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Morphometry, Embryonic Development and Fecundity of Freshwater Shrimp *Caridina gracilirostris* De Man, 1892 from Laguna de Bay, Laguna, Philippines

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Abstract

The Philippine lakes abound in crustaceans with both economic and ecological values. Recent findings on endemic freshwater shrimps in some Philippine riverine systems and the emerging interest on the potential of small freshwater shrimps for forage provided impetus for research on these species. In the present study, morphometric characters of small freshwater shrimp *Caridina gracilirostris* was examined together with embryonic and fecundity analysis. Result showed mean standard length at 18.05 ± 2.9 mm, carapace length at17.89 ± 1.8mm and mean rostral length at 22.70 ± 3.0 mm. The mean dorsal spine count was 7.43 ± 1.22; while the mean ventral spine count was 23.8 ± 1.76. The embryonic development of *C gracilirostris* lasted in 12 days under 7.4-degree Celsius temperature under laboratory condition. The eggs are oblong in shape with green color during early fertilization that later turned to yellow green and yellowish in the late stage until hatching. The mean fecundity of ovigerous female estimated at 323.4 ± 67.11 eggs. The individual fecundity ranges from 234, in ovigerous female with 2 cm total length, and 463 in ovigerous female with 2.7 cm total length. Positive linear correlation between the total length and the number of eggs of ovigerous females was also observed. The mean egg size in the long axis of *C gracilirostris* increased from stage I to stage II by 6.3, from 0.576 mm to 0.6128 mm and increased continuously until reaching stage III by 2.9% from 0.612 mm to 0.6308 mm; the short axis length changed from 0.364mm to 0.4506 mm upon reaching stage III.

Key words: Freshwater shrimps, Morphometry, Fecundity, Embryonic development, Laguna de Bay

INTRODUCTION

The freshwater shrimps of the Philippines have been reported by Holthuis (1980); Cai and Shokita (2006); Cai and Anker (2012). Expeditions and studies have been done on freshwater shrimps in the Philippines since 1852 (Cai and Anker 2004; Cai and Shokita 2006). De Grave et al. (2008) noted that many unidentified and probably mixed populations of small species of freshwater shrimp are heavily fished in the Philippines. Surprisingly however, no account of freshwater shrimp studies and freshwater decapod expedition for Laguna de Bay which is the biggest lake in the Philippines had ever been done or recorded.

These freshwater shrimps are supporting subsistence fishery and are eaten fresh or salted and made into a fermented product called *alamang*. Small-size freshwater species which has no currently commercial values, such studies are important to support the growing demand for live fish foods or high protein feed ingredients of the aquaculture industry. Taxonomical and morphometric studies are conducted to identify and properly characterize new species that could be used in biodiversity conservation and sustainable management of threatened and ecologically important species. In Laguna de Bay, despite the importance of its small freshwater shrimp population for supporting subsistence fishery and the degradation of the lake ecosystem which could affect its integrity and survival, knowledge on taxonomy and morphological characteristics of the shrimp population remains scanty. Despite the potential of the small freshwater shrimp as excellent fish food for aquaculture, until now there

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has been no reproductive study on this species in Laguna de Bay area and probably on similar lakes in the Philippines.

This paper identified the small freshwater shrimp found in Laguna de Bay Philippines and described its morphometry, embryonic development and fecundity

METHODOLOGY

Collection of Samples

Samples of *C. gracilirostris* were collected in Laguna de Bay, Philippines. Laguna de Bay, which has a volcanic and tectonic origin, is the largest Lake in the Philippines with a total area of 98,000 hectares and a shoreline of 220 kilometers (140 mi). It is situated at the heart of the Calamba-Laguna-Batangas-Rizal area and considered as a highly urbanized and developed center. The average depth of the Lake is 2.8 meters (9 ft. 2 in.) and an elevation of about 1 meter (3 ft. 3 in.). Samples were caught with hand nets (approximately 3-mm mesh size). The collected shrimp samples were placed inside an inflated plastic bag filled with lake water and tied with rubber band. The physico-chemical parameters of the water in each sampling site were measured and recorded using portable Hach® Kit for water quality measurement. The parameters measured include the following: temperature, dissolved oxygen and pH.

