arabia raising problems of identification which perhaps could be solved through Biochemical studies *L. hovarum* of Madagascar is treated as a distinctive species but this is doubtful in view of the extensive variation in the African species.

2.2 Distribution and Habitats of Freshwater Snail Intermediate Hosts

Trematode diseases are widely spread because their different snail intermediate hosts are as such broadly distributed. This is buttressed by Idris and Ajanusi (2002) when they observed that the distribution of snail intermediate hosts and the engagement of free swimming in many contaminated ponds and streams in the villages contributed to the high prevalence of infection.

Bulinus are widespread in Africa (Fig.4) including Madagascar (Stothard *et al.*, 2001) and the Middle East (Jorgensen *et al.*, 2007). This genus has not yet been established in the U.S.A, but it is considered to represent a potentially serious threat as a ‘pest’, an ‘invasive species’ which could negatively affect agriculture, natural ecosystems, human health or commerce. Therefore, it has been suggested that this species be given top national quarantine significance in the U.S.A (Cowrie *et al.*, 2009).

The distribution of the four species groups within the genus *Bulinus* is reviewed below:

***a. Bulinus africanus* group**

There are 10 species in this group (Brown, 1994), among which are *Bulinus globosus* – Morelet, 1866, *Bulinus africanus* – Krauss, 1848 and others were considered to be from intermediate populations (Mandahl-Barth, 1957).

***Bulinus globosus*:**

Bulinus globosus has the greatest range of any member of its species group. Globally, it is found in much of Africa South of the Sahara. The Northern Limits are in Southern Sudan, Lake Chad, the Middle Niger basin and the Senegal basin.

Its southern limits are the Okavango Delta (Oberholzer, 1970) and coastal plain of Eastern South Africa where it occurs in the Mpumalanga Lowveld, Southwards to Lakes Nhalabane and St. Lucia on the Coastal strip of Kwazulu-Natal (Appleton *et al.*, 2010).

Bulinus africanus

Bulinus africanus has a scattered distribution in Eastern and Southern Africa, unclear for many areas where critical comparison with *B. globosus* is needed (Brown, 1994). In Southern Africa, *B. africanus* inhabits cooler climate areas (de-Kock *et al.*, 2002) whereas *B. globosus* occurs only in the warmer parts (Brown 1966, Appleton, 1980).

In Nigeria, *Bulinus* is implicated and from the findings of Idris and Ajanusi (2002), *Bulinus globosus* is the species of the *B. africanus* group implicated. A number of species of non-marine molluscs are found in the wild in Nigeria which include *Bulinus globosus* (Ndifon, 1979) found in areas like Oyan Reservoir, Ogun State (Ofoezie *et al.*, 1991), some local government areas in Katsina State (Idris and Ajanusi, 2002), Agulu Lake area of Anambra State (Ekwunife, 2008), Ishienu Local Government Area in Ebonyi State (Okafor, 1990) to mention but a few indicating that *B. globosus* is the snail that has been more implicated in the Western, Northern and Southern Nigeria.

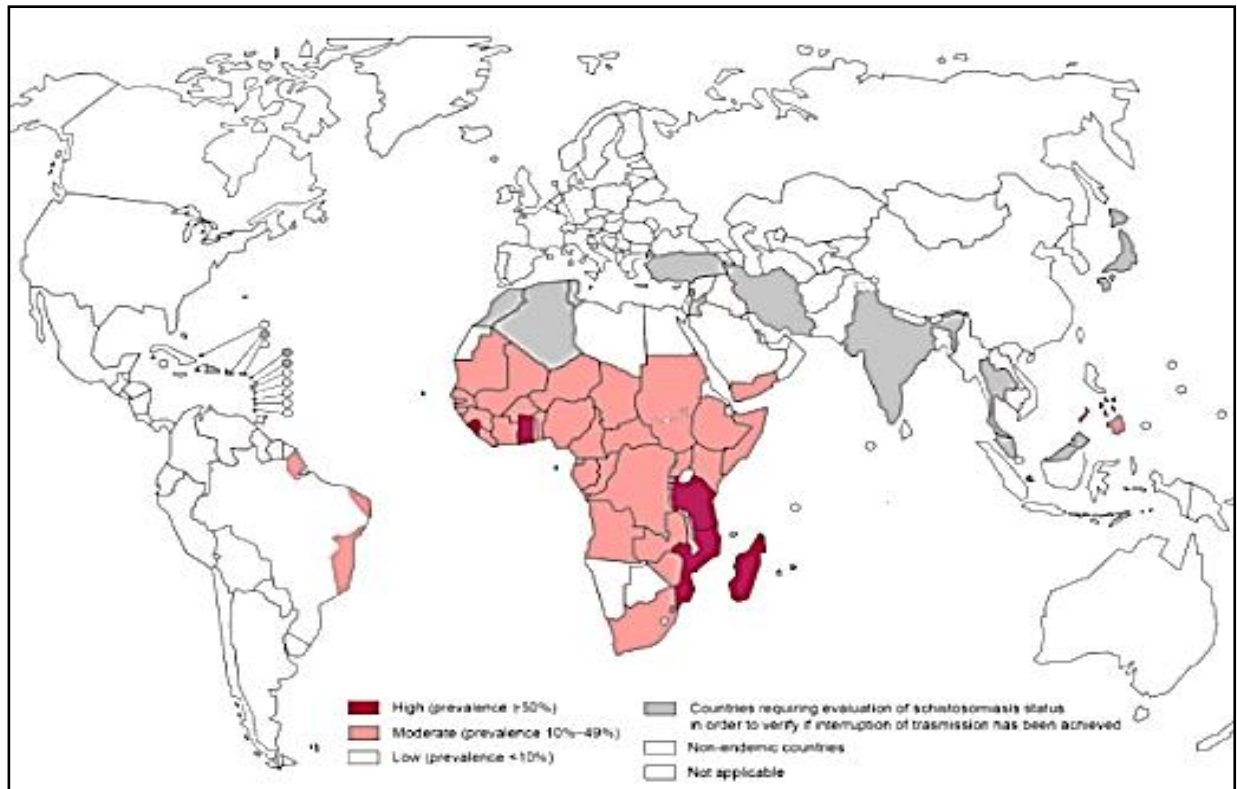


Figure 4: Worldwide Distribution Map of Schistosomiasis which is also areas where snail hosts are found

Source: WHO (2011)

***b. Bulinus forskalii* group**

There are 11 species in this group including *Bulinus barthi*, *Bulinus browni*, *Bulinus camerunensis*, *Bulinus forskalii* and *Bulinus scalarias* (Nalugwu *et al.*, 2010). Globally, *B. forskalii* is found to be widespread all over Africa. It is essentially an afro tropical species reaching the Mediterranean only in Lower Egypt. There is recent record in Lake Qaron (Fayoum, Egypt), northern region delta, and Nasser. Egypt is northern extent of species (Appleton *et al.*, 2010). The range of *B. forskalii* extends west to Senegal and Southwards to Eastern and Southern South Africa (Brown, 1994).

In Nigeria, the works of Idris and Ajanusi (2002) equally indicates that *B. forskalii* is implicated in the country. In Zobe Excavation areas and Kadda burrow pit of Katsina State, studies showed the presence of *B. forskalii* albeit, in small number (Idris and Ajanusi, 2002).

C. Bulinus reticulatus Group

Globally, this species is found from eastern and southern Africa. It is quite common in South Africa within the central area. In Eastern Africa, it is known from Kano plains in Kenya and Tanzania. In Northern Africa, it is present in Ethiopian (Brown, 1994). In Southern Africa, it is found on the Highveld Plateau (de Kock *et al.*, 2002). It is also found throughout Zimbabwe, east and north-east Zambia and northern Mozambique. There are also records in the Cuvelai drainage and the Nyae-Nyae area in Namibia.

Although the sample of *B. reticulatus* currently on record in the national freshwater snail collection shows that this species is well represented in the central parts of South Africa, it is an inconspicuous species, probably yet to be found at many more localities (Brown, 1994; de Kock *et al.*, 2002). As this snail is a specialized inhabitant of seasonal rain pools (Brown, 1994) and may not be active for more than a few months each year, it is unlikely to be found unless searched for, specifically at this time and should be much more common than indicated by its scattered records in South Africa (Van Eeden and Oberholzer, 1965), Ethiopia (Brown, 1967), Kenya (Brown, 1980), Tanzania (Mandahl-Barth, 1954; Brown, 1980) and Mozambique (Brown, 1980).

d. Bulinus truncatus group

Bulinus truncatus is a freshwater snail with the widest distribution of all *Bulinus species* (Brown, 1994). According to this author, it is found across much of Africa, the near east and the Mediterranean. It occurs in Cameroon, DR Congo and Gabon, Western Kenya, Ujiji, Kigomo in Tanzania, Lake Mutanda in Uganda, in Malawi, Sudan, Ethiopia, Somalia, South Egypt and possibly also in Eritrea and Djibouti. Its southern limits lie in Zaire and Malawi but it has also been recorded as rare in Zimbabwe. The main areas of distribution in Africa are Lower Egypt, Sudan and Westwards into Mauritania. It has been found in all Oases in Northern Africa and widespread in Western and Eastern Africa (Brown, 1994).

The northern limit of *B. truncatus* is known to be Portugal, Sardinia and Corsica, and the near east. It has also been recorded in the Mediterranean region and Southwest Asia eastwards to the Khuzistan region of Iran and found throughout the western parts of the Arabian Peninsula (Brown, 1980; Brown and Wright, 1980; Neubert, 1998). It is present in Saudi Arabia and Yemen, where it seems to be limited to few localities in Sana'a, Ibb, and Taiz districts (Al-Safadi, 1990) but not found in Oman. In Saudi Arabia, living populations have been found only in the western regions (Brown, 1994).

Habitats of *Bulinus globosus* are stagnant or slowly flowing waters with rich aquatic vegetation. In Ghana, the establishment of dense populations in stream within the forest seems to be favoured by human activities. However, despite its versality, *B. globosus* did not colonize Lake Volta in its early years (Odei, 1972).

Bulinus truncatus inhabits a wide range of water bodies including seasonal pools (Brown and Wright, 1974), irrigation system (Brown, 1994) and concentrate cisterns (Itagaki and Yasuvaoka, 1975). *B. truncatus* or closely related forms of the snail are successful also in Lakes, including the Agulu Lake in Nigeria (Emejulu *et al.*, 1992).

Bulinus* host of *Schistosoma intercalatum

Schistosoma intercalatum, a parasitic worm found in parts of western and central Africa, is one of the major agents of the rectal form of schistosomiasis and has two strains: the Lower Guinea Strain and the Zaire Congo Strain. Two species of freshwater snails make up the intermediate hosts, *Bulinus forskalii* for the Lower Guinea Strain and *Bulinus africanus* for the Zaire Strain (Tchuem *et al.*, 2003). The Lower Guinea Strain lives mainly in Cameroon (Bjornebe, 1978).

***Lymnaea* species**

Lymnaea species are found in Egypt and Sudan; common in the Nile Rivers, Somalia; Mijjarten district, Ethiopia (Lower Awash Valley) (Umechukwu, 2009). The most northerly living population found in recent years is near Zalingei, West of Jebel Marra and the Gambian River System. *Lymnaea* species are present almost throughout tropical Africa (Fig.5), but uncommon in the coastal region of the East Africa (Brown, 1965). Its limits in the southeast probably reflect the effect of both low rainfall and a comparatively cool climate (Van Eeden and Combrinck, 1966).

Habitat of *Lymnaea*

Lymnaea species mostly inhabit the permanent water bodies, including reservoirs and drains. Found also in very shallow though constantly seeping waters and rarely seasonally-filled pools (Bitakarimire, 1968). The abundance of *L. natalensis* in Ghana was greatly increased by the construction of permanent pools (McCullough, 1965), though aestivation for up to months is reported (Bitakaramire, 1968).

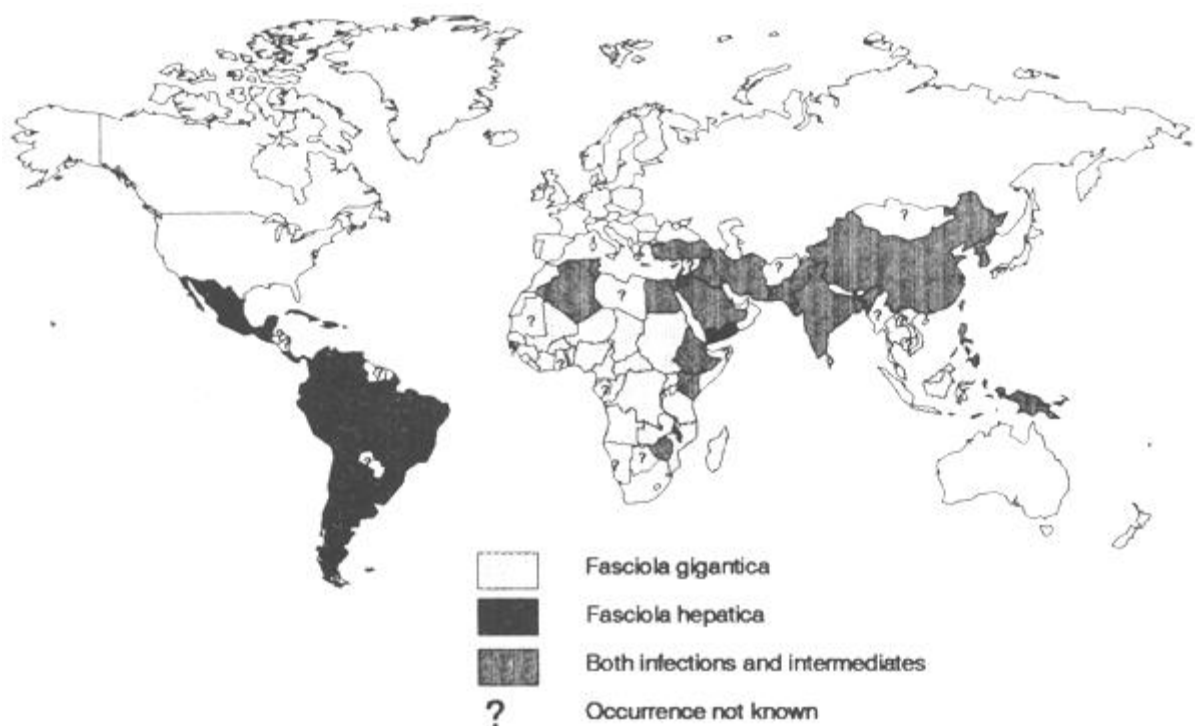


Figure 5: Distribution of fascioliasis which is also areas where *Lymnaea* hosts Are found

Source: WHO (2012)

The distribution and habitats of other freshwater snail intermediate hosts for parasitic trematodes have been emphasized by many workers (Okoji, 2017; McCullough, 1965; Okafor and Obiezue, 2015; Appleton, 1978).

2.3 Factors affecting the Abundance and Distributions of Freshwater Snails

Freshwater snail abundance and distributions are influenced by not only the availability of the snails' habitat, but also by some biotic and abiotic factors. Changing ecology, global warming and migration, may have led to changes in the prevalence and distribution of the disease in different parts of the state studied (Ekpo *et al.*, 2008).

