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Reproductive biology and physico-chemical parameters of the African Giant Prawn, *Macrobrachium vollenhovenii* from a tropical freshwater river

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Abstract

The African giant prawn, *Macrobrachium vollenhovenii* is the most abundant and commercially viable of the prawn species in Cameroon. Preliminary investigation into its reproductive biology from a tropical freshwater environment (Yoke River), Southwest Cameroon was done from April 2011- March 2012. The study also examined the physic-chemical parameters that prevailed in the river during the cycle as a means to determining conditions necessary for their culture. 1679 specimens (955 males and 724 females) were captured using local baited bamboo and wire mesh traps. The overall sex ratio during sampling was 1:0.76 (male to female) which differed significantly (P<0.05) from the expected ratio of 1:1. A total of 203 berried females carrying 6,060,256 eggs (mean: 29,854 ± 798.94) were examined. The fewest eggs were 6,847 and the most 58,866. Average egg volume increased significantly (P< 0.05) during embryogenesis from 0.041 to 0.065 mm3 (58.5% increase). Positive correlations existed between body weight, total length, carapace length, ovary weight and fecundity (0.94, 0.87, 0.85 and 0.97 respectively) with the strongest relationships occurring between fecundity and body weight (0.94) and fecundity and ovary weight (0.97). Spawning occurred during the peak rainy season (July – September) which corresponded generally with periods of lower temperature, higher pH and dissolved oxygen. These findings indicate that *M. vollenhovenii* can be successfully cultured in captivity. Efforts should be intensified to document and fully exploit its reproductive potential in other regions so that they could be bred as an African equivalent of the now widely cultured *Macrobrachium rosenbergii*.

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Introduction

Freshwater prawns of the genus *Macrobrachium* are decapod crustaceans belonging to the family Palaemonidae. They are distributed throughout the tropics and subtropics on all continents except Europe (Holthuis, 1980). Species of the genus *Macrobrachium* are ecologically and economically important. As a result of this, they support artisanal fisheries in many developing countries, especially in Africa (Nwosu and Wolfi, 2006). Holthius (1949) reported that *Macrobrachium* species occurs from Senegal to Angola along the Atlantic Coast and can be found in almost all types of freshwater habitats and estuarine environments. The external morphology of these prawns is adapted to life in these environments. A definite feature of *Macrobrachium* is that the second walking legs are modified to form the chelae. Significant differences exist between the male and female. Mature males are considerably larger than females and the second walking leg is much thicker. The cephalothorax is also proportionally larger in the male than female while the abdomen is narrower in the males. The genital pores of the male are between the bases of the fifth pair of walking legs while those of the females are situated at the base of the third pair of walking legs (New and Singholka, 1982).

Among the species of freshwater prawns of the genus *Macrobrachium* found in Africa, *Macrobrachium vollenhovenii* (Fig. 1) is the species of commercial interest. It is a viable fishery in most countries in African and it is widely known as the African giant prawn (Gabche and Hockey, 1995). It is cherished by indigenous consumers because of its large size, pleasant taste and nutrient value with crude protein values of 68.77 – 71.37%; carbohydrate content of 1.87 – 3.32%; lipid content of 3.53 – 5.60%; Ash content of 5.17 – 6.83%; moisture content of 77.58 – 82.64% and saturated fatty acid values that ranged between 28.87 – 34.81% (Dinakaran and Soundarapandia, 2009). It is the largest prawn in west Africa and individuals of greater than 20cm total length have been encountered (Okechukwu *et al.*, 2010). This interest has prompted scientific studies in many areas, including reproduction. Studies in this area were initially conducted to generate data that could be used in aquaculture, including studies on larval development and gonadal maturation (Alexandre *et al.*, 2010). Its growth and mortality have been reported and it has been recommended that this species should be used for aquaculture cultivation, as an African equivalent of the now widely cultured *M. rosenbergii* (Willfùhr-Nast *et al.*, 1993).

