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Study of fertility and oocyte characteristics of Macrobrachium sollaudii (DE MAN, 1912) (CRUSTACEA, PALAEMONIDAE) in a natural environment: Case of the lower sub-basin of the Tshopo River

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Abstract:

This article focused on the fertility and egg characteristics of Macrobrachium sollaudii in their natural environment, specifically in the lower basin of the Tshopo River. Data were collected using a net made from mosquito mesh, suitable for shallow areas. Collecting was often done at night, the time when the shrimp are active. After capture, all individuals were preserved in 70% alcohol and then taken to the laboratory for measurement and identification. Holthuis's dichotomous key (1980) was used for identification. The results show that the fertility of Macrobrachium sollaudii varies depending on the size of the females, and egg size increases with the embryonic stage. There is a positive correlation between the size of the females and that of the eggs. Finally, the comparison between the surveyed rivers shows significant differences in terms of fertility and oocyte size, likely linked to local ecological conditions.

Keywords:
Fertility; oocyte; Tshopo

I. Introduction

Macrobrachium is one of the most widely spread genera of freshwater shrimp in tropical and subtropical regions. To date, this genus includes over 200 species (IUCN, 2013), but only about ten are commercially exploited, mainly through fishing and aquaculture (Griessinger et al., 1991). The freshwater waters of the Congo (DRC) are home to around eight species of the Macrobrachium genus. These include Macrobrachium chevalieri (J. Roux, 1935), Macrobrachium vollenhovenii (Herklots, 1857), Macrobrachium dux (Lenz, 1910), Macrobrachium sollaudii (De Man, 1912), Macrobrachium macrobrachion (Herklots, 1851), Macrobrachium lujae (De Man, 1912), and Macrobrachium foai (Coutière, 1902) (IUCN, 2013). This group of shrimp is locally known by the common name 'Kosa Kosa.' However, this generic name doesn't allow for distinguishing the different species of the Macrobrachium genus that are harvested (Tshimuanga et al., 2022).

Macrobrachium sollaudii is one of the giant shrimp species found in the freshwater of the Democratic Republic of Congo. Highly valued in the local market because of its size and many culinary possibilities, it plays a role in the national economy. Its harvesting not only brings in foreign currency but also creates jobs and improves food security thanks to its protein richness for the population (Vincke and Philippart, 1984).). It seems to have fertility potential that is particularly favorable for aquaculture development (Lawal-Are and Owolabi, 2012; Kingdom and Erondu, 2013). Despite its economic and nutritional importance, Macrobrachium sollaudii remains one of the least studied species of the Macrobrachium genus from a biological standpoint, especially regarding fertility and oocyte characteristics.

A study on fertility and oocytes makes it possible to determine reproductive potential. It provides basic data on the size, number of eggs, and stages of oocyte maturation. This data serves as a baseline for understanding the reproductive cycle, assessing the population's renewal capacity, and guiding fishing management strategies as well as the potential development of aquaculture (De Man, 1912). This article aims to fill the gaps in knowledge about the fertility and oocyte characteristics of the species Macrobrachium sollaudii in the wild, with a view to possible domestication in a fish pond in Kisangani.

1.1 Objective

The general objective is to analyze the fertility and oocyte characteristics of the species Macrobrachium sollaudii in the lower basin of the Tshopo River.

II. Research Method

2.1 Study Area

This study was carried out in the waterways of the Lubuya-Bera area. Data were collected from the Alibuku River as well as from the Mai-Pembe and Agbukobe streams, located about 28 km from the city of Kisangani. The geographical coordinates of the sampling sites are as follows: the Alibuku River is located between 00°42'0813" N and 025°15'4962" E, at an altitude of 417 m; the Mai-Pembe stream between 00°41'6720" N and 025°15'7403" E, at an altitude of 414 m; and the Agbukobe stream between 00°14'8309" N and 025°15'3425" E, at an altitude of 404 m.

