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Original paper

MOLTING AND SPAWNING SYNCHRONY IN *Marsupenaeus japonicus* FROM TOKYO BAY

*Agung Setiarto**

Fisheries Department, Faculty of Fisheries and Marine Science Diponegoro University,
Jl. Hayam Wuruk 4a Semarang Indonesia

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ABSTRACT

This study was the first attempt to examine the molt stage and molting cycle in relation to the ovarian development of M. japonicus in Tokyo Bay. In particular, the study was done to reveal molting and spawning relationship, to predict reproductive peaks and thus larval recruitment and to investigate the possibility of multiple spawning within one molt cycle in natural condition. Only three out of five stages were represented in the samples since the early premolt (A) and ecdysis (E) occured in a very short time. There occured was a co-occurrence between ovarian maturation and molt stage, in which maturing ovaries occurred at B (late postmolt) to D_1 (early premolt) stage, mature ovaries at C (intermolt) to D_2 (late premolt), and spent ovaries at D_1 to D_2. Molting and spawning synchronization was observed in kuruma prawn, in which molting frequency was highest prior to and at the end of spawning season. There was a possibility of multiple spawning within single molt cycle in wild kuruma prawn, since there were enough time for spent ovaries to redevelop into mature ones.

Keywords: Marsupenaeus japonicus, molting, spawning, Tokyo Bay

Correspondence: Phone (024) 8311525, Fax. (024) 7474698

INTRODUCTION

Reproductive physiology of crustacean is significantly influenced by continued somatic growth, which is achieved through periodical molting in the adults (Adiyodi, 1985). The relationship between molting and reproduction is primarily evident in females, since vitellogenesis, the main feature of female reproductive cycle, and secretion of a new cuticle during molting, could affect the physiology of organisms by competitive utilization of reserve materials from storage organs (Subramoniam, 2000).

Studies on the relationships between molt and reproduction in crustacean have been conducted for decades (Adiyodi, 1985). Some important factors which potentially determine molt and reproductive patterns have been revealed. The proximate endocrine factors have received the most attention (Fingerman, 1987; Chang, 1995; Huberman, 2000; Subramoniam, 2000). In addition, the roles of environmental factors have also been widely studied (Aiken, 1973; Reaka, 1976; Nelson *et al.*, 1983; Justo *et al.*, 1991; Hoang *et al.*, 2003).

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Studies on the relationship between reproduction and molting in penaeids species have also been done on some closed-thelycum penaeids (*P. merguiensis*, Crocos and Kerr, 1983; *P. esculentus*, Crocos, 1991; *P. latisulcatus*, Penn, 1980; *P. indicus*, Emmerson, 1980). These have provided information on the coincidence of the various molt stages with given ovaries stages in laboratory and wild populations, and addressed the interrelationship of molt and reproduction in penaeid prawn. However, some questions related to the possibility of multiple spawning under natural condition are still unanswered.

In penaeid prawn with closed thelycum such as *M. japonicus*, mating occurs between a hard male and soft female (after molting) (Hudinaga, 1941), and active vitellogenesis (ovary stage III and IV) precisely occurs during premolt stage followed by ecdysis (Subramoniam, 2000), which indicates a high correlation between molting and reproduction. There also occurs a non-reproductive molt cycle when the ovary remains inactive throughout intermolt period resulting in consecutive molt cycles. In view of this, the ability to determine molting stage in relation to certain ovarian development stage would be highly useful in penaeid reproduction study. In addition, the possibility of multiple spawning under natural condition could also be investigated using this study. Furthermore, molt stage determination of adult females could be used for prediction of reproductive peaks and thus larval recruitment, which is very important for successful fisheries management.

So far, molt staging has not been described for *M. japonicus* and the relationship between molt cycles and reproductive stages through time in any field population has not been studied. This study was the first attempt to examine the molt stage and molting cycle in relation to the ovarian development of *M. japonicus* in Tokyo Bay. Specifically, the study was carried out to reveal molting and spawning relationship, to predict reproductive peaks and thus larval recruitment, and to investigate the possibility of multiple spawning within one molt cycle in natural condition.

