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Comparative studies on snail intermediate hosts of trematodes in ikwo and nkalagu of Ebonyi state

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Abstract
Freshwater snails share intimate relationship with parasitic trematodes because they serve as resource for development and vehicle for transmission to its next definite host. A cross sectional study was designed to establish the distribution, abundance and bionomics of freshwater snails; as intermediate host of trematodes in Ikwo and Nkalagu L.G.A of Ebonyi State. A field work was conducted for a period of four (4) months to study the ecology of these vector snails and identification of aquatic macrophytes where they thrive utilizing systematic random sampling at selected water bodies in the study area. Sampling was done using handpicking and scooping net method. A total of eight hundred and forty four (844) were collected in the study area which consist three families of Ampullariidae, Planorbidae, Lymnaeidae and the species identified includes *Lanistes varicus*, *Lymnae natalensis*, *Biomphalaria pfeifferi* and *Bulinus globosus. Lanistes varicus* recorded the highest species abundant 496(58.1%) followed by *Lymnae natalensis* 279(33.1%), *Bulinus globosus* recorded 49(5.8%) and lastly *Biomphalaria pfeifferi* recorded 20 (2.8%). Natural sunlight and artificial light were used to induce shedding of cerceriae. Identified cercariae includes Echinostome, Xiphidiocercous (Astiotrema *spp*), Distome (unidentified *spp*), Furocercous (*Pseudobiharziella spp*) other identified were immature sporocyts of *Schistosoma*, unidentified metacercariae, immature egg larvae of nematodes using crushing method. A descriptive test statistics was used to record the prevalence of infection in the snails. *Lanistes varicus* (42.9%), *Lymnae natalensis* (19.0%), *Bulinus globosus* (9.5%) and least was recorded for *Biomphalaria pfeifferi* (0.1%). In conclusion, snail surveilance is required to achieve efficient control of snail vectors thus enhancing rapid and effective reduction in transmission of snail-borne diseases in the study area.

Keywords: aquatic snails, trematodes, schistosomiasis, fascioliasis, cerceriae

Introduction
Freshwater molluscs are members of the phylum Mollusca which is the second largest phylum of the invertebrate animals, they are found in freshwater habitat and live in other ecological niches (Okafor and Ngang 2004) [28]. These snail serves as vectors and are important in the epidemiology of snail-borne diseases in the tropical and sub-tropical regions of the world thus they are known to serve as intermediate host of trematodes which are class of the phylum Platyhelminthes examined due to its medical and veterinary importance (WHO, 2010) [45]. The distribution of snails are required for trematode parasite to thrive and responsible for the diversity of these trematodes taxa which in turn result in continued transmission of schistosomiasis and other snail-borne diseases in endemic areas characterized with continued neglect, poor sanitation and lack of access to quality water sources and good infrastructural development (Hotez and Kamath, 2009; Stothard *et al*., 2009; Utzinger *et al*., 2011) [17, 38, 44]. Snails in the class Gastropoda which has been recently re-classified into eight (8) distinct subclasses which include Amphigastropoda, Archaeobranchia, Patellogastropoda, Neomphaliones, Vetigastropoda, Neritimorpha, Caenogastropoda, and Heterobranchia (Bouchet *et al*., 2017) [7]. There are eleven (11) different families of these class of snails among which are about 100,000 species described worldwide by Strong *et al*., (2008). Freshwater snails are rarely studied despite its niche and abundance in the ecosystem with many research to date limited to its focal bio geographical distribution, biodiversity, and inventory (Gelen *et al*., 2015) also with few studies on snail identification using varied conchological parameters and molecular methods, little or less data on snail host parasite interaction have been recorded about this neglected tropical diseases (NTDs). Freshwater snails (gastropods) convey a variety of diseases, including schistosomiasis, paragonimiasis, dicrocoeliasis, fascioliasis, faciolopsis, etc which affects millions of people and are endemic to Africa, Latin America, Southeast Asia (W.H.O, 2010) [45]. In Nigeria, schistosomiasis is endemic with estimated 11million people infected and 101.28million people at risk (W.H.O, 2010) [45]. In a layman's view, trematodes share an intimate relationship with their gastropods intermediate hosts which acts as the vehicle for the development and transmission. Some freshwater snails are intermediate hosts to parasitic flukes (helminthes) that have their definitive host in humans or other vertebrates (including invertebrates). The reality however is that all gastropod-borne diseases are neglected tropical diseases. This arise from little or no knowledge about their epidemiology, biology and ecology (Toledo *et al*., 2014) [41]. The distribution and existence of a particular parasite's snail intermediate hosts in an area can account for the prevalence of a parasitic disease in the inhabitants of a community (Geleta *et al*., 2015) [14]. For example *Fasciola hepatica* and *Fasciola gigantica* require the presence of the snail host *Lymnae* species for its development and life cycle, *Schistosoma haematobium* and *Schistosoma intercalatum* require the presence of *Bulinus* species for it development