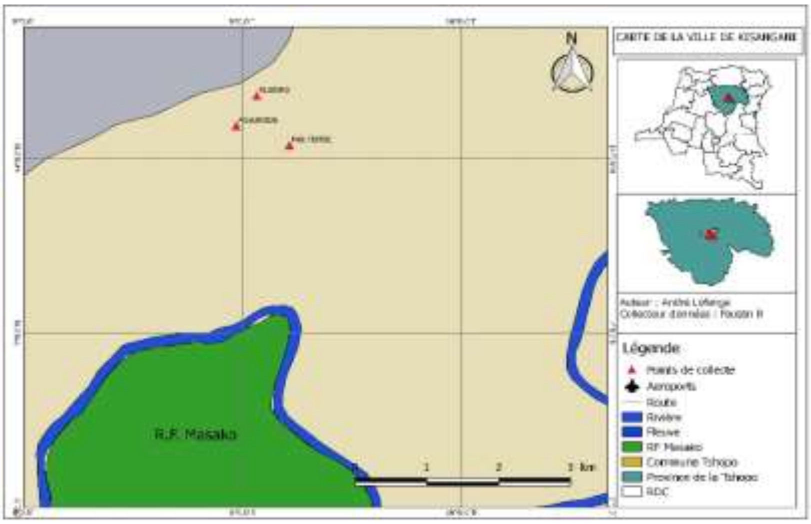

Figure 1. Map of the sampling waterways in the Lubuya-Bera area

2.2 Methods

a. Sampling

Data collection was carried out at night, a time when shrimp are most active. Individuals were captured using a net made from mosquito netting, suitable for shallow areas and quick movements. The collector used a headlamp to make it easier to observe the environment. The method involved gently dipping the net in shallow areas while disturbing the shrimp with a machete, which encouraged them to move toward the net, which was then quickly lifted. The contents were poured into a container to sort the individuals before transferring them to preservation jars. The shrimp were fixed in 70% ethanol, according to the protocol described by Cambridge and Reed (2004). At the end of each outing, the samples were taken to the hydrobiology and aquaculture laboratory of the Faculty of Natural Sciences for identification and measurements.

b. Identification and measurement

In the laboratory, the identification of *Macrobrachium sollaudii* specimens was carried out using Holthuis' (1980) dichotomous key, based on the observation of the morphology of the rostrum, claws, and telson. For specific analyses, ovigerous females were separated from non-ovigerous females to allow a comparative study of fecundity and oocyte characteristics. The total length of each ovigerous female was measured from the tip of the rostrum to the end of the telson using a measuring tape. The total weight of the individuals was determined using a SARTORIUS electronic balance (Celada et al., 1991). As for the fecundity analysis, the eggs were counted using a direct method, which involved manual counting. They were carefully detached from the pleopods using fine tweezers or a brush, following the technique described by Ching & Velez (1985). The oocytes were then placed in a dish and hydrated using a wash bottle to maintain their volume and prevent any deformation during observation. For the analysis of oocyte characteristics, a sample of five eggs per ovigerous female was taken. The length of each egg (in mm) was measured using a microscope equipped with an ocular micrometer, according to the method described by Celada et al., (1991). The observations were carried out with an OPTIKA brand microscope, using a 2X magnification.

c. Identification of Embryonic Stages

The eggs were classified based on their visual appearance, allowing us to distinguish three essential stages of development, which are:

1) Stage 1: the yellow color of the eggs, corresponding to the beginning of embryonic development.
2) Stage 2: the white color of the eggs, indicating a transitional phase of embryonic development.
3) Stage 3: the grayish color of the eggs with the appearance of eyes, characterizing an advanced stage where the embryo is clearly visible.

