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Reproductive Biology of the Sulu Shrimp *Metapenaeus suluensis* Racek and Dall 1965 (Crustacea, Malacostraca: Penaeidae) in Iloilo River, West Central Philippines

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Abstract

The reproductive biology of the Sulu shrimp *Metapenaeus suluensis* Racek and Dall 1965 in Iloilo River, West Central Philippines was studied over a 1-year period from June 2013 to May 2014 by examination of monthly samples of 60 shrimp from catches of fish corral and motorised pushnet. The smallest sexually mature female was 92 mm total length and the smallest mature male was 81 mm. Ovarian histology showed four developmental stages namely immature, previtellogenic, vitellogenic and redeveloping. Testicular histology revealed five developmental stages namely immature, early maturing, mature, partially spawned and redeveloping. No spent males or females were observed during the study. Gonadosomatic indices of both sexes show major spawning from March to April, with a secondary season in September to October. The interruptions to the progression of monthly modal size frequency distributions, along with the absence of spent individuals suggest that mature individuals emigrate to spawn outside the river.

Introduction

The Sulu Shrimp *Metapenaeus suluensis* Racek and Dall 1965 was first reported in the Sulu Sea, Philippines, where they inhabit muddy bottoms along the coastline at depths up to 40 m (Racek and Dall 1965). It has also been reported to occur in waters off Panay Island, West Central Philippines (Motoh and Buri 1984) and in the Gulf of Thailand (Naiyanetr 2007; Tangkrock-Olan et al. 2007). *Metapenaeus suluensis* is very similar to the Greasyback shrimp *Metapenaeus ensis* De Haan (1844), in external morphology but differs in fine structure of their petasma and thelycum, as well as the ischial spine of their first pereiopod (del Norte-Campos and Manuel-Santos 2014). The difficulty in detecting such fine differences in the field may lead to misidentification, and this may be the reason why, aside from those already mentioned, there have been no other reports of this species in the Philippines up to the present.

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The Iloilo River is a tidal inlet that cuts across Iloilo City on the south-eastern coast of Panay Island. A recent study on its fisheries showed that *M. suluensis* comprised 59.4% and 45% of landings from fish corrals and motorised pushnets, respectively, which in turn constitute 80% of the estimated total annual catch, making it the most important target species of fisheries in the River. In 2007, the reported annual catch of *M. suluensis* was 27, 205 kg valued at US$ 151,137. Because of its local commercial value, studies on the species' reproductive biology are important.

Penaeid shrimp typically have a longevity of 2-3 years, showing fast growth to maximum sizes that vary from 15-16 cm total length in smaller penaeids like *Metapenaeus*, up to 30 cm in *Penaeus* (Garcia 1988). Species of the genus *Metapenaeus* may possess entirely estuarine life cycles, with postlarvae moving upstream into lower salinities, while juveniles return to higher salinities in the lower estuary where they mature and breed (Dall et al. 1990). The Iloilo River is essentially a narrow estuary with limited freshwater inflow. While several shrimp species are caught in the River during the present study's sampling, *M. suluensis* makes up 90.4% of them.To ensure sustainability of this resource, local stocks need to be managed properly. Such efforts however require more information on the species' reproductive biology. This study describes aspects of the reproductive biology of *M. suluensis* in Iloilo River, West Central Philippines, including sex ratio, smallest size at sexual maturity, gonad development and spawning seasonality. This information can contribute towards rational management measures of local fisheries.

Materials and Methods

Study Area

The Iloilo River is a tidal inlet, which stretches about 10 km inland from the coast along Iloilo Strait (Fig. 1). It passes through highly urbanised Iloilo City, with both sides of the inlet in the seaward half of the River being developed and densely populated. The banks of the inner half, however, are still largely utilised as salt- and fish- ponds, with limited mangrove stands remaining. This is also the part of the inlet where motorised push nets and fish corrals operate in.

Field Sampling

Sampling was done on board a motorised pushnet operating at night (1800 to 0600 h) during the new moon phase of each month from June 2013 to May 2014. Pushnet operations typically covered the entire inner part of the inlet (Fig. 1) each night. Catches from fish corrals were then sampled at dawn after the pushnet operation from August 2013 to May 2014. On each occasion 60 individuals of *M. suluensis* were randomly selected from pooled catches of motorised pushnet and fish corral. All samples were collected from the same pushnet operator and the same fish corral. In situ measurements of surface and bottom water temperature (°C), salinity (ppt) and dissolved oxygen ($mg{\cdot}L^{-1}$) were made during pushnet operations using a Horiba multiparameter probe.