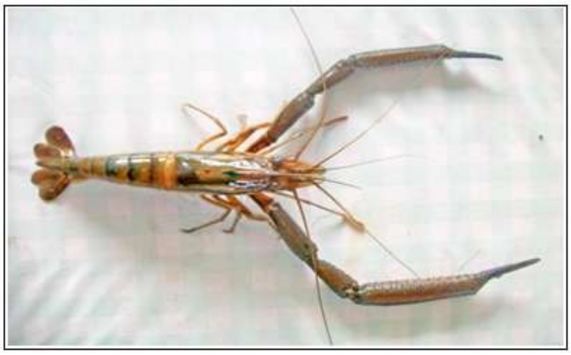

Fig. 1. Mature specimen of *Macrobrachium vollenhovenii* from Cameroon.

In Cameroon, *Macrobrachium vollenhovenii* lives in rivers that are directly or indirectly connected to the Atlantic Ocean. This is explained by their life cycle which includes larval stages that depend on brackish water for their survival. The lone published article on this species in Cameroon is that on its growth and mortality in River Lobe in Kribi, South Region by Gabche and Hockey (1995). The present lack of knowledge about the biology of many freshwater decapod carideans is worrisome, because of the intense fishing exploitation and subsequent changes in their habitats after their study. The objective of this study was to examine the reproductive aspects of *Macrobrachium vollenhovenii* in River Yoke, to determine aspects of its reproduction, including, breeding season, fecundity, sex ratio, egg size and egg volume and the water quality (physico-chemical) parameters that prevail in the aquatic environment during such activities. Such studies are expected to generate data that could be useful in aquaculture thus indirectly contributing to food security in the nation.

Materials and methods

Sampling site

The study was carried out in Yoke River in Muyuka of the South West Region of Cameroon (Fig. 2) for a period of one year (April 2011 – March 2012). The river is located between latitude 04°18'16.3" N and longitude 009°25'142" E. The river in its upper reaches flows through a secondary rainforest zone, while its terrain in the vicinity of Muyuka (site of study) is presently subjected to extensive land clearing and farming activities. The study site is a meandering stretch of about 1 km long. The riparian vegetation of this area could be described as farm bush. Land clearing activities for farming and sand dredging are the predominant form of land use. For most of its length, the river is flanked by Indian bamboo trees (*Bambusa* sp.), rubber trees (*Havea brasiliensis*) and palm trees (*Elaies* sp.) which provide a canopy. In areas where tree cover is absent, the marginal vegetation is composed of grasses such as *Panicum* sp. and ferns. The mid-channel of the river is mostly open and the current velocity is low. The substratum is predominantly clay and silt. The stretch could be described as non-tidal clear water.

Fig. 2. Map of coastal area of Cameroon showing geographical location of sampled site.

Water quality parameters

Some important water quality (physico-chemical) parameters of the river, namely temperature, pH, salinity and dissolved oxygen were determined monthly. They were measured according to the standard procedures for examination of water and waste water (APHA/AWWA/WEF, 1998). The water was collected at a depth of about 1m with the help of fishermen. The temperature and pH were determined with a pocket pH meter that also measures temperature (EXTECH, Model pH 60); the dissolved oxygen was determined with a portable dissolved oxygen (D.O.) meter (EXTECH, Model D.O 600) and the salinity with a portable refractometer (Model, RHS-10ATC).

Sampling for prawns

Specimens of the genus *Macrobrachium* were collected monthly from April 2011 – March 2012 (1 year) making a total of 12 collections. A total of 1679 specimens were collected (955 males and 724 females). The prawns were captured using different methods such as cast nets, hand nets, and small cage traps made up of either wire mesh or bamboo. These traps were baited with palm nuts, cassava tubers and coconut prior to casting in the water. The prawns were usually collected in the morning in an ice chest and taken to the laboratory for further analysis. Gravid females (egg bearing females) were placed in separate plastic bags to prevent egg loss during transportation. In the laboratory, sex differentiation was done by visual observation of the base of the fifth pair of walking legs (pereiopods) and by the means of the observation of the endopodite morphology of the second pair of pleopods, as proposed by Ismeal and New (2000). The samples were later separated and identified to the lowest taxon using classification keys proposed by Konan *et al.* (2008) and Monod (1980). Biometric measurements recorded for each prawn were Carapace length (CL), Abdominal length (AL) and Total length (TL) to the nearest 0.01mm using a digital calliper (range 0-200mm). The wet weights

(WT) of the prawns were also measured with an Ohaus electronic balance to the nearest 0.1g (Model CS 200, capacity 200g).

