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Malacological survey and dynamic of *Lymnaea natalensis* population intermediate host of *Fasciola gigantica* in the Douvar dam freshwater of Farth Nord region Cameroon

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Abstract
Malacological study was carried out by monthly manual harvests between April 2015 and March 2016 in the artificial reservoir of Douvar. The aim of this study was to identify mollusc's species and to determine the population dynamics of *Lymnaea natalensis*. At the end of investigation, 4005 molluscs were hardvested. These molluscs were belonging to seven species among which *Menaloides tuberculata* (39.05%) and *L. natalensis* (22.8%), while *Bullinus africanus* (7%) and *Bullinus truncatus* (9%) are less abundant. The population of *L. natalensis* presents a single annual generation which varies between October and July with an abundance of juveniles (<10 mm) and young (11-15 mm) (F = 7.72; df= 47; p =0.0003). It begins its appearance on September and their number evolves differently until July with a peak between the end of the dry season and the middle of the rainy season (March and June). In addition, the variation in relative abundance of this population is significantly influenced by rainfall (r = -0.699; n = 24; *p*<0.05) and ambient temperature (r = 0.484; df = 24; *p*<0.05). This study had also shown significant and positive correlations with pH (r = 0.622; df = 11; p = 0.031), very significant and positive with Salinity (r = 0.751; df = 11; P = 0.005) and very negative significant with conductivity (r = -0.918; df = 11; p < 0.0001) and with TDS (r=-0.894; df=11; p < 0.0001). The population of *L. natalensis* has significantly influenced fasciolosis in the butcher's shop of Douvar (r = 0.484; df = 24; *p*<0.05). The Douvar dam therefore constitutes a favorite reservoir for the sustainability of fasciolosis intermediate host and molluscs of medical interest in the Far North Cameroon Region.

Keywords: *Lymnaea natalensis*, population dynamic, Douvar dam, Far North Region, Cameroon

1. Introduction
The construction of dams in many countries of Africa has consequences for the environment. These structures are at the origin of the emergence and intensification of infectious diseases transmitted by insects and molluscs intermediate hosts of trematode [39; 35; 9]. Among these Limneas, the species of the genera *Biomphalaria* and *Bulinus* act as intermediate hosts of the Schistosomes while *Lymnaea nataenlsis* Krauss, 1848 is the intermediate host of *Fasciola gigantica* [9]. However, several studies have shown that in the absence of specific hosts to a parasite species, one of them can substitute for normal competence in the parasite cycle [44, 34, 2]. Snail habitats are diverse and the transmission of animal and human trematodosis is governed by their proximity to infested bodies of water [35]. In Cameroon, the construction of large water resource development projects in the Far North Region dates back to 1970 with the construction of the Douvar and Maga reservoirs as a result [27]. The distribution of *L. nataensis* deposits in this region has been described [45], but very little data is known about population dynamics especially in the Douvar Dam which plays an important hydrological role in the Mayo Tsanaga and Lake Chad [25]. This study is a contribution to the understanding of the epidemiology of cattle fasciolosis by studying of the variation of *L. nataensis* population and the impact of physicochemical and climatic parameters on their variations in order to contribute to the prediction of the transmission of fasciolosis and implements monitoring systems-response to prevent and control them.

2. Materials and Methods
2.1 Study sites
The Douvar reservoir commonly known as big dam of Mokolo, is an artificial water well dam built between 1975 and 1979 in a basin of the middle mountains at 5 km north of Mokolo [30, 26]

in the Mandara Mountains (Figure 1). This government project carried out upstream of Tsanaga mayo aimed to improve the water supply in the surrounding localities [26]. The structure was built with granite blocks at an altitude of more than 815 m between 10°47'10" East longitude and 13°47'10" North latitude on an area is estimated at 48.9 km^2 (Figure 1). The dam has a maximum capacity of 5.35 million m^3 and retains only an average of $3x10^{-6}$% of the average volume sold on the Tsanaga Mayo watershed [27]. The rocky substratum on which the dam rests is of a migmatitic nature with at its edges two rocky granitic massifs at the origin of said defile [27]. The vegetation consists of aquatic plants scattered in places on the siliceous soils of the banks because of the boulders [26].

