Timing and Synchronization of the Breeding Period in *Pilumnus vespertilio* (Crustacea: Pilumnidae) in Subtropical Okinawa, Japan

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Abstract: Observations on the temporal occurrence of copulating pairs, ovigerous females, monthly brooding periods, and embryo development stages in *Pilumnus vespertilio* Fabricius were carried out in the wild on Okinawa Island, Japan. The relationship between the female gonad and hepatopancreas during a reproductive period was also studied. These reproductive activities were observed in relation to the lunar cycles. An inverse relationship between mass of the hepatopancreas and development of the gonad was observed. Mating and breeding activities were perfectly synchronized with the lunar periodicity. Five broods from May to September were observed in a single breeding period, and the average brooding period was 21.2 days with an interlude of $8.5 \pm 3.1$ days between broods/months. The shortest interlude was between May and June (4 days). A sixth brood that started in October was not followed to the end because it started with very few ovigerous females. Embryo development time in days decreased with each stage and averaged 5.3 days per stage. Copulating activity and appearance of ovigerous females during successive broods (months) were clearly synchronized with the lunar cycle. Although copulating frequency was highest after the full moon, nearly 100% of females were ovigerous around the new moon. All females of any one sample carried eggs of the same development stage. All females released their larvae 1–3 days before full moon, coinciding with a high tide. Larvae are probably released during this time as a survival strategy against predators such as planktivorous fish and against adverse intertidal conditions during other times.

IN GENERAL, BRACHYURAN breeding periodicity can vary considerably; the major controlling factors appear to be latitude, temperature, larval food availability, and intertidal zonation (Sastry 1983). The tendency is toward extended reproductive seasons and continuous reproduction with decreasing latitude in benthic shallow-water crustaceans. With increasing latitudes, breeding seasons become more restricted to periods of higher water temperatures (Giese and Pearse 1974, Sastry 1983, Kyomo 1986). The effect of intertidal zonation on breeding periodicity has been shown in tropical brachyuran species *Sesarma messa*, *S. smithi*, and *S. erythroactyla*. These species do not breed all year round but for 3, 3, and 5 months of the year, respectively, and their reproductive strategies are related to their positions on the shore (Greenwood and Fielder 1988). *Macrophthalmus granidieri*, a shallow-water tropical species, breeds continuously year-round, whereas various species of *Uca* in the same area but of different zonation do not (Emmerson 1994). For one reason or another, most of the studies on brachyuran reproductive ecology have been reported on different species of only these families: Grapsidae (e.g., Hiatt 1948, Seiple 1979, Saigusa 1981, Kyomo 1986, Omori et al. 1997, Tsuchida and Watanabe 1997), Ocypodidae (e.g., Christy 1978, Zucker 1978,

Very few reproductive studies, however, have been done on species of the family Xanthidae; for example, *Neopanope sayi* (Swartz 1978, De Vries and Forward 1989), *Panopeus herbstii* and *Eurypanopeus depressus* (McDonald 1982), *Leptodius exaratus* (Watanabe et al. 1990), and *Eriphia smithii* (Tomikawa and Watanabe 1992). Knudsen (1960) described reproduction, life history, and larval ecology of four xanthids: *Lophopanopeus l. leucomanus, L. bellus diegensis, Paraxanthias taylori, and Cycloxanthops novemdentatus* in California. *Pilumnus vespertilio* Fabricius was a member of this family until very recently (Kyomo 1999, 2001). Notwithstanding, none of these studies on xanthids has reported on the relationship between breeding activities and the lunar cycles.

The only reported studies on *Pilumnus vespertilio* (Pilumnidae) include its geographical distribution (Sakai 1976), larval development (Lim and Tan 1981), feeding patterns and food habits (Kyomo 1999), and reproductive behavior (Kyomo 2001). The distribution of *P. vespertilio* ranges widely from Japan, central Pacific to Australia and New Zealand, various areas of the Indian Ocean, and the east coast of Africa (Sakai 1976). In Japan, this species is found in various localities of Sagami Bay and Okinawa Island (Sakai 1976). Preliminary surveys for this study on Okinawa showed that the species is found mostly in the northern (Motobu) and central (Maeda Misaki) parts of the island.

