Control of Hatching in an Estuarine Terrestrial Crab
I. Hatching of Embryos Detached From the Female and Emergence of Mature Larvae

MASAYUKI SAIGUSA

College of Liberal Arts & Sciences, Okayama University, Tsushima 2-1-1, Okayama 700, Japan

Abstract. Embryos of the terrestrial crab Sesarma hae-
matocheir hatch simultaneously just prior to their release into water. Larval hatching occurs synchronously when the embryos are attached to a female, and the role of the female in this synchrony has been investigated. Clusters of embryos (200-2000 berries in each cluster) were detached from ovigerous females, and their hatching was compared with that of embryos attached to the females. Of the detached embryos in a cluster, either all hatched, or none hatched. A remarkable feature was that the success of hatching of these detached eggs depended upon the time of hatching of the eggs still attached to the female. Clusters of embryos that were detached from the female within 48-49.5 h of the projected time of larval release all hatched successfully, and swimming zoeas appeared. But embryos that had been detached from the female for longer periods did not hatch at all, though they were obviously alive. These results suggest a hatching process different from the embryonic development process. The female may trigger this process. In addition, detached eggs hatched later than eggs attached to the female, and their hatching was less synchronized. These observations suggest that the female not only initiates hatching, but also enhances the synchrony of hatching.

Introduction

Studies over the past 50 years have demonstrated that many biological rhythms are driven by an endogenous pacemaker which, when coupled to an environmental cycle, adjusts the phase of the rhythm to local time. In contrast to the abundance of information about the timing systems in terrestrial animals, our knowledge of the mechanisms and ecological significance of such systems in marine organisms is severely limited. The reasons are evident: relatively inaccessible habitats, technical difficulties in monitoring the activity, and the noise that is often observed in locomotor activity patterns. Notwithstanding these restrictions, clearly-demarcated biological rhythms associated with reproductive activity have been demonstrated in a variety of marine animals; e.g., a polychaete (Franke, 1985), intertidal midges (Koskinen, 1968; Hashimoto, 1976), and estuarine crabs (DeCoursey, 1979, 1983; De Vries and Forward, 1989). This paper also concerns a rhythmical reproductive activity, focussing on the control of larval hatching in a marine crustacean.

Fertilized eggs of most marine crustaceans are attached by a funiculus to the abdominal appendages of the female, and are ventilated by the female during embryonic development. When the development is complete, the outer egg membrane breaks, and the larvae hatch. These larvae are released into water with a special fanning behavior of the female’s abdomen. Larval release is generally a short-lived event, and the timing is often correlated with such environmental periodicities as day-night, tidal, or lunar cycles. In lobsters (Ennis, 1973; Branford, 1978; Moller and Branford, 1979), for example, larvae hatch at the same time each night. The fiddler crab Uca also releases larvae for several minutes, in synchrony with nocturnal high water (DeCoursey, 1979, 1983). Such a short and precisely timed event implies that the timing of larval emergence from the egg capsule must be synchronized within each batch of embryos. Accordingly, we must ask whether the timing of hatching is controlled by the embryo itself, or by the female.

Previous studies of this problem have produced conflicting results. For the lobster Homarus gammarus, some investigators indicated that an endogenous factor in the
embryo itself sets the hatching time (Pandian, 1970; Ennis, 1973). Forward and Lehmann (1983) showed that embryos control the time of hatching, and suggested that the role of the female is to synchronize the development of the embryos. On the other hand, Branford (1978) showed that the female lobster controls the timing of hatching. This study is also aimed at this general problem, and the focus is on the distinction between the control of the hatching process and the control of embryonic development.

Females of the crab Sesarma haematocheir were used in these experiments. Each female incubates 20,000–60,000 eggs on her abdomen. While incubating, the females remain hidden under litter on the slopes of steep, wooded hills overlooking small estuaries. After the embryos have developed for a month, the female emerges, descends to the riverside, and releases her zoea larvae into the water. Larval release occurs at night. Release is synchronized with the time of high water (Saigusa, 1982, 1985), and the timing is endogenously controlled (Saigusa, 1986). Field observations (Saigusa, 1992) demonstrated that larval hatching occurs on the bank, just before the larval release, and not in the water.