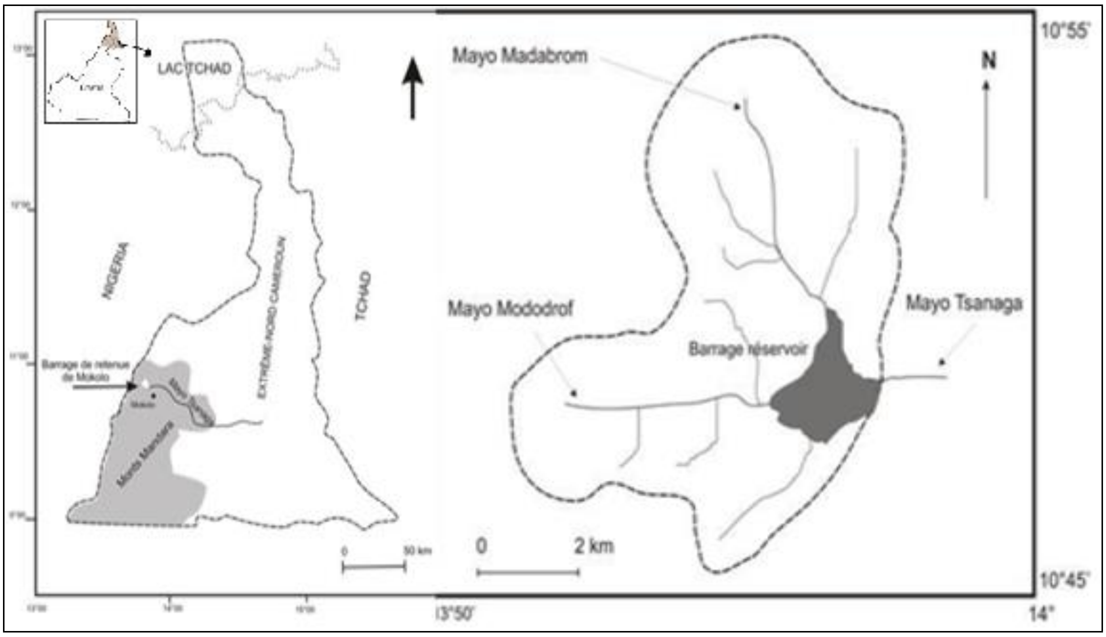

Fig 1: Location map of the Douvar reservoir (Lewa *et al*., 2014) [27]

The climate is Sahelian type with 7 months of drought and 5 months of rain. The mean annual precipitation is 1000 mm, the mean annual temperature is 26.0 °C and the ground air humidity is 48% [30]. Annual evapotranspiration (ETP) is around 2000 mm with a monthly minimum of 85 mm in July and a maximum of 235 mm in March [27]. Direct anthropogenic activities around the water resource are mainly agro-pastoral and more specifically market gardening [30]. The catchment area experiences intense agricultural and erosive activity during the rainy season (April to September) [26].

2.2 Assessment of *Lymnaea natalensis* population and molluscs

The population dynamics of *L. natalensis* was assessed by monthly harvesting of Limneas during the period from April 2015 to March 2016. These Limneas were harvested once a month using the manual collection technique to assess the density of population. This harvesting technique consists of collecting either directly by hand or using a landing net with a long handle to scrape the bottom and reach the molluscs on the roots of aquatic plants [32]. The density is estimated by the number of Limneas collected during one hour by a prospector who remained unchanged at the sampling site [41]. The collected snorkels are counted and measured on the spot according to the height of their shell using a graph paper or the ruler, and grouped according to the size ranges (≤10mm; 11-15mm; 16-20mm; ≥20mm). These shell size ranges correspond to the juvenile, young, adult and older age groups. After these inventories, these Limneas are returned to their natural biotope. However, during these harvests, the other species of Molluscs living with *L. natalensis* are also harvested, numbered and counted on the spot in a specific file. Some samples were kept in tubes containing alcohol at 70 °C and brought back to the laboratory for systematic identifications using the key from [3] and [6]. The count of species of molluscs allows us to assess the diversity of this environment by calculating the Simpson and Shannon indices according to the following formulas [43; 46].

Shannon index: $H' = -\sum_{i=1}^{s}(pi * lnpi)$

Simpson index: $D = \sum \frac{ni(ni-1)}{N(N-1)}$

H': biodiversity index D: Simpson index $p_i = n_i / N$
n_i is the number of individuals of the species; N is the total number of Individuals
Simpson's index will vary between 0 and 1. When the closer it gets to 0, there is a higher the chances of getting many individuals of different species.
Abundance is the ratio of the number of molluscs in a species collected and the total number of molluscs times [22].

2.3 Evaluation of physicochemical and climatic parameters

During each visit to the water point, the physicochemical parameters (pH, medium and water temperature, salinity, dissolved solute level, conductivity) are recorded using a multifunction pH meter. Precipitation measurements are taken near at each weather stations lodging.

3. Results

3.1. Diversity and abundance of molluscs

The harvested mollusc fauna (Table 1) consists of seven species of Gastropods, six of which belong to the Order of Pulmonata (*Lymnaea natalensis*, *Bulinus tropicus*, *Biomphalaria pfeifferi, Bulinus globosus, Bulinus africanus*) and one to the Order of Prosobranchia (*Melanoides tuberculata*). A total of 4005 specimens were counted, an average of 330 per month (Table 1). Among the harvested species, *Me. Tuberculata* (39.05%) and *L. natalensis* (22.8%) are more abundant while *B. africanus* (7%) and *B. truncatus* (9%) are less abundant. The monthly calculation of Simpson's diversity indices showed that they varied between 0.205 in January and 0.494 in August. But since these indices vary on average around 0 and are closer to 0, we can say in this deposit is very diverse in mollusc's species.