2.3 Statistical Analysis

a. Fertility Analysis

In biology, fertility is generally expressed by the total number of eggs carried by a brooding female (Pandian & Balasundaram, 1982). The most commonly used formula is

$F = N/n$

Where:

• F = average fertility per female
• N = total number of eggs counted in all brooding females
• n = number of brooding females analyzed

b. Weighted Variant (correlated with size or weight)

Fertility can also be expressed based on the female's biometrics:

$F = N/L$ or $F = N/P$

Where:

- L = total length of the female (from rostrum to telson)
- P = total weight of the female

This allows calculation of relative fertility (number of eggs per mm or per gram), which is useful for comparing individuals of different sizes.

c. Analysis of Oocyte Characteristics

To analyze oocyte characteristics, geometric and statistical relationships are often used to estimate the size, volume, and sometimes the surface of oocytes. The formulas used are:

d. Ellipsoid volume

To assess the volume of eggs, a standard formula is applied to the ellipsoid shape. This formula is as follows:

$$V = \frac{\quad}{6} * L * l^2$$

Where: V represents the volume (mm^3), L the length, and l the width of the egg (Corey & Reid, 1991; Anger & Moreira, 1998).

e. Proportional Volume

The proportional volume of the eggs was determined using a mathematical relationship expressed as follows:

$$V = K * L^3$$

Where: V represents the volume (mm^3), L the length of the egg, and k an empirical constant derived from morphological observations. 4.3.3. Length/width ratio the length/width ratio of the oocytes was calculated using the following formula:

$$R = \frac{L}{L}$$

III. Result and Discussion

The results of this study are presented in the form of tables and figures, allowing a clear and structured visualization of the data obtained.

3.1 Determination of the fecundity of ovigerous females

a. Number of eggs laid by females

The number of eggs laid by females is shown in Figure 2 and Table 1 below.

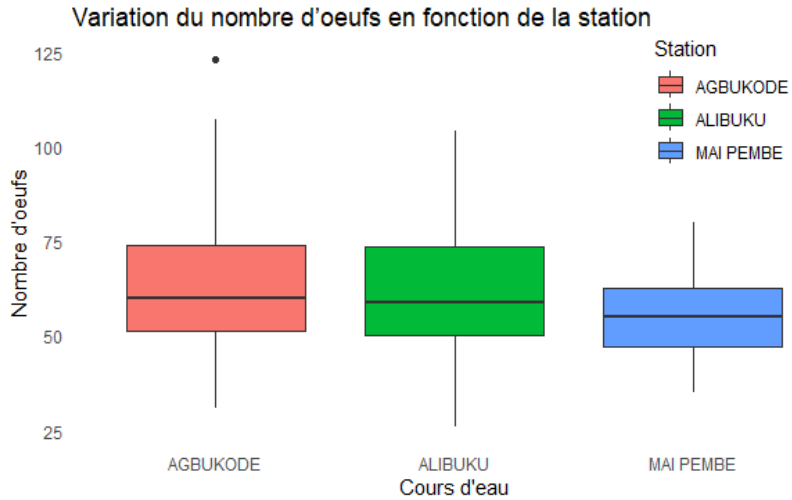

Figure 2: Variation in the number of eggs per female depending on the collection site

According to Figure 2, the number of eggs laid by females ranged from 26 to 123, with an average of 73.20±30.68 eggs. Statistical analysis showed a p-value of 0.02183, indicating a significant difference at the $p<0.05$ level.

The clear explanation of the figure above is actually given in Table 1, which shows the number of eggs per female.

Table 1. Number of eggs per female by sampling site

Site	**Min**	**rage±standard**	**deviation**	**Total**
AGBUKOBE	*31*	128	24,26 ± 33,25	2717
ALIBUKU	*26*	104	25,15 ± 32,63	2792
MAI PEMBE	35	80	13,71 ± 24,66	1727
Total	**26**	**123**	**20,73 ± 30,68**	**7236**

Table (1) shows that the AGBUKOBE and ALIBUKU sites have the highest fertility values, ranging from 104 to 128 eggs, while the lowest value is recorded in the MAI PEMBE stream. The highest average ± standard deviation values are noted in the Agbukobe stream and the Alibuku river (24.26 ± 33.25 and 25.15 ± 32.63), and the lowest value is recorded in the MAI PEMBE river.

b. Correlation between female size and oocyte dimensions

The correlation between female size and oocyte dimensions is shown in Figure 3.