and life cycle, *Schistosoma mansoni* require *Biomphalaria* species for its development and life cycle, *Paragonimus westermani* requires the presence of *Segmentina* species for its development and life cycle (Bereket *et al*., 2017) [6] also *Schistosoma japonicum* requires *Oncomelania* spp as intermediate hosts for development and transmission. Mollusc are responsible for the role they play in further transmission of infective stages (cercariae) and harboring the proliferative stages (which include sporocyst, rediae, metacercarie) thus examining these fresh water snails provide information on the trematode fauna present in the particular ecosystem also surveillance of these gastropod-borne diseases involves the identification of active and potential transmission sites. The intermediate host snails occupy varieties of natural habitats (Phiri *et al*., 2007), sometimes man-made habitats such as dams, lakes, ponds, pools, ditches, irrigation canals are colonize by these intermediate host snails (Oladejo and Ofoezie, 2006) [31]. Freshwater snails which acts as intermediates are of importance in the epidemiology of snail-borne diseases (WHO, 2010) [45] this is because of their crucial role in facilitating the development of infective larva stages (known as cercariae) which are of medical and veterinary importance. Fresh water snails provides resources for development, reproduction and means of transport by which these parasitic trematodes can reach their next host (Lockyer *et al*., 2004) [20]. There has been a long evolutionary history on the intimate association between snails and their digenetic trematodes (Cribb *et al.*, 2001) [12]. Trematodes exhibit apparent and marked relationship to temperature in their developmental stages and transmission cycle (Miller, 2007) [23]. This illustrations shows the stimulating effect of temperature in the shedding/release of cercariae from their snail hosts also owing to accelerative growth of different larva forms within snail hosts which are adaptive mechanisms which facilitate transmission of parasite from one host to another according to the report of Margolis *et al*. (2006) [21]. The transmission cycle of trematodes infection is prevalent where the snail intermediate host breed in areas contaminated by faeces or urine of infected persons (Miller, 2007) [23]. Freshwater snails harbors the asexual stages of the parasite while vertebrate (including birds, mammals) serves as definite host that harbors the sexual stage of their life cycle. Hence, this makes snail host studies a determining factor for the effective control of schistosomiasis and other snail-borne diseases in areas where it is endemic. This study is designed to provide information on the distribution, abundance and bionomics of various freshwater snails of medical and veterinary importance, their trematode infections, and their potential pathogenicity in endemic areas. It difficult to detect when and where transmission actually occurs without undertaking snail surveillance. Since snails are obligatory hosts for the larval stages of schistosomes their examination provides important information on active transmission foci therefore both the parasite and the vector must be targeted in order to break the cycle of transmission. This present study zero in on the knowledge of freshwater snails in the study areas as a measure in the control of snail-borne diseases in endemic area. Therefore, the aim of this study is Comparative studies on intermediate hosts of trematodes in Ikwo and Nkalagu of Ebonyi State. The distribution and abundance of freshwater snails according to locations, prevalence of infection within these aquatic snails, identification of cercariae shed by aquatic snails, identification of aquatic plants where these freshwater snails thrive and monthly variation in freshwater snails were determined.

Methodology

Study Area

Ebonyi State is located in the southeastern Nigeria. Ebonyi State lies approximately within latitudes 5° 40°' and 6° 45°' North of the equator and longitudes 7° 30°' and 8° 30°' East of the Greenwich meridian and is inhabited and populated by the igbo with the city of Abakiliki as its capital and largest city, other major townships include Afikpo, Onueke, Unwana, Ikwo, Ezzamgbo, Nkalagu etc. Ebonyi State prevailing climatic condition is primarily by two regimes which are rainy and dry seasons with the rainy season usually from April to October; while the dry season starts from October through to February thus this zone is described to have a bimodal rainfall pattern and annual rainfall between 1613.8mm to 2136.27mm. Ebonyi State is widely an agricultural region, it is a leading producer of rice, yam, potatoes and beans in Nigeria with rice predominantly cultivated. The State has several solid mineral resources, including lead, crude oil, etc.

Study Site

Two local government area were selected based on past/previous reports of prevalence of snail-borne disease endemic. For the purpose of this study, the sites include Nkalagu Community, a *Schistosomiasis* endemic area and Ikwo in Ebonyi State. Nkalagu Community is made up of five (5) villages: Ishiagu, Amanvu, Uwule, Imoha, and Akiyi. The geographical coordinates of the study area lies between longitude 54◦E and latitude 60◦N. The climate of the study area is tropical with mean daily temperature of 30 ± 5◦C for the most of the year. The annual rainfall is between 1900mm and 2200mm, with wet and dry seasons. The vegetation is typically rainforest. Several freshwater habitats intersect the study site, some of which include ponds, streams, dams, and rivers. These water bodies form the major source of water supply to the residents of the study site. The Study site of Ikwo Local Government Area is situated within coordinates of Longitude 800, 820E and Latitude 600, 620N. Ikwo is the largest local government area in Ebonyi State. It is situated on the eastern part of the state with land mass approximately 5,000 km2. It shares boundaries with Abakaliki and Ezza local government areas. The communities consist of Ndiagu Amagu, Ndiagu Echara, Ndufu Alike, Ndufu Echara, Noyo and Amanyima. Several fresh water habitats are found in these communities; some of these are man-made ditches, quarry pits as well as canal ditches. Members of these communities depend on streams, wells, ponds, and harvested rainwater for their water needs. The average annual rainfall is between 217cm to 240cm. The setting of the area is predominantly rural and the inhabitants are mainly farmers, they cultivate mainly rice, cassava maize, bambara groundnut and live in small farming settlements, a few kilometers (or even few meters) from the rivers/streams and dams and children as well are seen swimming in them mostly in the afternoon.

Map of Two Selected Local Government Area

Map of study area showing Nkalagu L.G.A and Ikwo L.G.A study sites (Fig.1) for snail sampling. The selected sample rivers and stream for this study were based on water contact

activities by residence of the villages for washing, swimming, drinking, and also farming practices. Sampling of site was done randomly twice a month during the period of this study.

