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Does Lunar Cycle Affect on Egg Hatching and Moulting Frequency in Freshwater CRAB,
Barytelphusa jucequemontii

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ABSTRACT- Evidence is that the Lunar cycle entrain crustacean biological rhythms in briefly reviewed. Egg laying and hatching undergo semilunar and monthly rhythms that appear to depend on endogenous clocks. The lunar cycle can be understood in terms of adaptation and life post larvae n = 150 hatched during March 2002 and rared artificially. Their moulting frequency was monitored from day 112 post hatch to day 204 (CL = 20mm + 1.55 SD.) Daily moults (n=25) recorded individually during July, August and September showed an average duration for the first and second inter moult internal of 34 to 36 days respectively. Daily moulting frequency distribution showed and trend characterized by peak values around new moon and full moon in the absence of any tidal condition. The pattern for commonly rared female crab is more natural water temperature affecting the rhythm. However it is model or many average values of over 24 hours sampling period support the presence of semilunar cycle. The cycle is maintained under laboratory could sublittroral population that are not rhythm may represent the powerful clock that is retained for synchronizing events throughout the life history of crustaceans. It results in reduced preparation risks for hatchery in dark new moon night & favour dispersed during spring tides due to tidal carrent. This may enhance mutual protection against habits simultenously juveniles.

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INTRODUCTION

Lunar phase associated tidal cycle strongly affect on the animals of intertidal zone. Every concrete species can use air or water environment only. It leads to forming special biological clocks which controls activity during day-night in the freshwater (and not only) organisms. Such kind of biological rhythms can be illustrated in the constant laboratory conditions. In this report we present result of 3 years observations. It was illustrated that animals uses different tidal rhythms associated physica factors for orientation. It is current water mass motion, light, level of water pressure, etc. Invertebrates have resistance to the stress influence to many stress factors except one which they use for orientation. This case they are quite sensitive and chages phase of native circatidal (near – tidal) rhythm very quickly. Most of detected species shows hard temperature compensation and maintain rhythmic patterns of behavior in the large diapason of water temperatures. It was shown that some species have age dependent stability of rhythmic activity. For example in the Gammarus family (Amphipoda) tidal associated current water mass

motions control of biological rhythms was observed. Most likely, Lunar phase associated tidal waves forming a water motion during the tidal cycle in the intertidal zone which strongly affects on the creation of circatidal rhythms. We changed native circatidal cycles of activity (12.4 h) of B. Jaquemontii in the laboratory aquariums by different water motion influences.

Ovigerous crabs were monitored in the laboratory to determine if the time of larval release is synchronoused and under endogenous control. To determine the time of larval release, ovigerous females were placed under a 14.10 light / dark cycle simulating the ambient photoperiod. Hatching was rhythmic, occurring as a quick burst lasting about 5 – 15 min shortly after the onset of darkness. An individual mole crab will release batches of larvae for up to three successive nights, suggesting that the rhythm is under endogenous control. Mole crabs monitored under constant low-level red light displayed the same release pattern with hatching occurring near the time of expected sunset, indicating the presence of a circadian rhythm in larval release. To investigate whether the female or the embryos control hatching, a portion of the egg mass (50 – 100 embryos) was separated from the female. The time of hatching of the detached embryos subjected to either a still or shaken treatment was compared with the hatching time of embryos still attached to the female. Detached eggs in both treatments hatched within 1.5-2 h of the time of the female attached eggs, which suggests that embryos control the timing of hatching.

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MATERIALS AND METHODS

Ovigerous females were collected in freshwater during July – September 2001 – 2002. After collection, crabs were immediately transferred to the laboratory and were kept in several plastic containers (70 cm X 50 cm X 40 cm, lenth X width X depth) containing a small quantity of diluted sea water (1-5%). The plastic containers were placed in the experimental room in which light and temperature were controlled.

The 24 h L. D. cycle is critical for maintaining the phase of the circatidal rhythm. In the field, the time of sunset shifts from 19:20 h to 18:15 h, and that of sunrise shifts from 05:00 h to 05:45 h, from early July and 12.5 h: 11.5 h L:D. in mid – September. In the laboratory, we employed similar photoperiods and phases to those observed in the field, i. e. a 15 h: 9 h L:D cycle (light off at 20.00 h and on at 05:00 h) or a 14 h : 10 h L : D cycle (light off at 19:00 h and on at 05:00 h). The intensity of illumination on the floor was 700-1200 lux in the light phase and <0.05 lux in the dark phase. Temperature was constant at $24 \pm 1^{O}C$. In these conditions, the larval – release activity of the population clearly shows the free-running tidal rhythm, the phase of which roughly coincides with the time of nocturnal high tide in the field in the field for at least one month after collection.