Fecundity

To estimate fecundity, all eggs were removed from the brood chamber of the gravid females and weighed using an electronic balance (Model Scout Pro SPU402, capacity 400g, precision 0.01g). Prior to weighing of the eggs, excess surface water was blotted using a paper tissue. Three egg subsamples were removed from each of the egg clutch, weighed and placed in 70% ethanol to segregate them. All the eggs in the 3 portions were later counted under a compound microscope and simple proportion was used to determine the total number of eggs per clutch (Dinh *et al.,* 2009). Egg development was categorized according to the criteria proposed by Luis and Ingo (2009).

Stage I: Berried females with orange eggs. Eggs recently extruded; yolk uniform; no eye pigments visible.

Stage II: Berried females with brown eggs. Eye pigments of eggs barely visible.

Stage III: Berried females with grey eggs. Eyes clearly visible and fully developed.

Egg size and egg volume

A total of at least 30 eggs were separated from ovigerous females in the different stages to measure the length and width under a compound microscope (Axiostar ZEISS) equipped with a calibrated ocular micrometer. Egg volume was calculated according to the following formula:

$v = \pi * l * (h)^2$ (Corey and Reid, 1991).

Where; "l" is length,"h" width in mm and $\pi = 3.14$.

Sex ratio

Sex ratio was obtained by the identification of the number of males and females in the monthly samples. These data were represented on a monthly chart throughout the study period. Chi-square test was used to test the significance of the sex ratio using the formula:

$$x^2 = \sum (O - E)^2 / E$$

Where; O = observed frequency, E = expected frequency.

Statistical analysis

All the data collected were entered into Microsoft Excel 2007. The Statistical Package for Social Science (SPSS) version 17 was used for the data analysis. The differences in sex ratio were analysed and tested for significant divergence from the expected 1:1 ratio by using a Chi-square (χ^2) goodness of fit test (Zar, 1999).

For each of the three embryonic stages, the average egg volume was calculated with standard deviation and ANOVA was applied to test for significance between the three stages (Luis and Ingo, 2009). Pearson's linear regression was used to assess the correlation between ovary weight and the fecundity, carapace length (CL) and fecundity, prawn weight and fecundity and finally, total length and fecundity. All statistical inferences were based on a significant level of $\alpha = 0.05$.

Results

Water quality (physico-chemical) parameters of river Yoke

During the period of the study, the monthly temperature ranged from 24.2 – 25.8°C with a mean of 25.2°C ± 0.15 (Table 1). The lowest temperature of 24.2°C was recorded in February and the highest was 25.8°C recorded in December. The amount of dissolved oxygen fluctuated between 5.90 – 7.66 mg/l with a mean and standard error of 6.80 and 0.14 respectively (Table 2). The lowest dissolved oxygen 5.90 mg/l was recorded in October and the highest dissolved oxygen 7.66 mg/l in September. Monthly salinity throughout the entire period was equal to 0 ppt. The peak reproductive period corresponded generally with a period of lower temperature, higher pH and dissolved oxygen.

Table 1. Mean and ranges of water quality parameters of the Yoke River from April 2011 to March 2012.

Parameter	Mean ± S.E	Range
Temperature (°C)	25.2 ± 0.15	24.2 – 25.8
Dissolved oxygen (mg/l)	6.80 ± 0.14	5.90 – 7.66
Ph	7.5 ± 0.10	6.8 – 7.9
Salinity (ppt)	00	00

Sex Ratio

During the 12 months of sampling, 1679 individuals of *M. vollenhovenii* from the Yoke River were captured, including 955 males and 724 females, resulting in a (M: F) sex ratio of 1: 0.76 in favour of males. A goodness of fit test was performed and the results showed that the ratio was significantly different from a 1:1 ratio (χ^2 = 31.82, P<0.001). The monthly chi-square values for *M. vollenhovenii* showed that there were significant differences from a 1:1 ratio except in October and March (Table 2). Males dominated the catch in 9 out of the 12 months of collection and the sex ratio varied between 1: 0.12 in May to 1: 2.65 in September.