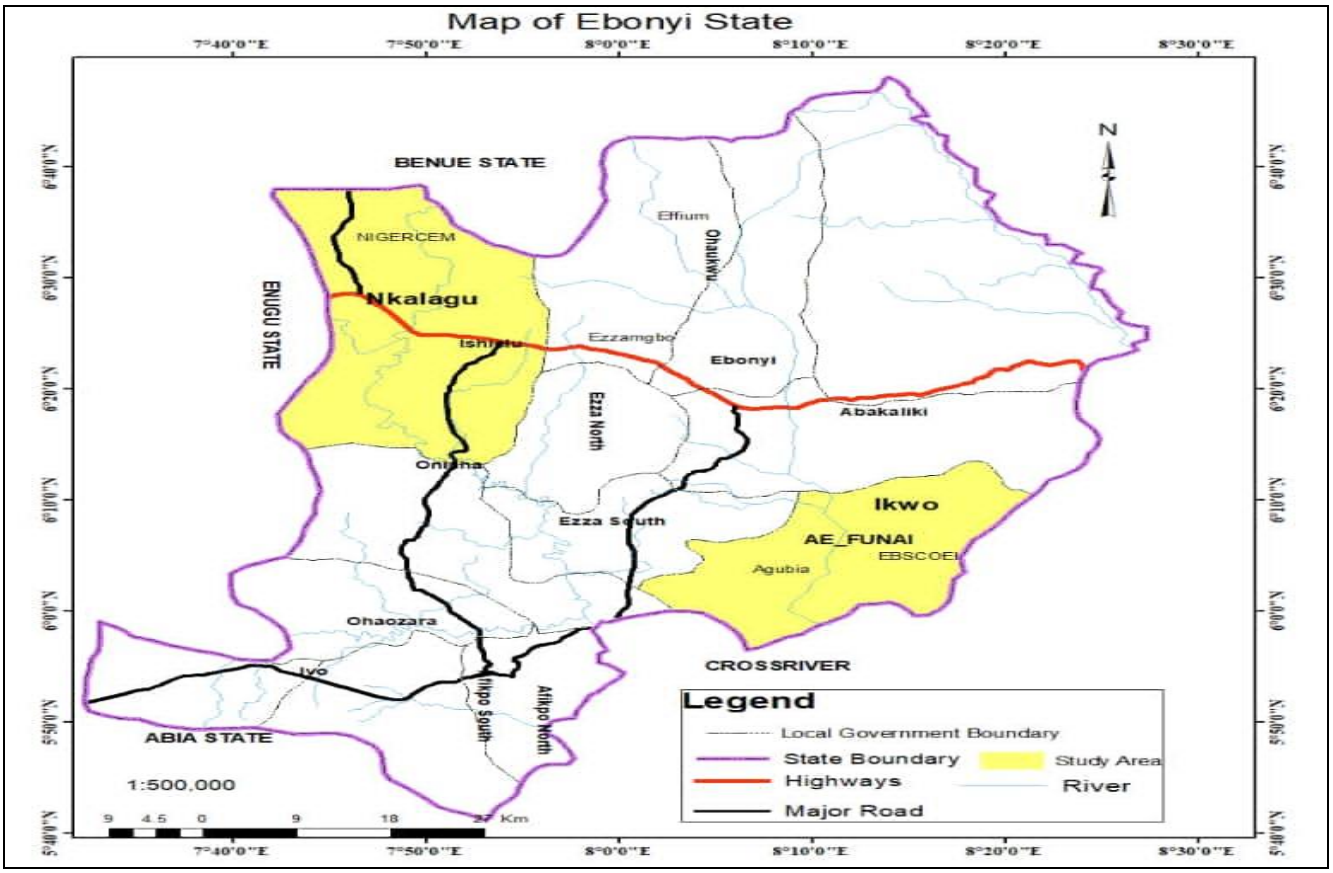

Fig 1: Fig. 1: Map of Ebonyi State showing Nkalagu and Ikwo Local Government Area

Study Design

A cross sectional study design which incorporates a field work carried out for a period of four (4) months from January to April, 2021 ranging from the peak of dry season to the onset of rainy season utilizing a systematic random sampling at selected water bodies in the study area. Weather data and climate predictions was collected from Nigerian Meteorological Agency, Abakiliki.

Ethical Clearance

Ethical approval and clearance was obtained from the research and ethics committee of Alex-Ekwueme Federal University Ndufu-alike, Ebonyi State. Ethical consent letter was addressed and submitted to the Ministry of Environment, Ebonyi State to seek for ethical clearance and permission to carry out research, the protocol of study was reviewed and given approval (Assigned number: EBSG/MENV/AD./36/167)

Snail Sampling and Identification

Sampling of snails were conducted for four months from January to April, 2021 in several fresh water bodies (including streams, dams, ponds, rice ditches, canals) found around various communities in study sites where there was high human contacts, fishing, grazing of animals also sampling of aquatic leaves and water lilies where snails was found. Snail samples were collected using improvised scoop net made up of rubber wire mesh 2mm in diameter and long wooden handle 1.4m long according to the work of Sharff *et al*. (2010). Collection time was done in the morning hours between 8:00am (minimum) to 12:00pm (maximum) and sampling was done per 20m² of each sampling sites once every two weeks in a month. Method of collection was done through visual search for snails on vegetative covers, debris, manual handpicking with forceps wearing protective hand gloves as protection against infection by cercariae and using scoop net at right angle to the banks of water bodies 2 meter deep as described by Oguoma *et al*., (2010) [26]. Furthermore, during sampling protective hand gloves, overall lab coats and wellington boots were worn to sampling sites as precautions against infection by cercariae (Agbolade and Odaibo, 1996) [2]. Collected snails were kept in a labelled take-away wide mouthed plastic container containing water from sampling site and transported to the laboratory of Department of Biology, Alex Ekwueme Federal University Ndufu-alike, Ebonyi State. The key methods of (Mendis and Fernando, 2002) [22] who provided guide for the identification of African fresh water snails of medical and veterinary importance were used in the identification of collected snails according to the morphological features of the shells. Measurement of shell, position of aperture, number of whorls also aided in the identification of snail to genus level (Brown, 1993) [8].