MATERIALS AND METHODS

Field observations on the reproduction and molting were made monthly for one year (March 2002 to April 2003). The samples were bought from Yokohama City Fisheries Cooperative Shiba Branch, which collected the prawn catch from local fishermen in Tokyo Bay area. *M. japonicus* was caught by small-sized bottom trawl and gill net at a depth of 10 – 40 m on sandy-mud substrate. Only females with developing ovaries and/or inseminated with spermatophore were used for this observation. Total number of animals used for this analysis were 82 individuals with the average weight of 59.62 ± 19.74.g and average carapace length (CL) of 48.93 ± 5.61 mm

Molt activity was assessed in two ways. First, microscopic molt staging was performed on females monthly. One uropod was examined on each animal. Uropods were excised and the edge of the inner part in the region adjacent to the telson tip was observed under a coverslip in filtered seawater. (**Fig. 1**). The animals were staged using degree of epidermal withdrawal (Smith and Dall, 1985) and the degree of development of new setae (Chan *et al.*, 1988). Photomicroscopy was carried out digitally using Pixera software installed in PC connected to Pixera CCD fitted to a Nikon stereomicroscope. The second procedure for estimating molt activity was a determination of the percent of soft animals present in the sample. Soft animals are in stage A and part of stage B. This second means of estimating molt

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activity served as a comparison for determinations of the percents of animals in early postmolt using microscopical observation.

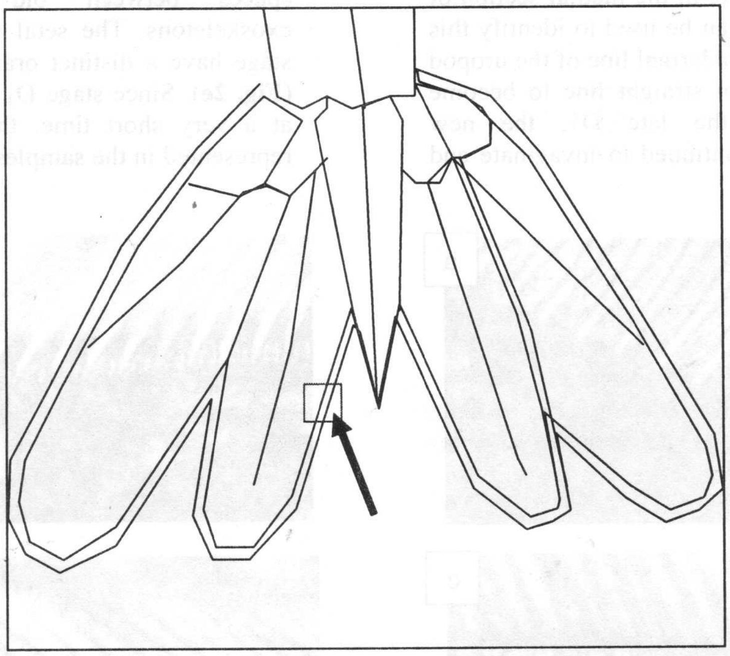

Fig. 1. Small area of the uropods that was sampled and observed for molt staging (arrow).

RESULTS AND DISCUSSION

Result

1. Molt staging

Although the actual molting cycle is divided into five main stages (Dall *et al.*, 1992), we could only identify three main stages in the samples. This is mainly due to the very short duration of the first (A) and the last stage (E) which is molting/ecdysis itself.

(1) Stage B (late postmolt). The cellular matrix is obvious within the setal lumen and begins to retract from the setal lumens towards the bases of the setae to form setal cones (SC). (**Fig. 2a**).

(2) Stage C (intermolt). This stage could be identified by the presence of setal cones (SC) in most of the setae in the uropods. Formation of setal cones is completed at the end of this stage (Chan *et al.*, 1988). Most of the setal lumens (SL) were clear of setal matrix (SM) at this time. The epidermis fills the bases of the setae and has a narrow border that extends round the setal nodes (SN) as well as the bases (**Fig. 2b**). This border is the cuticular edge of the uropod.

(3) Stage D (premolt). This stage by convention is divided into five substages, from D_0 through D_4. Early premolt (D_0) began with apolysis, the separation of the endocuticle from the epidermis. New cuticle was not present at this stage. As the epidermis retracted, it invaginated at the setal bases as new cuticle was deposited. The retraction continues until the epidermis has withdrawn out of the setal bases and forms a straight line below and parallel

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to the setal nodes (**Fig. 2c**). The period of development of new setae categorizes stage D1. The shape of epidermal line in the medial section of the uropod can be used to identify this stage. The epidermal line of the uropod changes from straight line to become wavy. At the late D1, the new epidermis continued to invaginate and new setae began to develop (**Fig. 2d**). At the D2, the epidermal retraction continued and resulted in large empty spaces between old and new exoskeletons. The setal shafts in this stage have a distinct orange-red color (**Fig. 2e**). Since stage D_3 and D_4 occur at a very short time, they were not represented in the samples.

