



## Full time mothers: daily rhythms in brooding and nonbrooding behaviors of Brachyuran crabs

Nathaly Ruiz-Tagle<sup>a</sup>, Miriam Fernández<sup>a,\*</sup>, Hans-Otto Pörtner<sup>b</sup>

<sup>a</sup>Estación Costera de Investigaciones Marinas and Center for Advanced Studies in Ecology and Biodiversity, Departamento de Ecología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Casilla 114-D, Santiago, Chile

<sup>b</sup>Alfred-Wegener-Institut, Columbusstr., D-27568 Bremerhaven, Germany

Received 27 March 2002; received in revised form 24 May 2002; accepted 8 June 2002

### Abstract

Daily rhythms in activity have been reported for marine and terrestrial organisms, including brooding behaviors which supply oxygen to the embryos of marine invertebrates. Considering that oxygen is a limiting factor in embryo masses of Brachyuran crabs and that female crabs play a critical role in supplying oxygen to the embryos, we studied and compared daily patterns of (1) brooding and nonbrooding behaviors in brooding females, (2) behaviors in nonbrooding females, and (3) oxygen provision to the embryos in a Xanthid crab (*Homalaspis plana*). We also experimentally evaluated whether daily patterns of nonbrooding behaviors were related to food intake. Behaviors were identified using video recordings and correlated with oxygen analyses in the center of the egg masses by use of microoptodes. According to  $PO_2$  recordings abdominal flapping was identified as the single, most important behavior supplying oxygen to the center of the egg mass, maintained at a constant rate during both day and night. Furthermore, the lack of a daily pattern in oxygen availability in the center of the embryo mass is consistent with the lack of daily pattern in abdominal flapping. In contrast, locomotor activity (walking) and maxilliped movements remained unrelated to  $PO_2$  oscillations and showed a pronounced diurnal pattern, increasing during the night. This strong diurnal rhythmicity in walking behavior decreased as embryos developed. The frequency of locomotor activity and egg ventilation increased as embryo development progressed. The increase in egg ventilation throughout embryo development may be a response to the increase in embryonic oxygen demand during development. The change in locomotor activity was unrelated to feeding activity, but may serve to make the ventilation process more efficient. These findings emphasize that

\* Corresponding author. Tel.: +56-35-43-1670; fax: 56-26-86-2621.  
E-mail address: mfernand@genes.bio.puc.cl (M. Fernández).

oxygen is a crucial factor during early development, affecting the normal rate of development of embryos. In consequence, females permanently provide oxygen to the brood despite daily cycles in other behaviors.

© 2002 Elsevier Science B.V. All rights reserved.

*Keywords:* Daily rhythms; Brooding; Oxygen supply; Brachyuran crabs

---

## 1. Introduction

Biological rhythms are well-known phenomena present in all kingdoms (Hastings et al., 1961; Pengelley and Asmundson, 1971; Palmer, 1973; Tauber and Tauber, 1981; Rathcke and Lacey, 1985). These rhythms include a wide repertoire of behavioral and physiological activities ranging from cellular division (Homma and Hastings, 1989) to complex processes such as sleep–wake (Winfrey, 1982) or hibernation cycles in mammals (Pengelley and Asmundson, 1971). Biological rhythms are triggered (or synchronized) by periodic environmental events (Hastings et al., 1961; e.g., circadian rhythms are synchronized by day–night cycles and tidal rhythms by low and high tides). Internal rhythms provide advance warning of upcoming changes, before signs of the changes appear (Cloudsley-Thompson, 1960; Pengelley and Asmundson, 1971) and are part of an adaptive process that allows individuals to increase their fitness (Maier, 1998).