Laboratory Examination of Snails and Identification of Cercariae

The snails were grouped based on genus level and kept in the plastic containers and fed with crushed lettuce, water melon and cucumber prior to exposure (Rosen, 2000) [36]. Using forceps and wearing protective hand glove snails

were placed individually into a 100ml beaker covered with a perforated foil nylon to prevent snails from escaping, the beaker was then exposed to natural sunlight for three (3) hours and during the absence of sunlight during the day artificial shedding light of 100watts electric bulbs was used for two (2) hours to stimulate the emergence of various forms of cercariae for further microscopy study (Okafor, 1990; Ivoke *et al*., 2014) [27, 18]. Snails which did not shed cercariae on the first day was re-exposed to the fourth day until further crushing to check for the presence of sporocyst forms, snails that shed cercariae was recorded positive while those that did not release cercariae were recorded negative (absence of trematode indicates absence of infection). Petri dish containing 100ml of water from specimen were observed for the presence of trematodes larva forms (Okoli and Iwuala, 2001) [29]. Cercariae shed by each different snail species in a petri dish were transferred in water solution onto a microscopic slide covered with a cover slip and observed under 10x and 40x objective lens of the light microscope. The observed cercariae were stained with a little quantity of iodine stain or neutral red according to work of Uthpala *et al*. (2010) [43]. Identification of different trematode larva forms released by snails to specie level was done using standard morphological characteristics and measurement as describe by Brown, 1994 [9].

Identification of Aquatic Plants Where These Fresh Water Snails Thrive

Sampling of macrophytes from these water bodies was done where these snails clustered or where attached during sampling periods also aquatic macrophytes where collected near river banks, surface of water and at the bottom of flowing streams and little water bodies. Samples of vegetation were collected from each sampling site at the time of snail collection. The specimen was then conveyed to the herbarium of Biology laboratory, Alex-Ekwueme Federal University Ndufu-alike, Ebonyi State and identified to specie level according to standard key of Arbonnier (2004) [3] and Uneke and Ekuma, (2015) [42] using reference specimen morphology and catalogue.

Mounting, Staining and Identification of Cercariae Shed By Aquatic Snail Species

The water from the 100ml beaker used for cercariae shedding by each freshwater snail species was examined and counted prior to microscopy. Live cercariae shed by each separated freshwater snail species were identified based on morphology and characteristics of movement. A hand pipette was used add little quantity of water onto a grease-free slide, and observed under 40x and 10x objective lens of the light microscope respectively. The observed different forms of trematodes were further stained with iodine. A cover slip was carefully placed on the stained slide and observed under the 40x objective of the light microscope. The morphological details of the live and stained sporocyst forms, metacercariae and effective larva stages were observed under the microscope. The number of metacercariae present on each glass slide were counted and recorded for identification purposes using standard key (Sharif, 2010; Uthpala, 2010) [37, 43].

Measurement of the freshwater Snails

Morphological parameters were measured using manual handheld vernier caliper. The selected measurable parameters were Shell Height (SH), Shell Width (SW), Aperture Height (AH), Aperture Width (AW), Spiral Length (SL) and Aperture Circumference (AC).

Data Analysis

Results gotten from research was subjected to statistical analysis using Chi-square to test for the significance and prevalence of infection in snails using (SPSS ver. 23). Collected raw data were entered into Excel 2013 spread sheet and descriptive statistics were used to summarize the data.

Results

The Distribution and Abundance of Freshwater Snails According to Locations

A total of eight hundred and forty four (844) snails were collected during the sampling period, all belonging to the sub-class Pulmonata, of three (3) families and three (3) species. The family consist of Planorbidae, Lymnaeidae, Ampullariidae. The four species identified were *Bulinus globosus*, *Biomphalaria pfeifferi*, *Lymnaea natalensis*, *Lanistes varicus* with increasing distribution percentage as follows *Biomphalaria pfeifferi* had 20 (2.4%), *Bulinus globosus* 20 (5.8%), followed by *Lymnae natalensis* 279 (33.1%) and *Lanistes varicus* 496 (58.8%) as shown in Table 1 and Plate 1.

Table 1: Showing the distribution of snail species in the study area

Snail species	Total number	Relative percentage (%)
Bulinus globosus	49	5.8
Lymnae natalensis	279	33.1
Lanistes varicus	496	58.8
Biomphalaria pfeifferi	20	2.4
Total	844	100

Spatial Distribution of Snail Species in Relation to Lgas in the Study Area

The spatial distribution of snails in Ikwo L.G.A shows that *Lymnae natalensis* 134 had the highest relative percentage of (48.0%) followed by *Lanistes varicus* 234 (47.8%) and no data was recorded for *Bulinus globosus* and *Biomphalaria pfeifferi* because they were not found in the study site while for Nkalagu L.G.A *Lanistes varicus* 262 had a high relative percentage of (52.8%), followed by *Lymnae natalensis* 145 with relative percentage of (52.0%) and *Bulinus globosus* 49 with relative percentage of (05.9%) and *Biomphalaria pfeifferi* 20 with relative percentage of (2.4%) respectively as shown in Table 2. Observation of the monthly abundance of snail densities shows that samples of *Lanistes varicus* were the most abundant snails throughout the four sampling months, followed by *Lymnae natalensis* and the least recorded was for *Bulinus globosus* and Biomphalaria *pfeifferi* which distribution was restricted to only Nkalagu L.G.A. In the descriptive statistics of the distribution of these aquatic snails vectors shows in relation to sampling sites in the study area shows that *Lymnaea natalensis* been the most abundant of these snails found in all sampling sites, followed by *Lanistes varicus* and *Bulinus globosus* and *Biomphalaria pfeifferi* was only sampled in Nkalagu L.G.A and this varied monthly and spatially. There was significant difference in the distribution and abundance of snails in the LGAs (p=0.00).