Larval development stages of xanthids, including *P. vespertilio*, have been studied as early as 1928 (Lim and Tan 1981). However, there have been no studies on the reproductive biology of *P. vespertilio*. This study first focused on the breeding activities of *P. vespertilio* with regard to the relationship between gonad and hepatopancreas activities. It was necessary to include the information on gonad activity because this organ is the primary indicator for the initialization of egg bearing and reproduction in crabs, and essentially growth and development depends on the food resources stored in the hepatopancreas (Adiyodi 1969, Kyomo 1986, 1988, Omori et al. 1997). Second, the study demonstrated how the occurrence of copulating pairs, ovigerous females, and the brooding period is synchronized with lunar cycles. Synchronized spawning in reproductive females was examined in terms of spawning time, monthly numbers of females becoming ovigerous, brooding periods, and larval release times.

**MATERIALS AND METHODS**

**Study Sites**

This study was carried out on Okinawa Island (26° 38' N, between 127° E and 128° 30' E), Japan. Although the island has subtropical characteristics, it experiences all four seasons of a temperate climate. Two sites, Bise (10 km from Sesoko Research Center) and Maeda Misaki (53 km from the Research Center), were used for sampling. Both sites are coral reef flats covered by water at high tide and are fully exposed only during very low tides of ≤60 m. The sites are also used as fishing and diving spots. Furthermore, at the Bise site two populations were studied, 1.5 km apart on the same stretch of reef. Preliminary surveys showed that *P. vespertilio* was distributed in patches ranging from two to four mature individuals per square meter. A mature female was defined as the smallest female carrying eggs at 15.0 mm carapace width (CW). A mature male was defined as the smallest individual of 16.0 mm CW found in copula. Individuals are most active during the ebbing tide, and particularly when there is still pool water in the burrow neighborhood.

**Ovary and Hepatopancreas Activities**

Ten to 20 mature females, irrespective of their reproductive condition (i.e., ovigerous or nonovigerous), were sampled once during full moon and once during new moon during daytime low tides, every month from April to October 1999. The sampling was alternated...
between the Bise and Maeda sites. Specimens were stored on ice and taken to the laboratory as soon as sampling was completed. Individuals were measured for carapace width and dissected to remove the gonads and hepatopancreas. The organs and bodies were weighed after drying at 60°C for 48 hr. Dry gonad and hepatopancreas indexes were then calculated as ratios of the weight of these organs to the total dry body weight (organs inclusive) \( \times 100 \) for each individual. These indexes have been referred to as the gonadosomatic index and the hepatosomatic index (Kyomo 1986). A mean was then calculated to determine the index for the month, irrespective of the reproductive condition of the female. The population of *P. vespertilio* was too small to collect a good sample size for each reproductive condition during the year.

**Active Numbers, Coupling, and Ovigerous Females**

Observations and sampling were done during daytime low tides of \( \leq 60 \) m within 2 hr before the lowest tide mark, from April to September. All mature and active individuals were captured and counted from transects 200 m long and from the low tide water’s edge to 35 m toward the reef center. An individual was regarded as active when its body was wholly emerged from the burrow/crevice irrespective of any movement. Gender and the condition of females were also determined. Copulating pairs in an embrace position were counted; individuals were turned to the ventral side of their body and their abdomen was lifted by forceps. An easily lifted and pliable abdomen, especially in males, shows that the male opens it for copulation. Otherwise, abdomens of uncopulated and particularly of immature males are held firmly to the sterna (Knudsen 1960). For escaping pairs, it was the female who entered the nearby burrow/crevice. The male was captured and the condition of the abdomen was checked. Seasonal changes in daily numbers of active individuals, copulating pairs, and ovigerous females were determined and related to the cycle of the moon. Successive observations and sampling were alternated between Bise and Maeda Misaki.