2.3.1 Abiotic Factors

Physical factors

Chemical factors

2.3.1.1 Physical Factors

Among the physical factors that influence the occurrence and distribution of the snail hosts are variations in rainfall, water temperature, water current and Elevation. The population dynamics of *Bulinus globosus* are negatively correlated with temperature (Woolhouse and Chandiwana, 1990). These authors observed in Zimbabwe that losses of *Bulinus* due to raining season floods ranged up to 99.1%. The relationship between recruitment and temperature was discovered by a bell-curve, with peak recruitment at 20.6⁰C. Recruitment rate varied between sites. Growth rate was positively correlated with temperature. *B. globosus* population has a temperature-dependent season cycle with rapid increases in abundance during period of favourable temperatures. Floods or drought caused variable and irregular reductions in abundance (Woolhouse and Chandiwana, 1990). Idris and Ajanusi observed that infection rate and prevalence of *Bulinus* tend to fluctuate during the months under study. The highest of which

was observed to coincide with the dry season, although towards the onset of the rainy season when the temperature range (20.6⁰C – 32.8⁰C) lies within the optimum limits for snail growth and reproduction (Idris and Ajanusi, 2002; Akogun and Obadiah, 1996).

Ezeugwu and Mafe (1998) attributed the increase in abundance of snail species during the dry season to the stability of the aquatic habitat in terms of water level and velocity. Etim *et al.*, (1998) during their studies in Cross River State, Nigeria observed that the snail population was highest in April, just before the onset of the rains but reduced at peak of the rainy season. Large empty snail shells were recovered in March, just before the population of all snail species showed marked and locally varying seasonal fluctuations and concluded that in general, snail populations fluctuated strongly (numbers decreased at the peak of the rainy season and further reduced during the dry season).

During a period of 2 years, the ecology of *Bulinus globosus* was studied in 8 habitats in two streams near Ifakara, South East Tanzania by Marti *et al.* (1985). The relative densities of *Bulinus* were followed monthly. *Bulinus* densities were constantly low throughout the year in the stream, but they showed distinct seasonal fluctuations in adjacent pools with a density-peak at the end of the small rainy seasons.

Furthermore, Marti *et al.* (1985) showed that fluctuations of the water level and fast increase of water velocity after heavy rains affect snails. Studying the ecological aspects of *Biomphalaria* in endemic areas in Brazil, South America, Marco and Alan (2012) observed that the positive sites for schistosomiasis were

those with predominant smooth undulated relief, with predominance of slopes below 35%, with softer slopes and flood plains. Flood plains are areas of materials from large reliefs, represented by the bottom of the more open valleys (Marco and Alan, 2012).

Moreover, Marco and Alan (2012) observed that during the period, comprising, in all locations, dry and rainy seasons, the smallest planorbid snails, whose shell diameters ranged from 1mm to 6mm, were found in greater numbers during the dry seasons suggesting a low dispensation in this season and higher during the rainy season, which favours the expansion of the distribution area of snails and in consequence of the disease. The bed of most breeding spots and foci was almost muddy and some were sometimes covered with fine mud, favouring the camouflage of snails (Marco and Alan, 2012).

Climatic change, along with other forms of global environment changes, alters the densities of freshwater snails and their schistosome dynamics. The net effect of climatic change on schistosomes' dynamics in Egypt is dependent on the full spectrum of the direct and indirect effects of climate on host and parasite life histories (Iman, 2013). The effects of rising temperature extend beyond simple changes in host or parasite geographical distribution to include significant shifts in the interactions between both, resulting in an increase or reduction in the severity of the disease (Paul and Johnson, 2001).

The habitats of *Oncomelania hupensis* in the middle and lower reaches of the Yangtze River include Lake/Marshland regions and hill regions, both of which have extensive physical connections with the Yangtze River through channels or in low flood plains beside Yangtze River. With frequent flooding of the Yangtze

River, snails in these habitats can be dispersed and subsequently deposited widely in various localities. The accumulation of mixed sources of snails can then generate genetically diversified populations of snails, leading to the existence of various haplotypes (Zhao *et al.*, 2010).

In Sichuan and Yunnan provinces in the upper reaches of Yangtze River, *O. hupensis robertsoni* are distributed in mountainous areas, and are not subjected to flood influence as much as in the middle and lower reaches of the River. It is interesting to see that a relatively lower number of haplotypes were found in the region as compared with *O. hupensis hupensis*. It appears likely that there have been certain degrees of isolation for these mountainous populations (Zhao *et al.*, 2010).

The effect of temperature on the survival rate, reproduction and growth of *Lymnae auricularia* has been shown in Turkey and Madagascar (Schmitt, 1973). Sturrock (1966) has shown that temperature exerts a marked influence on the fecundity of *Biomphalaria pfeifferi*. Optimum temperature of between 20.6°C to 32.8°C

Despommier *et al.* (2005) studied the four environmental factors that affect density and viability of snail populations as follows:

Water level controls snail population densities, and tend to vary considerably between years and seasons. Optimal snail habitat usually falls into a narrow zone of elevation above the mean low water level for any given region. Flooding can prove problematic, an annual flood in certain environments has been found to drown adult snails. In environment where flooding continually occurs, *O.*

hupensis lives above 1 year. In environments with less frequent or no flooding, the species can live at least twice as long, and often longer.

The current speed in riparian environments often determines the density of snail populations and during times of high water may serve to re-locate large populations down river. Flood-driven currents can also devoid areas of snails.

Temperature can determine whether or not snails can reproduce. Below 10⁰C, which occurs usually in early spring in sub-tropical environments, reproduction is severely inhibited. Both adults and eggs succumb at temperature that exceeds 30⁰C.

Elevation also plays an importance role in determining the density of snail populations, particularly for Marshlands that lie above the mean low water level of lakes and rivers. Optimal snail habitats are typified by expanses of flat, mid-level land with numerous dry season ponds and streams, and thick grass covering the ground.

Neotricula aperta is found only in shallow areas (typically 0.5 to 3m deep) of the Mekong River and some of its tributaries (Attwood and Upatham, 2012). The snails are restricted to areas where the current is moderate (around $2 \times 10^3 \text{m}^3 \text{s}^{-1}$), the water is clear and the bed rock forms platforms where algal *aufwuchs* is extensive. Such conditions exist only during the dry season in the lower Mekong (March to May) and so *Neotricula aperta* populations persist mostly by recruitment (from eggs laid on stones in the previous year) or re-colonization from other rivers, and transmission of *S. Mekongi* is seasonal (Attwood and Upatham, 2012).

2.3.1.2 Chemical Factors

The presence of chemical substances such as calcium, dissolved oxygen, pH, iron and dissolved carbondioxide in aquatic habitat affects the occurrence of snail intermediate hosts of human schistosomiasis. *Biomphalaria glabrata* removes calcium from the study water in a tank at a rate proportional to the absolute growth of the snails, it can therefore, become a limiting factor if the concentration drops below 40-80ugml⁻¹ (Thomas *et al.*, 1974). However, 80% of the calcium required by the snails is taken directly from the water (Thomas and Benjamin, 1974).

Chemicals released by snails may be concentrated in high-density tanks and have previously been shown to inhibit respiration. Coelho *et al.* (1975) noted a difference in the amount of ⁵⁹Fe uptake by snails in crowded tanks, which is necessary for snail growth. It then means that inhibition of snail growth can be caused by chemicals released by the snails as waste products and by a reduced capacity to uptake essential minerals. It is evident that *Oncomelania hupensis quadrasi* does not thrive in water with dissolved oxygen concentration as low as 0.08-3.6mg/litre. The dissolved oxygen concentration remained high throughout the year in all areas infested with *O. h. quadrasi*, the minimum value being 3.8mg/ litre and the maximum 9.85mg/litre (Rolando, 1972). It is known that highly acidic water affects shell formation (Brown, 2002). The results of the study show a moderate amount of free dissolved carbon dioxide (8.8 – 76.12mg/litre) in snail-infested habitat. On the other hand, the lower levels (0.88 – 60.72mg/litre) of free carbon dioxide associated with the very low dissolved oxygen concentration found in snail habitats may have been a limiting factor in the distribution of *Oncomelania hupensis quadrasi*. Muir-head-Thompson (1958)

claimed that low dissolved oxygen and high free dissolved carbondioxide are important for the survival of freshwater snails.

The hydrogen-ion concentration (pH) is closely related to the carbon dioxide complex. Unless values are extreme, which is unusual, most organisms in nature seem to have a wide tolerance to changes in pH. Welch's (1952) view that pH change is proportional to changes in the carbon dioxide level and is therefore a useful measure of the later, when alkalinity is constant, appears to be correct.

Although Komiya (1964) showed that 80% of *O. h. nosophora* died when they were immersed for 72 hours in water with a pH of 2.3, Abdel Malex (1958) believed that the pH of the water is not a major factor in the distribution of snails. The pH determinations in the field merely indicate the alkalinity or acidity of the water. However, Abel Malek (1958) stated, "it is necessary to be very circumspect in correlating pH measurements with vector distribution. Actually, the combined effects of other factors correlated with pH (alkali reserve, carbon dioxide content, sunlight, photosynthesis with its active removal of CO₂ and the production of O₂, and the character of the substratum) are more important than pH alone". Adetokunbo (2003) found that although *O. h. quadrasi* is relatively tolerant of a wide pH range, it appears to thrive better within a pH range of 6-8.

Earlier investigations by Azevedo *et al.* (1954) and Abdel Malek (1958) have indicated that in Mozambique, *Bulinus africanus*, *Planorbis (Biomphalaria) pfeifferi* and *Bulinus forskalii* thrive well in water having methyl orange alkalinities of 14-260mg/litre, 38-230mg/litre, and 54-100mg/litre respectively, measured as calcium carbon. These ranges approached those found in water

positive for *O. h. quadrasi* in North-eastern Leyte namely 28-200mg/litre (Rolando, 1972).

The alkalinity of all the natural water studied was due to hydrogen carbonates. In South Africa, de Meillon *et al.* (1958) observed that hydroxide (OH) alkalinity was never present and carbonates occurred in very low concentration in the Highveld and Lowveld streams where *Physopsis africanus* and *Planorbis pfeifferi* were present. They further observed that the alkalinity in both streams was caused by hydrogen carbonates. In other types of snails free habitats low dissolved oxygen concentration and the presence of hydrogen sulphide could have been harmful to the snails. Welch (1952) showed that hydrogen sulphide easily penetrates living cells in acid, neutral or slightly alkaline media, and causes intra-cellular acidity.

Oxygen has great influence on the distribution of snail intermediate hosts of human Schistosomes. Rolando (1972) observed that in aquaria both with and without aeration, the egg laying activity of *O. h. quadrasi* stopped temporarily for 37days after the start of the experiment. However, numerous young snails in subsequent counts during the first months following the acclimatization periods with aeration laid many eggs. The increased egg-laying capacity of the snails could have been the result of aeration.

The absence of *O. h. quadrasi* from certain habitat in north-eastern Leyte may have been caused by several factors including desiccation for long periods, the presence of hydrogen sulphide and other decomposition gases, and pollution. On the land, dissolved oxygen levels of 3.8 – 9.85mg/litre and adequate levels of dissolved carbon dioxide, favour the presence of *O. h. quadrasi* in a habitat

(Rolando, 1972). The high levels of calcium of the predominantly limestone environment of Virgem das Gracias appear to promote large *B. glabrata* populations in wells and springs (Tatiane *et al.*, 2014). Calcium has been associated with high fecundity of *B. glabrata* (Brown, 1994; Brown, 2002) and shell development (Brown, 1994).

2.3.2 Biotic Factors (Biological Factors)

Some of the activities engaged in by man and other animals and the existence of aquatic plants alongside the snails have contributed immensely to the occurrence and development of snail intermediate hosts of human schistosomes. Such practices as irrigation, construction of dams, cultivation, human-water contact pattern and the co-existence growth of aquatic plants have either flourished the snails' populations or diminished them. Fenwick *et al.* (2006) had this to say, "it is utmost important to recognize that, by extending snail habitats, agricultural projects intending to increase food production in underdeveloped countries have in many cases, created more misery than they have alleviated". For example, a 10-million dollar irrigation project in Southern Zimbabwe had to be abandoned 10 years after it was started because of schistosomiasis (Osmundsen, 1965). The Aswan High Dam in Egypt has had many of its benefits cancelled by the increase in disease it has caused. Restraints of the wide fluctuation in the water level of the Nile, although making possible for crops per year by perennial irrigation had also created conditions vastly more congenial to snails (Chistulo *et al.*, 2000).

The change from a hunting-gathering society to a more sedentary agricultural one, which occurred with the construction of canals, facilitated transmission of *Schistosoma*. During pharoanic times, the Egyptians utilized the Nile's annual flooding of basins as an irrigation system. This system was used to cultivate basic

crops such as wheat, barley and flax (Caminos, 1997). During the reign of Mohamed Ali (1805 – 1848), who advocated the cultivation of long staple cotton, perennial irrigation was extended which resulted in the flourishing of the aquatic snails (Kloos and David, 2002). Watts and El Katsha (1995) noticed that construction of barrages north of Cairo, the low Dam at Aswan after 1900 and the high Dam at Aswan in 1964 led to canal system and increase in perennial irrigation resulting in changes in Nile water velocity and a shorter winter closure period.

These changes have resulted in more stable snail habitats. Thus, *Biomphalaria* snails, which were previously restricted to the Delta, are now found in the Upper Egypt (Kloss and David, 2002).

The respective vectors of the two forms of bilharziasis in Egypt do not have the same ecological distribution. Both species are most abundant in the presence of aquatic vegetation, but they differ in their respective associations with the water hyacinth, *Eichhornia crassipes*. *Biomphalaria alexandrina* reaches maximum abundance in the presence of this plant, but *Bulinus truncatus* is as uncommon in the absence of plants as in the presence of *E. crassipes* (Dazo *et al.*, 1966).

2.4 General Life Cycle of Freshwater Snail Intermediate Host for Trematode Parasites

Most species of freshwater snail intermediate host(s) for parasitic trematodes, especially *Schistosoma haematobium*, *Fasciola gigantica* and *Fasciola hepatica* are hermaphrodites, possessing both male and female organs and being capable of self or cross fertilization. A single species can invade and populate a new habitat. The eggs are laid at intervals in batches of 5 – 40, each batch being enhanced in a

mass of jelly-like materials. Eggs of some snails like *Bulinus* and *Biomphalaria* spp are deposited in mass on plant or solid objects in water while the eggs of some other snail intermediate hosts are suspended in mass on the water surface. Usually a break occurs in the egg-laying of *B. globosus* after 243 days of egg laying. This break lasts for 15 days. When laying resumed, the egg masses laid were larger and contain higher number of eggs per mass (sometimes up to 30 eggs per mass) (Okafor and Anya, 1990). Hatching takes place under optimum temperature after 6 to 10 days and the young snails may reach maturity in 4 – 6 weeks. This is dependent on the species and the conditions of the environment. Such environmental conditions include temperature and availability of food which are among the most important limiting factors. The life cycle may last more than a year but the amphibious *Oncomelania* may last more than a year and have separate sexes. The female lays eggs singly near water margin (Brown, 1966).