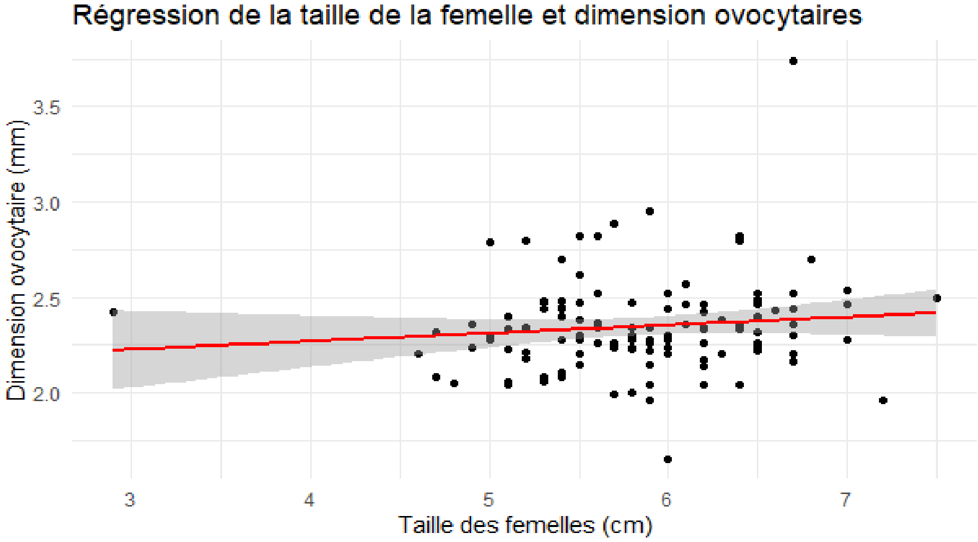

Figure 3: Regression of female size and egg dimensions

This figure shows that the correlation between female size and the average length of their eggs is positive, and the variation is not significant (r=0.01, p>0.05).

c. Body size-fecundity correlation

The body size-fecundity correlation is explained in the figure (4) below.

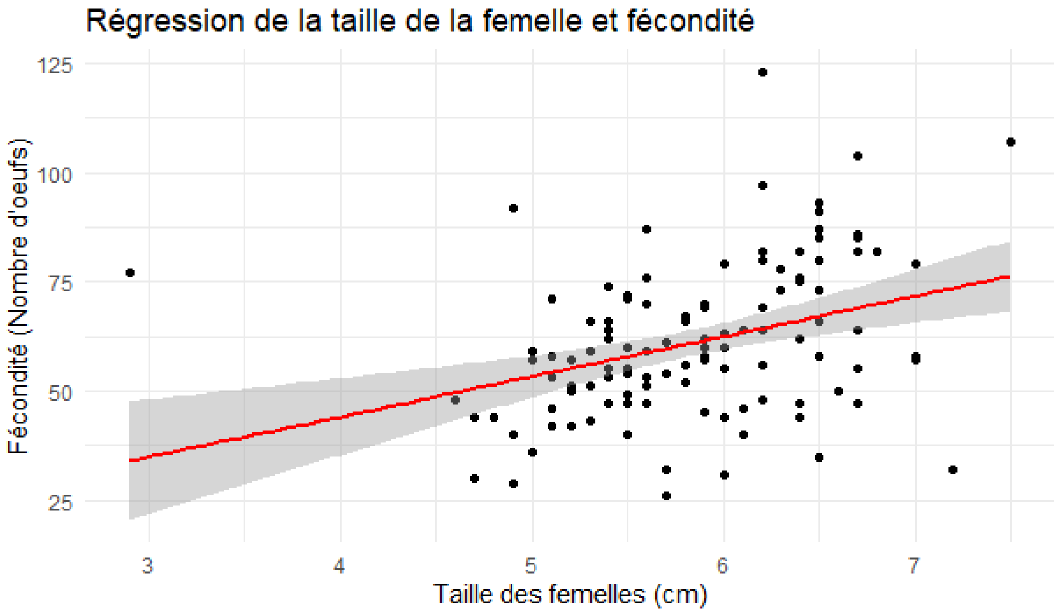

Figure (4): Body size-fertility correlation

It appears from Figure 4 that a linear regression analysis revealed a moderate positive correlation between the size of females and their fertility (r=0.12, p<0.05).

d. Body weight-fertility correlation

The correlation between the body weight of females and their fertility is shown in Figure 5 below.