Fecundity

A total of 203 berried females were captured. All the eggs of these berried females were counted resulting in a total of 6,060,256 eggs with a mean of 29,854 (± 798.94) eggs per female. The female with the fewest egg had 6,847 eggs (24.43 mm CL and 82.40 mm TL), and the one with more eggs carried 58,866 (39.78 CL mm and 133.16 mm TL). There was a significant relationship (P<0.05) between body weight and fecundity; total length and fecundity; carapace length and fecundity; and ovary weight and fecundity. The regression equations for the positive relationships were as follows: Y = 512 +1209, x and r = 0.94 (Figure 3), Y = – 60968 + 827.7, x and r = 0.87 (Figure 4), Y = – 46003 + 2361,x and r = 0.85 (Figure 5), Y = 471.8 +12313,x and r = 0.97 (Figure 6). There was a high correlation between total length/fecundity (r = 0.87) and carapace length/fecundity (r = 0.85) while a strong correlation existed between body weight/fecundity (r = 0.94) and ovary weight/fecundity (r = 0.97). Fecundity increases with the carapace length. The number of eggs also increases with an increase in weight and total length. Generally, fecundity was linear and a function of the body weight and carapace length.

Table 2. Chi square values for monthly sex ratio of *Macrobrachium vollenhovenii.*

Months	Numbers M	Numbers F	Sex ratio M: F	χ^2	Significance level
April	75	40	1: 0.53	10.652	***
May	93	11	1: 0.12	64.654	***
June	76	17	1: 0.22	37.430	***
July	75	120	1: 1.60	10.385	***
August	135	91	1: 0.67	8.566	***
September	69	183	1: 2.65	51.571	***
October	79	85	1: 1.08	0.220	___
November	70	40	1: 0.57	8.333	***
December	59	36	1: 0.61	5.568	**
January	108	30	1: 0.28	44.087	***
February	74	32	1: 0.43	16.642	***
March	42	39	1: 0.93	0.111	___

___ = not significant; * P= 0.025-0.05; ** P= 0.01-0.025; *** P= 0.001-0.01.

Egg Size and Egg Volume

A total of 386 eggs were measured collected from 30 ovigerous females. From this number, 131 eggs were in stage I, 130 eggs stage II and 125 eggs stage III. The average egg size increased from 0.26 at Stage I to 0.34 mm at Stage III (Table 3). Average egg volume increased significantly during embryogenesis ($P < 0.05$) from 0.041 to 0.065 mm^3 representing an overall increase of 58.5% (Table 3).

Table 3. *Macrobrachium vollenhovenii*. Average egg size (mm) and egg volume (mm^3) for the three embryonic developmental egg stages.

Egg stage	n	Mean egg length (mm)	±SD	Minimum	Maximum
I	131	0.26	0.018	0.22	0.30
II	130	0.30	0.023	0.26	0.35
III	125	0.34	0.037	0.28	0.41

Egg stage	n	Mean egg volume (mm^3)	±SD	Minimum	Maximum
I	131	0.041	0.007	0.022	0.050
II	130	0.052	0.008	0.038	0.063
III	125	0.065	0.014	0.047	0.101

n: number of observations; SD: Standard deviations.

Reproductive Period

Of the 724 females collected, 203 (28%) were ovigerous and 521 (72%) were non-ovigerous female. Ovigerous *Macrobrachium vollenhovenii* females were not collected throughout the entire year and the recurrence of non-ovigerous females was higher than ovigerous in eleven of the twelve months of collection. The ovigerous females were relatively small in number during the periods between May – June and October – December.