2.5 Snail Intermediate Hosts of Human Schistosomes

Three genera of freshwater snails serve as intermediate hosts for the three major schistosomes. They are *Bulinus*, *Biomphalaria* and *Oncomelania* which serve as hosts for *Schistosoma haematobium*, *Schistosoma mansoni* and *Schistosoma japonicum* respectively. In addition to the three major schistosomes are *S. intercalatum* and *S. mekongi* with snail hosts *Bulinus* and *Neotricula aperta* serving as intermediates respectively (Roberts and Janovy, 2010).

Bulinus, a genus of small tropical freshwater snails are aquatic gastropods molluscs in the family planorbidae, the ram's horns snails and their allies. The shell of the species of the genus *Bulinus* is sinistral (Fig.1). It has a very large

body whorl and a small spine. The genus is medically important because several species of *Bulinus* function as intermediate hosts for the schistosomiasis blood fluke (Kane *et al.*, 2008). Species within the genus *Bulinus* have been placed into four species groups: the *Bulinus africanus* group, *Bulinus forskalii* group, *Bulinus reticulatus* group and *Bulinus truncatus* group otherwise known as *Bulinus tropicus* complex (Brown 1994).

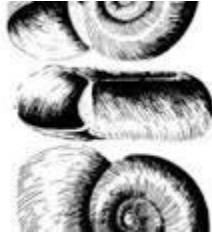


Figure 6: Shells of *Biomphalaria*

Source: Okafor and Obiezue (2015)



Figure 7: Snail of the *Biomphalaria* sp

Source: Okafor and Obiezue (2015)



Figure 8: *Oncomelania hupensis*

Source: Zhou *et al.* (2010)



Figure 9: *Oncomelania minima*

Source: Kameda and Kato (2011)

Biomphalaria, a genus of air breathing freshwater snails are aquatic pulmonate gastropod molluscs also in the family planorbidae, the ram'shorn snails and their allies. It is the type of genus of tribe biomphalariini. *Taphius* is a synonym for *Biomphalaria* (Bouchet and Kocroi, 2005). The shell of these species like all planorbids is sinistral in coiling, but is carried upside down and thus appears to be dextral (Fig.6 and 7). This genus of snail is medically important because the snails serve as intermediate host for the human parasitic blood fluke, *S. mansoni* that infects about 83 million people (Comptom, 1999). The International Association for Medical Assistance to Travelers, IAMAT (2012) describes *Biomphalaria* as a snail with a flat disc-shaped shell, dusky brown or reddish in colour with a diameter of 7-22mm.

Eighteen species of *Biomphalaria* are intermediate hosts for *S. mansoni*, seven species of the genus have not been tested for this susceptibility and nine species are resistant (Dejong *et al.*, 2001). There are 34 species in the genus *Biomphalaria* among which are 22 American species and 12 species in the Old World comprising Africa, Madagascar and the Middle East (Majora *et al.*, 2008).

Oncomelania is a genus of very small tropical freshwater snails, aquatic gastropod molluscs in the family Promatiopsidae (Fig. 8 and 9). The genus was named by Gredler (1881). *Oncomelania* snails are distantly related to the marine periwinkle and more closely related to the small marine species of the family Rissoidae. There are two species in the genus *Oncomelania* which are *Oncomelania hupensis* and *Oncomelania minima* (Kameda and Kato, 2011).

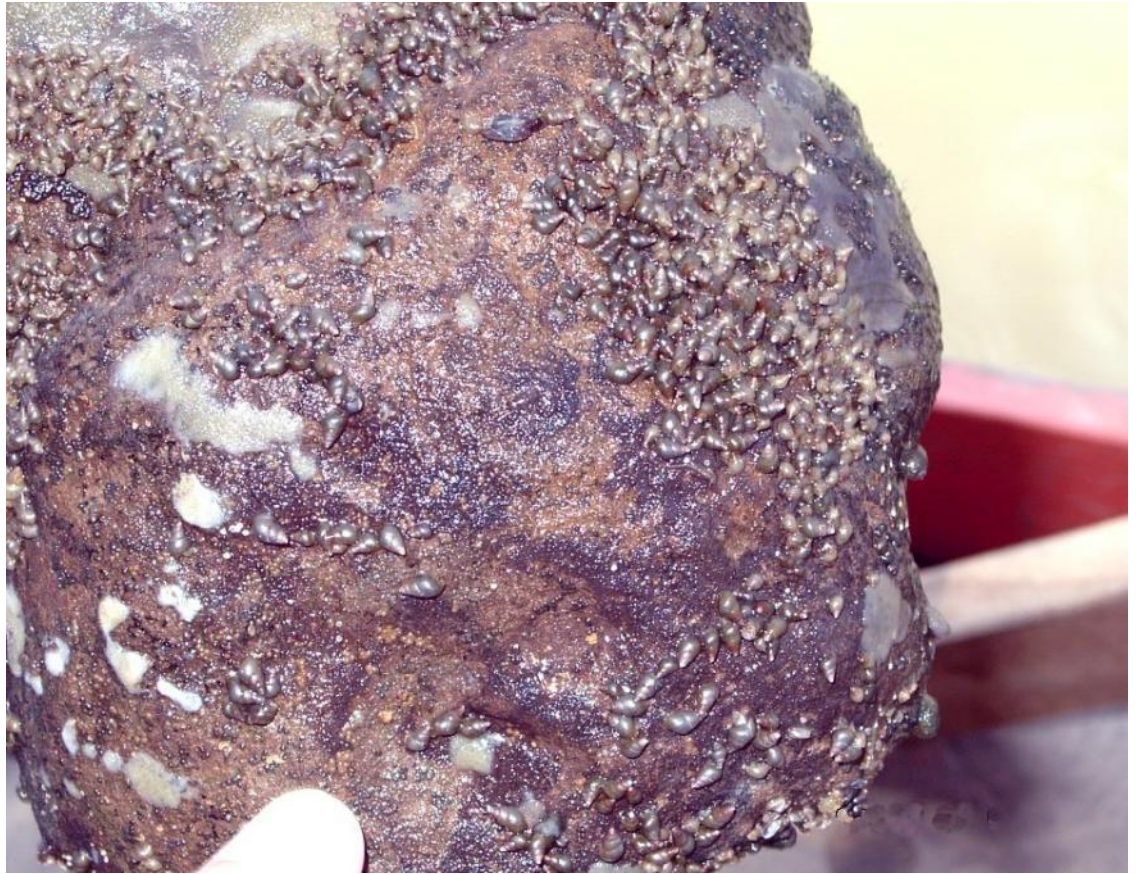


Figure 10: *Neotricula aperta* attaching themselves to a substratum

Source: <https://en.wikipedia.org/wiki/Neotricula-aperta>

O. hupensis, a polytypic species has a number of subspecies (Davis, 1979). Japanese Red List Data Book recognized also *Oncomelania lindoensis* while Japanese Red List Data Book recognizes also *Oncomelania shini* and *Oncomelania sakuyamai* (Attwood and Upatham, 2012). Various *Oncomelania* spp are significant medically because they can carry the blood fluke parasite and *Paragonimus* lung fluke parasite (Riley *et al.*, 2008). The snail intermediate hosts of *S. japonicum* (*Oncomelania* sp) and *S.mekongi* (*Neotricula aperta*) (Fig.10) are amphibious (water and land snails) (Cheesbrough, 2006).

Three strains of *Neotricula aperta* have been recognized (called α , β and γ), on the basis of shell size and body pigmentation. Although all the three strains are

susceptible to *Schistosoma mekongi*, only the Y-strain is epidemiologically significant (Attwood and Upatham, 2012).

2.6 *Schistosoma haematobium* Cercariae Transmission

When an infected man sheds eggs into the water body or its surroundings, the eggs under optimum conditions hatch into miracidia (singular=miracidium). The miracidia locate and penetrate *Bulinus* snails. Inside the snail host, they develop into mother sporocysts, each of which develops into daughter sporocysts. The fucocercous cercariae develop from daughter sporocysts and start to emerge from the host few weeks after initial penetration of the host by the miracidium. Cercariae alternately swim to the water surface and slowly sink toward the bottom, continuing to live this way for one to three days. If they come in contact with the skin of a prospective host, such as a human, they attach and creep about for a time as if seeking a suitable place to penetrate. They are attracted to secretions of the skin, showing a strongly positive response to the amino acid, arginine from post acetabular glands, thus attracting other cercariae in the neighbourhood (Granzer and Haas, 1986).

Cercariae require only half an hour or less to completely penetrate the epidermis and they can disappear through the surface in 10 to 30 seconds (Roberts and Janovy, 2010). Penetration is accompanied by a vigorous wriggling, together with secretion of products from the head organ. The tail drops off in the process. Within 24 hours the schistosomula (immature schistosomes) enter the peripheral circulation and are swept off to the heart.

Diagram of *Schistosoma mansoni* cercaria

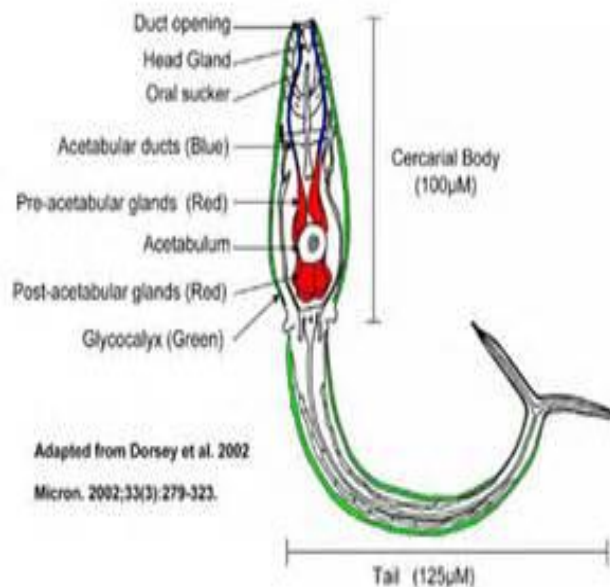


Figure 11: *Schistosoma haematobium* cercaria

Source: Arora and Arora (2010)

Some of the schistosomula may migrate through lymphatics to the thoracic duct and from there to the subclavian veins and heart. Their movement continues as they develop.

Useful information can be obtained by calculating some indices, especially those that help to determine the ecological and temporal aspects of the transmission patterns. The pattern and level of cercariae production in snails, in addition to factors such as the susceptibility of the snails to miracidial infections, and size structure of the snails, play important roles in the dynamics of schistosome transmission (Okafor, 1990). In some of the studies, human infections by this schistosome species depend on the density of cercariae at the transmission sites (Okwuosa, 1979; Tayo *et al.*, 1980). The temporal and spatial density of cercariae depends on their emergence, dispersal and their viability (Anderson,

1982). Climatic changes which affect the biology of both the snail and cercariae of schistosomes also influence the transmission (Okafor, 1990).

2.7 Control of Freshwater Snail Hosts for Schistosome Parasites

Control of snails was the major thrust of schistosomiasis control efforts before the advent of safe effective antihelminthics and it is still important to support chemotherapy campaigns with mollusciciding to reduce re-infection (Madsen, 1990). The control of intermediate host snails remains an essential component of integrated schistosomiasis control programmes. The multifold efforts in snail control discussed below have had great implication on the abundance and spatial distribution of intermediate host snails. The different control interventions reduce greatly the abundance and disrupt the spatial distributions of snails as exemplified in Sudan, Morocco and the Caribbean.

2.7.1 Action to Eliminate the Intermediate Host

In this control method, effort is to get the aquatic environment rid of the snail intermediate hosts. There are a number of ways of doing this:

7.7.1.1 Modification of Aquatic Environment

Aquatic environmental modification measures such as stream channelization, seepage control, canal lining, and canal relocation will deep-bury snails and prevent transmission.

Such actions helped prevent an increase in prevalence of schistosomiasis after construction of a lake and irrigation canals in Cameroun between 1879 and 1985 (Audibert *et al.*, 1990). This project included regular clearing of vegetation from

secondary and tertiary canals, use of only the concrete-lined primary canals for bathing and clothes washing, and provision of a clean source of drinking water. The spatial distribution of snails was greatly altered in Cameroun by such environmental practices as stated above.

Most snails died of starvation since their main source of food which was organic matter originating from decaying submerged or emerged vegetation, different species of algae, bacteria and fungi was cut off from them. Such practices also minimized or halted cercariae shedding since the snails were cut off from sunshine.

Aquatic vegetation makes a habitat more attractive for *Bulinus truncatus* because it provides shelter from high water flow velocities and excessive sun light, it offers support for egg masses and constitutes the ideal substratum for the algae that constitute the snail's main food.

Vegetation removal and clearing of irrigation canals lead to a substantial reduction in density of *B. truncatus* snails and their egg masses. Silt removal creates a synergetic effect as it renders snail habitats unsuitable for aquatic vegetation, which in turn affects availability of food and shelter for snails (Thomas and Tait, 1984). Canal clearing and sediment removal reduce resistance and increase water flow velocity (Oomen *et al.*, 1990). Increased water flow velocities hinder snail movement and development directly but also reduce the availability of food resources, thus reducing snail populations indirectly.

Vegetation removal and clearing have been applied under different conditions in Morocco and elsewhere to control schistosomiasis intermediate hosts. In an intervention study in Central Morocco the authors compared three different

environmental snail control measures that removed snail egg masses, aquatic plants and slits (Laamrani and Boelee, 2002). Intensified clearing of hydraulic structures led to a short term reduction, in the density of *B. truncatus* but the intervention could not be sustained by the farmers in the area and snail populations rapidly re-colonized the habitat (Laamrani *et al.*, 2002). In the Rahad irrigation scheme in Sudan, Meyer-Lassen (1992) observed the disappearance of *B. truncatus* after the removal of aquatic plants. In contrast, Hilali *et al.* (1985) reported less impressive results from the Gezira irrigation scheme, where mechanical weed removal alone did not lead to a significant reduction of the snail population because of the rapid re-colonization of the canals by weed.

Disadvantages of Environmental Modification Practices

Disadvantages of these methods may include:

- i Impracticality for economic or environmental reasons.
- ii Damage to fish populations (e.g. clearance of vegetation).
- iii Altering the environment such that it is then suitable for other disease organisms, for example, increasing rate of water flow may inhibit snail populations, but may then be suitable for colonization by simulium larvae, the vectors for river blindness (Roberts and Janovy, 2010).

2.7.1.2 Mollusciciding

Selective molluscicide treatment in snail-infested bodies of water at main human contact points is the preferred way to approach controlling snail populations. Metallic salts, such as copper sulphate, were among the first agents used and most effective when applied to standing bodies of water. Copper sulphate was introduced by dragging burlap sacks filled with large CuSO_4 crystals behind slow moving boats. This compound worked well enough, but it also limited alga

growth, that in turn affected growth patterns of fish that served as primary sources of protein (Roberts and Janovy, 2010).