Tableau 1: Distribution of malacological fauna

Periods	*Lymnaea natalensis*	*Bulinus globosus*	*Bulinus tropicus*	*Bulinus africanus*	*Bulinus natalensis*	*Biomphalaria pfeifferi*	*Melanoïdes tuberculata*	Total	H'	D'
April	201	13	87	37	21	76	236	671	-1,58	0,246
May	136	08	64	27	13	42	149	439	-1,58	0,244
June	27	09	41	33	09	75	175	369	-1,51	0,291
July	24	06	22	23	07	67	121	270	-1,51	0,283
August	00	00	00	00	00	32	36	68	-0,69	0,494
September	00	00	00	00	05	12	27	44	-0,90	0,451
October	36	00	06	06	01	22	79	150	-1,25	0,355
November	70	08	17	12	08	63	111	289	-1,54	0,256
December	87	13	10	30	01	79	134	354	-1,49	0,261
January	56	14	23	37	04	51	87	272	-1, 70	0,205
February	83	23	33	31	06	67	152	395	-1,63	0,236
March	193	27	72	34	12	89	257	684	-1,57	0,252
Total	913	121	375	270	87	675	1564	4005	-1,59	0,247
Abundance (%)	22,80	03,02	09,36	06,74	02,17	16,85	39,05			

Legend: H'= Shannon index; D' = Simpson index; %= percentage

3.2 Population dynamics of *L. natalensis*

The monthly counts show that the population of *L. natalensis* is larger in March (193 individuals) and nonexistent during the months of August to September (Figure 2). Between April and May, the Limneas are very numerous (136-201 individuals). But between August and September their number decreases considerably to the point that they completely disappear. They reappear in small quantities (36 individuals) in October and their number increases each month until reaching the first low peak (87 individuals) in December. After a slight decrease in January, their number increases very considerably between February and March (193 individuals) until May (201 individuals).

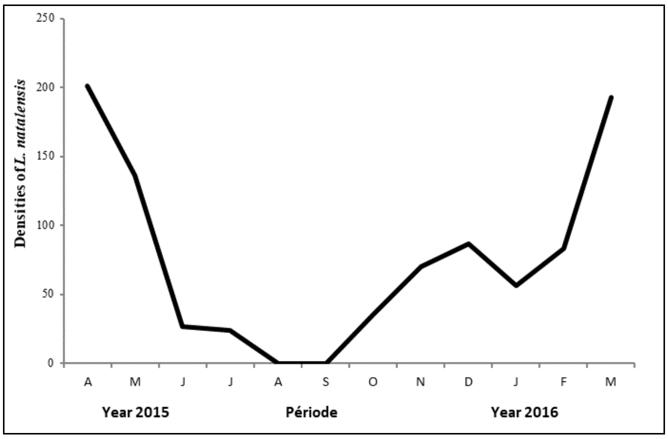

Fig 2: Monthly evolution of the *L. natalensis* population

3.3 Evolution of groups of individuals in the *L. natalensis* population

3.3.1 Average annual densities

The results reported in Figure 3 show that among the four groups of *L. natalensis*, the most dense are those whose shell height measures 11-15 mm (37.17 ± 27.86) while those measuring more than 20 mm are the very few. Limneas measuring ≥10 mm and 16-20 mm high are very little represented. The analysis of variance shows the existence of a significant difference between the average densities of these groups of individuals (F = 7.72; df = 47; p = 0.0003). The Duncan Test shows that individuals who measure 11-15 mm tall are significantly more abundant.

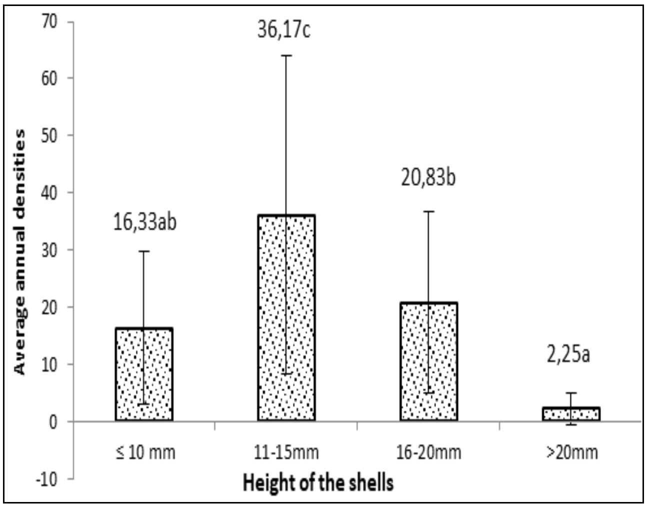

Fig 3: Annual average densities of individuals groups in the *L. natalensis* population

3.3.2 Dynamics of individuals groups in the *L. natalensis* population

The population of *L. natalensis* has a single annual generation which varies between October and July (Figure 4). Juveniles begin their appearance in September and their numbers evolve until July with a peak in March. Large peaks of juveniles are therefore observed during the months of February and March. In the other groups, the population evolves very differently depending on the height of their shell. Large Limneas (>20mm) are less numerous, and are observed between December and June with an abundance in May and June. Those measuring 11-15 mm begin to be visible in small quantities between October and January, but their number increases very considerably between February and May. *L. nataensis* measuring 16-20 mm and although less this numerous, show similar smalls amplitudes evolution to those of the young (11-15 mm).