Monthly rainfall data from the automatic rain gauge in Iloilo City were obtained from the Philippine Atmospheric, Geophysical and Astronomical Services Administration in Cebu. All shrimp samples were kept on ice in a cooler box in the field and transported to the laboratory for dissection.

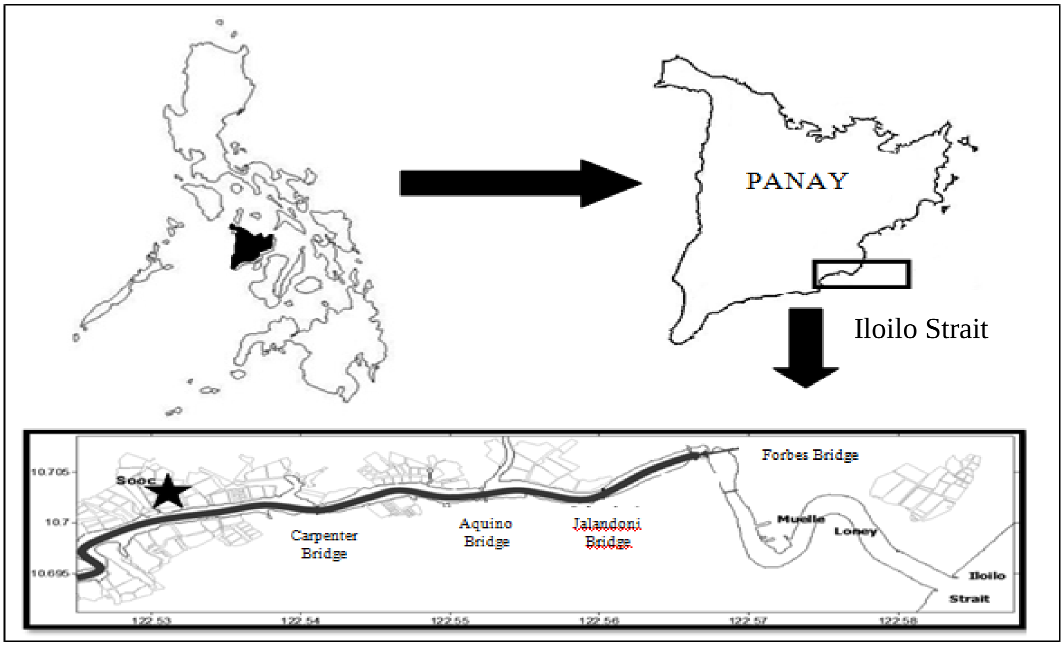

Fig.1. Map showing the study area, Iloilo River in Panay, West Central Philippines. Dark, bold, wavy line indicates the areas where motorised pushnets operate in. The star indicates the location of fish corral where samples were collected. Note: outline structures along both sides of the inlet are salt- and fishponds.

Laboratory Procedures

Total lengths (TL) (tip of the rostrum to the tip of the telson) and carapace lengths (CL) (post-orbital margin to the end of mid-dorsal carapace) were measured to the nearest millimetre using a calliper and ruler. Total weights (TW) and gonad weights (GW) were determined to the nearest 0.001g using a digital electronic balance. Each specimen was then dissected and the gonads were removed.

The physical appearance (size and colour) of the gonads was noted prior to fixation in Bouin's solution and histological processing. The process of dehydration, clearing, infiltration and embedding followed that of Humason (1972). The ovary (middle lobes and posterior lobes) and testes were sectioned transversely at 5 µm using a microtome. Sections were stained with hematoxylin and counterstained with eosin (Humason 1972). Gonads were staged under a compound microscope, based on predominant structures in the histological sections. Stages were described and compared with related literature (e.g. Tan-Fermin and Pudadera 1989; Singh and Roy 1994).

Table. 1. Comparisons of monthly and annual sex ratios of *Metapenaeus suluensis* from Iloilo River, West Central Philppines.

Month	Number of individuals		M:F ratio	x^2 Test	Binomial Test
	Male	**Female**		*p*- value	*p*-value
Jun-13	26	34	1:1.3	0.302	0.366
Jul-13	25	35	1:1.4	0.197	0.245
Aug-13	29	31	1:1.07	0.796	0.897
Sep-13	29	31	1:1.07	0.796	0.897
Oct-13	27	33	1:1.22	0.439	0.519
Nov-13	30	30	1:1	1	1.103
Dec-13	26	34	1:1.3	0.302	0.366
Jan-14	27	33	1:1.22	0.439	0.519
Feb-14	29	31	1:1.07	0.796	0.897
Mar-14	29	31	1:1.07	0.796	0.897
Apr-14	26	34	1:1.3	0.302	0.366
May-14	25	35	1:1.4	0.197	0.245
Annual	**328**	**392**	**1:1.2**	**0.017***	**0.019***