Newer molluscicides, such as nicotinanilide, or organotin dibromo-nitrazobenzene, sodium pentachlorophenate, Tritylmorpholine, Sodium dichloro-bromomphenol, niclosamide and acetamide analogs replaced copper sulphate, as these were deemed safer to the environment. Niclosamide is the only remaining commercially available molluscicides. While niclosamide is biodegradable, its “side effects” included the death of many fish species, as well as the targeted snail populations. It acts by depleting glycogen stores; its use is limited by cost. Plant-derived molluscicides are too variable in their effectiveness and are difficult to manufacture. It is worthy of note that in China, Taiwan, Philippines and Southeast Asia, molluscicidal control of *Schistosoma japonicum* is virtually ineffective because *Oncomelania* spp are amphibious and visit water only to lay eggs. Use of chemical molluscicides has met with some successes; but problems involved include determination and application of the proper quality in a given body of water, dilution, effects on other organisms in the environment, and errors in estimating the physical and chemical characteristics of water (Roberts and Janovy, 2010).

2.7.1.3 Biological Control

Biological agents have been considered for the last few decades as an alternative approach to chemical molluscicides (WHO, 2002) and numerous review papers have been written on the topic (Pointer and Jourdane, 2000; Ferguson, 1977; McCullough 1981; Jobin, 1970; Combes and McCullough, 1982; Pointer, 1983; Combes and Chang, 1986; Pointer and McCullough, 1989; Madsen, 1990, 1995). A great number of organisms have been considered for the biological control of

snails. They may be briefly categorized as micropathogens, predators, parasites and competitors. A list of micro pathogens of freshwater snails is available in the literature (Madsen, 1995). Micropathogens include viruses, fungi, protozoa and bacteria while very little work has been done in systemic screening of snails for micro pathogens, predators and competitors of freshwater snails have been studied more extensively (Pointer and Jourdan, 2000). Such predators belong to almost all groups of the animal kingdom including planarians, leaches, insects and larva of insects, crustaceans, fishes and mammals. It is worthy of note that most of the work available on freshwater snail predators are empirical observations and laboratory studies, field experiments remain scarce. The best-studied are potential competitive species of snails: *Marisa cornuarietis*, *Helisoma duryi*, *Thiara granifera* and *Melanoides tuberculata* (Jobin *et al.*, 1977; Jobin and Laracuenta, 1979; Prentice, 1983; Pointer *et al.*, 1989, 1991; Pointer and Guyard, 1992). In Martinique for example, *M. tuberculata* colonized rapidly after its introduction in January, 1983, and by October 1984, *Biomphalaria glabrata* and *B. straminea* had disappeared and have not been found since. Introduction of *M. cornuarietis* has successfully controlled *B. glabrata* in several habitats in Puerto Rico; *M. cornuarietis* not only competes with *B. glabrata* for food, but also preys on the vector snail (WHO, 2002). A North American Crayfish, *Procambarus clarkii*, was introduced into East Africa for aquaculture in about 1970 and has since then dispersed into all major drainages in Kenya. This field work, carried out in Kenya suggests that it is one of the most promising biological control agents in Africa (Hofkine *et al.*, 1991 a, b; Coker *et al.*, 1993; Mkoji, *et al.*, 1995). *Procambarus clarkii* feeds on *Biomphalaria spp* and under certain environmental circumstances can have a significant impact on Schistosome

transmission (Madsen, 1990). Snail eating fish (Molluscivorous fish) have been cultured and released in infected water with some successes (Roberts and Janovy, 2010). The cichlid fish *Astatoreochromis alluaudi* has been used in well documented field trials in Kenya and Cameroon. Small initial success was made using the fish but after a longer period the fish appeared to be ineffective in snail control. Moreover, the fish reproduces at a pace too slow to be of use in large scale biocontrol trials (Slootweg *et al.*, 1994). The failure of the fish in snail control was attributed to an observed reduction in the fishes pharyngeal jaw apparatus used to crush snails shells, results in a lower profitability of snails and as predicted by a simple foraging model, the prey preference of the fish shifts towards other more profitable prey items, such as benthic and pelagic macro fauna (Slootweg *et al.*, 1994). Other fish species such as *Tilapia melanopleura*, *Astronitus ocellatus*, have been used to control snails (Milward de Andrade and Antunes 1969; Kloos *et al.*, 2004).

Aquatic birds such as ducks (Michelson, 1957), Chelonian (Coelho *et al.*, 1975) have also been employed as snail predators. In addition, a number of other types of predators such as mosquito larvae have also been described (Berg, 1964). In the laboratory, a small leech, *Helobdella trise-rials lineata* and ostracods crustacia have been found to be good snail predators (Sohn and Hornicker, 1972; Guimar *et al.*, 1983). In the field however, these animals are found in snail breeding sites in an *ecological* equilibrium with the snails. The pathological action of bacteria such as *Bacillus pinotti* against *B. glabrata* has also been studied (Texera and Viente-Scorza, 1954) though follow up studies were not able to confirm the action.

Also, growth of certain plants on the sides of water ways has been used to control snail. The berries of endod (*Phytobacca dodecandra*) have been shown to be naturally molluscicidal when they fall into the water, and its presence on sides of rivers in Ethiopia has been shown to be associated with a reduction in local snail population (Guimar *et al.*, 1983).

2.7.2 Prevention of Infection of the Intermediate Hosts.

Without the infection of the snail host by the miracidia that hatch out of the schistosome eggs, there will be no emergence of cercariae that penetrate man. Therefore, the prevention of the snail host from being infected is a great success in the control of schistosomiasis. These control methods at present are principally, in this case, introduction of latrines, thereby reducing or eliminating contamination of the populations' water supplies with human urine and faeces containing eggs. The disadvantages of these methods include the large cost of implementing the schemes.

Infection of the snail with other larval trematodes, particularly those of echinostomes, has, interestingly, been shown to protect the snails from infection by larval schistosomes, and although these might not eliminate schistosomes for endemic areas, they may, to some extent, limit intensity of snail infections.

2.7.3 Integrated/Comprehensive Control of Snail Hosts.

A comprehensive approach to snail control or what is called integrated approach to snail control involves mollusciciding, environmental modification and introduction of biological agents. No single control strategy has proven effective on its own in the control of schistosomiasis (WHO, 1981; Sturrock, 2001). In

controlling schistosomiasis by controlling the snail host, an integrated approach, involving mollusciciding, environmental modification and introduction of biological agents simultaneously has proven very effective especially in the Moroccan context of schistosomiasis elimination. In the simultaneous approach, effort is geared towards deep burying the snails, removing the vegetation that favours the thriving of the snails, applying molluscicides, introducing snail-eating organisms and organisms that out-compete snail for food and space.

In the application of molluscicides, care should be taken not to use chemicals which are toxic to other useful aquatic animals including the organisms introduced to eliminate the snails.

A comprehensive approach was employed in the lower reaches of the Yangtze River, China between 2005 and 2008 to control snail host of *Schistosoma japonicum* (Le-Ping *et al.*, 2013). This was done by mollusciciding together with environmental modification. Over the 32 months study period, a total of 12671.52 hm² of snail habitats were treated with molluscicides like niclosamide and environmental improvement such as constructing fish ponds, digging new ditches, building fruit trees and filling of infested areas was carried out by health sections, together with water resources development and agricultural and foresting projects. 44 sluices were re-built for prevention of snail spread, and 217.4km long river banks were hardened using concrete. The result of this was that the spread of *Oncomelania hupensis* which was the major snail host of *Schistosoma japonicum* was halted and the number reduced to barest minimum. The 107 village habitats of the snail was reduced to 20 and the acute infections of *Schistosoma japonicum* have also been controlled for a successive 2 years since 2007 (Le-Ping *et al.*, 2013).

Integrated snail control has proven to be useful in the Caribbean area. The case of the rivers of the littoral central part of Venezuela is particularly clear. In this area of high transmission in the 1940s, an integrated control programme (a national control programme) based on chemotherapy, sanitation, health education, environmental improvements and application of molluscicides was initiated in 1943. The programme resulted in the near total interruption of transmission in the 1970s (Incani, 1987).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was carried out at Mmiri Ali in Nkalagu, Uzuru stream in Emuhu-Ali, River Ora in Amazu, Old Quarry Pits in Ezillo and Natural Pools behind “Over Rail” Settlement in Nkalagu, all in Ishielu Local Government Area of Ebonyi State (Fig.13). Mmiri Ali, Uzuru stream and River Ora are flowing water habitats while Natural Pools behind “Over Rail” Settlement and Old Quarry Pits are stagnant water habitats. Ishielu was purposely selected for the study because of the previous knowledge of the presence of snail intermediate hosts for parasitic trematodes (Okafor, 1990). The local government is in the South Eastern quadrant of the Federal Republic of Nigeria, in Ebonyi North Senatorial zone. Ishielu LGA is located between latitudes $6^{\circ}25^1\text{N}$ and $6^{\circ}35^1\text{N}$ and longitudes $7^{\circ}45^1\text{E}$ and $7^{\circ}55^1\text{E}$. In this area, there are several fresh water habitats including man-made pools, quarry pits, road ditches and irrigation canals. The climate of the area is tropical characterized by two distinct seasons, the wet and the dry seasons. The former takes place between May and October while the later spans through November to April. The rainy season month is sub-divided into rainy peak (in September) and a short break of two weeks in August (August break). The temperature of the area ranges from 25°C to 33°C for most of the year. The transitory temperature between the seasons is the highest in the year and occurs between March and April, preceding the rainy season. The lowest temperature occurs at the peak of harmattan in January. A cyclical change in the flood regimen of the area occurs annually consequent upon the rainfall pattern (Okafor, 1990).

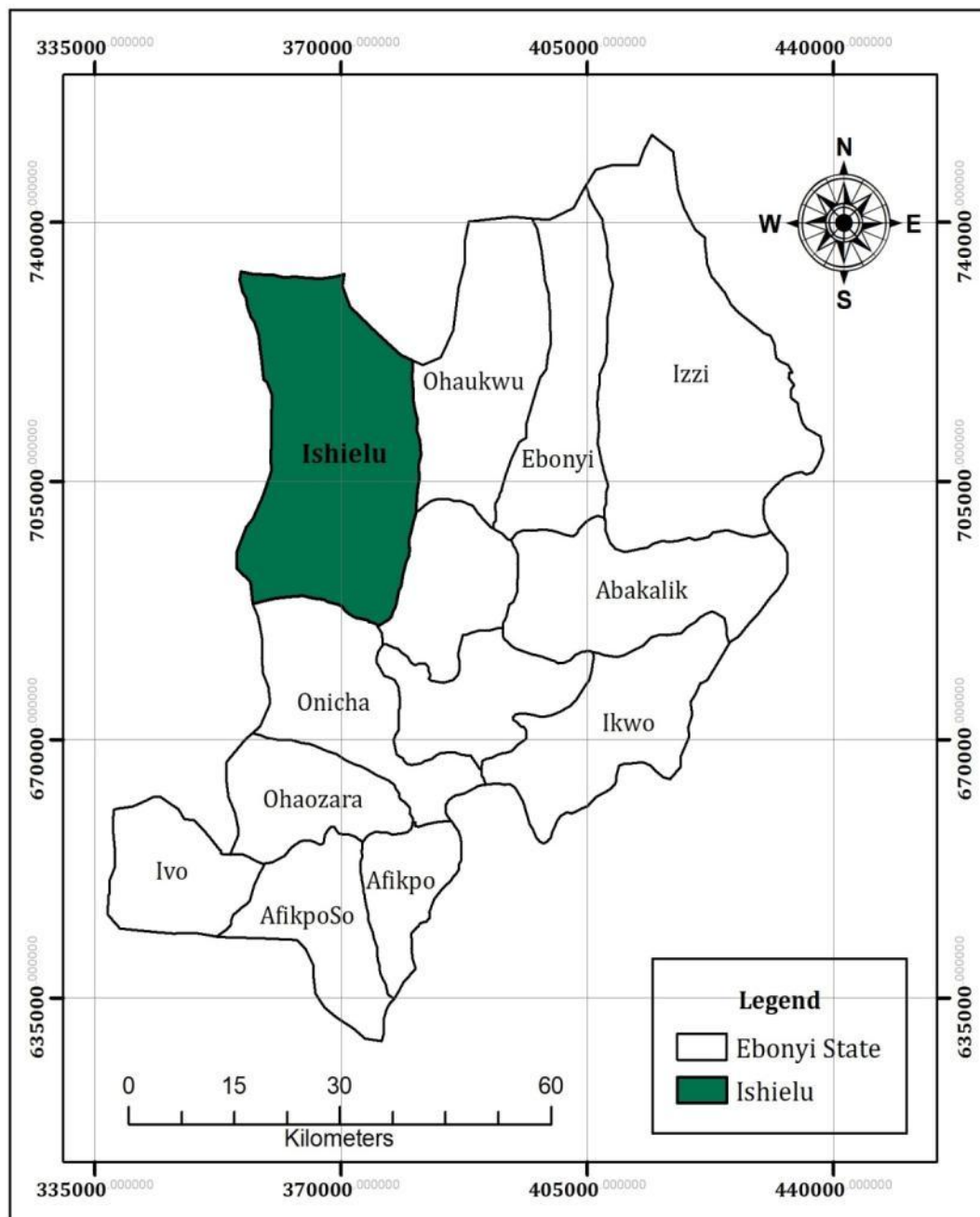


Figure 12: Map of Ebonyi State, Nigeria Showing the Study Area, Ishielu Local Government Area

Source: GIS Surveying and Geoinformatics, NAU, Awka, 2018.