They were kept separately in plaster contains, they were transferred to contain of (105 X 120 X 53 mm) light and 12 hrs 23 to 0.5 ca apair of eyestales of each were cut of at their bases with scissors on the following day after the fish laboratory moult occurred only earthworn food given to keep the uniformity of nutritive condition contain after initial moult. Crabs were fed at the schedule time and al. each feeding time enough food was given only the following 24 mm. In each experience either groups an wham which an fed every day (group A) every 3rd day (group B) every 5th day (group C) and warms to which no food is gives (group D). There own exoskeletons which were thrown off every molt however were crab removes from crab of all the group length of carapace days between each molt were measured through the three corrective molts.

The crab of body sizes 9.7.21.6 mm in length of carapace were classified into there groups after their initial moult crab which are for every day (group E) unfed (group F) a crabs which were dried one day after the initial molt. Their own exoskeleton were not fed to the crab of groups E & F. No. Food was given after the first moult till the dryness to eliminate the remained food in their digestive tract crab were dried of 60° c for more than 72 h dry weight was measured after cooling in desicator.

RESULTS

All hatching was monitored by the water exchange method. Female I frequently moved around in the plastic cage and immersed her body into the water from 01 : 30 h to 02:00 h on 3 September 2000, when 11.5% of crabs appeared in the water (top panel) This female liberated most crabs

(87.7%) by vigerous – release behavior between 02 : 00 h and 02 : 30 h. were all mature and swam. The median time of hatching distribution was 02:15 h on 3 September. The synchrony index (SI) was estimated as 43.9 in this female. A cluster if embryos that had detached from this female (detachment at 12:10 h on 2 September, middle panel) also hatched. Hatching peaked between 02: 30 h and 03: h (median 02:15 h) on 3 September. Hatching synchrony deteriorated to an SI of 6.7. A second embryo cluster that had detached from the same female (detachment at 19:00 h on 1 September, bottom panel) also hatched and all crabs swam. Hatching peaked at 02:30–03:00 h (median 02:45h) on 3 September. The SI further deteriorated to 3.7.

The hatching of embryos attached to Female 2 peaked at 02:00 – 02:30 h (median 02:15 h) on 1 September (87.5 %). Larvae were liberated by vigorous – release behavior. The remaining 12.5 % appeared in the water at 02:30 – 03:00 h on 1 September. (A small quantity of crabs often remains after vigorous release behavior. Such crabs are often liberated during a second episode of vigorous release behaviour). An embryo cluster that has detached at 13:35 h on 31 August all hatched (middle panel), with hatching peaking at 03: 45 h on 1 September, with all crabs swimming (bottom panel). However, their hatching was delayed and peaked at 05:45 h on 1 September (SI = 3.6). As shown in these two females, the embryos that detached at least 1 day before larval release all hatched and swam. In contrast, no embryo cluster that had detached more then 2 days before larval release hatched in aerated water (not shown).

This total wet weight of eaggs of 40 early-stage ovigerous females, i.e. with yellow eggs, from Cockburn sound and which covered a wide size rangs, was weighed to the nearest 0,001 g. The number of eggs in each of four replicate subsamples was weighted to the nearest 0.001 g. These dats were thenused to estimate the total number of eggs in each batch of eggs of each female. The relationship between batch fecundity (BF) and carapave width (CW) was described by using the equation:

$$InB [F.sub.j] = min C [W.sub.j] + b.$$

The number of batches of eggs produced by a full size range of mature females during the spawning period was estimated by determining the spawning period (SP), defined as the time (days) when > 5 % of all mature females were ovigerous, and the proportions of ovigerous females among all mature females in sequential 10 – mm CW intervals during the spawning period. The proportion of ovigerous females ([O.Sub.j.]) in the jth size class during this period also represents the average time a mature female in this size class is ovigerous during that period and takes into account the fact that an ovigerous female spawna at least once during a spawning period and that the brood period (BP) of an ovigerous female is about 18 days at 20 [degress] C (Meagher, 1971). Thus, the mean number of batches (N [B.sub.j]) produced by the mature

female crabs in the jth size class furing a spawning period (average water temperature 20.5 [degrees] C) can be estimated with the equation:

$$N [B.sub.j] = [O.sub.j] SP / BP.$$

The relationship between number of broods and carapace width was described empirically by fitting a modified logistic curve, NB = 1 + N [b. sub. Max] / [1 + exp [-in (19) (CW) – a) / (b-a)], ranging upwards form a minimum of one batch to a maximum of 1 + N [B.sub.max] batches, where a and b are parameters. The total fecundity of crabs at different carapace widths was calculated as the perodct of batch fecundity, BF, and the number of broods, NB, by using the relationships between BF and CW and NB and CW, as described above.

The molt interval were most prolonged at the later molt & the crabs which were fed the least (Table 1) In group C, the mean moult interval between the second & IIIrd moult was about two times as long as the interval between the initial & the first molts. It was also about two times longer than the interval between the second, the molts in group A.

This mean increase rate of carapace length, after each moult was the greatest in group A intermediate in group B & the smallest in groups C an F (Table 2). At the first moult, the mean rate of increase in carapace length was 20.4 in a group A but only 10.7 in group D. At the third moult. It was 13.9 in group A but 4.4" group C.