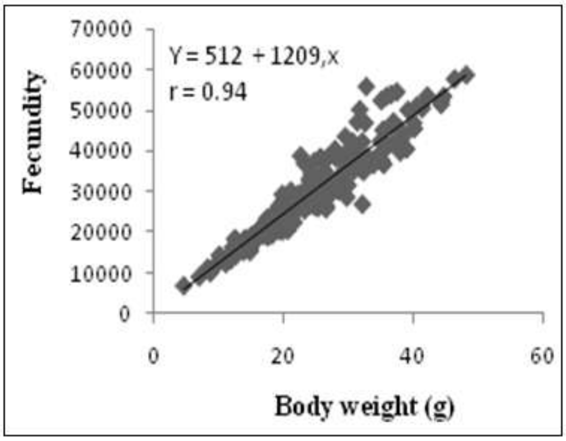

Fig. 3. Relationship between body weight and fecundity in *Macrobrachium vollenhovenii* from Yoke river.

These females were also completely absent in the months of January – April (Fig. 7). Despite the fact that ovigerous females were not captured in all the months, the gonads of the non- ovigerous females were usually found in advanced stage of development which indicated that the reproductive period was continuous.

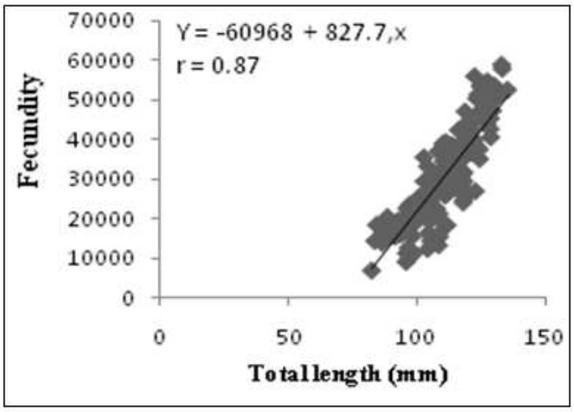

Fig. 4. Relationship between total length and fecundity in *Macrobrachium vollenhovenii* from Yoke river.

Discussion

The values of the water quality (physico-chemical) parameters observed in the study river were found to be within the ideal range cited by Sampaio and Valenti (1996) for *M. rosenbergii*, which were cultivated in ponds and Francis and Jacob (2007) for *M. dux* captured from their natural environment. The water quality parameters did not seem to have an influence on the variation in the natural population size and composition of *M. vollenhovenii* available for collection in the Yoke river. Although the numbers of males, non-ovigerous and ovigerous females available for collection varied considerably throughout the collection period, the physico-chemical parameters presented little or no variation over the same period. Ammar *et al.* (2001), while analyzing the reproductive biology of *M. olfersi* collected on the Island of Santa Catarina, Brazil, pointed out that salinity oscillation may have influenced the size of the population and variations of the individuals. The result differs from that obtained in this study, especially as the salinity observed throughout the study period was zero.

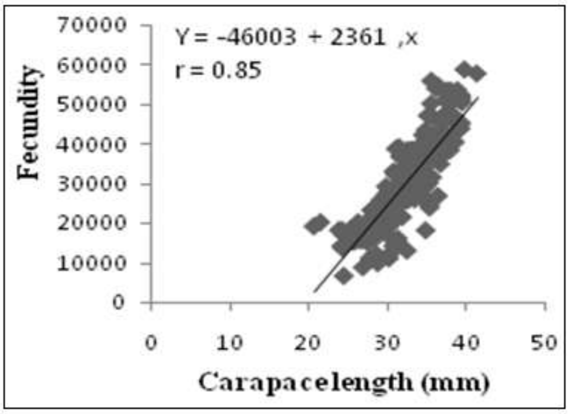

Fig. 5. Relationship between carapace length and fecunidy in *Macrobrachium vollenhovenii* from Yoke river.