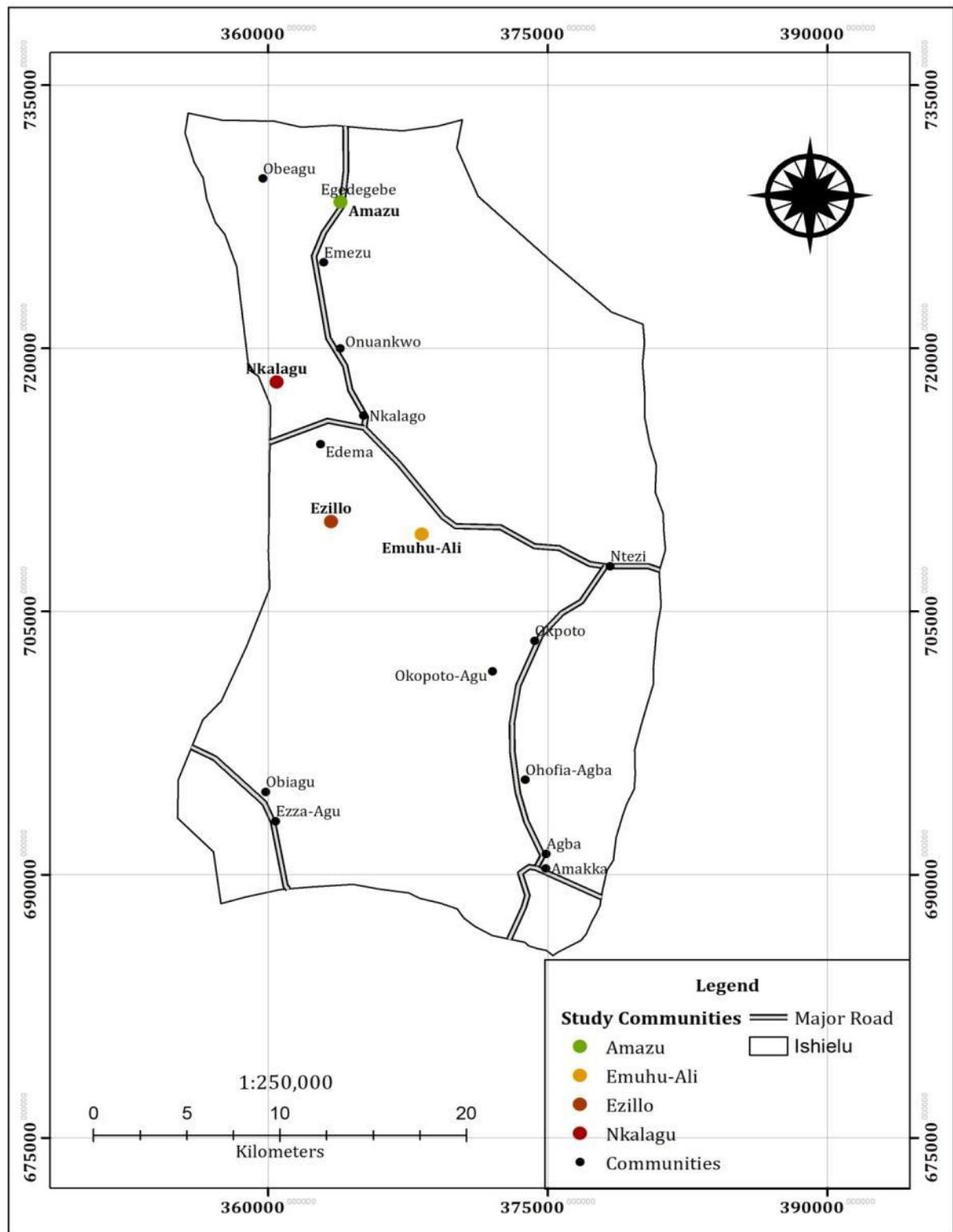


Figure 13: Map of Ishielu Local Government Area Showing the Study Communities

Source: GIS Surveying and Geoinformatics, NAU, Awka, 2018.

The vegetation of Ishielu Local Government Area is typically guinea savannah. The local government has an area of 872km² and a population of 151,048 (NPC, 2006). The Majority of the inhabitants are farmers and petty traders, with few civil servants, students and other professionals. It has four market days namely Eke, Orie, Afor and Nkwo with each community having at least one major market day. The farming activities have important bearing on the ecology of the area. More grasses and shrubs grow in areas with constant human activities. The leaves of these plants often fall into the water bodies and together with the existing water microorganisms and food substances arising from human processors provide adequate food for the snails and other aquatic organisms. (Okafor, 1990).

3.2 Advocacy

A letter of introduction was collected from the Head of Department, Parasitology and Entomology, Nnamdi Azikiwe University, Awka before embarking on the work (Appendix I). This letter was taken to the leaders of the communities where the study was carried out and their permission obtained before going to the water bodies, especially as there were clashes between Ezillo and Ezza Communities at that time. The Igwes of Ezillo and Emuhu-Ali provided guards well known in the communities who led me to the water bodies in the two communities.

3.3 Snail Collection

The water bodies were investigated for snails starting from June, 2014 to May, 2015. The collection of snails was done two times in a month at two weekly intervals and was made on the arms, banks and other parts of the water bodies which are accessible to human and animal activities. Snails collected each month from each site were counted and their numbers recorded.

Using a scoop net/dip net the standard area method of collection was employed (Okafor, 1990). A 10m² of site area was usually sampled in flowing water habitats; this was made at 100m intervals against the water current. In stagnant water habitats, the intervals between stations were 20 to 50m. Snail samples were taken by making swoops through water at right angles to the bank, collecting even floating vegetation. Table I shows details of the number of stations in each habitat sampled. The snails collected using the dipnet/long scooping net were hand-picked using protective gloves and forceps. They were transferred into a moistened cloth bag. To serve as food for the collected snails to prevent possible death, aquatic vegetation on which the snails clung including decaying raffia leaves were also introduced into the bag. A visual search for snails in the surrounding was also made. During each search, attempt was made to collect all snails seen. Snails on the underside of the leaves, aquatic macrophytes, wood trunks, raffia and on decaying palm trees were collected using hand protected with gloves or forceps depending on the location of the snails. All the collected snail specimens were transferred into the moistened bag and were taken to Environmental Biology Laboratory, Ebonyi State University, for size measurement, identification and test for shedding of cercariae of *Schistosoma haematobium*.

Table 1: Details of the number of stations in each site sampled

	Name of Habitat	No. of Stations
Flowing habitats	Uzuru	12
	Mmiri Ali	28
	River Ora	20
Stagnant habitats	Quarry Pits (Ezillo)	12
	Pools behind “Over Rail” Settlement	15

3.4 Identification of Snails

Snails collected were washed with a saturated solution of oxalic acid. Foreign matters that stuck to the snails after washing were removed with a stiff paint brush. The snails were identified using a snail identification guide by Frandsen, *et al.* (1980).

3.5 Infectivity of *Bulinus* Species Collected

After each collection, the sizes of the *Bulinus* species collected were first measured. They were checked for infection by observing for cercarial shedding in test tubes. This was done by putting each *Bulinus* snail in a 10ml test tube containing 2ml of water. The test tubes containing the snails were exposed for at least 2 hours to sunlight usually between 10.00am and 12.00noon (The cercarial shedding is at its peak at this time. Usually also at this time the shed cercaria easily locates its definitive host - man, in its natural habitat - water body). Marco and Alan (2012), Appleton (1978), and Ekwunife (2008) noted that this light source stimulates the snails and initiates them to shed cercariae, if infected. During rainy season, cercaria shedding was induced in the laboratory using bright bulb. After the exposure to light, the water from the various test tubes were put on a clean slide and viewed under the microscope for the presence of cercariae. Those found to be shedding human schistosome type cercariae (bifurcate cercariae) were recorded. After 12 days of observation, snails with no cercariae emergence were crushed individually and examined under the microscope. When at least one human schistosome type cercaria was seen, the crushed snails were considered infected. The number of infected snails for each month was recorded. The prevalence of infection in *Bulinus* (*Ph.*) *globosus* with *S. haematobium* and the cercarial output were determined and employed to establish the effect of season on transmission patterns.

The Infectivity or Infection rate of *Bulinus* spp was calculated as shown:

$$(a) \text{ Infectivity for each Habitat} = \frac{\text{No of } \textit{Bulinus} \text{ infected for each habitat} \times 100}{\text{No of } \textit{Bulinus} \text{ collected from that habitat}} \quad 1$$

$$(b) \text{ Total infectivity for Each group of habitats} = \frac{\text{Total no of infected } \textit{Bulinus} \text{ in each group} \times 100}{\text{Total no of } \textit{Bulinus} \text{ collected in that group}} \quad 1$$

$$(c) \text{ Monthly Infectivity} = \frac{\text{No of } \textit{Bulinus} \text{ infected for each month} \times 100}{\text{No of } \textit{Bulinus} \text{ collected in that month}} \quad 1$$

(Okafor, 1990; Rudge *et al.*, 2013)

3.6 The Relative Abundance (R.A) of Snail Species

The monthly Relative Abundance (R.A) (%) was calculated thus:

Number of each snail species collected in a month (n) divided by Total number of snails collected in that month (N) expressed as a Percentage,

$$\frac{n}{N} \times \frac{100}{1}$$

The Relative Abundance (R.A) (%) of each snail species during the twelve (12) months of study was calculated as follows:

Number of each snail species collected from all habitats (n) divided

By total number of snails from all habitats (N) expressed as

Percentage,

$$\frac{n}{N} \times \frac{100}{1}$$

3.7 Determining the Monthly and Seasonal Transmission Potentials

The monthly and seasonal Transmission Potentials as developed by Chu and Dawood (1970) were adopted as shown below:

$$(a) \text{ Monthly T.P.} = \frac{\text{Monthly No of infected snails in each group of habitats}}{\text{Sum of infected snails in that group}} \times \frac{100}{1}$$

(b) Seasonal T.P. = Addition of the T.P.s of the months in a season.

No = Number

T.P. = Transmission Potential

3.8 Data Analysis

The data collected were analyzed using Statistical Programme for Social Sciences (SPSS) software, version 20.0. Also test of significance was carried out using chi-square (χ^2). A value of $P < 0.05$ was regarded as significant. Pie charts and bar charts were equally used to elucidate data.

CHAPTER FOUR

RESULTS

4.1 Identification of the Freshwater Snail Species in Ishielu Local Government Area

A total of 923 freshwater snails were collected and identified during the study periods. The snails comprised three different species, the *Lymnaea natalensis*, belonging to the family lymnaeidae, the *Bulinus globosus* belonging to the family Planorbidae and the *Bulinus truncatus* also belonging to the family planorbidae (Plates 1, 2 and 3).

4.2 Monthly Snail Species Abundance in Ishielu Local Government Area

Out of the 923 snails, *L. natalensis* was the most abundant (625(67.7%)), followed by *B. globosus* (213(23.1%)) while few *B. truncatus* were encountered (85(9.2%)). Each of the snail species was most abundant during the rain with a peak in July (*L. natalensis* 150(71.4%), *B. globosus* 45(21.4%), *B. truncatus* 15(7.1%)) whereas, during the dry season months, the snails were less with the lowest abundance during the onset of rain in April (*L. natalensis* 8(61.5%), *B. globosus* 3(23.1%) and *B. truncatus* 2(15.4%)). The monthly collections of snail species in Ishielu Local Government Area were statistically different ($P < 0.05$) (Table 2). There were no snails collected for all the species of snails in March.

Table 2: Monthly Abundance of each snail species collected from all the habitats in Ishielu Local Government Area

Month		<i>L. natalensis</i> No (%)	<i>B. globosus</i> No (%)	<i>B. truncatus</i> No (%)	TotalNo(%)
June,2014		110 (70.5)	35 (22.4)	11 (7.1)	156 (16.9)
July,	„	150 (71.4)	45 (21.4)	15 (7.1)	210 (22.6)
August,	„	72 (69.2)	25 (24.0)	7 (6.7)	104 (11.3)
September,	„	60 (77.9)	12 (15.6)	5 (6.5)	77 (8.3)
October,	„	45 (76.3)	10 (17.0)	4 (6.8)	59 (6.4)
November,	„	40 (64.5)	13 (21.0)	9 (14.5)	62 (6.7)
December,	„	31 (62.0)	11 (22.0)	8 (16.0)	50 (5.4)
January,		35 (45.5)	30 (39.0)	12 (15.6)	77 (8.3)
2015					
February,	„	19 (44.2)	17 (39.5)	7 (16.3)	43 (4.7)
March,	„	0 (0)	0 (0)	0 (0)	0 (0)
April,	„	8 (61.5)	3 (23.1)	2 (15.4)	13 (1.4)
May,	„	55 (76.4)	12 (16.7)	5 (6.9)	72 (7.8)
Total		625 (67.7)	213 (23.1)	85 (9.2)	923 (100)

(P<0.05, df=11, $\chi^2 = 2.656$)

4.3 Relative Abundance of Snail Species

Study on the Relative Abundance of each of the freshwater snail species collected from all the habitats during the one year period of study has the result represented on the pie-chart shown in Figure 14

L. natalensis with total number of 625 has the highest relative abundance of 67.7% while *B. globosus* with total number 213 and *B. truncatus* with total number 85 have relative abundances of 23.1% and 9.2% respectively. The different snail species collected were significantly different ($P < 0.05$).

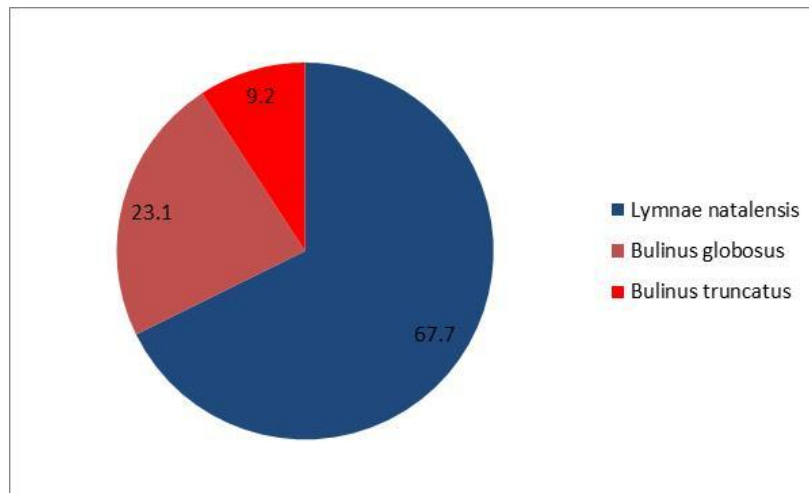


Figure 14: Relative abundance of the snail species encountered

4.4.1 Changes in the Population of *Bulinus* Species Found in Different Sampling Habitats

Observations on the total number of *Bulinus* snails collected from each sampling habitat as represented in Table 3 show that for the flowing water habitats, the highest number of *Bulinus* out of the total 136 was collected from Mmiri Ali at Nkalagu 76(55.9%), 45(33.1%)) were collected from Uzuru at Emuhu-Ali and the lowest number of snails 15(11.0%) were collected from River Ora at Amazu. For stagnant water habitats, 25(15.4%) out of the total 162 were collected from Old Quarry Pits at Ezillo while 137(84.6%) were collected from the Natural Pools behind “Over Rail” Settlement at Nkalagu. There was a significant difference in the number of *Bulinus* snails collected from the sampling habitats ($P < 0.05$).

Table 3: Total number of *Bulinus* snails collected from each sampling habitat for both groups of habitats.

Flowing water habitats(FWH)		Stagnant water habitats(SWH)	
Habitat	No. of <i>Bulinus</i> spp collected	Habitat	No. of <i>Bulinus</i> spp collected
Mmiri Ali (Nkalagu)	76 (55.9)	Quarry Pits (Ezillo)	25 (15.4)
Uzuru(Emuhu-Ali)	45 (33.1)		
River Ora (Amazu)	15 (11.0)	Natural Pools behind “Over Rail” Settlement (Nkalagu)	137 (84.6)
Total	136 (100.0)		162 (100.0)
FWH (P<0.05, df=4, $\chi^2=16.389$) SWH (P< 0.05, df=4, $\chi^2=16.389$)			

4.4.2 Seasonal Changes in the Number of *Bulinus* Species Collected from all the Habitats during the Twelve Months.

The number of each of the *Bulinus* species collected for the twelve (12) month period for flowing and stagnant water habitats is presented on Table 4. For flowing water habitats, the number of *Bulinus* snails was high in dry season from October to February. In stagnant water habitats the number collected was high during rain as from May to September, with a peak in the month of July. More *B. globosus* were collected in each season than *B. truncatus* for both groups of habitats. The monthly collections of *Bulinus* species for each group of habitats were significantly different ($P < 0.05$).