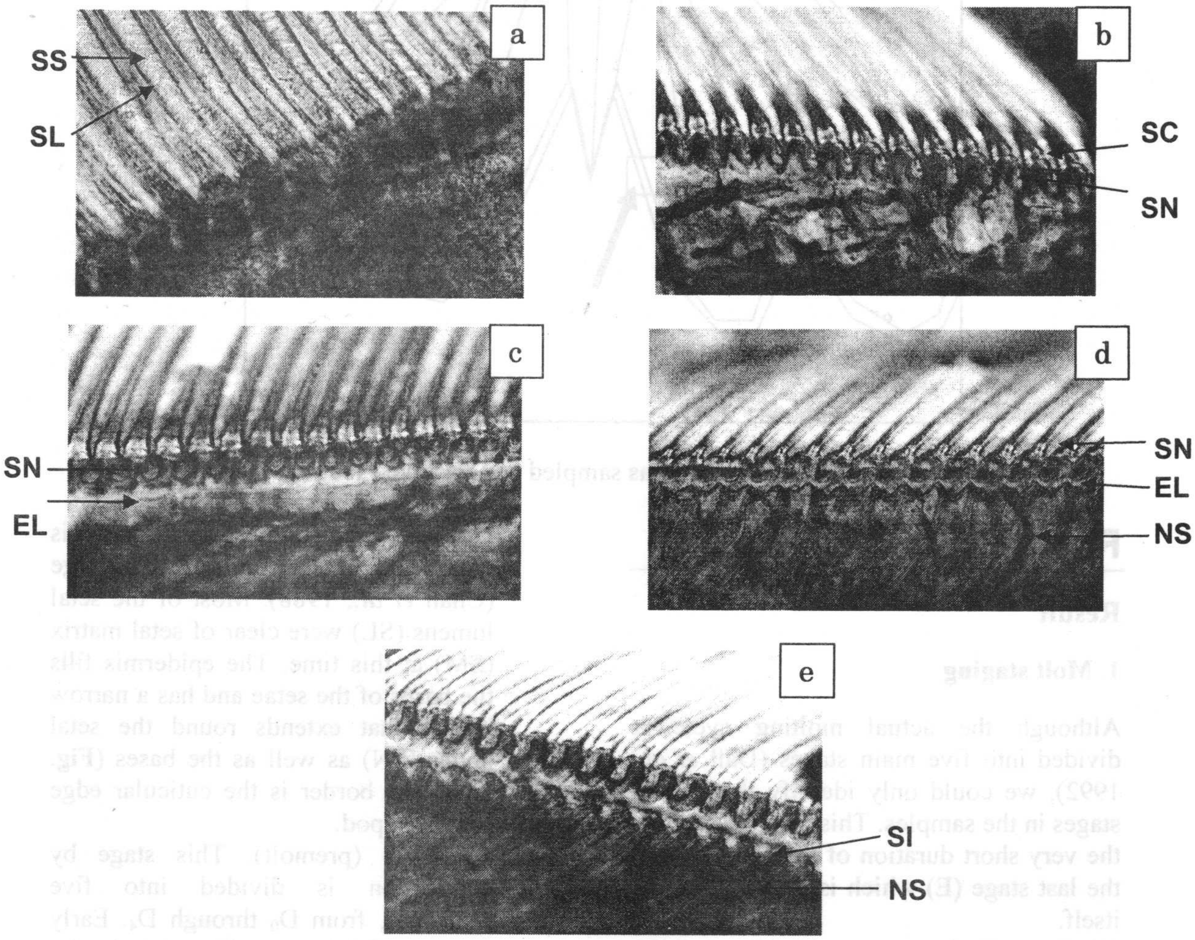

Fig. 2. Molt staging in kuruma prawn. (a) stage B: postmolt;(b) stage C: intermolt; (c) stage D_0: early premolt; (d) stage D1: early premolt; (e) stage D2: late premolt. SS: setal shaft; SL: setal lumen; SC: setal cone; SN: setal node; EL: epidermal line; NS: new setae; SI: setal invagination

2. Reproduction

The spawning season was delineated by determining the frequency of ripe animals and the mean gonadosomatic index. Frequency of ripe animals closely paralleled the profile of mean GSI (Gonadosomatic Index). A period of low GSI was evident in the winter months from October to April. Occasionally, animals with developed ovaries were seen at this time of the year, but they were not as well developed as during the peak season. GSI began to increase in May and this continued through summer into September. This was the period when the first vitellogenesis of the season occurred. During May, August and September high mean GSI was evident. A large drop in the number of ripe animals occurred in October and the final spawning of the season occurred at this time.