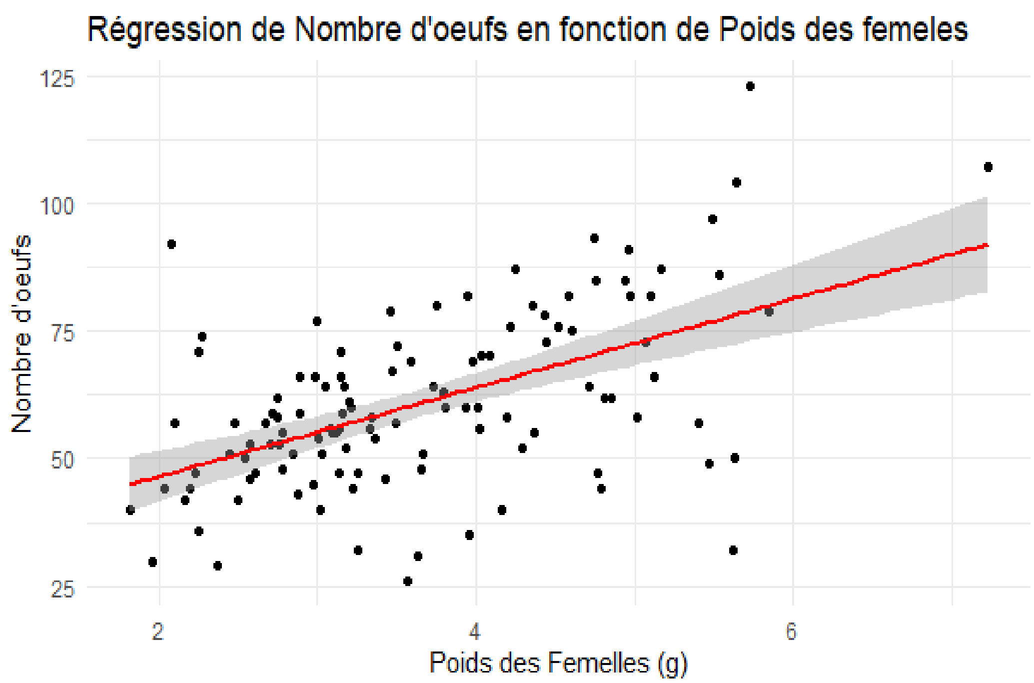

Figure (5): Body Weight-Fertility Correlation

Figure 5 shows a positive correlation between female body weight and their fertility ($r = 28\%$, $p < 0.05$).

4.2 Stages of Embryonic Development

a. Number of Eggs According to Embryonic Development Stages

The number of eggs according to the embryonic stage, across the different sites, is shown in Figure 6 below.