Table 4: Number of each of the *Bulinus* species collected from Each Group of habitat during the 12 months of study

	<i>Flowing water habitats</i>		<i>Stagnant water habitats</i>		
	No. of <i>B. globosus</i>	No. of <i>B. truncatus</i>	No. of <i>B. globosus</i>	No. of <i>B. truncatus</i>	
June, 2014	0	0	35	11	Wet Season
July, „	2	2	43	13	
August, „	3	2	22	5	
September, „	7	3	5	2	
October, „	8	3	2	1	Dry Season
November, „	11	8	2	1	
December, „	10	8	1	0	
January, 2015	29	11	1	1	
February, „	17	7	0	0	Wet Season
March, „	0	0	0	0	
April, „	3	2	0	0	
May, „	0	0	12	5	

FWH ($P < 0.05$, $df=8$, $\chi^2=-0.628$) SWH ($P < 0.05$, $df=8$, $\chi^2=0.682$)

4.5 Prevalence of *Schistosoma haematobium* in *Bulinus* Species Collected From the Habitats in Ishielu Local Government Area.

The Prevalence (Infectivity rate) of *S. haematobium* infections in both *Bulinus* spp collected from the sampling habitats was measured. The detailed monthly prevalence was also measured and the results are shown on Tables 5 and 6.

The data show that there was no significant difference in prevalence of *S. haematobium* infection in *Bulinus* spp in both flowing water habitats and stagnant water habitats in the study area ($P > 0.05$). Out of 136 *Bulinus* snails collected for flowing water habitat, 43 were infected (31.62%) whereas, for stagnant water habitat, 30 were infected out of the total 162 (18.51%). Prevalence rates also varied between the different sampling habitats (Table 5). The monthly prevalence of *S. haematobium* infections (Table 6) in *Bulinus* snails was not statistical difference ($P > 0.05$) and showed that for flowing water habitats, infections in *Bulinus* spp was very low during the rainy period and high during the dry period whereas for the stagnant water habitat, infections in *Bulinus* spp was low during dry period and high during rain with a peak (26.79%) in July.

Table 5: Prevalence of *S. haematobium* infections in *Bulinus* spp collected from the Sampling Habitats

Flowing water habitats(FWH)			Stagnant water habitats(SWH)		
Habitat	No. of <i>Bulinus</i> spp collected	No. infected (%)	Habitat	No. of <i>Bulinus</i> spp collected	No. infected (%)
Mmiri Ali (Nkalagu)	76	25(32.9)	Quarry Pits (Ezillo)	25	2(8.0)
Uzuru (Emuhu-Ali)	45	17(37.8)	Natural Pools behind “Over Rail” Settlement (Nkalagu)	137	28(20.4)
River Ora (Amazu)	15	1(6.7)			
Total	136	43(31.6)		162	30(18.5)

FWH ($P>0.05$, $df=2$, $\chi^2=1.266$) SWH ($P>0.05$, $df=1$, $\chi^2=-1.468$)

Table 6: Monthly prevalence of *S. haematobium* infections in *Bulinus* spp collected from Flowing and Stagnant Water Habitats

	<i>Flowing water habitats</i>			<i>Stagnant water habitats</i>		
	No. of <i>Bulinus</i> spp collected	No. infected	Infection rate (%)	No. of <i>Bulinus</i> spp Collected	No. infected	Infection rate (%)
June, 2014	0	0	0	46	9	19.6
July, 2014	4	0	0	56	15	26.8
August, 2014	5	0	0	27	4	14.8
September, 2014	10	0	0	7	1	14.3
October, 2014	11	3	27.3	3	0	0
November, 2014	19	6	31.6	3	0	0
December, 2014	18	8	44.4	1	0	0
January, 2015	40	15	37.5	2	0	0
February, 2015	24	11	45.8	0	0	0
March, 2015	0	0	0	0	0	0
April, 2015	5	0	0	0	0	0
May, 2015	0	0	0	17	1	5.9
Total	136	43	31.6	162	30	18.5

FWH ($P > 0.05$, $df=8$, $\chi^2=-2.373$) SWH ($P > 0.05$, $df=8$, $\chi^2=1.894$)

Monthly infection rate= $\frac{\text{No. of } \textit{Bulinus} \text{ infected for each month in each group}}{\text{No. of } \textit{Bulinus} \text{ collected in that month}} \times \frac{100}{1}$

4.6 Monthly and Seasonal cercarial Transmission Potentials in Flowing and Stagnant Water Habitats

The monthly transmission potentials in flowing and stagnant water habitats were calculated and the results are shown on the bar-charts on figure 15. The chart shows that the Transmission Potential varies with month. For flowing water habitats, like the infective rate, T.P increases from rainy months to dry months with a peak in core dry months of January and February (34.9% and 25.6% respectively) and decreases with the onset of rain. The months with no bar indicate zero transmission potential. In the stagnant water habitats, T.P increases from dry months to rainy months with the highest T.P occurring in July (50%). The months with no bar have zero transmission potential.

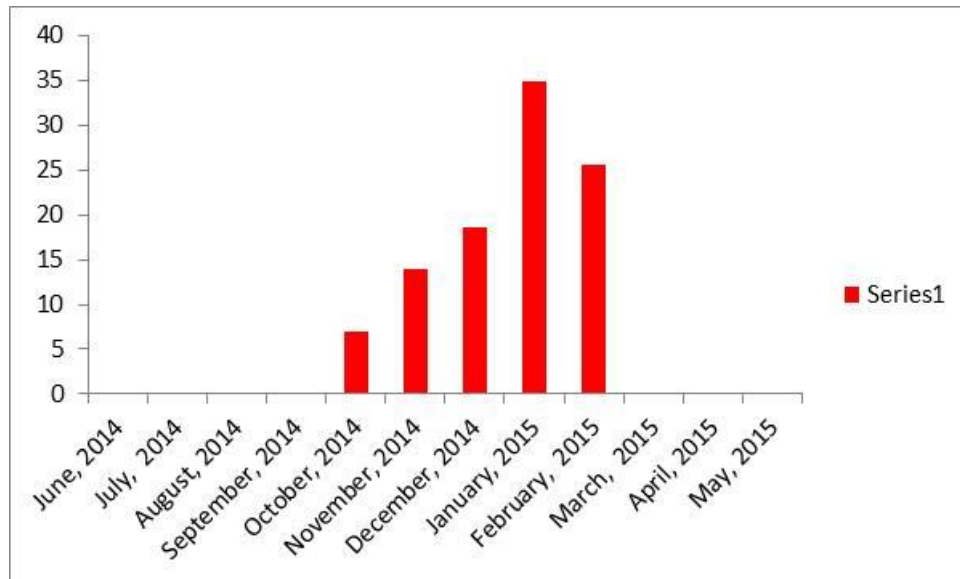


Figure 15a: Monthly transmission potential based on the number of infected *Bulinus* species per month for flowing water habitats

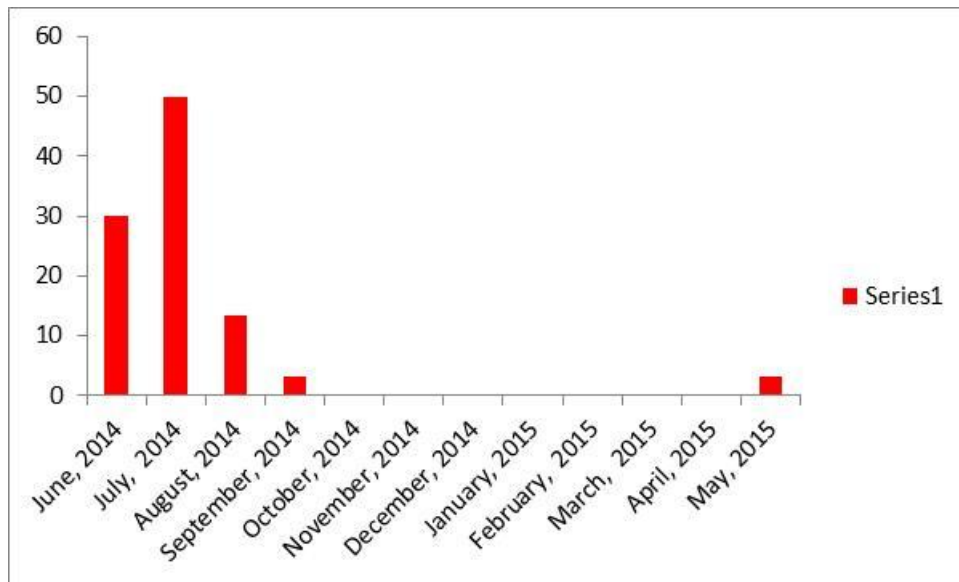


Figure 15b: Monthly transmission potential based on the number of infected *Bulinus* species per month for stagnant water habitat

Furthermore, the results of the seasonal transmission potentials in flowing and stagnant water habitats are presented in Table 7. The table shows that the T.P varied with season. It also varied with the habitat types. It is shown to be very high during the dry season (a total of 93.0%) and low in wet season (a total of 7.0%) for flowing water habitats. On the other hand, for stagnant water habitats, transmission potential becomes very low during the dry season months (a total of 0%) and high in the wet season months (a total of 100.0%).

Table 7: Seasonal transmission potential (T.P) based on the number of infected *Bulinus* spp collected from all habitats.

Month	Flowing water habitats					Stagnant water habitats				
	No. of <i>Bulinus</i> spp collected	No. infected	Infection rate (%)	Monthly T.P (%)	Seasonal T.P (%)	No. of <i>Bulinus</i> spp collected	No. infected	Infection rate (%)	Monthly T.P (%)	Seasonal T.P (%)
June, 2014	0	0	0	0	Wet season 7.0	46	9	19.6	30	Wet season 97.0
July, 2014	4	0	0	0		56	15	26.8	50	
August, 2014	5	0	0	0		27	4	14.8	13.3	
September, 2014	10	0	0	0		7	1	14.3	3.3	
October, 2014	11	3	27.3	7.0	Dry season 93.0	3	0	0	0	Dry season 0
November, 2014	19	6	31.6	14.0		3	0	0	0	
December, 2014	18	8	44.4	18.6		1	0	0	0	
January, 2015	40	15	37.5	34.9		2	0	0	0	
February, 2015	24	11	45.8	25.6	Wet season 0	0	0	0	0	Wet season 3.0
March, 2015	0	0	0	0		0	0	0	0	
April, 2015	5	0	0	0		0	0	0	0	
May, 2015	0	0	0	0		17	1	5.9	3.3	
Total	136	43	31.6			162	30	18.5		

CHAPTER FIVE

DISCUSSION

The presence of three freshwater snails (*Lymnaea natalensis*, *Bulinus globosus* and *Bulinus truncatus*) suggests that some pools and rivers in Ishielu Local Government Area are suitable habitat for these snails, hence supporting the development and transmission of their respective trematode parasites. This finding is similar to the report of Emejulu *et al.* (1992) who studied the gastropod fauna of Agulu Lake and the surrounding water bodies and discovered the presence of the three freshwater snails and three other genera of gastropod. Umechukwu (2009) in a study at the same Agulu Lake reported the presence of three genera of freshwater intermediate hosts namely *Lymnaea natalensis*, *Bulinus truncatus* and *Bulinus globosus* which is very similar to the findings of this work. Ibikounle *et al.* (2009) in a similar study in Republic of Benin (West Africa) found four species of human schistosome transmitting snails: *Bulinus forskalii*, *Bulinus globosus*, *Bulinus truncatus* and *Biomphalaria pfeifferi* and seven species of non-human schistosome transmitting snails.

Studies on the relative abundance of the three snail species show that *Lymnaea natalensis*, the snail intermediate host for the causative agents of fascioliasis in West Africa namely, *Fasciola hepatica* and *Fasciola gigantica*, has the highest relative abundance, followed by *Bulinus globosus* and then *B. truncatus*, the last two snails being the intermediate hosts for *Schistosoma haematobium*, causative organism for urinary schistosomiasis in West Africa (Nnochiri, 1975). This relative abundance is comparable to that obtained by Umechukwu (2009) in which *Lymnaea natalensis* has relative abundance of 50% followed by *B. truncatus* (33.3%) and *B. globosus* (16.7%). One notices that the relative abundances of *L. natalensis* in this study and in Umechukwu's were higher than that of the *Bulinus*

species in both studies. But for the two *Bulinus* species, the findings of Umechukwu were the reverse of the findings of this work. This can be attributed to the environmental factors like increased sunshine, less rain, rocky terrain and hills, guinea savannah vegetation, slightly higher temperature in Ishielu than in Agulu which may have favoured *B. globosus* in the area than *B. truncatus* in terms of number.

More *Bulinus* snails were found at Mmiri Ali in Nkalagu than were found in Emuhu-Ali and Amazu for flowing water habitats whereas, for stagnant water habitats, more snails were found at Natural Pools behind Over Rail Settlement in Nkalagu than where found in Ezillo (table 3). This conforms to the findings of Okafor (1990) where Nkalagu produced greater number of *Bulinus* snails than every other community studied. This can be explained in the light of the nature of the surroundings and the banks of the water habitats at Nkalagu which have more vegetative cover (detritus, rotten leaves and raffia) which serves as food for the snails and also helps to maintain optimum temperature.

The findings of this study showed that some of the *Bulinus* snails were infected by *Schistosoma haematobium*. The presence of *Bulinus* species and its role as the snail host of *Schistosoma haematobium* in Nigeria has been previously recorded (Okafor, 1984; Okafor, 1989; Okafor, 1990; Emejulu *et al.*, 1992; Akogun and Obadiah, 1996; Obianika, 1998; Ezeugwu and Mafe, 1998; Idris and Ajanusi, 2001; Ekwunife, 2008; Ekwunife *et al.*, 2004; Ekwunife *et al.*, 2008; Ekwunife *et al.*, 2009; Umechukwu, 2009; Ikpeze *et al.*, 2012; Ukoji, 2017; Eze, 2017).

The dynamics of cercarial transmission is very likely to be influenced by the infection rates of snails in individual habitats. This in turn, would affect the

epidemiology of schistosomiasis in a geographical area (Okafor, 1990). Thus, seasonal population changes in snail hosts and their larval schistosome parasites are of great importance in the study of the epidemiology of the infection. The pattern and level of cercarial production in snails, in addition to factors such as the susceptibility of the snails to miracidial infections, and size structure of the snails, play important roles in the dynamics of schistosome transmission (Okafor, 1990). In some of the studies on this subject, human infections by this schistosome species depends on the density of cercariae at the transmission sites (Tayo *et al.*, 1980). The temporal and spatial density of cercariae depends on their emergence, dispersal and their viability. Climatic changes which affect the biology of both the snail hosts and cercariae of schistosomes also influence the cercarial transmission dynamics (Okafor, 1990).

In this study, the infectivity rates of the *Bulinus* species were generally low for stagnant water habitat as only very few snails were infected (table 5). This is also similar to studies made in stagnant waters by Obianika (1998), Emejulu *et al.*, (1992), Umechukwu (2009) and Okafor (1990) who found infectivity rate of *Bulinus* species to be generally low with *B. truncatus* (1.9%), *B. globosus* (1.8%) (Obianika, 1998); *B. truncatus* (1.9%), *B. globosus* (1.8%) (Emejulu *et al.*, 1992); *B. truncatus* (3%), *B. globosus* (4%) (Umechukwu, 2009); *B. globosus* (9.91%) (Okafor, 1990). However, the infectivity rate obtained in this work is noticeably higher than the ones cited above. The difference can be attributed to low intervention schemes. Public health enlightenment campaign and control of the vector snails are at low ebbs unlike at places like Agulu where a lot of progress on enlightenments has been made so far.