3. Interaction between Molting and Reproduction

The one-year profile of female molt activity based on the frequency of soft animal is given in **Fig. 3**. A high and broad peak of molt activity occurred from May to October and April the following year, no soft females were observed during January to March. in October and April the frequency of soft females was relatively high. This indicates a very high frequency of molting in this period, and might also imply molt synchrony in wild population.

Individual records on the field collected animals revealed that vitellogenesis occurred in molt stages B through D_2, and mature females (with cortical crypt ovary) occurred in stages C through D_2 **(Fig. 4)**. Females with spent ovary were observed in molt stages D_1 and D_2, while inseminated females were recorded in molt stage C to D_2 **(Fig. 5)**.

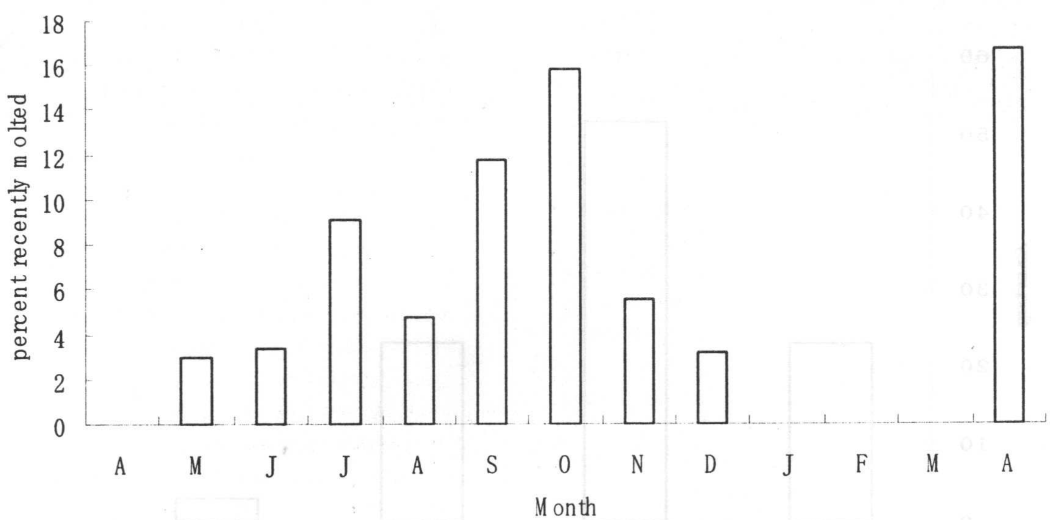

Fig. 3. Monthly variation of recently molted female kuruma prawn

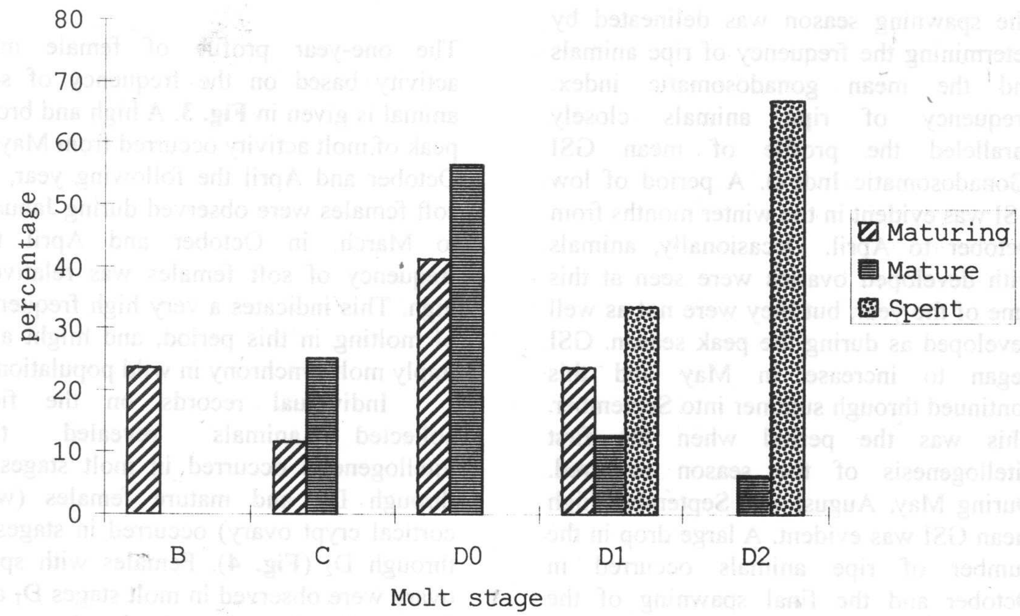

Fig. 4. Co-occurrence of ovarian maturation and molt stage in female kuruma prawn

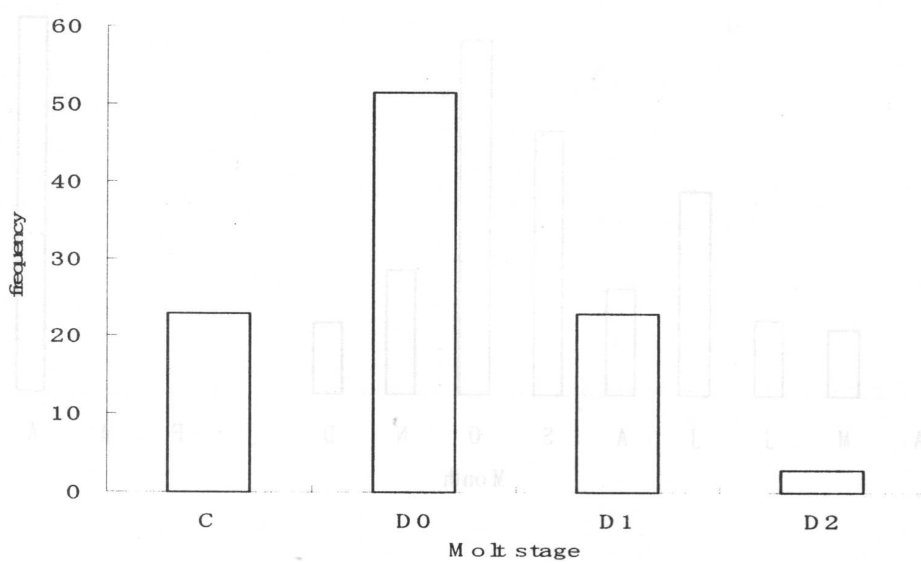

Fig. 5. Frequency of inseminated females in various molt stages

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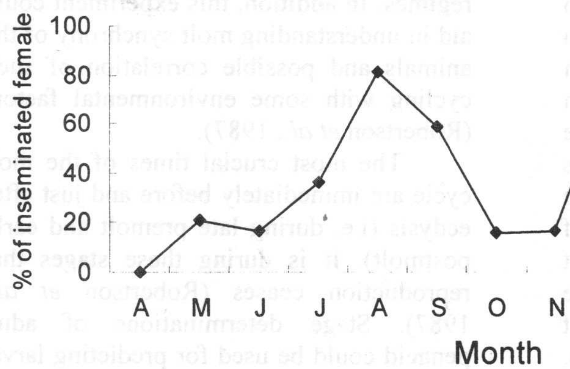

Fig. 6. Monthly changes in the percentage of inseminated females

Discussion

1. Multiple spawning

The frequency of cortical crypts stage oocytes in ovaries of *M. japonicus* was used as an index of spawning activity in the field (Minagawa *et al.*, 2000). Laboratory determination in *Sicyonia ingentis* (Anderson *et al*., 1984) revealed that the time from the onset of cortical crypts phase to spawning ranges from 1 to 9 days with a mean of 4 days. Minagawa *et al.