Crabs surviving through the third moult after the removal of egestalle initial moult 14, 17 and 6 of 24 crabs at the time of the third moult and they were exculpated froms the present data. All of the crab in the group D completed the first. Moult but 3 of there died after the first moult & all of the others died at the time of the second moult.

The mean rate of increase in compare length after each molt was plotted against the das following the initial moult.

Eggs recorded for a single batch of eggs under the abdomen of a female, ranged from 68, 450 in a crab with a CW of 84 mm to 324,440 in a crab with a cw of 154 mm. The relationship between batch fecundity (BF) and carapace width (CW) is described by the following equation:

$$I BF = 1.82081 nCW + 3.2862.$$

The vast majority of previous estimates of the fecundity of crustaceans have been based on the number of eggs borne by females at a particular time which, in the case of multiple spawners, does not take into account the fact that larger crabs can produce two or more batches of eggs within a spawning period. The few previous attempts to obtain the total fecundity of crustaceans have involved tracking the number of batches of eggs borne by particular individuals at different times (e.g. Chubb *et al*.). The advantage of the approach developed during the current study is that it uses a combination of batch fecundity and an estimate of the number of batches produced during the spawning period by female of different carapace widths to determine the relationship between the total fecundity and body size of this spacies in a given population. Because the older

crabs have a far longer intermolt period between copulation and egg extrusion than younger crabs, i.e. eight versus four months, they have a far greater amount of time to accumulate the energy reserves required to produce eggs. This difference accounts for the greater number of egg batched produced by larger than small crabs.

Table 1: Moulting frequency in *B. Jaquemontii* on full Moonday

Molt	Group A Fed Every day 14	G-B Fed Every 3rd day 17	G-C Fed Every 5th day 5	G-D Unfed 21
Initial to I & Ist	59 $\pm$ 0.1	6.8 $\pm$ 0.2	7.0 $\pm$ 0.0	7.4 $\pm$ 0.3
1st to 2nd	601 $\pm$ 0.2	0.1 $\pm$ 0.3	11.2 $\pm$ 0.5	15.8 $\pm$ 0.0
2nd to 3rd	7.7 $\pm$ 0.3	12.2 + 0.6	14.8 $\pm$ 1.1	-

The mean days of the moult intervals.

Data were obtained from the crab, which completed the three corrective molts excepts. For the unfed (D) in this group all crabs died at the time of second molt. The numbers of individuals measured.

Table 2: The average compares length (molt) after each moult in crab as well as eyestalles crab on New Moon day

Moult	Group A Fed Every day	G-B Fed Every 3rd day	G-C Fed Every 5th day	G-D Unfed 2
Initial	10.8 $\pm$ 0.2 $\pm$ 3	11.1 $\pm$ 0.0	1.2 $\pm$ 0.3	11.2 $\pm$ 0.2
1st	13.0 $\pm$ 0.3 (20.4)	12.9 $\pm$ 0.2 (16.2)	12.4 $\pm$ 0.3 (15.3)	12.4 $\pm$ 0.2 (20.7)
2nd	15.1 $\pm$ 0.4 (15.2)	14.4 $\pm$ 0.3 (11.6)	13.6 $\pm$ 0.3 (9.7)	-
3rd	17.2 $\pm$ 0.4 (13.9)	15.3 $\pm$ 0.3 6.3	14.2 $\pm$ 0.4 4.4	-

DISCUSSION

Hatching of embryos shows a tidal rhythm, and the timing of hatching is controlled by an endogenous clock or pacemaker in the female. This study focused on the localization of the circatidal clock in the opticpeduncle of the eyestalk in the female. The treatments and their effects on hatching and hatching synchrony are summarized. No effects indicated that neither hatching nor hatching synchrony were different from those of the control group (embryos attached to the female in. In these females, hatched larae were liberated by vigorous begaviour. Synchrony with the nocturnal high tide was also maintained in these females.

Male and female B. Jaquemontii that was eyersall ablated in the spring on same, developed gonads larger than the intact contrils. This was con-

vured with the observed season of remoduration in the field. That both sexes were responsive to eyestalle ablation is not unusual & is supported by field observation of seasonal gonadal development. For this species as well as other species (Lindbay 1955, Berry 1971). The female on experienced responded to eyestalle ablation in a size (age) dependend manner vitellogenesis occurred only in eyestalle ablated animals greater than 70 mm carapoel length. There result indicates that –70 mm length rocyhly apportunities the point of potential reproductive competemy. Field studies in collection region reveal that relatively few females below 70 mm catopael length bean external egg (warver et as 1977 Davis 1975) others have reported ovarian development. In crustalequs taken place in stepwise manner first ovarian growth then vitelloyeneris both of which are controlled by eyertalle faltors (Charnia are cotton 1960). Furthnmens the sequenent pf ovanian development has been compaired to the somatic growth counterponl limit gennation where also controlled by eyestalle fastus (Adiyodi & Adiyodi 1970). Thus, sequential Stepown Sowade growth may be similar to development & vitellogenesis in the overles.

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