Furthermore, settlements are predominantly rural, with few semi-urban settlements around governmental projects. Many rural dwellers still visit pools and streams for various activities and the possibility of defecating in and around the water bodies cannot be ruled out. Ecological factors such as irrigation and construction of dams which are evident in the area may also have contributed.

Analysis of the size distribution of all *Bulinus* species infected by *Schistosoma haematobium* collected from the study areas suggests that the size of the snail affects its infection by the parasite as the trend is such that greater numbers of larger snails were infected. This finding lends credence to the report of Okafor (1990) that snail size affects its infection. The larger size of some snails than others found in this study can be explained in the light of the site in which the snails were found. Snails found at water banks or edges with more vegetation especially with more detritus, rotten leaves and raffia were large in size whereas those found in less vegetation areas were diminished in size suggesting that the large snails fed better and enjoyed more optimum temperature than the diminished snails. The direct proportionality of infection in relation to size can be explained as thus: *Schistosoma haematobium* may have preferred and thrived more in large snails where penetration of the miracidium is more likely to be successful and encystations, formation of sporocysts and cercariae are more efficient.

Infection rate and prevalence of both *Bulinus* species tend to fluctuate during the months under study for both habitats. The highest prevalence of *Bulinus* and infection rates were observed to coincide with the dry season for the flowing water habitats with a peak prevalence of snail in January and a peak infection rate in February (Table 6). This increase in abundance of snail species during the hot season can be attributed to the more stability of the aquatic habitat in terms of water level and velocity. Incidentally, during the hot weather there was an increase

in human-water contact activities in all the sites studied in Ishielu Local Government Area as most people had used up their stored-up domestic water and needed the water bodies to continue to carry out their tasks. There was need to cool the body equally especially in the afternoon and evening time. A similar observation was made by Idris and Ajanusi (2002) when they stated that the highest infection rates were observed to coincide with the dry season although towards the onset of rainy season when the temperature range (26.0°C to 32.8°C) lied within the optimum limits for snail growth and development. Ezeugwu and Mafe (1998) made a very similar finding and attributed the increase in abundance of snail species during the hot season to the stability of the aquatic habitat in terms of water level and velocity. For the stagnant water habitats, the highest prevalence of *Bulinus* and infection rates coincided with the rainy season with a peak in July whereas, during the dry season, both prevalence of snail and infection by the *S. haematobium* were low. This is in line with the observations of Okafor (1990) that the number of snails and infection rates were high in flowing water habitats from October to February and for stagnant water habitats they were high from May to October with a pick in July. Barsoum (2013) related the seasonal density fluctuation of the intermediate host population which accompanied *Schistosoma* infection rate to rainfall and habitat volume. The observation was found to be similar to the findings of this study for stagnant water habitats and the findings of Akinboye *et al.* (2011) as the least number of snails were found in the period between February and April, when there was little or no rainfall.

The significance difference in prevalence of infection in *Bulinus* species between flowing water habitats and stagnant water habitats observed in this work reinforces the report of Okafor (1990) who stated that there was a significant difference in prevalence of *S. haematobium* infection in *B. (Ph.) globosus* between flowing

water habitats and stagnant water habitats in the area studied. The monthly transmission potential (T.P) of flowing water habitats was high during the dry season months with its highest occurring in January. However, the monthly transmission potential of stagnant water habitats was high in rainy season with its highest in July. Also, seasonal transmission potential in flowing water habitats was very high during the dry season whereas in stagnant water habitats, it was low. During the rainy season the transmission potential was low in flowing water habitats and high in stagnant water habitats. This observation and calculations made during this work agree almost completely with the findings of Okafor (1990) who obtained the highest monthly transmission potential of flowing water habitats in January and that of the stagnant water habitats in July. It also shows seasonal transmission potential in flowing water habitats to be very high during the dry season and low in rainy season and in stagnant water habitats, seasonal transmission potential was high during rain and low in dry season. Meaning that, the ability of water to flow or otherwise its stagnancy, among other factors, taking into consideration the season in question, determine Schistosome parasite transmission in freshwater habitats.

CONCLUSION

Identified freshwater snails in Ishielu L.G.A are *Lymnaea natalensis*, *Bulinus globosus* and *Bulinus truncatus* of which some of the *Bulinus* are infected with *schistosoma haematobium*. Both season and habitat type have clear influence on the abundance of the snails, prevalence and transmission of cercariae, a knowledge which can help in the study of the epidemiology of the schistosomiasis infection in the study area, because they demonstrate when and how the human populations

can be at the greatest risk of infections, a great tool for planning snail control programmes so that the scarce resources can be optimized and massive results achieved.

RECOMMENDATION

- 1.** Government authorities should establish a programme for Mass Chemotherapy of residents in the study area.
- 2.** Health awareness programme especially on the role of the snails as vectors in the transmission of the disease should be conducted at school and community gathering by health educators.
- 3.** Identification of freshwater bodies in Ishielu L.G.A as flowing waters or stagnant waters should be done first before any control measures targeted at the snail intermediate hosts can be embarked upon, as this could help in knowing appropriate period(s) of the year, for each habitat, when control of snail could be very effective and the scarce resources optimized.
- 4.** Residents of Ishielu communities should be educated on periodic removal of aquatic vegetation like detritus, rotten leaves and raffia from the banks or edges of the water bodies to discourage the snails from feeding well and enjoying optimum temperature, thereby diminishing them since snails found in these areas of high vegetation were large with high infection rate.

5. Some careful work should also be done to ascertain, reliably, why flowing waters support high prevalence of snail vector and high infection rate during the dry season whereas stagnant waters encourage high prevalence of snail and high infection rate during rain.

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PLATES



Plate 1: *Bulinus globosus*
(With less-developed spire)



Plate 2: *Bulinus globosus*
(Aperture view)



Plate 5: *Lymnaea natalensis*



Plate 3: *Bulinus truncatus*
(With well-developed spire)



Plate 4: *Bulinus truncatus*
(Aperture view)



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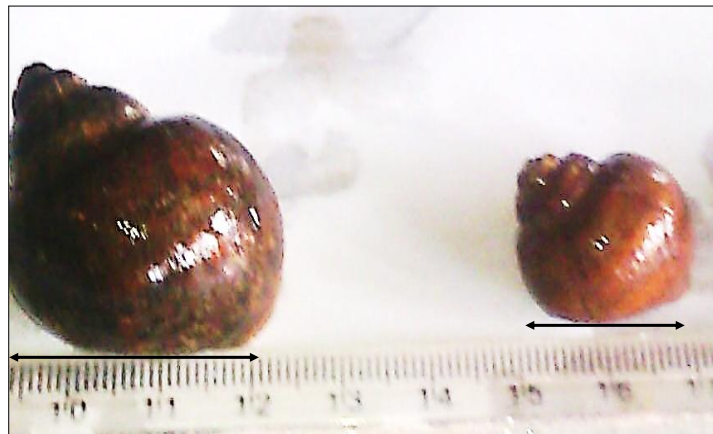


Plate 6: Size and Aperture Measurements (mm) of *Bulinus*



Plate 7: Vegetation around Mmiri Ali



Plate 8: Snail Collection at the Vegetation around Mmiri Ali



Plate 9: Snail Collection at the Vegetation around Uzuru River



Plate 10: Shallow Part River Ora

APPENDIX II

Monthly Abundance of Each Snail Species Collected from all the Habitats in Ishielu Local Government Area

Count

		snail species caught			Total
		L. natalensis	B. globosus	B. truncatus	
monthly collection of snail species	June,2014	110	35	11	156
	July	150	45	15	210
	August	72	25	7	104
	Sempt	60	12	5	77
	October	45	10	4	59
	November	40	13	9	62
	December	31	11	8	50
	January 2015	35	30	12	77
	Febuary	19	17	7	43
	April	8	3	2	13
	May	55	12	5	72
	Total	625	213	85	923

Chi-Square Tests

				Monte Carlo Sig. (2-sided)			Monte Carlo Sig. (1-sided)		
				Sig.	95% Confidence Interval		Sig.	95% Confidence Interval	
	Value	df	Asymp. Sig. (2-sided)		Lower Bound	Upper Bound		Lower Bound	Upper Bound
Pearson Chi-Square	45.389 ^a	20	.001	.002 ^b	.000	.005			
Likelihood Ratio	43.166	20	.002	.003 ^b	.000	.007			
Fisher's Exact Test	43.775			.002 ^b	.000	.005			
Linear-by-Linear Association	7.054 ^c	1	.008	.010 ^b	.003	.016	.007 ^b	.001	.012
N of Valid Cases	923								

a. 4 cells (12.1%) have expected count less than 5. The minimum expected count is 1.20.

b. Based on 923 sampled tables with starting seed 624387341.

c. The standardized statistic is 2.656.

Analysis of variance for species of snails caught

Variate: No_of_snails_caught

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Snail_species	2	13270.2	6635.1	9.83	<.001
Residual	33	22264.1	674.7		
Total	35	35534.3			

Tables of means

Variate: No_of_snails_caught

Grand mean 25.6

Snail_species	B. globosus	B. truncatus	L. natalensis
	17.8	7.1	52.1

Least significant differences of means (5% level)

l.s.d. 21.57

Analysis of variance for the months of snail collection

Variate: no_of_snails_caught

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
month	11	12381.0	1125.5	1.17	0.359
Residual	24	23153.3	964.7		
Total	35	35534.3			

Tables of means

Variate: no_of_snails_caught

Grand mean 25.6

month	February 14.3	March 0.0	April 4.3	May 24.0	july 70.0
month	August 34.7	September 25.7	October 19.7	November 20.7	December 16.7
month	January--2015 25.7	June--2014 52.0			

APPENDIX III

Total Number of *Bulinus* Snails Collected from Each Sampling Habitat For both Groups of Habitats

Count

	Bulinus spp					Total
	Mmiri Ali (Nkalagu)	Uzuru (Emuhu-Ali)	River Ora (Amazu)	Quarry pits (Ezillo)	Natural pools behind Over rail settlement (Nkalagu)	
water habitat flowing water	76	45	15	0	0	136
stagnant water	0	0	0	25	137	162
Total	76	45	15	25	137	298

Chi-Square Tests

				Monte Carlo Sig. (2-sided)			Monte Carlo Sig. (1-sided)		
					95% Confidence Interval			95% Confidence Interval	
	Value	df	Asymp. Sig. (2-sided)	Sig.	Lower Bound	Upper Bound	Sig.	Lower Bound	Upper Bound
Pearson Chi-Square	298.000 ^a	4	.000	.000 ^b	.000	.010			
Likelihood Ratio	410.844	4	.000	.000 ^b	.000	.010			
Fisher's Exact Test	389.639			.000 ^b	.000	.010			
Linear-by-Linear Association	268.615 ^c	1	.000	.000 ^b	.000	.010	.000 ^b	.000	.010
N of Valid Cases	298								

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 6.85.

b. Based on 298 sampled tables with starting seed 2000000.

c. The standardized statistic is 16.389.

APPENDIX IV

Number of each of *Bulinus* Species Collected from each Group of Habitat during the 12 Months of Study

A. Flowing Water Habitats

Count

		Species		Total
		B. globosus	B. truncates	
month of collection	July 2014	2	2	4
	August 2014	3	2	5
	September 2014	7	3	10
	October 2014	8	3	11
	November 2014	11	8	19
	December 2014	10	8	18
	January 2015	29	11	40
	Feb 2015	17	7	24
	April 2015	3	2	5
Total		90	46	136

Chi-Square Tests

				Monte Carlo Sig. (2-sided)		Monte Carlo Sig. (1-sided)			
				Sig.	95% Confidence Interval		Sig.	95% Confidence Interval	
	Value	df	Asymp. Sig. (2-sided)		Lower Bound	Upper Bound		Lower Bound	Upper Bound
Pearson Chi-Square	3.351 ^a	8	.910	.912 ^b	.864	.959			
Likelihood Ratio	3.305	8	.914	.926 ^b	.883	.970			
Fisher's Exact Test	3.909			.875 ^b	.819	.931			
Linear-by-Linear Association	.394 ^c	1	.530	.500 ^b	.416	.584	.250 ^b	.177	.323
N of Valid Cases	136								

a. 8 cells (44.4%) have expected count less than 5. The minimum expected count is 1.35.

b. Based on 136 sampled tables with starting seed 624387341.

c. The standardized statistic is -.628.

B. Stagnant Water Habitats

Count

		Stagnantwater		Total
		B. globosus	B. truncates	
month of collection	June, 2014	35	11	46
	July	43	13	56
	August	22	5	27
	September	5	2	7
	October	2	1	3
	November	2	1	3
	December	1	0	1
	January 2015	1	1	2
	May 2015	12	5	17
Total		123	39	162

Chi-Square Tests

				Monte Carlo Sig. (2-sided)		Monte Carlo Sig. (1-sided)			
				Sig.	95% Confidence Interval		Sig.	95% Confidence Interval	
	Value	df	Asymp. Sig. (2-sided)		Lower Bound	Upper Bound		Lower Bound	Upper Bound
Pearson Chi-Square	2.156 ^a	8	.976	.985 ^b	.965	1.000			
Likelihood Ratio	2.273	8	.971	.985 ^b	.965	1.000			
Fisher's Exact Test	3.665			.926 ^b	.883	.970			
Linear-by-Linear Association	.465 ^c	1	.495	.456 ^b	.372	.540	.213 ^b	.144	.282
N of Valid Cases	162								

a. 10 cells (55.6%) have expected count less than 5. The minimum expected count is .24.

b. Based on 136 sampled tables with starting seed 957002199.

c. The standardized statistic is .682.

APPENDIX V

Prevalence of *Schistosoma Haematobium* infections in *Bulinus* Species Collected from the Sampling Habitats

A. Flowing Water Habitats

Count

		Bulinus spp infected		Total
		positive	Negative	
flowing water habitat	Mmiri Ali (Nkalagu)	25	51	76
	Uzuru (Emuhu-Ali)	17	28	45
	River Ora (Amazu)	1	14	15
Total		43	93	136

Chi-Square Tests

				Monte Carlo Sig. (2-sided)			Monte Carlo Sig. (1-sided)		
				95% Confidence Interval			95% Confidence Interval		
	Value	df	Asymp. Sig. (2-sided)	Sig.	Lower Bound	Upper Bound	Sig.	Lower Bound	Upper Bound
Pearson Chi-Square	5.166 ^a	2	.076	.088 ^b	.041	.136			
Likelihood Ratio	6.419	2	.040	.074 ^b	.030	.117			
Fisher's Exact Test	5.456			.074 ^b	.030	.117			
Linear-by-Linear Association	1.603 ^c	1	.206	.213 ^b	.144	.282	.103 ^b	.052	.154
N of Valid Cases	136								

a. 1 cells (16.7%) have expected count less than 5. The minimum expected count is 4.74.

b. Based on 136 sampled tables with starting seed 92208573.

c. The standardized statistic is 1.266.