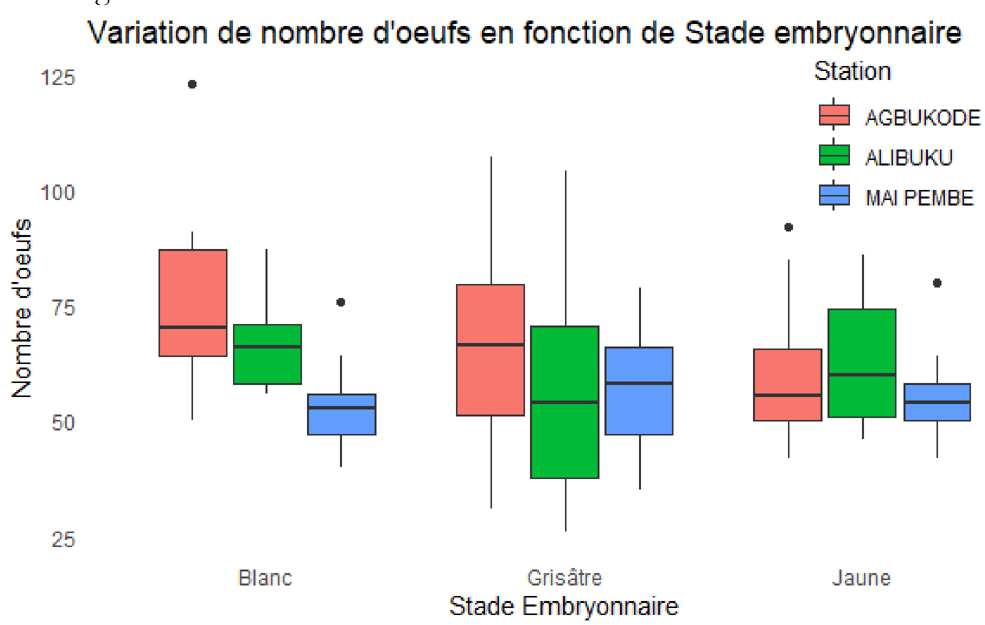

Figure (6): Variation in eggs according to embryonic stage by site

Figure 6 shows that at embryonic stage 2, characterized by a whitish coloration of the eggs, a high number of eggs was recorded in the Agbukobe stream. It was followed by embryonic stage 3, marked by a grayish coloration, and then by embryonic stage 1, where the eggs have a yellowish coloration and a relatively low number of eggs. However, these differences are not statistically significant ($p>0.05$).

b. Comparison of average size and weight of ovigerous females between watercourses

The results of the comparison of the size and weight of the females are shown in Figures (7) and (8) below.

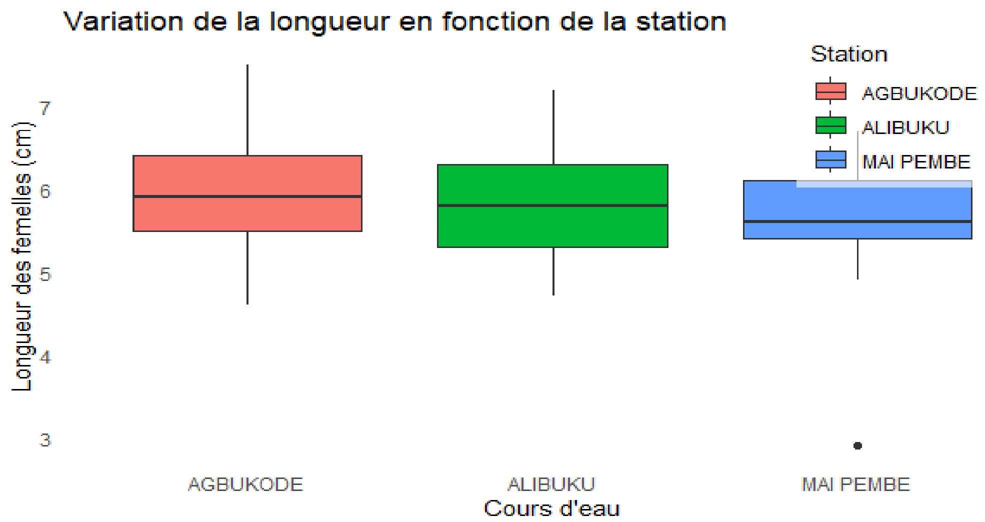

Figure 7: Comparison of the average sizes of ovigerous females between rivers.

Figure 7 shows that the average sizes of ovigerous females do not show a statistically significant difference between the different rivers surveyed ($p > 0.05$).