In the present study, the range of absolute fecundity observed was 6,847-58,866 eggs and the mean number of eggs per female was 29,854 ± 798.94 in *M. vollenhovenii* from Yoke river. A strong correlation existed between the fecundity and body weight of berried females (r = 0.94). The high egg count observed in this study might be related to the highly prolific nature of these prawns and the favourable environmental factors that prevailed in the study site (temperature, pH and dissolved oxygen). Similar trends in correlation was observed in other shrimps studied such as *Penaeus latisculatus* (Penn, 1980); *Macrobrachium vollenhovenii* (Udo and Ekpe, 1991) from Nigeria and *M. nipponense* (Masshiko, 1990). Reports on the number of eggs borne by a berried *M. vollenhovenii* female varies tremendously throughout the world. Ville (1970) and Miller (1971) from Nigeria reported 300-1,000 eggs and 12,000-45,000 respectively while Marioghae and Ayinla (1995) reported 49,979-401,212. These numbers greatly differ from what was obtained in this present study. These differences may be due to interaction between the different environmental and climatic conditions prevailing in the regions where these aquatic bodies are located. The high correlation between female weight and fecundity reported for *M. vollenhovenii* was however similar to that reported by Ovie (1986) for *M. macrobrachion*. This may suggest that there are differences in the pattern of allocation of food energy by the animals at different sizes. Usually in larger individuals which have lower growth rates, much of the available energy is devoted to egg production as compared to smaller individuals where a large fraction of the energy may be devoted to growth rather than egg production (Cox & Dudley, 1968).

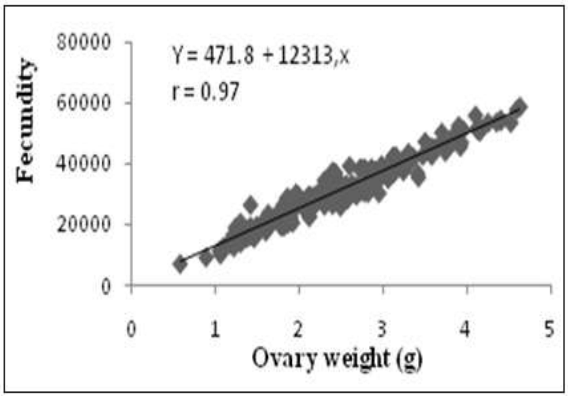

Fig. 6. Relationship between ovary weight and fecundity in *Macrobrachium vollenhovenii* from Yoke river.

The egg length of *M. vollenhovenii* from the Yoke river increased from 0.22 - 0.41mm and the egg volume increased significantly (P<0.05) from 0.022 - 0.101mm³. The increase in egg length and volume during the incubation period of the prawns might be as a result of gradual water intake during embryogenesis. The swelling of these eggs properly permitted the development of the embryo. The overall egg volume increased was 58.5%. Similar increase in egg volume was also reported for other *Macrobrachium* species: *M. amazonicum* (Heller, 1862): 33.4% (Odinetz-Collart and Rabelo, 1996); *M. olfersi*: 30.7% (Mossolin and Bueno, 2002) and *M.*

potiuna: 28.0% (Nazari *et al*., 2003). The size of eggs of *M. vollenhovenii* recorded from the Yoke River were smaller when compared to the results of Rutherford (1971) who reported the egg diameter of between 0.5 and 0.7 mm in Cape Coast, Ghana. Deekae and Abowei (2010) who reported 0.40 and 0.78 mm from Luubara creek, Nigeria.

The male to female sex ratio of 1: 0.76 in this study was significantly different (P< 0.05) from the expected 1:1 sex ratio. This could be as a result of the location of the sampling river which was inland far away from the estuarine areas that usually harbours the females during their reproductive periods. Similar results were obtained by Ammar *et al.* (2001) studying *M. olfersii* population of the coast of Santa Catarina and Mossolin and Bueno (2002) studying samples of the same species collected in the coastal waters of the state of São Paulo who found a predominance of males over females at a proportion of 1: 0.71 and 1: 0.23, respectively. Cases where females predominated over males were also encountered by other authors as was the case in Inyang (1981) studying the population of *M. felicinum* and Marioghae (1982) studying the populations of *M. vollenhovenii* and *M. macrobrachion,* all of which have a sex ratio of male to female as 1: 1.2.