* (2000) also reported that more than 75% of *M. japonicus* with cortical crypts may spawn within a single day. Result of our study support the above report, in which females with this cortical crypt ovary were found in stage C (intermolt) up to D_2 (late premolt) and females with spent ovary were observed in stage D_1 and D_2. If one single molt cycle takes one month and the duration from stage C until the next ecdysis is approximately 80% of the molting cycle (Smith and Dall, 1985), it means that there are approximately 24 days available before the spawning. Considering the above evidences, it seems that there is a possibility of repeated spawning (multiple spawning) within a single molt cycle. Emmerson (1980) and Beard and Wickins (1980) provided evidence that multiple spawning of both eyestalk-ablated and intact prawns can occur within a single molt cycle under culture condition, but no observation has been made for wild population. Hoang *et al.* (2002) reported that molting interval of ablated *P. monodon* can extend to 30 days or more when the spawners produce up to eight-nine spawns within an intermolt period. However, Crocos and Kerr (1983) and Crocos (1991) suggested that there was no evidence of multiple spawning within a single molt cycle under natural conditions since ovary stages III (early ripe) and IV (ripe) coincide with molting stages C and D respectively, and it is unlikely that within particular molting cycle another ovarian maturation occurs after spawning (Crocos and Kerr, 1983). The different condition between wild and cultured population may be responsible for this discrepancy and further investigation is needed to reveal this difference.