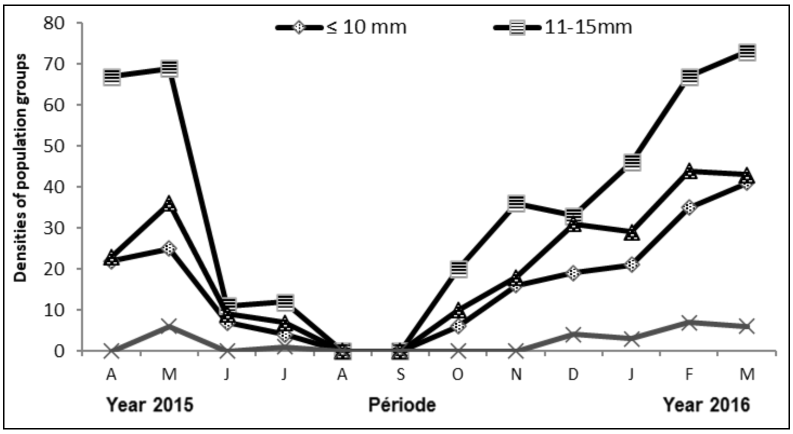

Fig 4: Monthly variation of groups of individuals in the *L. natalensis* population

3.3.2.4 Impact of climatic parameters on the population of *L. natalensis*

3.3.2.4.1 Influence of the ambient temperature

Comparison of ambient thermal readings with that of the population of *L. nataensis* shows that between June and September when temperatures are average (25-30 °C) their density is very low (Figure 5). But between October and February, as temperatures gradually drop, their numbers increase slightly. In the middle of the dry season (March-May) when temperatures reach the peak, the population is more abundant. Comparison of ambient thermal readings with that of the population of *L. nataensis* shows a weakly positive correlation (r = 0.484; df = 24; p<0.05).

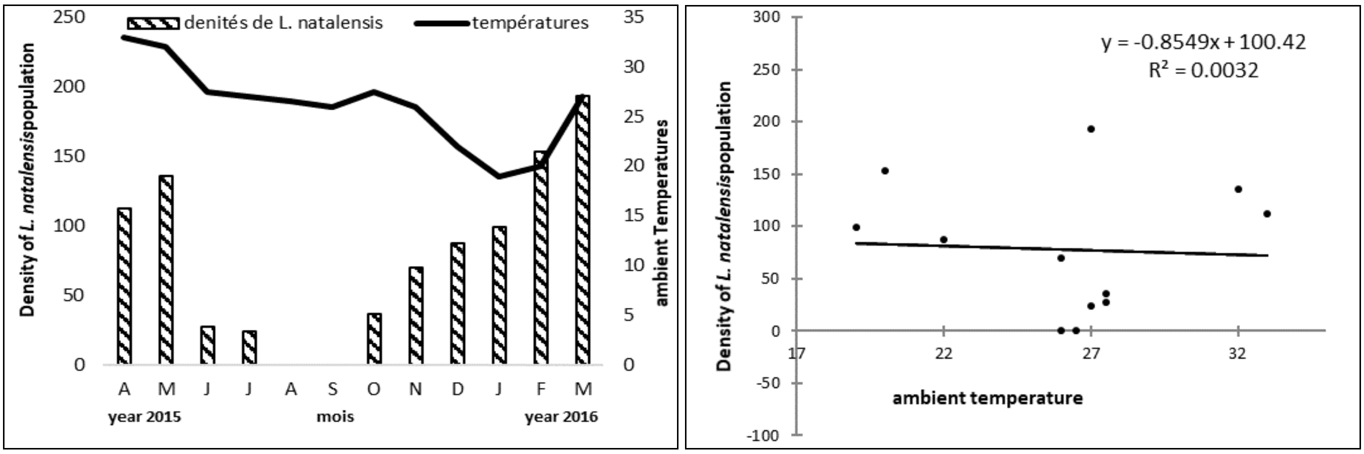

Fig 5: Variation of the *L. natalensis* population as a function of ambient temperatures and their linear regression curve

3.3.2.4.2 Influence of precipitation

The records of precipitation and density of *L. nataensis* in the Douvar reservoir, there is a sharp drop in the density of mollusc's in the rainy season between June and September as precipitation becomes more significant (Figure 6). The population of *L. natalensis* is most abundant in the dry season until the start of the rainy season (December and May) when there is little or no rainfall. The correlation test between the evolution of precipitation and that of Limneas is negative (r = -0.699; df = 24; p<0.05), which means that precipitation has a negative influence on the population of *L. natalensis*.