Identification of the Species and Taxonomic Description

Samples were identified to the species level and described following Holthuis (1980), Cai and Shokita (2006); and Cai and Anker (2012) and checklist to the identification of freshwater shrimps.

Identification of Sexes

The sexes were identified through the presence or absence of *appendix masculina* and different morphotypes. Female shrimps were also identified as either ovigerous or non-ovigerous.

Morphometric Variables

Relevant measurable characters were considered as morphometric parameters, while the teeth of the rostrum were counted as meristic parameters. Measurements and counting of these measurable characters was done with the aid of rulers, Vernier caliper and Motic® microscope. Morphometric measurements were taken to the nearest 1.0 mm.

Thirty morphometric characters were measured and analyzed namely: total length (TL), body height (BH), carapace height (CH), carapace length (CL), rostrum length (RL), body length (BL), telson length (TelL), uropod in (UroIn), uropod out (UroOut), scaphocerite length (SL), two antennule length (AntL), antenna length (AL), six abdominal segments length; first abdominal segment (seg1), second abdominal segment (seg2), third abdominal segment (seg3), fourth abdominal segment (seg4), fifth abdominal segment (seg5), sixth abdominal segment (seg6), maxilliped (MaxL), first pereopod (PerL1), second pereopod (PerL2), third pereopod (PerL3), fourth pereopod (PerL4), fifth pereopod (PerL5), first pleopod (PleL1), second pleopod (PleL2), third pleopod (PleL3), fourth pleopod (PleL4), fifth pleopod (PleL5), eye (E).

Determination of Embryonic Development and Fecundity

Fifteen ovigerous females were separated for each sample population for embryonic development of the eggs. The eggs from ovigerous sample species were carefully removed from the pleopods using small brush or fine forceps and needle. They were then carefully blotted on a filter paper to drain extra water. The eggs were weighed in a laboratory weighing balance to determine the total egg mass (W). The egg size was measured digitally using the installed Motic measuring computer software in the microscope from the shortest and longest diameter.

Gravid samples were used to determine the stages of development. Egg samples were carefully removed using fine forceps. The collected eggs were placed into one liter plastic bottles with dechlorinated tap water and maintained with 27 degree Celsius temperature and controlled aeration.

Developmental stages were monitored daily under stereo microscope. Measurement and photographs were taken using Motic® microscope and Sony G Cyber-shot ® 10x optical zoom.

Analysis of Data

Morphometric characters of the sample species were analyzed using descriptive statistics. Spearman bivariate correlation was employed to determine the degree of relationships between metric counts.

The significant difference in morphometric characters between male and female of each species were tested using the t-test.

The egg volume (V) was calculated following:

$$V = \pi \cdot l \cdot h2/6 \quad (1)$$

where l = longest diameter (length), and h = shortest diameter (height).

The following equation was used to estimate the fecundity of the individual:

$$F = Fs \cdot W/w \quad (2)$$

where F is the estimated fecundity of an individual, Fs is the number of eggs in a sample, W is the total weight of the egg mass, and w is the weight of the sample egg mass.

The mean of fecundity was estimated for stages I, II and III, and pooled fecundity of the three stages. Pearson's linear regression was used to assess the correlation between CL and the number of eggs in pooled stages.

RESULTS AND DISCUSSION

Morphometric and Meristic Characters

Mean standard length of *C. gracilirostris* was recorded at 18.05 mm with a standard deviation of 2.9; the mean body height was 9.00 mm with a standard deviation of 0.7; mean carapace height was 12.24 mm with 1.5 standard deviation; while the mean rostral length measured 22.70 mm with 3.00 standard deviation. Other morphometric measurements are presented in Table 2. The mean dorsal spine count was 7.43; while the mean ventral spine was 23.8. with standard deviation ranging between 1.22 and 1.76 (Table 3).

Table 2. Morphometric characters of *Caridina gracilirostris* species from Laguna de Bay.