**Temporal Spawning and Brooding**

The determination of spawning dates and the number of brooding days needed to be done daily and this was done on the Bise populations only. Sampling at Maeda Misaki was stopped temporarily during the determination of the spawning dates. During spawning and brooding times, temperature of the air and pool water was noted. An intensive search for ovigerous females was done on every sampling day. A small sample was “pinched off” from the egg masses of 10 to 15 ovigerous females and their embryonic development stages were observed under a microscope. Development stages were modified from Henmi and Kaneto (1989) and categorized as follows. Stage I(a): egg mass (orange) fully filled with a compartmentalized yolk, leaving no open space on the margins; stage I(b): compartments have disappeared but yolk appears as oil globules with 5–10% space on the margins of the egg mass; stage II: yolk looks like an assemblage of oil globules and is slightly pulled off the spherical margin, leaving 10–35% open space at one point or around the entire egg mass, and two dotlike red eyespots may be seen; stage III: two elliptical, light brown eyespots, surrounded by an orange color, and yolk is reduced to \( \leq 50\% \) of the egg mass; stage IV: two distinctive elliptical, dark brown eyespots, and developing blood vessels and abdomen may be seen; the yolk is reduced to \( \leq 20\% \) of the total egg mass, which now appears whitish. Spawning dates, brooding period, and time interval (interlude) between broods were determined from the successive appearance of these breeding activities. The spawning date was set for individuals carrying eggs of development stage I(a). A 1-day interval was observed between stages I(a) and I(b), and females carrying eggs of stage I(b) or later had their spawning date backdated accordingly. All females of any one sample carried eggs of the same development stage.

Monthly means of numbers of individual crabs and gonadosomatic and hepatosomatic indexes were calculated. These were analyzed using Spearman’s correlation coefficients and t-tests (SYSTAT).
RESULTS

Relationship between the Gonadosomatic Index and the Hepatosomatic Index

The gonadosomatic index (GSI) started at a low level of 0.5% in April before reproduction started, with the hepatosomatic index (HSI) about three times higher. As the reproductive period started in May, there was a sharp rise of the GSI and this continued to a very significant \( t = 4.87, P < 0.001 \) high peak in July. The HSI increased, though not significantly \( t = 1.36, P > 0.05 \), from April to May and then started decreasing steadily to a significantly \( t = 4.56, P < 0.05 \) lower level in July. There was a direct relationship between the declining of the HSI from May (2.5%) to the July minimum (1.2%) and the increase of the GSI from its lowest level (0.5%) in April to the July maximum (4.5%). Between July and October the HSI again increased steadily and significantly \( t = 4.43, P < 0.05 \) while the GSI decreased significantly \( t = 9.08, P < 0.001 \) to levels of 2.4 and 0.1%, respectively (Figure 1).

Coupling and Ovigerous Females Related to Lunar Cycle

Coupling was observed mostly around full moon, and the highest peak occurred 4 days after the full moon of every month in May, June, and July. In August, the peak occurred on the sixth day after the July full moon. The only two copulating pairs in September occurred 7 days after full moon (Figure 2). The coupling frequency increased from May and peaked in July. During that period, peaking times were shorter than from August to September when coupling was also decreasing. Although the number of active individuals fluctuated from day to day, it was slightly greater on the peak copulating days than on other days of every month except July. Sep-
Figure 2. Temporal occurrence and distribution of number of copulating pairs (open bars), males (hatched bars), and females (solid bars) of Pilumnus vespertilio (●, new moon; ○, full moon).
tember had the least number of copulating pairs \( (n = 2) \) compared with May \( (n = 23) \), June \( (n = 82) \), July \( (n = 52) \), and August \( (n = 34) \). Although the number of copulating pairs in May was not significantly different \( (t = 0.532, P > 0.05) \) from that in August, this number was significantly \( (P < 0.05) \) lower than that of June and July. There was no significant difference \( (P > 0.05) \) in the number of copulating pairs in June, July, and August.

Figure 3 shows that practically no ovigerous females could be seen between full moon and 5-10 days after full moon of each month. The interlude between brooding periods was 8.5 ± 2.4 days. For copulating pairs, the average number of daily active ovigerous females increased steadily from 11 in May, to 28 in June, to 45 in July, and began decreasing to 32 in August and 16 in September. Of the daily active number of mature females during the brooding period of each month, nearly 100% were ovigerous in July and August (Figure 3). The total number of ovigerous females in May and September was significantly \( (P < 0.05) \) smaller than the total number in either June, July, or August. However, there was no significant difference \( (P > 0.05) \) in the number of ovigerous females in June, July, and August.