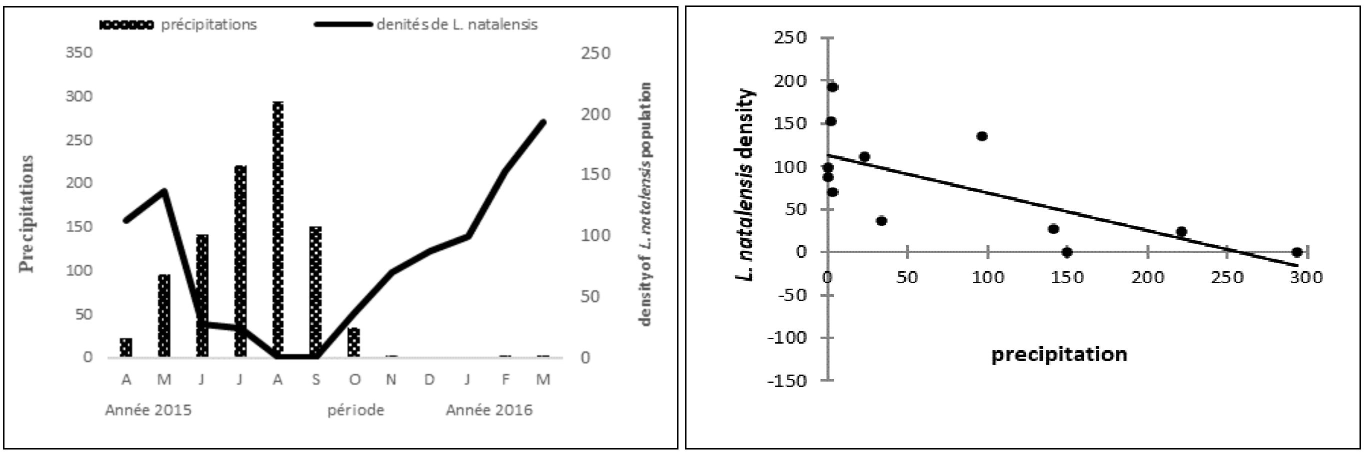

Fig 6: Variation of the *L. natalensis* population as a function of precipitation and their linear regression curve

3.3.2.5 Influence of physicochemical parameters on the *L natalensis* evolution

3.3.2.5.1 Physicochemical parameters of the water in the deposits

The information's putting in the Table 2 presents the values of pH, salinity, conductivity, dissolved solute levels (DSL) and temperatures of the water sampled at each harvest in the deposit. The pH is generally not very acid (06.61 ± 1.00), the salinity and the conductivity are also low (63.01 and 124.37 respectively). The average dissolved solutes (DSL) levels are very low (90.95 ppm) while the water temperatures vary on average around 23 °C.

Table 2: Physicochemical parameters of water studied during every *L. natalensis* population collection

Periods	Population	P^H	Salinity (mg/l)	Conductivity ($\mu S.cm^{-1}$)	DSL (ppm)	T° water (°C)
April	112	8,24	71,47	105,6	77,51	28
May	136	8,12	71,28	118,21	85,3	28
June	27	7,46	65,27	137,32	102,2	27
July	24	5,32	60,34	136,24	105,4	26
August	0	5,14	57,34	138,2	111,3	25
September	0	6,01	58,23	137,58	102,4	26
October	36	5,98	56,21	135,24	98,31	23
November	70	6,56	59,02	134,32	87,14	24
December	87	6,23	62,41	128,4	81,01	19
January	99	6,13	62,25	112,5	82,4	18
February	153	6,97	66,27	109,6	85,1	21
March	193	7,12	68,12	99,27	73,37	22
Mean	78,08 ±62,81	06,61±1,00	63,01±5,257	124,37±14,42	90,95±12,34	23,92 ± 3,37
Correlation's		0,622*	0,751***	-0,918***	-0,894***	-0,305

The study of the relationships between these parameters and the population of *L. natalensis* (Table 2 ; Figure 7) shows the existence of a significant and positive correlation with the pH (r = 0.622; df = 11; p = 0.031), a very significant and positive correlation with Salinity (r = 0.751; df = 11; p = 0.005) and two very significant negative correlations with conductivity (r = -0.918; df = 11; $p < 0.0001$) and DSL (r = -0.894; df = 11; $p < 0.0001$).