***$p < 0.05$** is significantly different from 1:1

Data Analysis

Monthly and annual sex ratios were computed and compared using the Chi Square test (Zar 1984) and the binomial test (Grabowski et al. 2014). The reproductive patterns were determined based on the quantitative gonadosomatic index (GSI). The GSI (%) of each individual was computed using the equation (Oh and Hartnoll 2004):

$$\textbf{GSI (\%) = GW/ TW x 100}$$

Monthly mean GSI's were computed, plotted against time and compared by means of one-way ANOVA and multiple comparisons test (Games-Howell procedure, α=0.05) (Zar 1984). Assessment of the annual spawning periodicity was based on both monthly GSI values and monthly frequencies of gonad development stages. For both sexes, the size of the smallest individual possessing a ripe gonad was taken as the smallest size at sexual maturity (Villarta and del Norte- Campos 2004).

Results

Sex Ratio

A total of 720 *M. suluensis* individuals were examined with 392 females and 328 males. Monthly sex ratios are shown in Table 1. While females outnumbered males in most months, monthly sex ratios did not differ significantly from 1:1. The overall (annual) sex ratio (M: F= 1: 1.2), however, was significantly different.

Gonadal Maturation Stages

Female

Ovarian maturity was categorised into four stages based on the ovary colour, size and histological analysis namely: immature, previtellogenic, vitellogenic and redeveloping (Fig. 2A-D). The spent stage was not encountered in any of the samples. The smallest size of sexually mature females was 92 mm TL.

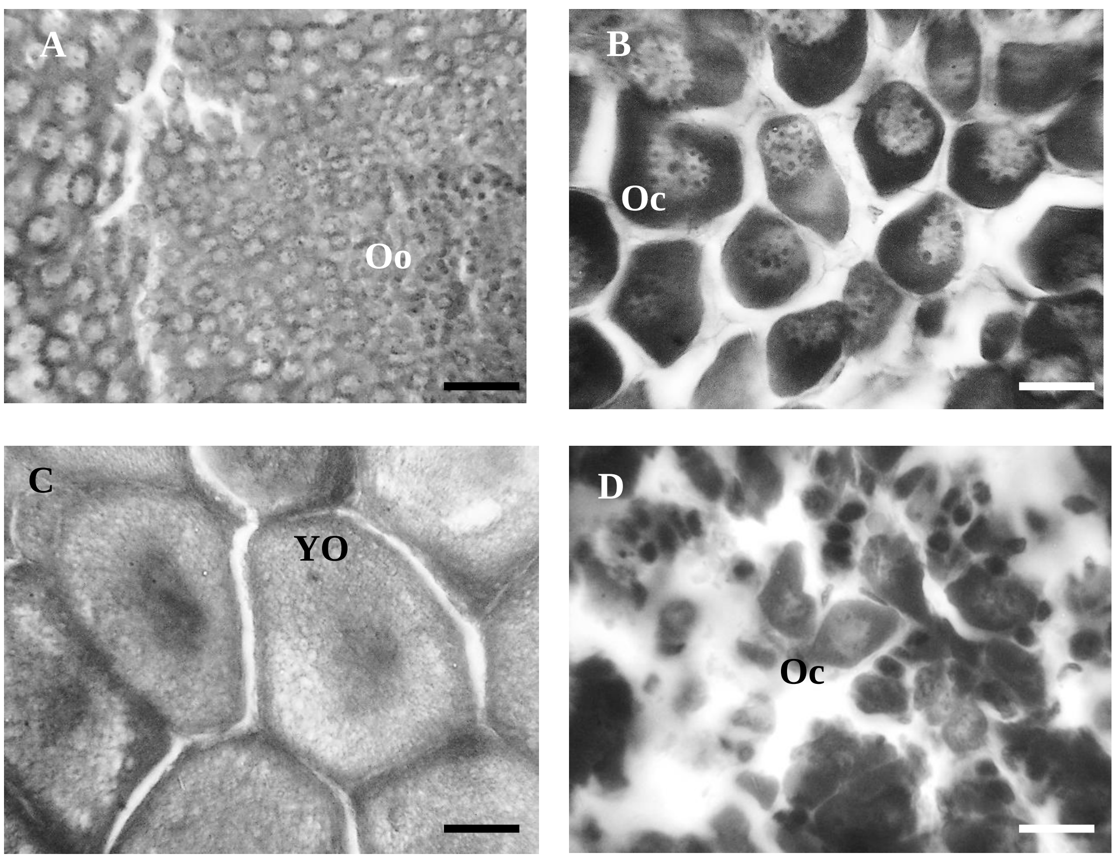

Fig. 2A- D. Ovarian maturation stages of *Metapenaeus suluensis* in Iloilo River, West Central Philippines. **A**) Immature stage with oogonia (Oo) **B**) Previtellogenic stage with 1º oocytes (Oc); **C**) Vitellogenic stage with yolky substances (YO) in the ooplasm; **D**) Redeveloping stage with irregularly shaped 1º oocytes (Oc) dispersed in the lumen. Magnification= 1000x. Scale bar= 20µm.