**Spawning and Brooding Period Related to Lunar Cycle**

Females of *P. vespertilio* started spawning on 11 May, about 8 days after the first mating and copulating date on 3 May. This first spawn of May was brooded for 21 days, and all ovigerous females released their larvae 1 day before full moon. The incubation periods were 23, 21, 20, and 21 days in June, July, August, and September, respectively, and the larvae releasing events took place 2–3 days before the day of the full moon. This gives an average incubation period of 21.2 days in *P. vespertilio* (Table 1). The incubation period between months was not significantly different. However, embryonic development stages were incubated for different lengths of time (days) and the number of developing days in advanced stages decreased in all months (Table 1). The embryonic development of stage I took the longest time (6.2 days) to develop to stage II, whereas stage IV had the shortest duration (only 4 days to develop to larval stage and release time). The number of developmental days between stage I and II was not significantly different \( (t = 1.00, P > 0.05) \). However, other comparisons of development stages were significantly different: between stage I and III \( (t = 5.19, P < 0.05) \), stage I and IV \( (t = 8.66, P < 0.05) \), stage II and III \( (t = 5.00, P < 0.05) \), stage II and IV \( (t = 9.00, P < 0.05) \), and between stage III and IV \( (t = 6.00, P < 0.05) \).

**Air and Pool Water Temperatures Versus Brooding**

Both air and pool water temperatures increased from the lowest (26.7°C and 25.3°C) in April to the highest (31.6°C and 33.7°C) in July, respectively (Table 2). Variations in air and pool water temperatures occurred between months. These variations were either significantly different (e.g., between May and June [air (A), \( t = 4.429, P < 0.05 \); pool water (PW), \( t = 6.804, P < 0.05 \)]) or not significantly different (e.g., between June and July [A, \( t = 1.247, P > 0.05 \); PW, \( t = 1.275, P > 0.05 \)]). Temperature seemed not to have any influence on the variations of embryonic development stages and on the duration of brooding period between months (Table 2). For example, females in June, which had pool water temperature equally as high as in August but relatively lower than in July, incubated eggs for the longest period (23 days).

**DISCUSSION**

A high hepatosomatic index at the beginning of the reproductive period was followed by a high gonadosomatic index and therefore a large number of ovigerous females. In this and other cited studies, the hepatosomatic index declines and always stays at lower levels during the reproductive period when organic sources are being mobilized for vitellogenesis (Kyomo 1986, 1988, Omori et al. 1997, Tsuchida and Watanabe 1997). Most probably, food acquisition (i.e., feeding) does not compensate for the organic materials utilized
FIGURE 3. Occurrence and distribution of number of ovigerous females (hatched bars) and mature nonovigerous females (solid bars) in relation to the lunar cycle (new and full moon as in Figure 2). Bars and roman numerals below the dates for each month indicate brooding periods and embryonic development stages, respectively.
### TABLE 1
Number of Days of Development of Embryonic Stages of *Pitumnus vespertilio* in Each Month

<table>
<thead>
<tr>
<th>Stage</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>September</th>
<th>October</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6 (6)</td>
<td>7 (13)</td>
<td>6 (38)</td>
<td>6 (29)</td>
<td>6 (20)</td>
<td>6 (3)</td>
<td>6.2</td>
</tr>
<tr>
<td>II</td>
<td>6 (18)</td>
<td>7 (36)</td>
<td>6 (54)</td>
<td>5 (50)</td>
<td>5 (17)</td>
<td>-</td>
<td>5.8</td>
</tr>
<tr>
<td>III</td>
<td>5 (17)</td>
<td>5 (39)</td>
<td>5 (56)</td>
<td>5 (-)</td>
<td>6 (-)</td>
<td>-</td>
<td>5.2</td>
</tr>
<tr>
<td>IV</td>
<td>4 (11)</td>
<td>4 (19)</td>
<td>4 (40)</td>
<td>4 (22)</td>
<td>4 (-)</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Total (days)</td>
<td>21</td>
<td>23</td>
<td>21</td>
<td>20</td>
<td>21</td>
<td>-</td>
<td>21.2</td>
</tr>
<tr>
<td>Mean number of days of development per stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5.3</td>
</tr>
</tbody>
</table>

Note: Mean number of active ovigerous females of each embryonic development stage is shown in parentheses. Only two ovigerous females with stage IV eggs were active on 21 September following a strong typhoon, and only three females became ovigerous in October (see Figure 3). October females are not included in the determination of the mean.