Thus, release behavior is caused by the stimulus of hatching, i.e., what is actually controlled is the timing of larval hatching from each egg, not the release behavior itself. Hence, the main questions to be answered are: (1) how do the larvae hatch simultaneously; (2) how is the timing synchronized to the time of high tide at night; and (3) does the female, or do the embryos, control the timing of hatching? To answer all of these questions, many experiments will be necessary. But as a first step, the hatching time and hatching synchrony of detached embryos were compared with those of embryos attached to the female.

**Materials and Methods**

Female Sesarma haematocheir bearing eggs that appeared likely to hatch within 1–10 days (see Saigusa, 1988 for signs) were collected from the field at Kasaoka, Okayama Prefecture (Saigusa, 1982), and brought into the laboratory. The dates of collection were: 23 and 27 September 1988; 18 and 29 July 1989; 6, 14, and 28 August 1989; 31 July 1990; and 5 September 1990. The females were maintained in experimental rooms where light and temperature were controlled. A 15 h light:9 h dark photoperiod, similar to that in the field, was employed (light-on at 5:00; light-off at 20:00). Temperature was maintained constant at 23 ± 1.5°C. Groups of 1–5 females were selected and placed in four experimental rooms under the same conditions of light and temperature. Embryos were then detached from these females. Hatching of the larvae from these eggs, the swimming ability of the larvae, and the timing of these events were compared with embryos still attached to the female. (The terms ‘egg’ and ‘embryo’ are used synonymously in this paper. The embryo is considered as such until it hatches; it is then called a zoea larva.)

Detached eggs were prepared as follows. A cluster of embryos (200–2000 berries) was removed from the female together with a portion of the ovigerous hairs. This cluster was then suspended—by cotton thread tied to a horizontal nylon thread—in the center of a small plastic container (usually with a diameter of 8 cm and a depth of 6 cm). This container was then placed into a 1 l glass beaker containing about 600 ml of diluted, clean seawater. (The seawater was sterilized by boiling, and diluted with distilled water to a salinity of 10%). The water was strongly aerated with an air stone placed in the bottom of the container (for a figure, see Saigusa, 1992).

The embryo cluster was detached from the female at various times of day for experimental purposes (e.g., Fig. 2). In the 1988 experiments, detachment from, and binding to the nylon thread were carried out with a small handheld flashlight during the dark period. The intensity of the light was very much reduced. The procedure was difficult with a hand-held light, so a head-attached light covered with a few sheet of red cellophane was used in the experiments of 1989 and 1990. To reduce the influence of light as much as possible, the manipulation of each sample was completed rapidly, i.e., within 5 min. Thus, each female experienced the light at different times, and only briefly (Fig. 2c). Almost all of the crabs released their zoeas within 4-5 days after having been placed in the experimental rooms. Therefore, such a weak and brief light is not likely to have affected the timing of hatching, either of the isolated embryos, or of the embryos attached to the females.

Detached eggs that did not hatch were placed in a beaker for 7–10 days, and if vigorous aeration and turbulence were provided, these isolated embryos remained alive. Detached eggs hatched on the same night as those still attached to the female, and all of the larvae emerged from their egg cases on a single night, or by noon of the following day, at the latest. After the larvae escaped into the water, the empty egg cases still remained attached to the ovigerous hairs. Therefore, I easily determined whether hatching had occurred and what proportion of the swimming larvae had emerged. This examination was mostly carried out during the light period, and a stereo-microscope was used as required.

In some of the experiments, the hatch time of detached eggs was monitored. At intervals of 30 min or 1 h, the plastic container in which an egg cluster was being maintained was transferred to another beaker with a similar quantity of diluted (10%) seawater. The original beaker was then removed from the experimental room, and the number of swimming zoeas was counted with the help of
a pipette. This exchange of beakers was made during the dark phase under the illumination of a hand-held flashlight covered with red cellophane.

Each female from which some embryos had been detached was also monitored, and the time of larval release recorded. The recording system consisted of a sensor unit (infrared source-receiver) and a controller unit with a photoelectric switch. The sensor unit was placed in the experimental room, and the controller unit was set outside the room. The larval release could thus be monitored without a change in ambient light. The female was confined in a perforated plastic cage suspended from the rim of a 1 or 2 l glass beaker containing diluted, clean seawater (500 or 750 ml; salinity at about 10 %). Only the bottom of the beaker was immersed in the water. When the larvae were released, they fell through the perforations and into the beaker, where they triggered the photoelectric switch. The output of this system was monitored by an event recorder (Saigusa, 1986). Females release their larvae as soon as hatching is completed, so the time of release clearly marks the completion of hatching.