Male

Five stages of testicular maturity were observed based on physical appearance and histology of the testes, namely: immature, early maturing, mature, partially spawned and redeveloping (Fig. 3A-E). As in the females, no male specimen in the spent stage was observed. The smallest size of sexually mature males was 81 mm TL.

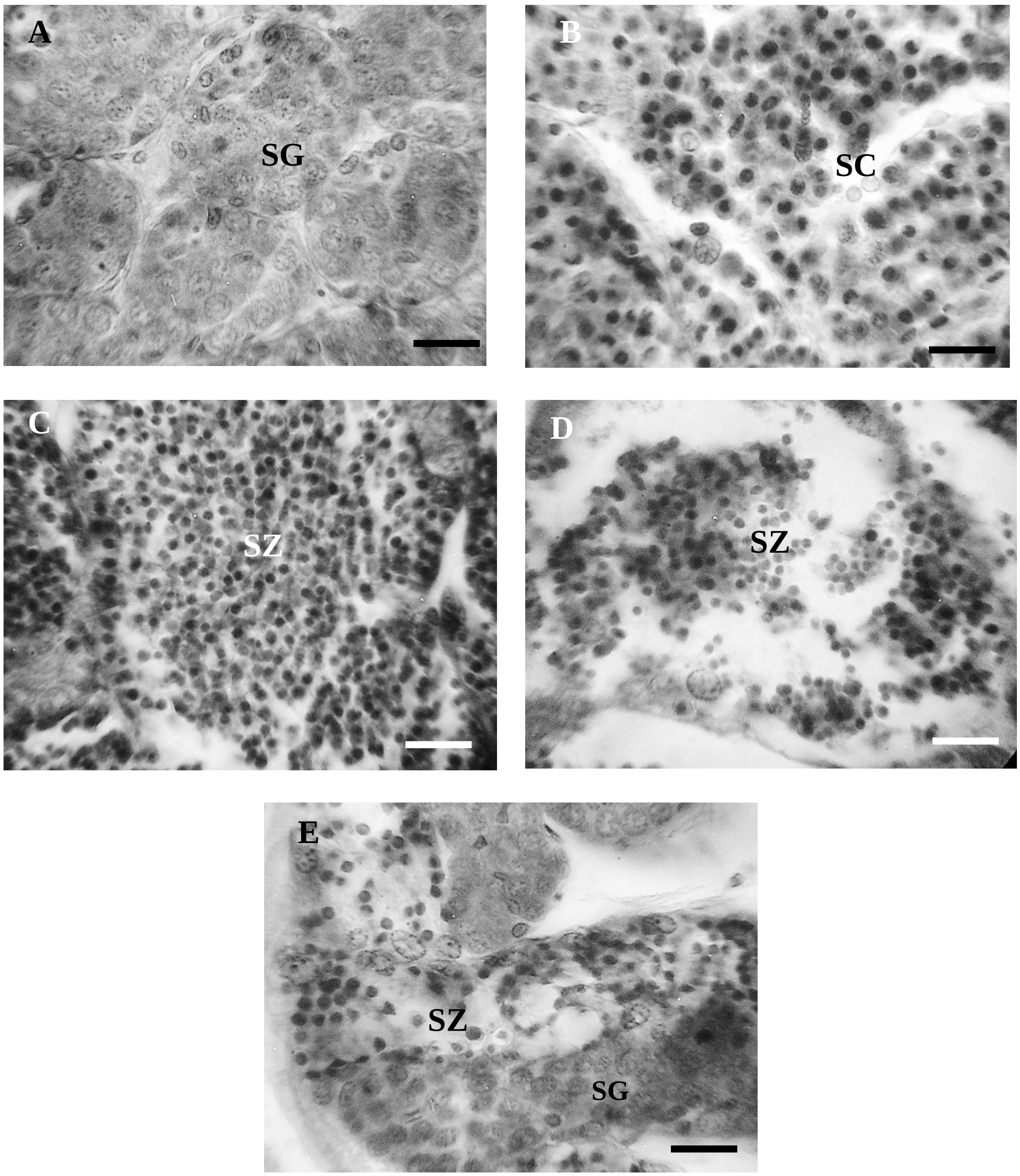

Fig. 3A- E. Testicular maturation stages of *Metapenaeus suluensis* in Iloilo River, West Central Philippines. **A**) Immature with spermatogonia (SG) inside the tubules; **B**) Early maturing with spermatocytes (SC); **C**) Mature stage packed with spermatozoa (SZ); **D**) Partially spawned stage with spaces in the lumen and spermatozoa (SZ); **E**) Redeveloping with residual spermatozoa (SZ) and spermatogonia (SG). Magnification= 1000x. Scale bar= 20µm.