Table 2: Showing the spatial distribution of snail species in relation to L.G.A in the study area

Snail species	Total collected (%)	Ikwo L.G.A (%)	Nkalagu L.G.A (%)
Bulinus globosus	49 (5.8)	0	49 (5.8)
Lymnae natalensis	279 (33.1)	134 (48.0)	145 (52.0)
Lanistes varicus	496 (58.8)	234 (47.2)	262 (52.8)
Biomphalaria pfeifferi	20 (2.4)	0	20 (2.4)
Total	844	368 (43.6)	476(56.4)

Monthly Distribution of Snail Species in the Study Area

In Table 3, the monthly distribution of different snail species in the study area with the month of April having the highest snail collection and diversity followed by February, March and January. The highest occurrence 262(31.0%) of freshwater snail was recorded in April followed by February 209(24.8%) and March 199(23.6%). The least abundance 174(20.6%) was recorded in January. The four species of freshwater snails' collected (*Bulinus. globosus, Biomphalaria pferifferi, Lanistes varicus* and *L. natalensis*) occurred in the four months of the study. *B. globosus, Lanistes varicus* and *L. natalensis* occurred in all the months of the study while *Biomphalaria pferifferi* occurred on only April. There is significant difference in the monthly distribution of snails p=0.00.

Table 3: Showing the monthly distribution of snail species in the study area

Sampling month	*Bulinus globosus* (%)	*Lymnae natalensis* (%)	*Lanistes varicus* (%)	*Biomphalaria pfeifferi* (%)	Total (%)
January	17 (9.74)	56 (32.18)	101 (58.05)	0	174(20.6)
February	11 (5.26)	61 (29.19)	137 (65.55)	0	209(24.8)
March	0	79 (39.70)	120 (60.30)	0	199(23.6)
April	21 (8.03)	83 (31.7)	138(52.17)	20 (7.6)	262(31.0)
Total	49 (5.8)	279 (33.1)	496 (58.8)	20 (2.4)	844

Spatial Distribution of Freshwater Snail Species Sampled from Different Habitat Sites

The spatial distribution of freshwater snail species sampled from different sites in 2020/2021 was shown in Table 4. Of the six sites sampled during the study period, Site C (Dam) recorded the highest number of freshwater snails 283 with overall percentage abundance of 33.5%, followed by Site F (Marshypools/ponds) (197:23.3%). The lowest snails' population was documented at Sites D and E (74(8.8%) and 79(9.4%)). This table also revealed that (*Lymnae natalensis*) was present at all sites. *Biomphalaria pferiffer* (20) was present only in Uzuru river (Site D). *Bulinus globosus* was not found in site A, B and C while there was the presence of *B. globosus* in sites D, E and F. The highest population of *Lanites varicus* 220(77.7%) was recorded in Dam. However, no species of *L. varicus* was recorded in Uzuru and Abashi sites.

Table 4: Showing the spatial distribution of freshwater snail vectors distributed across the different sampling sites in the study area

L.G.A	Sampling sites	*Bulinus globosus* (%)	*Lymnae natalensis* (%)	*Biomphal-aria pfeifferi* (%)	*Lanistes varicus* (%)	Total (%)
Ikwo	Ako river (A)	0	36 (43.4)	0	47 (56.6)	83(9.8)
	Ebonyi river (B)	0	35 (27.34)	0	93(73.8)	128(15.2)
	Dam (C)	0	63 (22.6)	0	220(77.7)	283(33.5)
Nkalagu	Uzuru river (D)	28(37.8)	26 (48.15)	20(37.0)	0	74(8.8)
	Abashi river (E)	14(17.7)	65 (82.3)	0	0	79(9.4)
	Marshypools/ponds(F)	7 (3.55)	54 (27.41)	0	136(69.0)	197(23.3)
Total	6	49	279	20	496	844

Identification of Aquatic Plants where these Fresh Water Snails Thrive

The identified plants associated with freshwater snails in this study include Typha grass (Typha species), Water lillies (*Nymphae odonata* and Water Hyacinth (Pistia species), Touch and die (*Mimosa pudica*), Wild rice (*Orya longistaminata*). The Table 5 represents the abundance and distribution of macrophytes across selected sampling sites in the study area using the following KEY: Present +, Abundance ++, More abundance +++, Most abundance +++++.

Table 5: Showing the list of plants distributed across the L.G.A in the study area

List of plants	Ikwo LGA	Nkalagu LGA
Water lilies (*Nymphae odonata*)	+++	+++++
Water Hyacinth (*Pistia spp)*	+	++
Touch and die (*Mimosa pudica)*	++	+++++
Wild rice (*Oryza longistaminata*)	+++++	+++

The Prevalence of Infection within these Aquatic Snails.

The prevalence of trematode infection in samples of freshwater snails collected at various sampling sites surveyed are shown in Table 6. The overall prevalence of trematodes infection in these snails were recorded for *Lanistes varicus* (47.1 %) followed by *Lymnae natalensis* (32.6%) and the lowest prevalence was recorded for *Bulinus globosus* (18.0%) and *Biomphalaria pfeifferi* (2.3%).

Lanistes varicus was observed to harbor the immature sporocyst, ova of different nematodes and metacercariae stage of different trematodes after crushing method and dissection of the hepato-pancreas tissues and seminal gland in the upper part of the body within the shell (Plate 1).