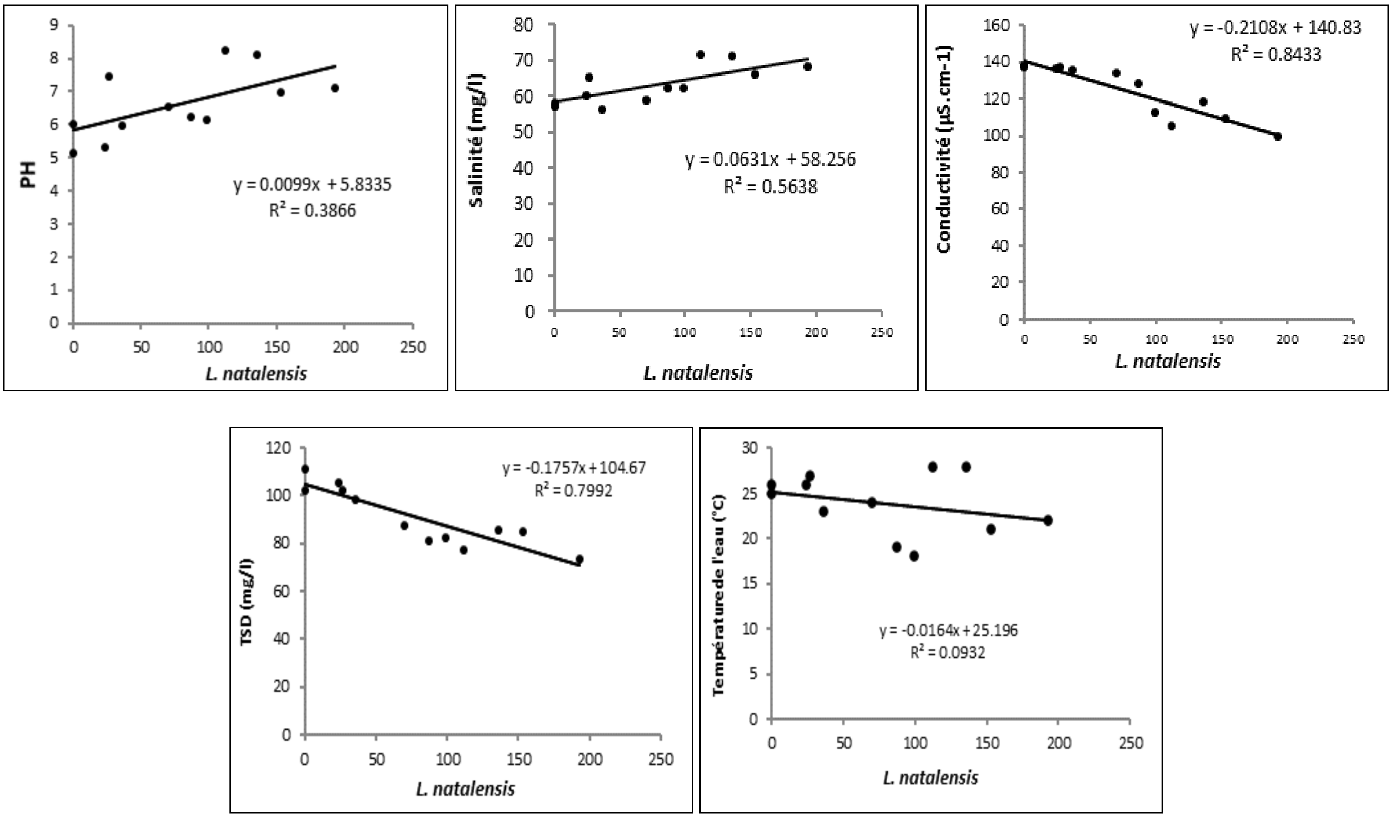

Fig 7: Linear's regression curve between *L. natalensis* density and physicochemical parameters of water

3.3.2.6 Relationship between evolution of *L. natalensis* population and fascioliasis dynamics of butchers near the roosts

Data analysis of the monthly frequencies of bovine fascioliasis in the main butcher's shop in Mokolo with the density of the population of *L. natalensis* in the Douvar dam show that (Figure 8), when the population of Limneas increases slightly between September and October, the infestation rates increase slightly and makes a first weak peak in November. But when the population of Limneas increases between January and May, the infestation increases and forms significant peaks between March and June.

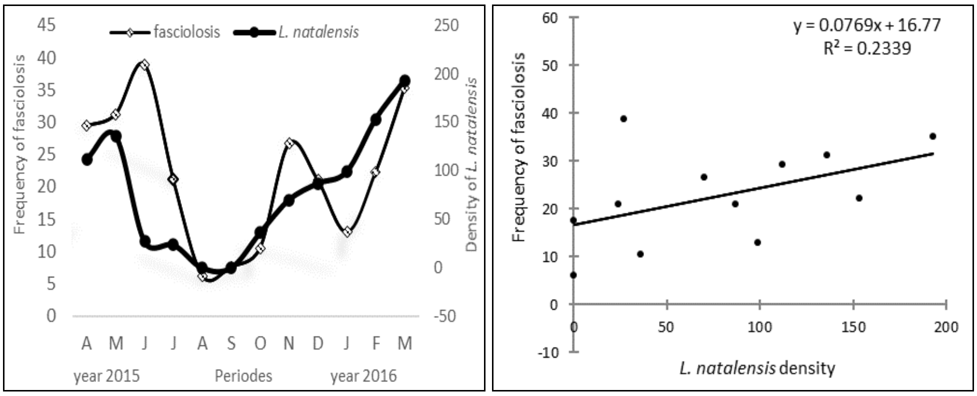

Fig 8: Variation between *L. natalensis* population and fascioliasis in butcher shops and their linear regression curve

The comparison of these results shows the existence of a positive correlation between the population of *L. natalensis* and the fascioliasis of the Douvar butcher's shop (r = 0.484; df = 11; $p<0.05$).