The time of day that each female released her attached larvae was compared with the time of hatching from the eggs that had been detached from her. However, the day during which a female released her larvae could not be precisely estimated. The signs of hatching only appear on the day of larval release (i.e., a few zoeas begin to swim in the glass beaker only a few hours before larval release). Many of the data were like those set out in the upper two sections of Table I. Hence, many comparisons of detached and attached embryos were necessary; i.e., 250 samples from 110 females were examined.

Results

Figure 1a indicates the hatching profile of the eggs attached to a female. The female moved about in the perforated cage, and her body was frequently soaked in water in the glass beaker. On 5 September, as the time of larval release approached, several hatched larvae escaped from the female's abdomen, and were observed swimming in the beaker (23:30–24:00; Fig. 1a). Shortly after a rapid increase in the number of swimming zoeas in the beaker, the female vigorously vibrated her body to release all of the remaining larvae into the water; this triggered the photoelectric switch (0:40 on 6 September). These observations indicate that almost all of the eggs attached to the female hatch within an extremely brief period. The exact time required for the completion of hatching could not be determined because the eggs are attached in a mass, but it was not more than 1 h in this female.

Embryos separated from females before larval release all hatched on the same night as those attached to the female (Fig. 1b). A feature of such instances was that the hatching of detached embryos was not as simultaneous as that of attached embryos; i.e., detached embryos started hatching shortly before the time of larval release by the female and continued for over 7 h. In the experiment shown in Figure 2, eggs were detached from the female at different times on the day of larval release, and the times of hatching were compared. These embryos all hatched, but as in the study illustrated in Figure 1b, hatching extended for 5–6 h. Figure 2c gave no evidence that the red light, on for 5 min, affected the time of hatching of the detached eggs. The time of hatching of eggs detached on the day of larval release (Fig. 3a) were compared with the time of hatching of eggs separated one day before that release (Fig. 3b). The eggs all hatched on the same night as the larval release in this case, too. And as in Figures 1b and 2, the simultaneity of hatching deteriorated. Thus, the eggs detached from the female on the...
day of larval release and one day earlier all hatch regardless of light conditions.

In contrast to these results, most embryos detached more than 2 days before larval release did not hatch. When such detached embryos were placed in a beaker for a week or longer, hatching occurred sporadically. Those larvae that did emerge had poorly developed telsons, dorsal spines, and abdominal appendages. Thus, they were premature larvae (i.e., prezoaeas), and they almost never developed into zoaeas. These prezoaeas sank to the bottom of the beaker.

Embryos enclosed in the egg membrane remained alive, if they were aerated. The heart beat was clearly recognizable under the microscope, and movements of the embryos were often observed through the transparent egg capsule. These movements are a common feature of embryos attached to the female. Fungi and protozoans were never observed to overgrow the surface of eggs. Nevertheless, the local environment of a detached egg cluster may differ from that of an attached cluster and may be less suitable for development. In that case, the induction of hatching and the appearance of swimming larvae should be dependent on the interval between detachment and natural (female-attached) hatching. If this possibility is accepted, then the hatching rate of the isolated eggs should gradually decrease as the time of separation from the female increases.

As a test of this hypothesis, embryos were detached at various times of day before the larval release by the female, and the hatching success rate was recorded. For this purpose, many ovigerous females that were expected to release larvae within a few days were collected from the field and brought to the laboratory. They were set individually in the apparatus used to record larval release. Eggs were detached from these females several times per day, and the success of their hatching was monitored. Table I summarizes the results from three specimens that released their larvae on 16, 20, and 18 August, respectively. Eggs separated from female B–5 all hatched on the same night as the larval release by the female, but the embryos detached from another female, A–15, all failed to hatch. Finally, of the embryos from female C–14, most failed, but the embryos contained in the last cluster of eggs to be detached did hatch. The time of each trial, from detachment of the eggs to the larval release by the mother female, is listed in Table I.