There are several types of periodic events that trigger biological rhythms in coastal marine ecosystems, among them, tidal, lunar and light/dark cycles, and there are also several reported cases of rhythmic biological activities for marine invertebrate taxa. Intertidal species are affected by tidal cycles, which trigger rhythms in motor activity (e.g., crustaceans and gastropods, Palmer, 1973, 1995; Zann, 1973; Pino et al., 1994), affect oxygen consumption rates (e.g., *Carcinus maenas*; Arudpragasam and Naylor, 1964) and influence filtration rates in mussels (*Mytilus californianus* and *Mytilus edulis*, Rao, 1954). In subtidal species, rhythmic activities are mostly synchronized by light/dark cycles (Palmer, 1973, 1995; Forward, 1987; Naylor et al., 1997). Reproductive behavior also appears to be triggered by periodic events in most animal taxa, including marine organisms. Cycles in reproduction can be a response to long-term (e.g., temperature, light, rainfall, or food conditions) or short-term (e.g., tidal and lunar cycles, day–night cycles, Palmer, 1973, 1995; Babcock et al., 1986; Lessios, 1991) environmental fluctuations, and enhance offspring survival (Morgan and Christy, 1994, 1995). The reproductive rhythms in marine invertebrates include spawning, mating and larval release following lunar rhythms or tidal cycles, among others (Brown et al., 1954; Gifford, 1962; Babcock et al., 1986; Lessios, 1991; Zeng and Naylor, 1997). As of yet, the adaptive significance of reproductive behavior in response to short-term environmental fluctuations (e.g., daily) is not clear.

Daily (or shorter term) fluctuations in oxygen availability in embryo masses of brooding marine invertebrate species of several taxa have been shown (Cohen and Strathmann, 1996; Naylor et al., 1999a). In the center of benthic, gelatinous egg masses of gastropods and polychaetes, oxygen concentration may vary between day and night,

due to oxygen production (photosynthesis) and consumption (respiration) of microorganisms associated with the egg masses (Cohen and Strathmann, 1996). Strong temporal variations in oxygen levels inside the egg mass have also been found among Brachyuran crabs, where embryos are exposed to levels of air saturation between 0% and 100% within minutes (Fernández et al., 2000). In this case brooding female crabs play a critical role in setting the patterns of oxygen provision to the egg mass (Naylor et al., 1999a). Furthermore, females seem to adjust their behavior according to the oxygen demand by the embryos (Naylor et al., 1999a; Baeza and Fernández, 2002; Fernández et al., in press), suggesting that development of the embryos greatly depends on female behavior. Naylor et al. (1997) suggested that female crabs show a daily rhythm in activity, and preferably ventilate the embryos at night. Considering that oxygen is such a critical factor during embryo development (Strathmann and Chaffee, 1984; Strathmann and Strathmann, 1995) and that females play a very important role in providing oxygen to the embryos, daily rhythms in brooding behavior could have important effects on embryo development, if in fact females show strong daily rhythms in the ventilation of embryos.

We addressed this problem experimentally, using a Xanthid crab (*Homalaspis plana*) as a model. Considering that oxygen is likely a limiting factor in embryo masses of Brachyuran crabs and that female crabs supply oxygen to the embryos (Naylor et al., 1999a; Fernández et al., 2000; Baeza and Fernández, 2002), we assumed that brooding behaviors are exclusively those that help to provide oxygen to the embryos. We therefore distinguished behaviors, which support brooding from activities not associated with brooding, by relating the frequency of occurrence of each behavior with the patterns of oxygen availability to the egg masses. Then, we studied and compared daily patterns of (1) brooding and nonbrooding behaviors in brooding females, (2) behaviors in nonbrooding females, and (3) oxygen provision to the embryos. We also experimentally evaluated whether daily patterns of nonbrooding behaviors were related to food intake.

## 2. Material and methods

Brooding and nonbrooding female crabs of *H. plana* (Milne Edwards, 1834) (Decapoda, Brachyura) were collected by divers in the study area (Central Chile, 32–33°S) during their reproductive period (May to August 1999 and 2000). Experimental females were kept in holding tanks (3 m diameter, 0.5 m high) with circulating water and constant aeration at the Estación Costera de Investigaciones Marinas of Las Cruces (Central Chile), where the experimental work was conducted. Brooding female size (carapace width (CW)) ranged between 80.4 and 127.5 mm ( $\bar{x}$  = 102.5 mm CW, SD = 16), and the mean diameter of the embryo masses was 53.1 mm in diameter (SD = 7.03). The mean wet weight of brooding females of this size range varied between 146.7 and 418.8 g ( $\bar{x}$  = 275.9 g, SD = 112). We sorted females according to the stage of development of the embryos in early (embryos with uniformly distributed yolk, absence of cleavage and eyes, yolk reduced to not less than 75% of embryo volume, ca. 25 days of development) and late (between less than 75% of embryo volume and hatching). We also used control females

(no eggs), ranging in size between 95 and 129 mm ( $\bar{x}$  = 112.4 mm CW, SD = 13). All laboratory experiments were carried out at 14 °C.