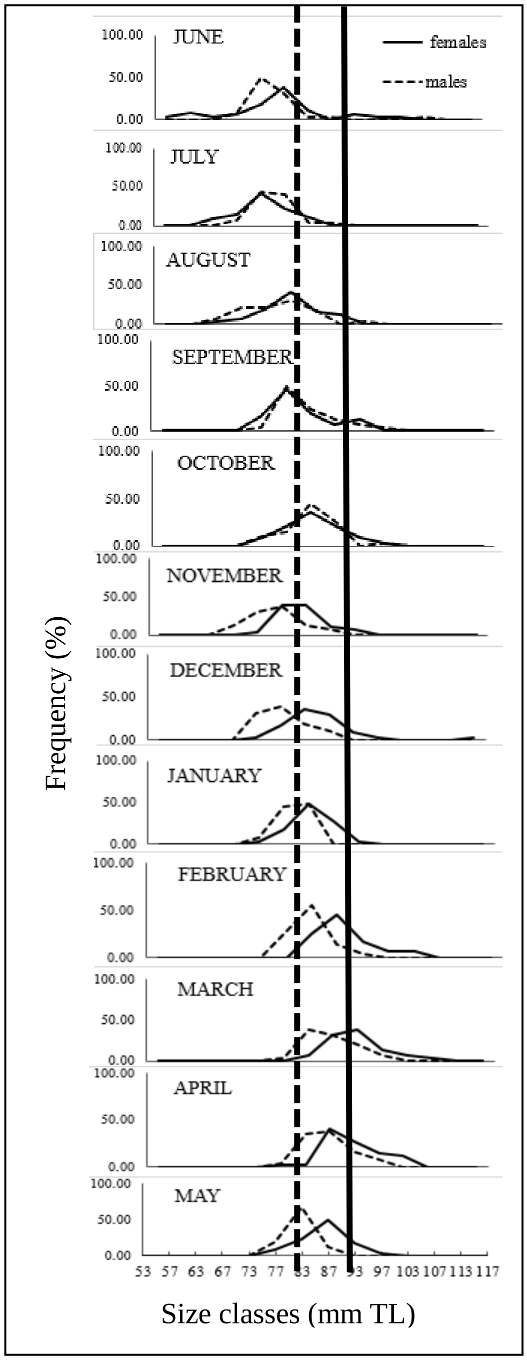

Fig. 4. Monthly size frequency distributions of male and female *Metapenaeus suluensis* in Iloilo River, West Central Philippines from June 2013 to May 2014. The minimum sizes at sexual maturity of female (92 mm TL) and male (81 mm TL) are shown as vertical lines.

Size Structure

The monthly size frequency distributions of both males and females show comparable modal sizes progressively increasing from June to October 2013. Modal sizes in both sexes stagnated from November to December 2013, increased somewhat again in January to March 2014, before stagnating again from April to May 2014 (Fig. 4). While sex- specific modal sizes were most different during the latter half of the year, with generally larger females, the proportions of males and females larger than their respective minimum sizes at sexual maturity were also highest during these months. For males, over 50% were larger than 81 mm TL from February to April. During the same months, the proportion was lower in females, but still higher than in other months. The only other months when both sexes had individuals larger than their minimum size at maturity were in September and October 2013.

Reproductive Cycle

Female

Mean GSI (%) values from June 2013 to May 2014 ranged from 0.062- 0.142% (Fig. 5). The highest mean GSI values for female *M. suluensis* were recorded in February to May 2014, representing the major peak in spawning, September appears as a secondary peak. One-way ANOVA ($F_{11,380} = 12.146$, $p < 0.05$), followed by Games- Howell post hoc multiple comparisons tests showed mean GSI to be significantly higher from March to May than in other months of the study period. The major peak in GSI values coincides with the months showing the highest proportions of previtellogenic and vitellogenic gonads (Fig. 6). While individuals with immature and previtellogenic gonads were present all year round, a few vitellogenic females were observed in the months of June (2.94%), September (3.23%), December (2.94%) and March (6.45%) while redeveloping females were present only in June (2.94%).