Morphometric Characters	Mean	SD
SL	18.05	2.9
BH	9.00	0.7
CH	12.24	1.5
BL	46.16	2.7
CL	17.89	1.8
RL	22.70	3.0
TelL	13.24	1.3
UroIn	13.58	1.4
UroOut	17.16	1.7
AntL	52.06	15.9
AL	64.17	17.4
ScapL	13.76	2.9
AbdS1	5.51	1.0
AbdS 2	12.17	1.4
AbdS 3	9.21	1.3
AbdS 4	6.67	1.5
AbdS 5	5.69	0.8
AbdS 6	12.19	1.3
MaxL	24.72	3.4
PerL1	24.14	3.4
PerL2	25.28	3.3
PerL3	26.59	3.6
PerL4	27.61	3.8
PerL5	28.44	4.7
PleL1	14.96	4.2
PleL2	14.97	3.6
PleL3	14.30	3.1
PleL4	13.23	2.8
PleL5	11.50	3.0
E	3.60	0.9

Table 3. Meristic characters of Caridina gracilirostris species from Laguna de Bay.

Metric Characters	Mean	SD
Dorsal Rostral Spine	7.43	1.22
Ventral Rostral Spine	23.8	1.76

Embryonic Development

Embryonic development of *C. gracilirostris* last for 9-11days at 23oC, 7.3 pH, proper aeration and good quality of water. The eggs are oblong, green in color during early fertilization and turned into yellow green and yellowish in the late stage until it hatched and attached through pleopods. The changes of the stages occurred day by day and synchronous in development but not uniform in size (Fig. 1). The embryonic development of *Caridina sp*. is divided into nine stages:

* Stage I. Fertilized Egg (Day 1)
* Stage IV- Gastrulation with depression (5 days)
* Stage V- Early Nauplius (7 days)
* Stage VI- Mid-Nauplius (Day 8-9)
* Stage VII- Late Nauplius with Eye pigmentation (10 days)
* Stage VIII- Eye Condensation (10-11 days)
* Stage IX- Pre- hatch embryo (12 days)

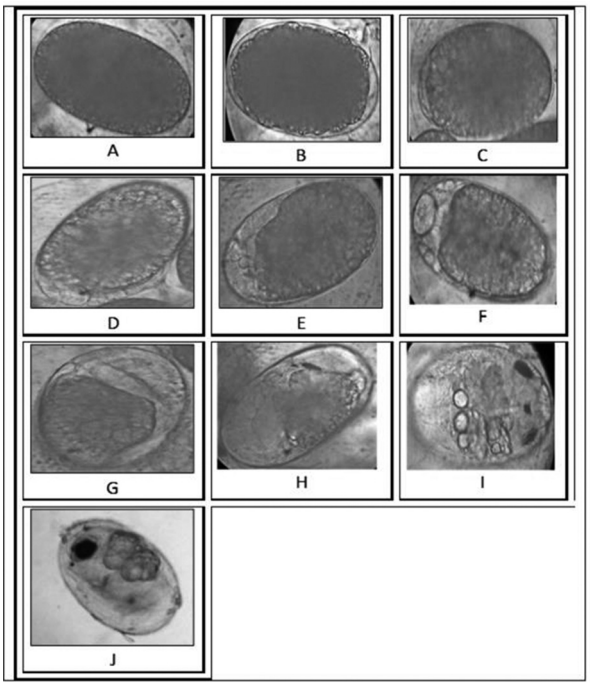

Figure 1. Images of embryonic development of Caridina gracilirostris. : (A) stage 1, fertilized egg; (B) stage II, Cleavage; (C) stage IIIa, Gastrulation with Blastopore; (D) stage IV , early nauplius;(E) stage V, Mid-nauplius;(F) stage VI, Late-nauplius; (G) stage VII, post nauplius with eye pigmentation; (H) stage VIII, Post-nauplius with eye condensation;(I-J)stage IX, Pre-hatch embryo.

Fecundity

The total length (mean ± standard dev.) of fifteen ovigerous *Caridina gracilirostris* collected from Laguna de bay was 2.287 ± 0.1846 mm (range: 2-2.7). The mean fecundity of fifteen ovigerous female was 323.4 ± 67.11 eggs. The individual fecundity ranges from 234, in an ovigerous female with 2 cm in total length, and 463 in ovigerous female with 2.7 cm in total length (Table 4). Figure 2 shows the correlation between total length and number of eggs of ovigerous females of *Caridina gracilirostris*.