Experiments similar to those presented in Table I were carried out with 250 samples from a total of 110 females. These experiments demonstrate that induction of hatching

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Figure 2. Comparison of the hatching from the eggs detached from a single female at different times on the day of larval release. Times of removal (downward arrows): (a) 15:12; (b) 18:06; (c) 21:25 on 28 August 1989. In figure 2c a red light was used for 5 min to remove the egg cluster. Larval release of the female: 22:20 on 28 August 1989 (upward arrow). N: total number of larvae emerged.

Figure 3. Comparison of the hatching from eggs detached from a single female on the day of larval release (a) with the hatching from eggs detached one day before the larval release (b). Times of removal (downward arrows): (a) 16:20 on 7 August 1989 (hatching was monitored in dark conditions after detachment); (b) around 16:00 on 6 August 1989. N: total number of larvae emerged. Larval release of this female: 0:55 on 8 August 1989 (upward arrow).
LARVAL HATCHING OF A TERRESTRIAL Crab 405

Table I

Hatching profiles of detached embryos and larval release by the female

<table>
<thead>
<tr>
<th>Female identification number</th>
<th>Time of day of detachment</th>
<th>Larval release by the female</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-5</td>
<td>14 Aug. 21:07 (O), 23:06 (O) [27:13] [25:14]</td>
<td>16 Aug. 00:20</td>
</tr>
<tr>
<td></td>
<td>15 Aug. 21:27 (O) [02:53]</td>
<td></td>
</tr>
<tr>
<td>A-15</td>
<td>15 Aug. 22:17 (X) [99:23] [95:39] [74:40]</td>
<td>20 Aug. 01:40</td>
</tr>
<tr>
<td></td>
<td>16 Aug. 02:01 (X), 23:00 (X) [72:02] [70:31]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>17 Aug. 01:38 (X), 03:09 (X) [72:02] [70:31]</td>
<td></td>
</tr>
<tr>
<td>C-14</td>
<td>14 Aug. 20:17 (X), 22:56 (X) [76:43] [74:04]</td>
<td>18 Aug. 01:00</td>
</tr>
<tr>
<td></td>
<td>15 Aug. 21:10 (X) [51:50]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16 Aug. 00:17 (X), 02:35 (O) [48:43] [46:25]</td>
<td></td>
</tr>
</tbody>
</table>

O: A cluster of eggs from which all larvae hatched
X: A cluster of embryos which did not hatch. Hatching time of the embryos detached from the female is expressed as the time of larval release by that female. The female identification number is that of the apparatus in which larval release was monitored.

depends upon the time of larval release by the female, not on the time of egg detachment (Fig. 4 top panel). Those embryos that had been separated from the female for more than about 48 h prior to the larval release exhibited neither hatching nor swimming zoeas. Clos examination of the data presented in Figure 4 (top panel) shows that the drastic change in hatching success occurred within a critical interval: 48–49.5 h (the shadowed interval in Fig. 4 bottom panel).

A question that still remains is whether clusters of embryos detached successively from a single female would hatch or not depending on the time of larval release. To answer this question, I examined the hatchability of embryos obtained from at least two egg clusters that were taken from a single female within several hours before or after the 48 h interval leading up to larval release. The resulting data (similar to those for female C-14 in Table I) were selected from among the values in Figure 3b. All 14 instances showed the same tendency (Table II). A comparison of the time at which each embryo cluster was detached from a female, with the time of larval release by that female, led to the hypothesis that there is a critical interval at 48–49.5 h before the time of larval release.

Discussion

A major question raised by these observations is whether the timing of hatching in crustaceans is controlled by the embryo itself, the female, or both. Previous studies have led to different conclusions. Pandian (1970) suggested that a clock that sets the hatching time is within the egg. Ennis (1973) also proposed an endogenous factor controlling the timing of hatching, but was inconclusive about whether it is in the embryo or in the mother.

More direct evidence derives from the experiments in which a portion of embryos were separated from a female, and the time of hatching of those detached embryos was compared with that of the embryos still attached to the female. In some crustaceans, the embryos can complete their development and hatch as viable larvae even when they are separated from the mother. Branford (1978) reported that Homarus eggs removed more than 10 days prior to larval release still hatched. Similarly, in the estuarine crab Rhithropanopeus, hatching occurred when the embryos were separated 1–5 days before larval release by the female (Forward and Lohmann, 1983). Detached embryos of Homarus hatched rhythmically in a 24 h LD cycle, but arrhythmically under constant light (LL) or constant dark (DD) conditions (Branford, 1978). This might suggest an exogenously cued hatching rhythm. On the other hand, when the ovigerous female was kept in DD conditions, larval hatching showed a marked 24 h rhythmicity, so Branford (1978) concluded that any endogenous component of the rhythm is located in the female. The results of Forward and Lohmann (1983) were somewhat different from those of Branford (1978): embryos removed from the female within two days of larval release hatched at a similar time to the larvae released by the female; hatching synchrony deteriorated with longer removal times. From these results, Forward and Lohmann (1983) concluded that the timing of hatching is controlled by the embryo, and that the role of the female is to synchronize embryo development.