### 3. Female behavior and the relationship with oxygen availability in embryo masses

Laboratory experiments were conducted in order to determine the relationship between female behavior and oxygen availability in embryo masses of *H. plana* carrying early stage embryos. In this experiment, we used only females carrying early embryos because previous studies have shown that several behaviors are performed simultaneously by females carrying late embryos, and it is not possible to assess the relationship between each female behavior and oxygen availability (Baeza and Fernández, 2002). This experiment also allowed us to discriminate nonbrooding from brooding behaviors (related to oxygen provision). We analyzed all behaviors exhibited by berried females using 24-h video recordings and concurrently monitored oxygen tensions in the center of the embryo mass. Females carrying early embryos ( $n$  = 4 per stage) were placed individually in a transparent plastic tank (25 × 25 × 25 cm) filled with a layer of shell hash, large rocks and well-aerated seawater. At all times, air saturation was maintained by using air pumps. The light/dark cycle (12:12 h, L/D) was simulated with white and red light, respectively, using an automatic switch system. We used a Sony (time-lapse) video recorder and a vigilance camera to monitor female behavior for 24 h. As female behavior was videotaped, oxygen availability (% air saturation) in the center of the brood masses was recorded with optic fibers (Presens microoptodes, Regensburg, Germany). Both instruments were set to the same times. The optic fiber was placed in the center of the embryo mass through a small hole drilled in the fifth abdominal segment of each brooding female (see Fernández et al., 2000 for more details of the methodology). Females were allowed to acclimate for 1 h before oxygen availability and behavior started to be recorded. Prior to the start of the experiment the microoptode was calibrated (0% air saturation: solution saturated with Na<sub>2</sub>SO<sub>3</sub>, 100% air saturation: aerated water from the tank where the experiment was conducted). Oxygen partial pressure (% air saturation) was recorded on a computer once every 5 s during the 24-h experiment.

Female behavior was observed by registering the most frequent brooding and non-brooding behaviors: maxilliped movements, abdominal flapping, and locomotor activity (Baeza and Fernández, 2002). We measured the duration of each specific behavior from the videotapes, and at precisely the same time, we were able to determine oxygen conditions in the center of the embryo mass (difference in oxygen availability immediately before, and right after, each behavior was performed). For each female the duration of each behavior and the level of oxygen availability to embryos were registered six times (randomly selected from the video analyzed). In order to eliminate the effect of any other behavior (performed previously), or combination of behaviors, we used only the cases where each behavior was performed in isolation from other behaviors, and for this reason we could use only females carrying early stage embryos. Different time ranges were used for each behavior since the duration of each was completely different (from seconds, in the case of abdominal flapping, to minutes, for locomotor activity or maxillipeds). Correlation analyses (Spearman) were conducted to discriminate the behaviors related to oxygen provision (brooding behavior, see below) from those unrelated behaviors (nonbrooding).