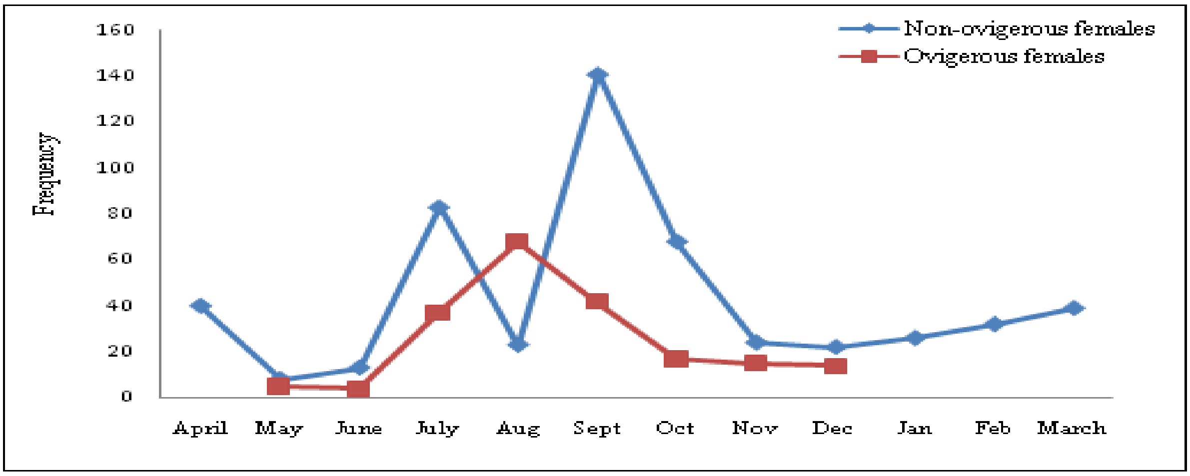

Fig. 7. Monthly frequency distribution of ovigerous and non-ovigerous females of *Macrobrachium vollenhovenii* collected from Yoke river.

The occurrence of ovigerous *M. vollenhovenii* from the Yoke river during the collection period indicates that this species is capable of reproducing throughout the year. Despite the fact that these ovigerous females were not collected in all the months, they are known to have a continuous reproduction because of the mature ovaries that were seen throughout the year. The predominance of non-ovigerous females during most of the months of collection may be related to the difficulty in catching ovigerous females, since during the reproductive phase, the ovigerous females migrate to estuary regions where egg incubation occurs or they may look for shelter in protected areas, that is, in marginal plants to avoid predation pressure. This observation was similar to the one by Chaves and Magalhães (1993); Bragagnoli and Grotta (1995); Bialetzki *et al.* (1997); Porto (1998) and Silva *et al.* (2002) for samples of *Macrobrachium* species collected from different parts of the world. Pinheiro and Hebling (1998) assert that the reproductive period of freshwater decapods crustaceans is closely associated to the rainy period and to the photoperiod of the region where it happens. Chaves and Magalhães (1993), Porto (1998) and Silva *et al.* (2002) observed that ovigerous *M. amazonicum* females were more abundant in the rainy season than other times of the year. This was similar to the situation that prevailed with *M. vollenhovenii* collected from the Yoke river during the study period with a peak collection of ovigerous females in August.

Conclusion

The present study has revealed strong relationships between fecundity and carapace, fecundity and total length, fecundity and ovary weight, as well as between fecundity and body weight in *Macrobrachium vollenhovenii* from the Yoke river. The results showed that there is a tendency for fecundity to increase with prawn size, suggesting that there are differences in the pattern of allocation of food energy by the animals at different sizes. The increase of fecundity with body size seems to be a rule that is applicable to many crustaceans. From the findings in this investigation, *M. vollenhovenii* in Cameroon can be cultured in captivity like some other species of the genus, although smaller in size than *M. rosenbergii*, which has been widely studied in other parts of the world. Efforts should therefore be intensified to fully document and exploit the reproductive output of pond cultured broodstock of this species.

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