4. Discussion

Seven species of Gastropod Molluscs were identified in Douvar dam. This result is similar to that of [31] in the Oyan dam in Nigeria, but it differs from that of [41] and [7] which have counted 11 species respectively in the Gho-Manhasan and Jammu rivers in Egypt (belonging to the Classes of Gastropods and Bivalves) and in the dams of the center of the Ivory Coast. The absence of Bivalves in this deposit would be an indicator of the organic pollution of this environment because, according to [4] and [47], Bivalves only like good quality water while Gastropods can survive in polluted waters.

Planorbidae are more widespread and more abundant because they are fond of alkaline fresh water in the Sahelian regions [29]. Their importance is also linked to their capacity as intermediate hosts for trematodes [17]. The dominance of *Me. tuberculata* followed by *L. natalensis* and *B. pfeifferi* is similar to that observed by [33] in the Eleye Dam in southwestern Nigeria. But it differs from that [8] report in the Mara river basin in Kenya and Tanzania, and that [31] report in the Oyan reservoir in southwestern Nigeria (dominance of *L. natalensis*, *B. pfeifferi* and *B. africanus*). Their abundance is linked to their epidemiological role in the transmission of human and animal trematodes [38] and to their affection for freshwater located at high altitude [48] which would facilitate the dissemination of Limneas in all the plains to Lake Chad through the Mayo-Tsanaga hydrographic network [27]. The Shannon-Weiner index obtained is lower than that obtained by [8] in the Tiger river (H '= 0.518) and greater than that obtained in Pulangui lake (H' = 0.144) in the Philippines and shows that this breeding ground is very diverse in species of Molluscs.

The population of *L. nataensis* is highest between the end of the dry season and the middle of the rainy season with two peaks of high amplitudes in December and April, but decreases sharply or even completely after the middle of the rainy season. [40] also observe an increase in the population of Limneas at the beginning of the rainy season and a decrease during the long rainy season along the shores and pools of Africa. Many author have reported that the higher temperature cause death of molluscs. But [5] report in the middle of the dry season and a sharp decrease at the end of the latter in the vicinity of Bunia in Ituri region of Democratic Republic of Congo. In the Oued Aïn Chkef valley in Morocco, [13] observe an increase in the population in late December, then a peak in late February and a gradual decrease until early September. [36] report on some Nigerian river that the population of *L. natalensis* appears between the end of January and the beginning of February, increases to reach its maximum in March and April, and decreases rapidly depending on the time of withdrawal of water from the vegetation. In Egypt, [15] observe a maximum of gastropods in the fresh waters of Beheira in March and April, while in Nigeria the highest densities are observed between September and October and the lowest densities between June and July [38, 33]. The absenteeism period is between August and September corresponds to the period of floods characterized by heavy precipitation and high flow water currents [24, 26]. According to [1], floods and floods prevent the harvest of Limneas between August and October in Parakou in Benin.

In this Limneas population, young and juveniles (whose shells measures 11-15 mm high are seconded by those measuring at least 10 mm) are more numerous. This result reflects a very high birth rate and strong growth within the population. According to [22], in conditions of extreme stress, Limneas multiply very quickly and accelerate their growth to at least reproduce several times before dying. But the low density of older people would reflect their low life expectancy linked to the long period of drought coupled with extreme ambient heat and a rainy season coupled with the rise of torrential waters carrying most of the molluscs towards the Mayo-Tsanaga [52, 19].

The general observation of the study of the population of *L. natalensis* shows that it presents a single annual generation. Similar observations have been observed in the river of Niger valley in Benin [1], in Nigeria [31, 36,], in Victoria lake in Kenya [37] and in the small rivers of Lwiro with its surroundings in eastern Democratic Republic of Congo [42, 23]. However, [13] and [12] observe two annual generations in the populations of *L. truncatula* in Morocco. This difference is linked to climatic conditions [38, 14]. Juveniles appear very quickly in the Douvar reservoir because of the short duration of the floods which ends with the rains in September, which allows the mollusc's to repopulate the edges of the rock and the vegetation as of October [24]. The low density of aquatic plants favors the development and rapid emergence of Limneas [9].