#### 4. Daily patterns of female behavior

After abdominal flapping was identified as the key brooding behavior (see Results), we determined the daily patterns in brooding and nonbrooding (locomotor activity) behaviors in control females (without eggs) and in females carrying embryos at different stages of development. In this experiment, two factors were analyzed: (1) female condition, for which we used females carrying early and late embryos, as well as control females, and (2) time of the day, for which we included six levels (4-h time blocks during 24 h). The response variables were percentage of occurrence of abdominal flapping (brooding behavior, see Results) and locomotor activity (nonbrooding behavior). We considered brooding behaviors those that were significantly correlated with oxygen provision (abdominal flapping, Baeza and Fernández, 2002; and Results). Nonbrooding behaviors were denominated as those that did not show any correlation with oxygen provision (e.g., locomotor activity; see Results). We did not include maxilliped movements in this analysis of daily patterns (see Results) because females were not always facing the camera in all 4-h time blocks ( $\bar{x}$  = 33.5% of total time,  $SD$  = 23). Thus, a full factorial design was not possible using maxilliped movements since we had missing data for some times of the day. Using the same experimental protocol (and videotapes) described above, we analyzed 18 females (six early, six late, and six controls (without eggs)) over a 24-h period. The presence or absence of each behavior per minute was registered. Using this information, the percentage of occurrence (presence or absence) of each behavior per hour was estimated (adding all the minutes where the behavior was present over 60 min) for females carrying early and late stage embryos, and for control females (no eggs). This percentage of occurrence does not indicate whether the behavior lasted a full minute, but that it was simply present. The hourly data were averaged over 4-h time blocks starting at 20:00 (when the light was switched to red light) and data are reported for time periods of 20:00–24:00, 24:00–04:00, 04:00–08:00, 08:00–12:00, 12:00–16:00, and 16:00–20:00 h. The percentage of occurrence of each behavior in brooding and nonbrooding females was compared among the six 4-h time blocks using two-way ANOVAs. Data were transformed (see Table 1) in order to meet the assumptions of the ANOVA model (factors were fixed). Multiple range tests were used to identify significant differences between treatments.

In order to assess whether daily patterns of nonbrooding behaviors were related to factors such as food intake, a laboratory experiment was conducted for 70 days. Females carrying early eggs and control females (no eggs) were placed in individual tanks, conditioned with a layer of shell hash and a shelter. All tanks had a similar flow of running seawater throughout the experiment. Mean size of brooding females was 96.3 mm carapace width ( $SD$  = 4.3) and of nonbrooding females was 95.4 mm ( $SD$  = 3.0). Five brooding and five nonbrooding females were used. No difference in mean size between the two groups was detected ( $p > 0.05$ ). After 48-h starvation, crabs were fed on fresh mussels (5 per tank, with replacement as the prey were consumed). Every other day, the number of prey consumed was estimated and the daily proportion of mussels eaten was calculated for each female condition (brooding and nonbrooding). Since the experiment ran until the embryos hatched and the proportion of the egg occupied by yolk was monitored every 3 days, brooding females were classified according to the developmental stage of the embryos (early and late), following the same approach that was described above. A two-

Table 1

Results of the two-way ANOVAs conducted to test for differences in the mean percentage of the occurrence of brooding (abdominal flapping) and nonbrooding (locomotor activity) behaviors in brooding and non-brooding females of *H. plana* throughout the day (six 4 h time blocks)

Factor	F-ratio	df	p
<i>(A) Non-brooding behavior (locomotor activity after ln transformation)</i>			
Female condition	7.22	2	0.0013
Time block	4.28	5	0.0017
Interaction	0.77	10	0.642
Error		97	
<i>(B) Brooding behavior (abdominal flapping after square-root transformation)</i>			
Female condition	192.36	2	< 0.00001
Time block	0.10	5	0.99
Interaction	0.41	10	0.94
Error		97	

way ANOVA (with repeated measures) was used to compare daily food intake rate (in number of prey) during the first 25 days of the experiment (corresponding to females carrying early embryos) and the rest of the experimental period (corresponding to females carrying late embryos), and between female conditions (brooding and nonbrooding). Data were square root transformed to meet the assumptions of the model.

## 5. Daily patterns of oxygen availability

For the determination of daily patterns of oxygen availability in brooding females carrying early and late embryos, laboratory experiments were conducted. Oxygen in the embryo mass was monitored using optic fibers, as described above. The two factors included in this experimental design were: (1) stage of development of the embryos (early and late), and (2) time of the day, for which we included six levels (4-h time blocks during 24 h) as above. The response variable was the percentage of time that embryos in the center of the egg mass were exposed to three ranges of oxygen partial pressures (<39.7, 39.7–79.4, and >79.4 mm Hg). Patterns of oxygen availability (expressed in oxygen partial pressure) were analyzed in six 4-h blocks (20:00–24:00, 24:00–04:00, 04:00–08:00, 08:00–12:00, 12:00–16:00, 16:00–20:00). We report the patterns of oxygen fluctuations (using one female for each stage of development as an example), and also the mean estimates of oxygen availability in the center of the embryo mass for females carrying early and late embryos for each time block. We used the percentage of time that the embryos from the center of the mass were exposed to various levels of oxygen saturation as a proxy for oxygen availability. For each female, and each 4-h time block, the percentage of time that we recorded oxygen levels from within the three ranges was estimated. Between four and six females were used for each combination of treatments. Three independent two-way ANOVAs were used in order to test for differences in the mean percentage of time that the embryos in the center of the mass were exposed to different levels of oxygen availability between different stages of development and among the six 4-h time blocks. We used independent two-way ANOVAs for each level