Table 4. Fecundity of *C. gracilirostris* in Laguna de Bay. (TL: Total length of ovigerous female, TW: Total Weight ovigerous female, EW: Egg Weight of ovigerous female carried) (n = 15).

	Total Length (cm)	Total Weight (g)	Weight of Egg Mass (g)	Fecundity
Mean	2.287	0.071	0.0040	323.4
Std. Dev	0.185	0.012	0.0021	67.11
Min	2	0.05	0.002	234
Max	2.7	0.09	0.0098	463
Range	0.7	0.04	0.0078	229

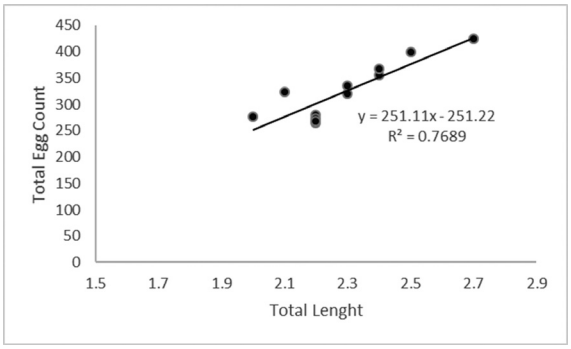

Fig. 2. Relationship between total length and total egg counts of ovigerous female *Caridina gracilirostris* from Laguna de Bay.

DISCUSSION

This paper described the morphometric characters in *C. gracilirostris* of Laguna de Bay. This data set provides key initial information on the species for future works. It also described the embryonic development of shrimp species using key insights from accomplished studies on *Palaemonidae* by Nazari *et al*. (2000). The embryonic development of *C. gracilirostris* was described based on eight different major events or stages which was also similar to the observation of Muller (2003) in *Macrobrachium olfersii* with no fixed duration and separated by morphological events. However, some findings are not analogous in other congeneric species of decapod crustacean. This study showed that the development of *C. gracilirostris* embryo lasted for 12 days under 27.4℃ temperature. This is much shorter than the observations made by Ketse and Muller (2004) which lasted for 17 days under a temperature of 24°C. It should be noted that *C. nilotica* belongs to the same family and genus. In *Macrobrachium americanum*, Garcia-Guerrero and Hendricks (2009) observed its embryonic development completed in 18 days under a temperature gradient of 24°C. Different species express different embryonic development stages at different temperature gradients. Samples from warmer water environment have typically shorter embryonic development (Yamaguchi, 2001). This explains the relatively short stage of embryonic development in *C. gracilirostris*. The fact that crustaceans belong to ectothermic species whose body temperature varies with the environment strengthens this observation. Literature elsewhere articulated the strong dependency of embryonic development of ectotherms on differential expression of certain genes and temperatures (Ojanguren and Brana, 2003) as their biological functions are very dependent on environmental temperature. Embryogenesis of crustacean is difficult to distinguish and some events may start before the ending of a previous one, making the separation into events unclear when the process is a continuum (Garcia- Guerrero, 2003) and Habshy *et. al* (2012).

The fecundity in berried *C. gracilirostris* increases with the body length of the species. This is mostly true and applicable to crustaceans than fish (Sastry, 1983). This observation was also confirmed in the studies done by Patra (1976) and Penn (1980), who observed that the heavier the shrimp, the more number of eggs are carried by the ovigerous shrimps. The increasing of size and volume of eggs were observed in the samples. In decapod shrimps, the increasing of size of eggs are commonly attributed to increase of water content and changes in biochemical composition during embryonic stage (Clarke, 1993).

CONCLUSION AND RECOMMENDATION

This paper demonstrated that *C. gracilirostris* in Laguna de Bay possesses both distinct and similar morphometric and embryological development features with Caridinid species in the current literatures. The variations in the observed characteristics of the species could be attributed to the distinct limnological features of Laguna de Bay and the tropic environment of the study site. The information generated will provide the baseline in future studies on the species that are crucial in providing deeper understanding on its ecological significance and potential economic importance. The fresh

information on its fecundity provides insights for the management of the lake ecosystem and the rational exploitation of population stock of *C. gracilirostris*. More serious studies on *C. gracilirostris'* population ecology, reproductive biology, abundance and distribution in the Laguna de Bay need to be conducted in the future to support these directions.

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