Analysis of the relationships between ambient temperatures and the population of *L. natalensis* reveal the existence of a positive correlation and a significant influence of temperatures on the life and development of Molluscs. This result agrees with that of [28], who observes experimentally, optimal development of Limneas between 20 and 25 °C and lethal extremes between 0 °C and 40 °C. But the Limneas are more abundant between March and June on the banks of the deposit while the temperatures are very high (30-40 °C) because of the sharp drop in the water level in the ponds and causing a high concentration of Limneas [4, 28, 18]. However, the extrapolation of laboratory results with those from the field giving precise information on the effect of temperature requires nevertheless some caution. Temperature in the nature is not constant and undergoes both daily and seasonal variations [20]. However, according to [21] and [37], seasonal variations in the growth of molluscs, their survival rate and fecundity are more attributed to seasonal changes in biotopes and water levels than to the influence of temperature.

The population of *L. natalensis* is higher at the end of the dry season and at the middle of the rainy season and decreases considerably in August while the rains are higher. The decrease in the number of Limneas would be due to the phenomenon of leaching in view of the repeated precipitation. By comparing the data on the monthly densities of the Limneas with the average annual precipitation of the different sites, a negative correlation emerges. These observations agree with those of [50] and [49] who report an increase in the population of Limneas along the rivers of the ponds and flood plains during the rainy season and their decrease when the precipitation becomes more intense. This difference would not only be linked to the level of water which rises with the rains and especially to the speed of the currents [37]. In this reservoir, precipitation and torrential water descending from the mountains causing an overflow of water beyond the normal bed [19]. This causes a violent discharge of water with large quantities of debris and animal organisms (fish and Limneas ...) in the mayo-Tsanaga [25, 19]. According to [17] and [9], precipitation exerts two different actions during the rainy season. The first during the period from March to June when the stormy type of precipitation and spaced over time, on the contrary favor their growth by enriching the lodgings in nutritive substances and their laying by a renewed supply of water because of the long periods of sunshine and soil water deficit. The second action takes place between July and October when the rains are more abundant and more frequent, less sunshine and more saturated soils. The rains then cause large variations in the level of rivers and an acceleration of the current, which is detrimental to the maintenance of populations of molluscs.

The pH exerts a significant influence on the population of *L. natalensis*. [38] also report positive correlations between pH (6.9-7.5) and *Potadoma* sp in Nigeria. But [20] and [15] report negative correlations with *Biomphlalia* sp., and *B. unicolor* respectively. Positive correlations with *C. bulimoides*, *M. tuberculata* and *T. niloticus* have also been observed in Egypt [15]. But [31] and [16] found insignificant correlations in Nigeria. These results show that pH is a weak limiting factor in the distribution and abundance of freshwater Limneas.

DSL exerts a significant and negative influence on the population of *L. natalensis*. This result could be explained by the speed and the types of water flowing in the deposit. In fact, in this deposit, the water flow is not stable all year round. During the floods, the speed of the water currents increases more than normal, with results in high turbidity and a decrease in the population of Limneas. According to [11], molluscs are abundant in low turbidity waters. According to [21] and [20], the fertility of molluscs is higher in biotopes with low turbidity than in those with high turbidity. The numerical values of turbidity recorded in our study areas oscillate within acceptable limits to such an extent that they have no influence on the population dynamics of molluscs and the transmission dynamics of the parasite.

The significant negative correlation observed between the population of *L. natalensis* and conductivity similar to that of [31] who observes positive correlations with *P. marmorata*, *B. globosus* and *P. liberiensis* in the Eleyele dam in Nigeria. [16] also observed positive correlations between the conductivity and the population of the Limneas of the littoral part of Lake Alau in Maidougouri in Nigeria. However, [38] observes non-significant correlations between the shellfish population and the conductivity.

The salinity significantly influences the population of *L. natalensis* in the reservoir. [15] also report similar correlations between salinity and the population of *Cleopatra bulimoides* and *Biomphalaria alexandrina* in Egypt. However [16] report positive correlations in Nigeria. According to [11], Limneas have a very wide spectrum of tolerance towards salinity which allows them to live in unstable environments such as seguias and drainage canals.

The analysis between the evolution of the *L. nataensis* population and the monthly fascioliasis beef cattle infestations of those close to the roosts shows that fascioliasis rages all year round with positive correlations. These results are similar to those observed by [10] and [2] who show that fascioliasis is endemic in farms close to permanent biotopes. According to [40] and [4], in permanent biotopes the density of gastropods remains constant while the pathology rages permanently without visible clinical signs.

5. Conclusion

At the end of this study, we noticed that the Douvar reservoir is a very diverse breeding ground for Mollusc species. The population of *L. natalensis*, which presents a single annual generation, is more abundant between the end of the dry season and the beginning of the rainy season and less abundant in the middle of the rainy season. The abundance of young people and juveniles reflects the fact that this lodging is a reservoir for their proliferation and their sustainability in the water points of the region characterized by a hot and arid climate. In the days to come, it would be wise to determine the periods of great transmission of fasciolosis while determining the responsibility of the intermediate hosts.

6. Reference

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