Table 2

Results of two-way ANOVAs conducted to test for differences in the mean percentage of time that embryos spent at different oxygen partial pressures and different time blocks during the day. Oxygen availability was recorded in the center of the embryo mass of females of *H. plana* carrying early and late embryos

Factor	F-ratio	df	p
<i>(A) Low PO<sub>2</sub> (&lt; 39.7 mm Hg, after ln transformation)</i>			
Stage of development of the embryos	373.21	1	<0.00001
Time block	0.49	5	0.78
Interaction	0.56	5	0.73
Error		63	
<i>(B) Intermediate PO<sub>2</sub> (between 39.7 and 79.4 mm Hg after square-root transformation)</i>			
Stage of development of the embryos	7.96	1	0.0067
Time block	0.79	5	0.56
Interaction	1.50	5	0.21
Error		63	
<i>(C) High PO<sub>2</sub> (&gt; 79.4 mm Hg after square-root transformation)</i>			
Stage of development of the embryos	55.23	1	<0.00001
Time block	0.32	5	0.90
Interaction	0.22	5	0.95
Error		63	

of oxygen partial pressure (instead of a three-way ANOVA) because data were not independent. Data were transformed (see transformation for each case in Table 2) in order to meet the assumptions of the ANOVA model (Model I). Multiple range tests were used to identify significant differences between treatments (Zar, 1996).

In order to determine the effect of optic fibers on female behavior, the behavior of brooding females with and without the optic fiber was compared. Using the same experimental set up as above, the behavior of females carrying early and late embryos, with and without optic fibers was recorded. Between four and six females were used for each combination of embryo stage and experimental condition (with and without optic fibers). Using the video recordings, we randomly selected 1-h time blocks, and during this hour the presence or absence of abdominal flapping and locomotor activity was registered per minute (as explained in Daily patterns of female behavior). We used two-way ANOVAs to compare the percentage of occurrence of each behavior (locomotor activity and abdominal flapping) between brooding females carrying embryos at early and late stages and the presence or absence of the optic fiber.

## 6. Results

### 6.1. Female behavior and the relationship with oxygen provision

Two different behaviors were consistently observed in all brooding (early and late stage embryos) and nonbrooding females (without eggs) analyzed: locomotor activity and