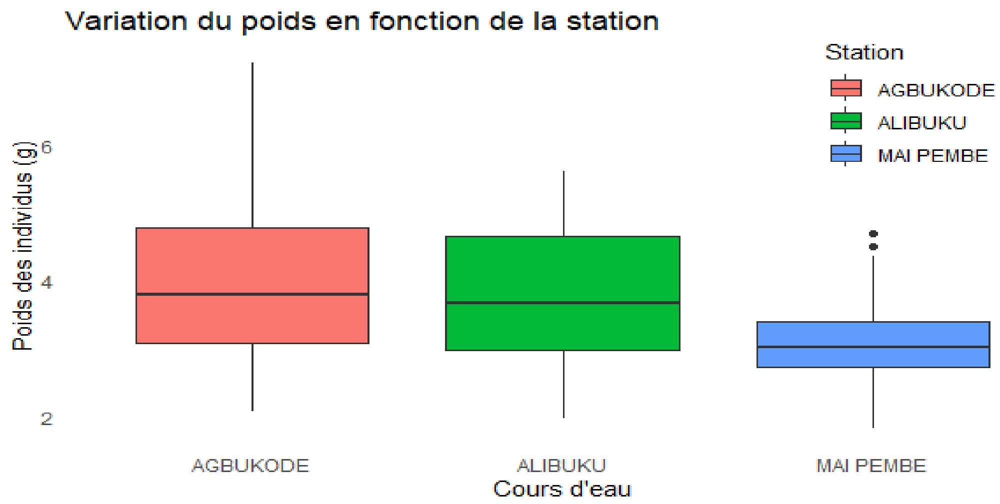

Figure 8: Comparison of the average weights of ovigerous females across waterways

Figure 8 shows that the average weights of ovigerous females are statistically significantly different in the Mai pembe stream, compared to the Agbukode and Alibuku rivers.

c. Size class, number of individuals, and weight of ovigerous females

Table 2. shows the distribution of size classes, the number of individuals observed, and the associated average weight.

Size class (cm)	Number of individuals	Average Wight (g)	Standard deviation (g)
Classe 1 (2,9 - 3,9)	1	3	-
Classe 2 (3,9 - 4,9)	4	2,25	0,37
Classe 3 (4,9 - 5,9)	55	2,98	0,65
Classe 4 (5,9 - 6,9)	52	4,3	0,76
Classe 5 (6,9 - 7,9)	5	5,8	0,85
Total	**117**	**18,4**	**1,07**

Table 2 shows that 117 egg-carrying females were collected. These individuals are spread across five size classes, with classes 3 and 4 being the most represented, having 52 and 55 individuals respectively, while the other classes are sparsely represented. The highest average weight is recorded in classes 4 and 5, with values ranging from 4.3 to 5.8 g.

d. Size class, average egg length and volume

Table (4)3. Size class, average egg length and volume.

Size class (cm)	Average lenght (mm)	Average egg volume (mm^3)
Classe 1 (2,9 - 3,9)	2,42	7,37
Classe 2 (3,9 - 4,9)	2,16	5,30
Classe 3 (4,9 - 5,9)	2,35	6,88
Classe 4 (5,9 - 6,9)	2,36	7,15
Classe 5 (6,9 - 7,9)	2,35	6,89

Table 2 shows that classes 1 and 4 include egg-carrying females with large eggs, ranging from 7.15 to 7.37 mm^3, with an average length between 2.36 and 2.42 mm. Classes 3 and 4 come next with slightly smaller eggs, ranging from 6.88 to 6.89 mm^3. Finally, class 2 is made up of females carrying smaller eggs.

4.3 Discussion

a. Fecundity and Body Size of Females

The results highlight a positive correlation between the body size of females (total length and weight) and their fecundity. This relationship suggests that larger individuals produce a higher number of eggs, which aligns with observations reported for other species in the genus Macrobrachium. Bhuiyan et *al.* (2009) found a positive correlation between body size and fecundity in *Macrobrachium dayanum*. Similarly, Anger and Moreira (1998) observed that, in species of the genus Macrobrachium, larger females invest more in reproduction, thus producing a higher number of eggs.