Table 6: Showing the prevalence of trematodes infections within the snail samples

Snail specie	No. of snails Collected (%)	No. positive of snails (%)	Prevalence of infection (%)
Bulinus globosus	49 (5.8)	21 (42.9)	31 (18.0)
Lymnae natalensis	279 (33.1)	53 (19.0)	56 (32.6)
Lanistes varicus	496 (58.8)	47 (9.8)	81 (47.1)
Biomphalaria pfeifferi	20 (2.4)	2 (10.0)	4 (2.3)
Total	844	123 (14.6)	172

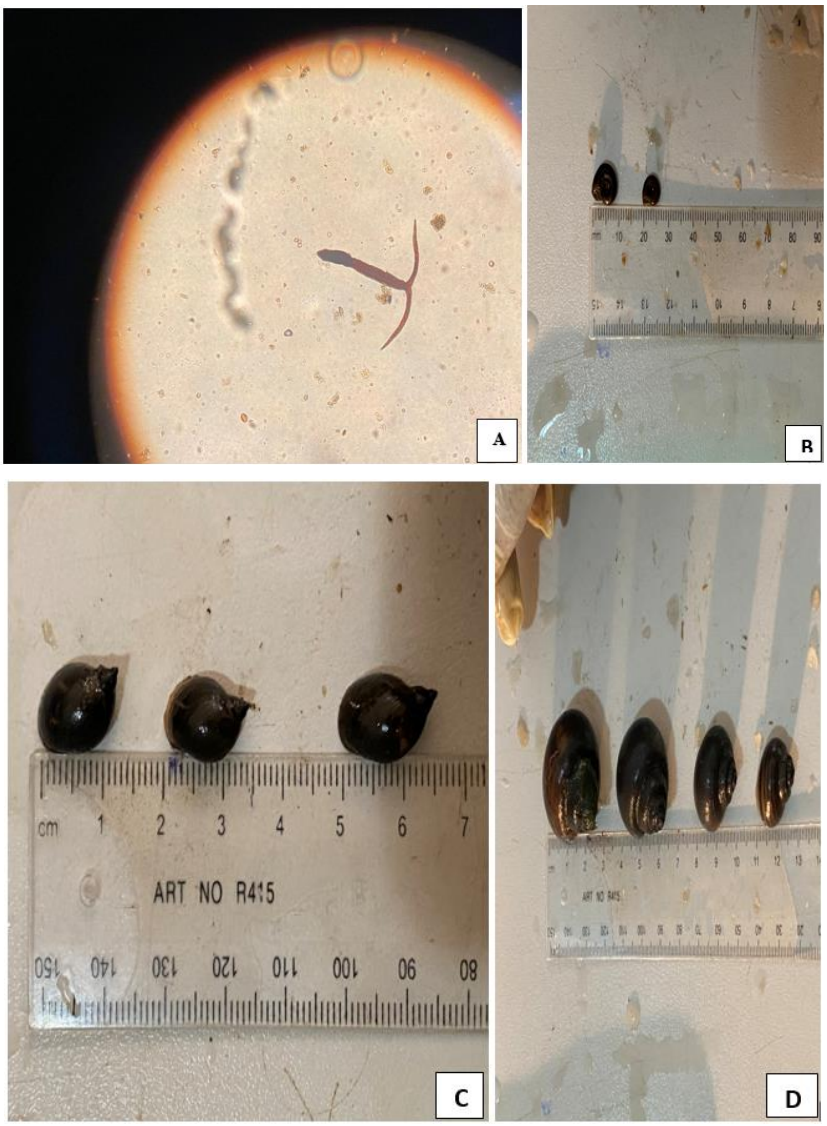

Plate 1: A=Cerceriae of *Schistosoma mansoni* from *Biomphalaria* snails, B= *Biomphalaria* snails, C= *Lymnae natalensis* snails, D= *Lanistes varicus* snails

Mounting, Staining and Identification of Cercariae Shed by Aquatic Snail Species.

The identified cercariae are members of the family Virgulate xiphidiocercercariae, Longifurcate-pharyngeate distome, Brevifurcate cercariae, Echinostome, Furcocercous (Pseudobiharziella *spp*), Amphistome, Distome (unidentified *spp*) others include immature sporocyts of *Schistosoma* spp, larvae of different parasitic nematodes, ova of nematode specie obtained by direct crushing and dissection of the hepato-pancrease gland. Images of different trematode larva forms was taken using iphone 11 pro tripple camera.

Measurement of the Freshwater Snails

The average mean morphometric data gotten from twenty (20) random samples of *Lanistes varicus* are described in Table 7 as follows shell height (6.82±0.13), shell width (5.65±0.24), aperture height (3.35±0.04), aperture width (2.12±0.08), spiral length (2.26±0.08) and aperture circumference (6.18±0.15). Morphological parameters measured on twenty (20) samples of *Lymnae natalensis* are described accordingly, the shell height had a mean value of (9.28±0.61), shell width (6.33±0.44) aperture height (5.12±0.48), aperture width (4.42±0.24), spiral length (3.38±0.18) and aperture circumference (6.62±0.28)

respectively. The average mean morphometric data gotten from twenty (20) random samples of *Bulinus globosus* are described as follows shell height (4.46±0.22), shell width (3.65±0.14), aperture height (2.15±0.10), aperture width (2.22±0.04), spiral length (3.06±0.13) and aperture circumference (4.38±0.10). The mean measurement of *Biomphalaria* recorded shell height (6.54±0.20), shell width (6.45±0.08), aperture height (1.35±0.28), aperture width (1.10±0.18), spiral length (2.06±0.32) and aperture circumference (3.18±0.26).