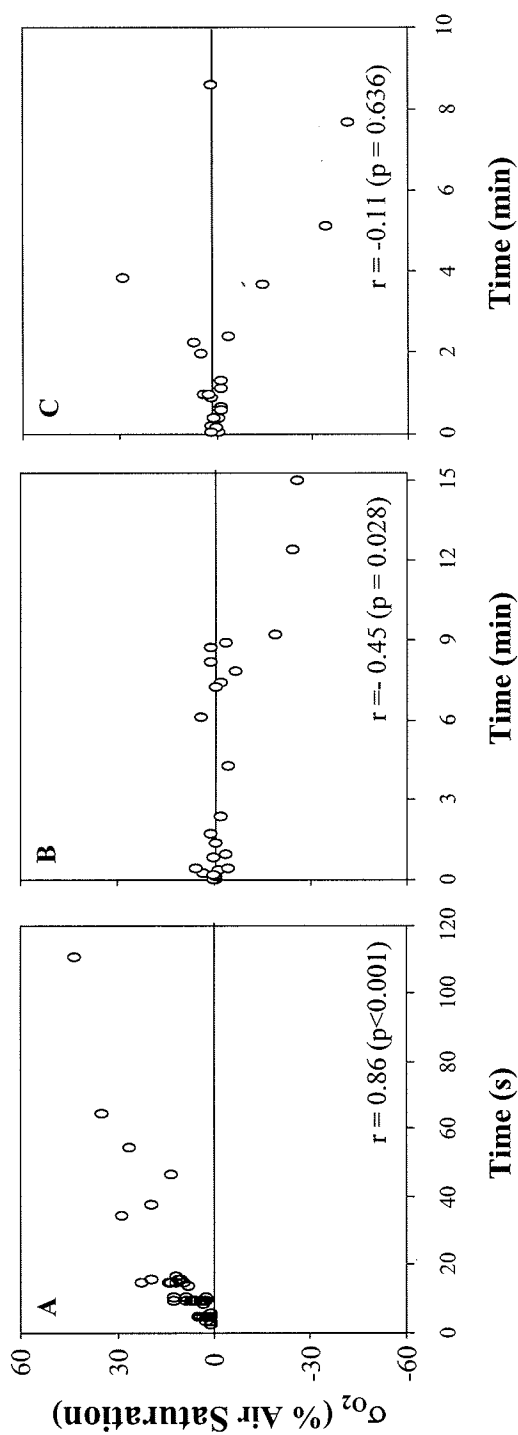


Fig. 1. Relationship between the time spent performing a behavior and the corresponding change in oxygen availability in the center of the egg mass of *H. plana* at the time the behavior was performed. Three behaviors were studied: (A) abdominal flapping, (B) maxilliped movement, and (C) locomotor activity (walking). Results of Spearman correlations are included in the text.



maxilliped beating. In addition, all brooding females exhibited abdominal flapping. We recorded locomotor activity when females moved along the bottom floor of the aquarium using the walking legs, and maxilliped movements when these appendages were opened or closed. We recorded abdominal flapping when females extended their abdomen backwards and forwards. The duration of abdominal flapping (s) was positively correlated with positive changes in oxygen availability in the embryo mass (Fig. 1A) in females carrying early embryos. The duration of maxilliped beating (min) was negatively correlated with negative changes in oxygen availability in the embryo mass (Fig. 1B). Locomotor activity was not at all correlated with changes in oxygen availability in the center of the embryo mass (Fig. 1C). From now on, we will refer to abdominal flapping as brooding behavior (movements present only in brooding females and correlated positively with oxygen availability in the embryo mass). Locomotor activity will be considered as nonbrooding behavior, firstly because it was not used by females to provide oxygen to the embryo mass and secondly because it was exhibited by both brooding and nonbrooding females. Maxilliped movement was not used any further because it does not show a positive relationship with oxygen availability and also for the reasons given above. However, maxilliped beating may be associated to oxygen supply and serve to detect oxygen conditions in the embryo mass (Baeza and Fernández, 2002).

## 6.2. Daily patterns of female behavior

From now on, we compared only two behaviors: one brooding (abdominal flapping) and one nonbrooding (locomotor activity). These two extreme behaviors (in terms of presence in brooding and nonbrooding females, and in relation to oxygen availability in the embryo mass) allowed to address the question of rhythmicity without interference with other factors. Females of *H. plana* showed contrasting daily patterns in the two studied behaviors. The nonbrooding behavior showed daily variations in the percentage of occurrence while brooding behavior did not exhibit any change over time (Fig. 2). The nonbrooding behavior was affected by both factors included in the experiment (Table 1A). The percentage of occurrence of nonbrooding behavior was significantly higher in females carrying late embryos than in those carrying early embryos or in nonbrooding females during both day and night ( $p < 0.05$ ). Nonbrooding behavior, in general, was highest between 24:00 and 08:00 than during any other time block ( $p < 0.05$ ; Fig. 2). Within the day, no differences were detected ( $p > 0.05$ ). The diurnal pattern was less expressed in those females carrying late eggs with a significant level of locomotor activity during the day. In contrast, brooding behaviors were never affected by the time of day (Table 1B), although the percentage of occurrence of abdominal flapping was evidently increased in females carrying late embryos to a level significantly higher than in females carrying early embryos ( $p < 0.05$ ; Fig. 2; Table 1B). Abdominal flapping was absent in control (nonbrooding) females. In both cases, the interaction term was not significant (Table 1A and B).