In Sesarma haematocheir, when some eggs were detached from the female, and their hatching was compared with the eggs left attached to the female, the hatching synchrony of the detached eggs decreased (Fig. 1). These features have also been observed in other crabs inhabiting estuaries: Neopanope sayi, Uca pugilator, and Sesarma cinereum (De Vries and Forward, 1991). Desynchronization and delay of hatch time increased when the eggs were aerated at 15°C (unpub. data). So temperature is clearly one of the factors affecting the timing of hatching in detached eggs. But the data of Figure 1 cannot be explained in terms of temperature alone; the desynchronization and delay of hatch time could be due to the absence of some cue from the female.

Furthermore, if the embryos of Sesarma haematocheir were detached from the female sooner than 48–49.5 h before larval release, then only sporadic hatching (delayed by a week) occurred, and the larvae produced did not swim (Fig. 4 and Table II). To explain the failure of
hatching and swimming in prematurely detached embryos. I postulated a process of hatching that, once initiated, requires 48-49.5 h until the motor activity of the embryo becomes high enough for swimming. Indeed, this process and the actual timing of hatching are controlled by the embryo itself under detached conditions (Fig. 4, top). However, the initiation of the hatching process is not likely to be determined within each embryo, as well. If this were the case, eggs removed more than two days before the actual time of hatching would all hatch. Hatching of detached eggs always followed an “all-or-nothing” pattern, with a drastic cut-off at about 48 h before larval release (Fig. 4, bottom). These results suggest that the timing that triggers the start of the hatching process is determined by the female.

Larval release by *S. haematocheir* coincides with the time of high water at night. The phase of this rhythm is endogenously controlled, and the internal period is about 24.5 h (Saigusa, 1986, 1988). Since larval release occurs as a result of synchronous hatching, the timing of hatching would actually be under 24.5 h circa-tidal control. As mentioned above, the hatching process might start between 48 and 49 h before the larval release. In the field, this would occur roughly two nights before the time of high tide, although the physiological mechanisms underlying the commencement of hatching are not yet known.

The embryos of most crustaceans are surrounded by two principle membranes: an outer egg-capsule, and a very delicate inner membrane investing the embryo. The common properties of hatching in the Crustacea are that...
the rigid outer membrane bursts first, and the inner membrane then emerges from the outer membrane. Emergence of this thin membrane occurs gradually in some species and very quickly in others (Marshall and Orr, 1954). Different hatching mechanisms have been proposed. Osmotic hatching has been suggested in a number of Crustacea (Yonge, 1937; Marshall and Orr, 1954; Davis, 1959, 1965). On the other hand, in the lobster Homarus, Davis (1964) proposed that breaking of the egg capsule is caused by the swelling of the embryo itself, due to an uptake of ambient water. Furthermore, in the amphipods Corophium (Fish and Mills, 1979), spines on the embryonic cuticle of the telson are used to break the membranes in hatching.

The outer membrane of S. haematocheir embryos, like that of other Crustacea, is also ruptured by pressure from within. The pressure responsible for bursting could be brought about by the larva, which swells, possibly due to the absorption of ambient water (Saigusa, 1992). This type of hatching makes the synchronization of the large number of embryos attached to the female even more difficult to understand. The temporal correlation between the hatching of the embryos attached to the female, and that of the detached embryos, suggests that a physical or chemical stimulus, transmitted from the female to each embryo, must be responsible for establishing hatching synchrony. De Vries and Forward (1991) postulated a hatching enzyme released by the embryo, and suggested that the timing of its release is controlled either by the embryo itself, or by the mother. In S. haematocheir, the stimuli that induce the hatching process and enhance hatching synchrony are unknown. Nor is it even clear that a hatching enzyme is released by the embryo.

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Literature Cited


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