### TABLE 2
Monthly Air (A) and Pool Water (PW) Temperatures (°C) (Mean ± SD) during the Observed Spawning and Brooding Periods at the Bise Study Site

<table>
<thead>
<tr>
<th>Monthly Temperatures (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
</tr>
<tr>
<td>April</td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>August</td>
</tr>
<tr>
<td>Range</td>
</tr>
<tr>
<td>Mean</td>
</tr>
</tbody>
</table>

Note: Data were collected on only 10 days in October and therefore its mean temperatures were not included.

from the hepatopancreas at a rate fast enough to meet the reproductive activities (Kyomo 1988, 1999). When the breeding period ends, there is a rise in the index level of the hepatopancreas because more organic materials are stored again and ready for the beginning of the next reproductive period. This shows that the hepatopancreas plays a vital role in the breeding periodicity of crabs. The gonadosomatic index, a more important indicative parameter for breeding periodicity, depends very much on the organic materials stored in the hepatopancreas. This index is high when the hepatosomatic index is low, and vice versa.

At a local population level, environmental physical factors such as temperature may control and trigger the activity and timing of reproduction in crabs (Sastry 1983, Kyomo 1986). However, in some cases such factors may not be more important for continuous breeding periods than food availability can be (e.g., Kyomo 1986, Henmi and Kaneto 1989, Emmerson 1994). If food is considered a more important factor than the physical environment for the initialization of reproduc-
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CONCLUSIONS

For many marine brachyurans, and probably for other species of marine organisms, breeding periodicity is synchronized with environmental cycles such as phase of the moon (e.g., Forward et al. 1982). Most intertidal animals...
release their larvae during high tides of the full moon (Forward et al. 1982, Christy 1986). But lunar periodicity in hatching of brachyurans is just one and the last event of the egg-carrying period. Data in this study support the thesis that copulation, spawning, and incubation periods in *P. vespertilio* are also synchronized with the lunar cycle so that each episode leads to the production of offspring during appropriate hatching conditions: high tide. This breeding period in *P. vespertilio* seems to be synchronized within individuals too because all reproducing females in successive broods become more or less ovigerous on the same day, even when taken from separate populations. Furthermore, 100% of ovigerous females of any one sample and time carry eggs in the same development stage. This synchrony indicates that some kind of an endogenous control mechanism is present in all individuals. This mechanism has also been implicated in controlling the breeding cycle of *Neopanope sayi* as observed in the laboratory (De Vries and Forward 1989). The mechanism most likely also triggers all reproductively active females of *P. vespertilio* to attract copulating partners and start spawning on a day that will allow the brooding time to end at the beginning of the next full moon of the lunar cycle.

Ultimately it is the larvae that must be produced during the appropriate time if the population of a particular species is to grow and avoid death in the environmentally adverse conditions of the intertidal habitats. There are evolutionary advantages for the larvae to be released according to the lunar cycle and during high tide. These include allowing increased dispersal from the parent population to avoid competition for food (Christy 1978, Saigusa 1981) and allowing larvae to be carried to deep water, decreasing predation by planktivorous animals and minimizing mortality from adverse changes in conditions such as temperature and salinity in shallow water. For a species with low population density, cyclical reproduction must be accompanied by a high rate of oviposition (Omori et al. 1997). Most of these results and arguments for other species seem to be applicable in describing the life cycle of *P. vespertilio* in this study. A breeding period synchronized with the lunar cycle at both the individual and population levels has been shown in *P. vespertilio*, the only pilumnid crab studied in such detail so far.

**ACKNOWLEDGMENTS**

I thank M. Murai of the Tropical Biological Research Center, Sesoko, for reading the initial version of the manuscript and the anonymous reviewers for their valuable comments that greatly improved the manuscript.

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