B. Stagnant Water Habitats

Count

		Bulinus spp infected		Total
		positive	Negative	
stagnant water habitat	Quarry pits (Ezillo)	2	23	25
	Natural pools behind Over rail settlement (Nkalagu)	28	109	137
Total		30	132	162

Chi-Square Tests^d

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	2.168 ^a	1	.141	.171	.112	
Continuity Correction ^b	1.422	1	.233			
Likelihood Ratio	2.554	1	.110	.171	.112	
Fisher's Exact Test				.171	.112	
Linear-by-Linear Association	2.154 ^c	1	.142	.171	.112	.082
N of Valid Cases	162					

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 4.63.

b. Computed only for a 2x2 table

c. The standardized statistic is -1.468.

d. For 2x2 crosstabulation, exact results are provided instead of Monte Carlo results.

APPENDIX VI

Monthly Prevalence of *Schistosoma Haematobium* Infections in *Bulinus* Spp Collected from Flowing and Stagnant Water Habitats

A. Flowing Water Habitats

Count

	Bulinus spp infected		Total
	Positive	Negative	
monthly collection of snails from July, 2014	0	4	4
flowing water August, 2014	0	5	5
September, 2014	0	10	10
October, 2014	3	8	11
November, 2014	6	13	19
December, 2014	8	10	18
January, 2015	15	25	40
February, 2015	11	13	24
April, 2015	0	5	5
Total	43	93	136

Chi-Square Tests

				Monte Carlo Sig. (2-sided)		Monte Carlo Sig. (1-sided)			
				Sig.	95% Confidence Interval		Sig.	95% Confidence Interval	
	Value	df	Asymp. Sig. (2-sided)		Lower Bound	Upper Bound		Lower Bound	Upper Bound
Pearson Chi-Square	15.446 ^a	8	.051	.015 ^b	.000	.035			
Likelihood Ratio	22.366	8	.004	.000 ^b	.000	.022			
Fisher's Exact Test	14.649			.022 ^b	.000	.047			
Linear-by-Linear Association	5.632 ^c	1	.018	.007 ^b	.000	.022	.000 ^b	.000	.022
N of Valid Cases	136								

a. 8 cells (44.4%) have expected count less than 5. The minimum expected count is 1.26.

b. Based on 136 sampled tables with starting seed 1993510611.

c. The standardized statistic is -2.373.

B. Stagnant Water Habitats

Count

		Bulinus spp infected		Total
		Positive	Negative	
monthly collection of snails from stagnant water	June, 2014	9	37	46
	July, 2014	15	41	56
	August, 2014	4	23	27
	September, 2014	1	6	7
	October, 2014	0	3	3
	November, 2014	0	3	3
	December, 2014	0	1	1
	January, 2015	0	2	2
	May, 2015	1	16	17
Total		30	132	162

Chi-Square Tests

				Monte Carlo Sig. (2-sided)			Monte Carlo Sig. (1-sided)		
				Sig.	95% Confidence Interval		Sig.	95% Confidence Interval	
	Value	df	Asymp. Sig. (2-sided)		Lower Bound	Upper Bound		Lower Bound	Upper Bound
Pearson Chi-Square	6.743 ^a	8	.565	.566 ^b	.483	.649			
Likelihood Ratio	8.688	8	.369	.426 ^b	.343	.510			
Fisher's Exact Test	5.322			.662 ^b	.582	.741			
Linear-by-Linear Association	3.587 ^c	1	.058	.081 ^b	.035	.127	.044 ^b	.010	.079
N of Valid Cases	162								

a. 10 cells (55.6%) have expected count less than 5. The minimum expected count is .19.

b. Based on 136 sampled tables with starting seed 475497203.

c. The standardized statistic is 1.894.

APPENDIX II

Monthly collection of snail species * snail species caught Cross tabulation

Count

		Snail species caught			Total
		<i>L. natalensis</i>	<i>B. globosus</i>	<i>B. truncatus</i>	
Monthly collection of snail Species	June,2014	110	35	11	156
	July	150	45	15	210
	August	72	25	7	104
	September	60	12	5	77
	October	45	10	4	59
	November	40	13	9	62
	December	31	11	8	50
	January 2015	35	30	12	77
	February	19	17	7	43
	April	8	3	2	13
	May	55	12	5	72
Total		625	213	85	923

Chi-Square Tests

				Monte Carlo Sig. (2-sided)			Monte Carlo Sig. (1-sided)		
					95% Confidence Interval			95% Confidence Interval	
	Value	df	Asymp. Sig. (2-sided)	Sig.	Lower Bound	Upper Bound	Sig.	Lower Bound	Upper Bound
Pearson Chi-Square	45.389 ^a	20	.001	.002 ^b	.000	.005			
Likelihood Ratio	43.166	20	.002	.003 ^b	.000	.007			
Fisher's Exact Test	43.775			.002 ^b	.000	.005			
Linear-by-Linear Association	7.054 ^c	1	.008	.010 ^b	.003	.016	.007 ^b	.001	.012
No of Valid Cases	923								

a. 4 cells (12.1%) have expected count less than 5. The minimum expected count is 1.20.

b. Based on 923 sampled tables with starting seed 624387341.

c. The standardized statistic is 2.656.

APPENDIX III

Analysis of variance for species of snails caught

Variate: No_of_snails_caught

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Snail_species	2	13270.2	6635.1	9.83	<.001
Residual	33	22264.1	674.7		
Total	35	35534.3			

Tables of Means

Variate: No_of_snails_caught

Grand mean 25.6

Snail_species	<i>B. globosus</i>	<i>B. truncatus</i>	<i>L. natalensis</i>
	17.8	7.1	52.1

Least significant differences of means (5% level)

l.s.d. 21.57

APPENDIX IV

Analysis of variance for the months of snail collection

Variate: No_of_snails_caught

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
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Month	11	12381.0	1125.5	1.17	0.359
Residual	24	23153.3	964.7		
Total	35	35534.3			

Tables of means

Variate: no_of_snails_caught

Grand mean 25.6

Month	February	March	April	May	July
	14.3	0.0	4.3	24.0	70.0
Month	August	September	October	November	December
	34.7	25.7	19.7	20.7	16.7
Month	January—2015	June--2014			
	25.7	52.0			

APPENDIX V

Water habitat * *Bulinus* spp Crosstabulation

Count

	<i>Bulinus</i> spp					Total
	Mmiri Ali (Nkalagu)	Uzuru (Emuhu- Ali)	River Ora (Amazu)	Quarry pits (Ezillo)	Natural pools behind Over rail settlement (Nkalagu)	

Water habitat	Flowing water	76	45	15	0	0	136
	stagnant water	0	0	0	25	137	162
Total		76	45	15	25	137	298

Chi-Square Tests

				Monte Carlo Sig. (2-sided)			Monte Carlo Sig. (1-sided)		
				Sig.	95% Confidence Interval		Sig.	95% Confidence Interval	
	Value	df	Asymp. Sig. (2-sided)		Lower Bound	Upper Bound		Lower Bound	Upper Bound
Pearson Chi-Square	298.000 ^a	4	.000	.000 ^b	.000	.010			
Likelihood Ratio	410.844	4	.000	.000 ^b	.000	.010			
Fisher's Exact Test	389.639			.000 ^b	.000	.010			
Linear-by-Linear Association	268.615 ^c	1	.000	.000 ^b	.000	.010	.000 ^b	.000	.010
No of Valid Cases	298								

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 6.85.

b. Based on 298 sampled tables with starting seed 2000000.

c. The standardized statistic is 16.389.

APPENDIX VI

Monthly collection of snail species for flowing water * species Crosstabulation

Count

		species		Total
		<i>B. globosus</i>	<i>B. truncatus</i>	
Month of collection	July 2014	2	2	4
	August 2014	3	2	5
	September 2014	7	3	10
	October 2014	8	3	11

	November 2014	11	8	19
	December 2014	10	8	18
	January 2015	29	11	40
	Feb 2015	17	7	24
	April 2015	3	2	5
Total		90	46	136

Chi-Square Tests

				Monte Carlo Sig. (2-sided)			Monte Carlo Sig. (1-sided)		
				Sig.	95% Confidence Interval		Sig.	95% Confidence Interval	
	Value	df	Asymp. Sig. (2-sided)		Lower Bound	Upper Bound		Lower Bound	Upper Bound
Pearson Chi-Square	3.351 ^a	8	.910	.912 ^b	.864	.959			
Likelihood Ratio	3.305	8	.914	.926 ^b	.883	.970			
Fisher's Exact Test	3.909			.875 ^b	.819	.931			
Linear-by-Linear Association	.394 ^c	1	.530	.500 ^b	.416	.584	.250 ^b	.177	.323
No of Valid Cases	136								

a. 8 cells (44.4%) have expected count less than 5. The minimum expected count is 1.35.

b. Based on 136 sampled tables with starting seed 624387341.

c. The standardized statistic is -.628.

APPENDIX VII

Monthly collection for stagnant water * Crosstabulation

Count

	Stagnant water		Total
	<i>B. globosus</i>	<i>B. truncatus</i>	
Month of collection June, 2014	35	11	46
July	43	13	56
August	22	5	27
September	5	2	7
October	2	1	3
November	2	1	3
December	1	0	1

	January 2015	1	1	2
	May 2015	12	5	17
Total		123	39	162

Chi-Square Tests

				Monte Carlo Sig. (2-sided)			Monte Carlo Sig. (1-sided)		
				Sig.	95% Confidence Interval		Sig.	95% Confidence Interval	
	Value	df	Asymp. Sig. (2-sided)		Lower Bound	Upper Bound		Lower Bound	Upper Bound
Pearson Chi-Square	2.156 ^a	8	.976	.985 ^b	.965	1.000			
Likelihood Ratio	2.273	8	.971	.985 ^b	.965	1.000			
Fisher's Exact Test	3.665			.926 ^b	.883	.970			
Linear-by-Linear Association	.465 ^c	1	.495	.456 ^b	.372	.540	.213 ^b	.144	.282
No of Valid Cases	162								

a. 10 cells (55.6%) have expected count less than 5. The minimum expected count is .24.

b. Based on 136 sampled tables with starting seed 957002199.

c. The standardized statistic is .682.

APPENDIX VIII

Flowing water habitat * *Bulinus* spp infected Crosstabulation

Count

	<i>Bulinus</i> spp infected		Total
	positive	Negative	
Flowing water habitat Mmiri Ali (Nkalagu)	25	51	76
Uzuru (Emuhu-Ali)	17	28	45
River Ora (Amazu)	1	14	15
Total	43	93	136

Chi-Square Tests

				Monte Carlo Sig. (2-sided)			Monte Carlo Sig. (1-sided)		
				Sig.	95% Confidence Interval		Sig.	95% Confidence Interval	
	Value	df	Asymp. Sig. (2-sided)		Lower Bound	Upper Bound		Lower Bound	Upper Bound

	Value	df	Asymp. Sig. (2-sided)	Sig.	Lower Bound	Upper Bound	Sig.	Lower Bound	Upper Bound
Pearson Chi-Square	5.166 ^a	2	.076	.088 ^b	.041	.136			
Likelihood Ratio	6.419	2	.040	.074 ^b	.030	.117			
Fisher's Exact Test	5.456			.074 ^b	.030	.117			
Linear-by-Linear Association	1.603 ^c	1	.206	.213 ^b	.144	.282	.103 ^b	.052	.154
No of Valid Cases	136								

a. 1 cells (16.7%) have expected count less than 5. The minimum expected count is 4.74.

b. Based on 136 sampled tables with starting seed 92208573.

c. The standardized statistic is 1.266.

APPENDIX IX

Stagnant water habitat * *Bulinus* spp infected Crosstabulation

Count

	<i>Bulinus</i> spp infected		Total
	positive	Negative	
stagnant water habitat Quarry pits (Ezillo)	2	23	25
Natural pools behind Over rail settlement (Nkalagu)	28	109	137
Total	30	132	162

Chi-Square Tests^d

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	2.168 ^a	1	.141	.171	.112	
Continuity Correction	1.422	1	.233			
Likelihood Ratio	2.554	1	.110	.171	.112	
Fisher's Exact Test				.171	.112	

Linear-by-Linear Association	2.154 ^c	1	.142	.171	.112	.082
No of Valid Cases	162					

- a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 4.63.
b. Computed only for a 2x2 table
c. The standardized statistic is -1.468.
d. For 2x2 crosstabulation, exact results are provided instead of Monte Carlo results.

APPENDIX X

Monthly collection of snails from flowing water * *Bulinus* spp infected Crosstabulation

Count

		<i>Bulinus</i> spp infected		
		Positive	Negative	Total
Monthly collection of snails from flowing water	July, 2014	0	4	4
	August, 2014	0	5	5
	September, 2014	0	10	10
	October, 2014	3	8	11
	November, 2014	6	13	19
	December, 2014	8	10	18
	January, 2015	15	25	40
	February, 2015	11	13	24
	April, 2015	0	5	5
Total		43	93	136

Chi-Square Tests

			Monte Carlo Sig. (2-sided)			Monte Carlo Sig. (1-sided)		
				95% Confidence Interval			95% Confidence Interval	
Value	df	Asymp. Sig.	Sig.	Lower	Upper	Sig.	Lower	Upper

			(2-sided)		Bound	Bound		Bound	Bound
Pearson Chi-Square	15.446 ^a	8	.051	.015 ^b	.000	.035			
Likelihood Ratio	22.366	8	.004	.000 ^b	.000	.022			
Fisher's Exact Test	14.649			.022 ^b	.000	.047			
Linear-by-Linear Association	5.632 ^c	1	.018	.007 ^b	.000	.022	.000 ^b	.000	.022
No of Valid Cases	136								

a. 8 cells (44.4%) have expected count less than 5. The minimum expected count is 1.26.

b. Based on 136 sampled tables with starting seed 1993510611.

c. The standardized statistic is -2.373.

APPENDIX XI

Monthly collection of snails from stagnant water * *Bulinus* spp infected Crosstabulation

Count

		<i>Bulinus</i> spp infected		Total
		Positive	Negative	
Monthly collection of snails from stagnant water	June, 2014	9	37	46
	July, 2014	15	41	56
	August, 2014	4	23	27
	September, 2014	1	6	7
	October, 2014	0	3	3
	November, 2014	0	3	3
	December, 2014	0	1	1
	January, 2015	0	2	2
	May, 2015	1	16	17
Total		30	132	162

Chi-Square Tests

			Monte Carlo Sig. (2-sided)			Monte Carlo Sig. (1-sided)		
				95% Confidence Interval			95% Confidence Interval	
Value	df	Asymp. Sig. (2-sided)	Sig.	Lower Bound	Upper Bound	Sig.	Lower Bound	Upper Bound

Pearson Chi-Square	6.743 ^a	8	.565	.566 ^b	.483	.649			
Likelihood Ratio	8.688	8	.369	.426 ^b	.343	.510			
Fisher's Exact Test	5.322			.662 ^b	.582	.741			
Linear-by-Linear Association	3.587 ^c	1	.058	.081 ^b	.035	.127	.044 ^b	.010	.079
No of Valid Cases	162								

a. 10 cells (55.6%) have expected count less than 5. The minimum expected count is .19.

b. Based on 136 sampled tables with starting seed 475497203.

c. The standardized statistic is 1.894.