b. Correlation between female size and oocyte dimensions

Our analyses highlight a correlation between the body size of females (total length or weight) and the size of the eggs they carry. This relationship suggests that larger individuals produce bigger eggs. Sahoo et *al.,* 2011, had noticed the same thing in their study on Macrobrachium rosenbergii, showing that oocyte dimensions gradually increase over the course of embryonic stages. This observation supports the conclusions of Anger and

Moreira (1998), who studied the morphological and reproductive traits of tropical caridean shrimp and found that, in some species of the genus Macrobrachium, larger females invest more in egg quality, producing bigger eggs, which are often linked to higher larval survival rates. Similarly, Corey and Reid (1991), in their study on Orconectes virilis, reported a significant correlation between female size and egg volume. This link could reflect a reproduction strategy aimed at maximizing larval success in environments with variable conditions, like those found in natural settings. Our data analyses revealed a positive correlation between the body length of ovigerous female *M. sollaudii* and the size of their oocytes. This observation supports the hypothesis that larger females tend to produce very large oocytes, likely better supplied with yolk reserves. This observation has also been made in other species of the genus. Alvarez et *al.,* (2000) showed that in *Macrobrachium americanum*, large females not only produce a higher number of eggs but also eggs with a greater volume, which improves larval survival. Similarly, Gonzalez et *al.,* (2011), working on *Macrobrachium tenellum*, observed that oocyte size increases significantly with the body size of females. This relationship is often seen as an evolutionary advantage, because bigger females have the physiological ability to develop richer eggs, which maximizes embryonic variability.

c. Changes in oocyte size according to developmental stage

Analyses show a significant increase in egg size as embryonic development progresses, from the initial stage to the final stage. This biological variation is consistent and results from water accumulation in the eggs, the development of embryonic tissues, and the increase in internal pressure (Cavalli et *al.,* 2000). Previous studies have highlighted an increase in egg size during embryonic development in various species of the Macrobrachium genus. For instance, Sahoo et al. (2011) showed that in Macrobrachium rosenbergii, egg size increases steadily until hatching, with an average growth estimated between 15 and 20%. Similarly, Nazari et al. (2003) observed a gradual increase in the eggs of Macrobrachium acanthurus at each embryonic stage.

Morphological changes are often observed during physiological preparation for hatching. The increase in size reflects the development of internal organs and the emergence of visible structures like the eyes.

d. Correlation between body weight and fertility

Our analyses show a similar relationship between the weight of females and the number of eggs laid. This was observed by Bhuiyan et a*l.,* 2009, who reported a strong positive correlation between body length and fertility in *Macrobrachium dayanum*, but also between body weight and fertility. These results support the idea that female size and weight are good indicators of the number of eggs produced. Vargas-Ceballos et al., (2018) described the stages of embryonic development, the size of eggs at each stage, volume, and the fertility-size correlation, separating non-eyed eggs from eyed eggs. They concluded that the volume of eggs increases significantly between the embryonic stages.

e. Influence of the environment on the reproductive parameters of *M. sollaudii*

The analyses show a notable variation in the fertility and oocyte sizes of M. sollaudii across the three rivers studied. This variability could be due to local ecological differences such as water quality, food availability, temperature, or human impact. This observation was also made by Lima et al. (2014), who stated that the fertility of M. amazonicum varied from one region to another in Brazil, which affects freshwater shrimp reproduction. These authors highlight that more productive or better-oxygenated habitats promote higher fertility. These results support the trends observed in this study, where females from the less disturbed river had higher fertility and larger oocytes compared to those from more impacted environments.

IV. Conclusion

This study is a contribution to the understanding of fertility parameters and oocyte characteristics of the shrimp species Macrobrachium sallaudii in natural settings. The results obtained in this study highlighted several key aspects of the reproductive biology of Macrobrachium sollaudii. The data show that the fertility of Macrobrachium sollaudii varies depending on female size, and oocyte dimensions increase with the embryonic stage. There is a positive correlation between female size and oocyte size. Finally, the comparison between the surveyed rivers reveals notable differences in terms of fertility and oocyte size, likely linked to local ecological conditions.

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