Table 7: Showing the mean measurement of different snail species

Snail Species	Shell length	Shell width	Aperture length	Aperture width	Spiral length	Circumference
Biomphalaria pfeifferi	6.54±0.20	6.45±0.14	2.15±0.10	2.22±0.04	3.06±0.13	4.38±0.10
Bulinus globosus	4.46±0.22	3.65±0.14	2.15±0.10	2.22±0.04	3.06±0.13	4.38±0.10
Lymnae natalensis	9.28±0.61	6.33±0.44	5.12±0.48	4.42±0.24	3.38±0.18	6.62±0.28
Lanistes varicus	6.82±0.13	5.65±0.24	3.35±0.04	2.12±0.08	2.26±0.08	6.18±0.15

Discussion

Snails collected from all freshwater contact sites were pooled each month to obtain the monthly snail population. Lanistes varicus and Lymnae natalensis occur to be the most abundant snail species in the study area as a result of bond conditions that are favorable to the species although the former haven't been confirmed for its specific trematode parasite the later has been confirmed for facioliasis and faciolopsis in animals respectively compared to its counterparts Bulinus Spp, Biomphalaria Spp which have been reported for its role in Urinary schistosomiasis, intestinal schistosomiasis and hepatic schistosomiasis in endemic areas (Barbosa and Barbosa, 1994) [4]. Their low abundance are attributed to the fact Biomphalaria Spp and Lymnae Spp snails do not maintain stable population due to fluctuations in changes of human activities and seasonal weather variations. The resulting variation in the number of snails collected monthly may therefore be due to changes in abiotic and biotic factors during the dry season comparably more snail species were collected in the early months of wet season (March to April) when there was minimum rainfall in the study area with similar observations by Ngele *et al*. (2012) [24] in their implemented in Abia state which showed the highest number of snails collected at the beginning of the wet season when there was no heavy rainfall. The monthly variations of infections in infected snails are used to determine the availability of cercariae and identify periods when human populations are at greatest risk of acquiring schistosomiasis and other trematodes infection at selected sampling sites. Monthly collection of snails during the late dry season to the onset of wet season showed great correlation with snail densities due to seasonal fluctuations. Seasonal variations and environmental changes have proved to modify the pattern of distribution of freshwater snails and trematodes larvae stages shed can be used to access the environmental impact and infections in endemic areas. Cercariae diversity and prevalence of infection in freshwater snail during the onset and peak of dry season was very low, and this correlates to the research of (Uthpala, 2010; Mendis and Fernando, 2002) [43, 22] compare to reports of high cercariae infections especially in children living at endemic areas during the wet season (Nwosu *et al*., 2006, Okpala *et al*., 2004) [25, 30]. This confirms reports of freshwater snails known as Bulinus globosus, Biomphalaria pfeifeiri and Lymnae natalensis identified in the transmission of urinary schistosomiasis, intestinal schistosomiasis and facioliasis was predominantly present during this survey. Analysis of seasonal rainfall and temperature data show direct influence on the availability of aquatic snails this is similar to observations that have been reported (Sturrock, 200; Clercq 1999; Belot, 1993) [11, 5] to demonstrate the usefulness of rainfall in the increase or decrease of snail populations. During cercariae shedding, Biomphalaria pfeifferi was observed to shed Furcocercous cercariae and Longifurcate-pharyngeate cercariae, also the numerous rediae shedding was observed in Bulinus globosus and sporocyst at the time of crushing, a higher diversity of cercariae of Faciola species was observed in Lymnae natalensis which include Echinostome, Distome. Virgulate xiphidiocercariae and Amphistome cercariae was observed in Lanistes varicus also presence of metacercariae and larvae of Ascaris spp is unique for further research this also corresponds to the work of Cazzaniga, (2002) [10] who reported such for cause of angiostrongyliasis. Similar result of Amphistome cercariae and Xiphidiocercariae found in Lanistes varicus has been reported by (Radomayos *et al*., 1994) [34] and this corresponds with findings of Olsen (1974) that Ampullarids snails such as Lanistes varicus and Pila ovate acts as repository host and intermediate host in the life cycle of parasitic nematode and trematodes. Result of metacercariae and encysted larvae of different trematodes species in aestivated freshwater snails from transmission sites showed reduced growth, fruitless development and is based hypothetically on the abortive transmission to the next definite host which may be as a result of unfavorable conditions (such as increasing temperature, mortality of infected snails, hibernation of snails, dearth, waterlessness etc). Studies have shown that high and increasing temperature had effect on the survival of infected snails (Uthpala, 2010) [43]. Increasing temperature is directly proportionally to mortality rate of infected snails. This gives a feasible lead in the reduction of the different trematodes forms and cercariae released and in the disease risk during the dry hot seasons when population density and size of snails is reduced (Manyangadze, 2016). This wet season provide a significant evident when freshwater snails are capable of repopulating their natural habitat because this period provide conducive environmental process for snail flourishment and promote growth of aquatic plants such as water lilies (Nymphaeaceae family), water hyacinth, and other macrophytes on which snails feed and oviposit during reproduction period (Kristensen *et al*., 2001; Afshan *et al*., 2013; Ejehu *et al*., 2017; Raheem *et al*., 2008) [19, 1, 13, 35]. During sampling of macrophytes found in these snails habitat the abundance of these macrophytes are showed in Table 5 above it was observed that in Nkalagu Lanistes varicus was observed to be an invasive snail specie found in all habitat all high percentage of it was collected in habitat that are more eutrophic and was found to coexist in habitat where Lymnae natalensis was found, Biomphalaria pfeifferi was found to coexist with Lanistes varicus and Lymnae natalensis in habitat with more abundant macrophytes such