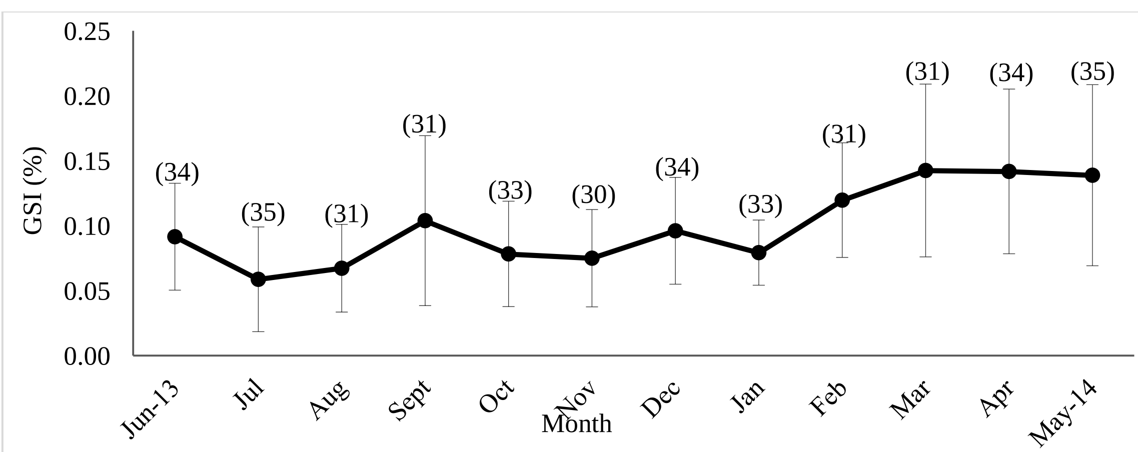

Fig. 5. Ovarian gonadosomatic index (%) of *Metapenaeus suluensis* sampled from June 2013 to May 2014 in the Iloilo River, West Central Philippines. Vertical bars indicate +/- 1 standard deviation. Sample size (n) is enclosed in parentheses.

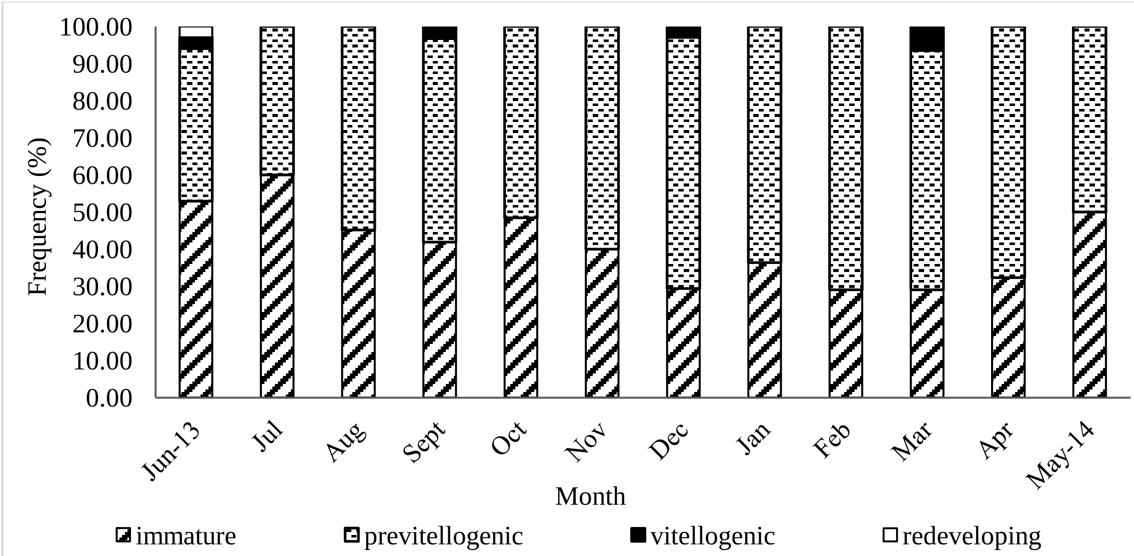

Fig. 6. Frequency distribution of gonad development in female *Metapenaeus suluensis* sampled from June 2013 to May 2014 in the Iloilo River, West Central Philippines.

Male

Mean GSI values ranged from 0.060 to 0.145% (Fig. 7). The highest mean GSI values for male *M. suluensis* were recorded from February to May, representing the major peak, and in September and October 2013, which appears as a secondary peak. One-way ANOVA ($F_{11,316}$ = 20.123, $p < 0.05$) followed by Games- Howell tests showed significant differences in mean GSI only between March (highest) and January (lowest), with no differences with and among the rest of the months. In terms of stages of gonad development, males with mature and older staged gonads were most abundant (> 50%) in March and April, with redeveloping gonads recorded only from March to May (Fig. 8). Mature and older males showed a secondary peak in abundance (15- 20%) in September and October 2013.