2. Molt and reproduction synchrony

Monthly analyses of soft females for *M. japonicus* populations revealed molting pattern that vary with changes in reproductive status and with season. Molt frequency was highest in the spring and fall months when ripe animals were rarely found. Molt frequency was especially high in the mid spring and mid autumn, prior to and after the spawning season. From October the majority of the females began to progress synchronously through a single molt cycle in the winter, which was completed prior to the next spawning season. This is the first documentation of molt synchrony in this species. This result agrees well with that reported by Boddeke *et al.* (1978) who demonstrated molt synchrony in wild penaeid populations, and also with that by Shigueno (1975) who observed molt synchrony under pond condition.

In lobsters and crayfish, molt cycle appears to be overriding importance with ecdysis occurring during various stage of ovary development (Emmerson, 1980). The penaeid appears to be similar in that molting is of prime importance, but differ in that there is never ovarian development during ecdysis. Read and Caulton (1980) have shown that ovary maturation corresponds to water loss in wild *P. indicus*. Ecdysis has long been associated with water uptake in Crustacea (Passano, 1960) and since those functions are antagonistic, it may be possible that water uptake prior to ecdysis inhibits ovarian development causing resorption of the ovary (Emmerson, 1980).

The evidence that many inseminated females were found during winter (December and January) could suggest that females mated after molting in October and retained the spermatophore until the end of the season (**Fig. 6**). This is supported by the data on the molt stage of inseminated females which showed that they were found in stage C to D_2 (**Fig. 5**), which means that insemination might occur several days before. These data further indicate variability in length of the molt cycle according to different environmental condition. Further research in laboratory is necessary to know the absolute period of molting cycle of kuruma prawn under different environmental regimes. In addition, this experiment could aid in understanding molt synchrony of the animals and possible correlation of such cycling with some environmental factors (Robertson *et al.*, 1987).

The most crucial times of the molt cycle are immediately before and just after ecdysis (i.e. during late premolt and early postmolt). It is during these stages that reproduction ceases (Robertson *et al.*, 1987). Stage determinations of adult penaeid could be used for predicting larval recruitment, since open thelycum species do not mate and closed thelycum species do not spawn during late premolt or early postmolt. Emmerson (1980) found that most spawns occurred during early premolt (D_{0-1}) and intermolt (C), and at late premolt (D_2-D_3) ovaries of *Penaeus indicus* resorb to an undeveloped stage. Anderson *et al.* (1985) also found similar evidence for *Sicyonia ingentis*. In our study, we observed that females with spent ovary were found in stages D_1 and D_2, which indicates that the spawning occurs during these stages. By using this information, we could predict the larval recruitment, which usually occurs 3 - 4 months after spawning in penaeid prawn (Garcia and Le Reste, 1981). This is a common period for penaeid prawn to grow from nauplii into juvenile stage.

CONCLUSIONS

1. This study was the first to describe molt stages in wild kuruma prawn (*M. japonicus*) in Tokyo Bay. Only three out of five stages were represented in the samples since the early premolt (A) and ecdysis (E) occur in a very short time.
2. There was a co-occurrence between ovarian maturation and molt stage, in

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which maturing ovaries occurred at B to D_I molt stage, mature ovaries at C to D_2, and spent ovaries at D_I to D_2.

3. Molting and spawning synchronization was observed in kuruma prawn, in which molting frequency was highest prior to and at the end of spawning season.
4. There was a possibility of multiple spawning within single molt cycle in wild kuruma prawn, since there seemed enough time for spent ovaries to redevelop into mature ones.

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