as Pistia spp and dead decaying leaves of trees found around the water bodies. A large population of lymnae natalensis was found around pheripheral water bodies while Lanistes varicus was found in deep bottom water that are less eutrophic this similar observation was made by Okafor and Ngang (2002). Eggs of snails was seen on leaves of Nymphae odonata and dead decaying leaves in the water bodies. Less population of aestivated bulinids were picked from rice field and marshy pools that are more eutrophic also presence of root of rice and water lilly was observed and this may be due to prolonged dry season and drying up of their natural habitat. In Ikwo L.G.A a large population was found in almost all the habitat of the water bodies. Lymnae natalensis coexist Lanistes varicus in habitat more abundant with Mimosa pudica, Nymphae odonata other dead plants and leaves. No bulinids or Biomphalaria spp was collected in this Locality. Human behavioral activities including farming may also provide ample vegetation that serves as oviposition, niche, shelter, feeding substrate and protection against natural predators (Gortmark *et al.*, 2008). In the measurement of the different species of snails the conchological parameters where observed as follows Lymnae natalensis has conical shape with dextral opening, the height of the shell is larger than the width and the spine is cone shape, Biomphalaria pfeifferi has discoid shape, the shell is coiled in one plane, Lanistes varicus has hyperstropic shell with dextral opening, the width of the shell and height are nearly the same and also Bulinus globosus has globuse shell with sinistral opening, the height and width of the shell are about the same. A representative random sample was measured and used to derive the average parameters as shown in Table 7 above.

Conclusion

The study was designed to provide information on the distribution of various freshwater snails of medical and veterinary importance, their trematode infections, and their potential pathogenicity in endemic areas. This present research was able to achieve the distribution of snail vectors causing diseases of schistosomiasis and facioliasis in the study area, in this regard snail control is essential for reducing the prevalence of diseases such as Schistosomiasis, Facioliasis, Echinostomiasis etc in humans and animals. Although praziquantel and bithionol have been effective drugs in the treatment of schistosomiasis and facioliasis respectively, important and effective control measures from potential re-infection of diseases in endemic areas is by proper health education by health authorities to provide information on the safety of open waters, installation of good water supply and sanitations facilities to obstruct the transmission mechanism of these parasites to its definite host. In conclusion evaluation of snail ecology is invaluable to achieve efficient control of snail vectors thus enhancing rapid and effective reduction in the transmission of snail-borne diseases.

Recommendation

Most importantly wearing of boots and protective gloves by the rice farmers, occupational fish farmers and also provision of good water sources and sanitation infrastructures to avoid inhabitants from contaminating the water bodies with infected stool and urine, not excluding inspection and regulation open grazing of cows along water bodies of public use and also the use of molluscicides will help in checkmating the snail vectors. Snails can also be controlled indirectly by reducing their habitat or directly by removing them; In the indirect method, the use of molluscicides is effective against the freshwater species of the genera Bulinus and Biomphalaria, removal of aquatic vegetation or macrophytes which serves as food, shelter, substrate for snails and it's reproductive cycle, elimination of breeding sites such as borrow pits, irrigation canals, small pools and this includes treatment of rice fields which are found to be important site for the transmission cycle of trematodes and other zoonotic infections. Implementations of snails control depends on several factors such as infection level in the definite hosts (humans and animals), freshwater snail habitat, transmission pattern, snail species and other ecological concerns. Further research in future are needed to categorize the prevalence of trematodes in snails from different localities of endemic areas, also use of more accurate methods to identify different cercariae at species and promote clear understanding of the epidemiological conditions of these infection in endemic suburban areas. Control measures should concur with the time of greater abundance and conditions optimal for freshwater snails survival.

Limitations

This study had some limitations. In the comparative study of the abundance and seasonal climate variations of freshwater snails ranging from the peak of the dry season to the peak of wet season and virulence of trematodes infections in snail was not exhaustively carried out due to duration of research time as a result of the Covid-19 pandemic lockdown.

Significance of Findings

The results are vital and essential for planning snail control in endemic areas of Ebonyi State. Snail control prior to the onset of wet season (From late March to early May) would serve as dual benefit in curbing out related snail-borne diseases. Firstly by reducing or eliminating the abundance and population density of these aquatic snails after most of it populations have recovered from its aestivation period due to prolonged hot season and secondly it would be cost effective control program because there is small volume of water and in some cases water bodies have been dried off. This also confirms the presence of *Biomphalaria pfeiferri* which is a causative agent for transmission of *Schistosoma mansoni* responsible for intestinal schistosomiasis in one of the selected water bodies of Nkalagu L.G.A. Furthermore future research should focus more on search for these freshwater snails of *Biomphalaria* species for polymerase chain reaction characterization of specie level and examination of intensity of infection in stools of inhabitants living around marked locality. In this course, monthly variation analysis is needed to target when this snail species are highly abundant and also more on the physio-chemical parameters responsible for optimal growth and propagation of these snail vectors which have been identified to be of medical and veterinary importance in the study area. No *Bulinus spp* or *Biomphalaria spp* was found in Ikwo L.G.A during the course of study which may be due to its geographical distribution. Low abundance of *Bulinus globosus* and *Biomphalaria pfeifferi* during the peak of dry season confirms seasonal variation in their abundance and that *Bulinus* and *Biomphalaria* snails do not maintain stable populations due to the fluctuation occurring non-seasonally

and physio-chemical parameters within the water bodies can have effect on snail population. Most importantly further research should be carried out on the findings of metacercarie, larvae and eggs of parasitic nematodes in the *Lanistes varicus* snails although this was not a major focus of study such occurrence may also contribute to Ascariasis diseases in the study area.

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Authors' contributions
The study was conceived and designed by COA while all authors read and approved the final version of the manuscript.

Competing interests
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