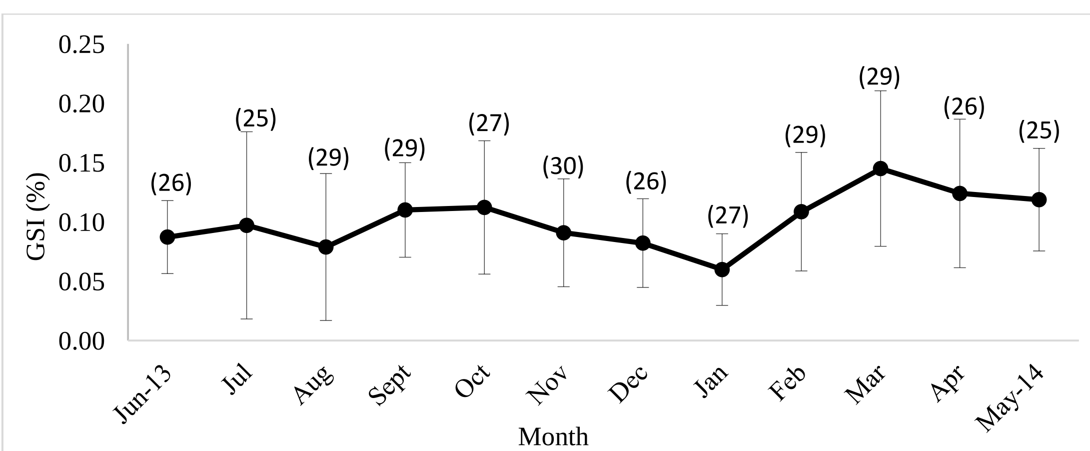

Fig. 7. Testicular gonadosomatic index (%) of *Metapenaeus suluensis* sampled from June 2013 to May 2014 in the Iloilo River, West Central Philippines. Vertical bars indicate +/- 1 standard deviation. Sample size (n) is enclosed in parentheses.

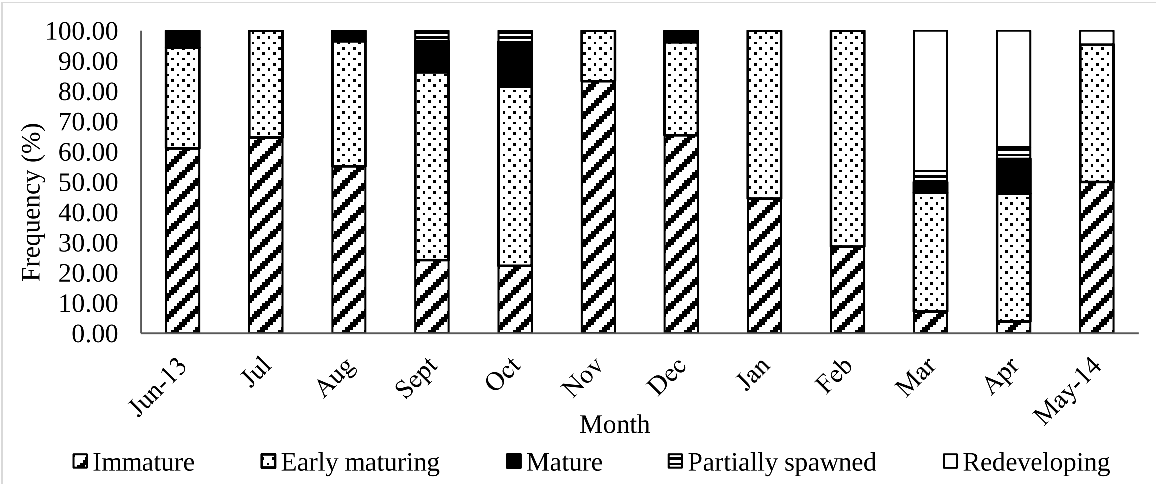

Fig. 8. Frequency distribution of gonad development in male *Metapenaeus suluensis* sampled from June 2013 to May 2014 in the Iloilo River, West Central Philippines.

Physicochemical Parameters

Rainfall in Iloilo City was highest from June to September (8-16 mm), during which surface and bottom salinities in the River were also lowest (16-18 ppt). In contrast, salinity was highest from December to May (21-28 ppt) when rainfall was lowest (1-5 mm). Water temperature in the River was lowest in January (22 °C) and highest in May (33 °C). Water temperature in the rest of the months was intermediate. Surface dissolved oxygen was highest from June to December (3-5 $mg \cdot L^{-1}$), and lowest from January to May (1-4 $mg \cdot L^{-1}$), while bottom DO showed the opposite trend, with highest values during the north-east monsoon (March 2014). Female GSI was positively correlated to surface (r = 0.592, p = 0.043, n = 12) and bottom (r=0.588, p = 0.044, n = 12) salinities, but was negatively correlated with surface dissolved oxygen (r= -0.613, p = 0.034, n = 12). Male GSI did not show any significant correlation with any of the abiotic factors measured.

Discussion

In this study, the predominance of mostly immature and developing *M. suluensis* in the river strongly indicates that they emigrate to deeper waters outside to spawn and complete their life cycle. Most penaeids spawn offshore, while earlier stages from post-larvae to juveniles utilise estuarine or inshore habitats, oftentimes including mangroves, to complete growth (Dall et al. 1990). The Iloilo River tidal inlet fits in well with this model and likely serves as a feeding ground (nursery) for juvenile shrimp. Its inner portion is still bordered by extensive areas of salt- and fish- ponds (Fig. 1), which were likely developed from dense mangrove forests along the banks some 50-60 years ago. Emigration from the River is supported by the monthly size frequency distributions (Fig. 4).

Increasing modal sizes in both sexes from June to October is consistent with growth and development of juveniles in the River, while their relative stagnation from November to December, and again from April to May is not due to a cessation of growth, but rather to the emigration of larger (older) individuals from the River. These months of interrupted size progression correspond to the major spawning season based on gonad size (GSI) (Figs. 5 and 7) and development (Figs. 6 and 8). Size frequencies for females show substantial portions of the population that are larger than their minimum size at maturity (92 mm TL) during these months (Figs. 4). Smaller but still recognisable portions can also be seen in September to October, the secondary spawning season. For males, individuals larger than 81 mm TL were present in all months, but these proportions were higher during the major and secondary spawning seasons.

Subsequent settlement of early juvenile shrimp in the River would likely take place around April to May. The appearance of 50-60 mm long juveniles in June 2013 (Fig. 4) is consistent with this timeframe, and corresponds well with the onset of monsoon rain (June) in the vicinity of the study area. Increasing rainfall leads to nutrient enrichment inside the river from bordering land and pond areas, creating conditions that favour growth and development of early stages. This timing of events is also well supported by the moderate correlations between GSI and salinity.

Other species of *Metapenaeus* show similar life cycles, such as *Metapenaeus kutchensis* P.C. George, M.J. George and Rao 1963 whose juveniles make up almost the entire shrimp fishery in the Kutch Estuary, India (Deshmukh 2006) and *M. ensis* in Hongkong and Australia, which are abundant as juveniles in months of low salinity but are found only in higher salinities of 33-34 ppt as adults (Cheung 1964 as cited in Van Viet and Sakuramoto 2012; Crocos et al. 2001). In the Zhujiang Estuary, China, Chu (1995) showed that mature *Metapenaeus joyneri* (Miers 1880) migrated to deeper waters of the estuary to spawn.

The absence of spent males or females in the River is also consistent with emigration to mate and spawn outside. However, the presence of partially spent and redeveloping individuals (Figs. 6 and 8) indicate that spawning is very rapid and the gonads redevelop immediately after spawning, which is a characteristic of tropical penaeids (Heldt 1938 and Fujinaga 1963 as cited in Rao 1968). Furthermore, this also suggests that *M. suluensis* returns to the River after spawning outside. Hence, the species appears to spend most of its life cycle within Iloilo River, making this area a key habitat for a relatively uncommon (Carpenter and Niem 1998), yet locally valuable species.

Female *M. suluensis* attain larger sizes than males, particularly at the onset of the major spawning season (Fig. 4). This suggests faster growth in females, which is consistent with known growth estimates for various species of tropical penaeids (Villarta et al. 2006). Females need more energy reserves, and are hence larger, so that they can allocate surplus energy to gamete production after reaching a certain size. This ensures that females are ready for reproduction at the time the males are mature.

Conclusion

The results of the study provide enough information to describe the life cycle of *M. suluensis* in the study area. The species has a limited distribution in the country's coastal waters, but is abundant in the Iloilo River. This area is a key habitat because the reproductive biology of the species suggests that it spends most of its life cycle within the River. Given this, fisheries in the Iloilo River tidal inlet should be managed properly to attain sustainability. To this end, *M. suluensis* would serve as a good indicator of habitat conditions and status of fisheries in the River. To complete our understanding of the life cycle of the species, future studies should include verification of identification of shrimp species outside of the River and determination of their population dynamics.

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