Feeding rates were analyzed to test for the role of locomotor activity in foraging. Our tank experiments showed that, clearly, all crabs fed on the mussels offered at the same rate, since no significant differences between female conditions were found (ANOVA;  $F = 1.3$ ,  $df = 1, 32$ ,  $p = 0.26$ ). However, at the beginning of the experiment (when brooding females carried early eggs) food consumption was significantly higher than towards the end of the

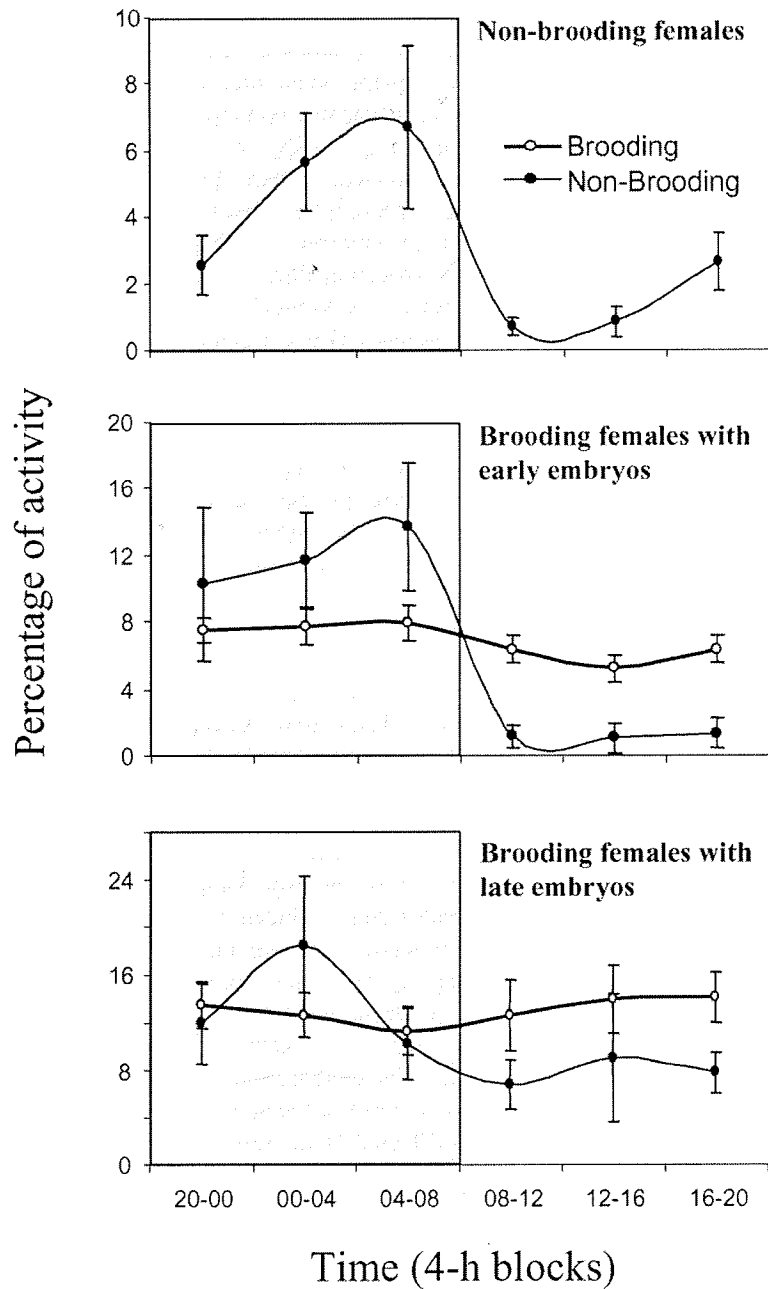


Fig. 2. Percentage of the occurrence of brooding (abdominal flapping; broken lines) and nonbrooding (locomotor activity; solid lines) behaviors in control animals (without eggs) and brooding females (carrying early and late embryos) of *H. plana* during 24 h under a light/dark cycle. Behaviors were analyzed in 4-h blocks, and the mean reported. The period of darkness is indicated by shadowed areas. Vertical lines indicate one standard deviation.

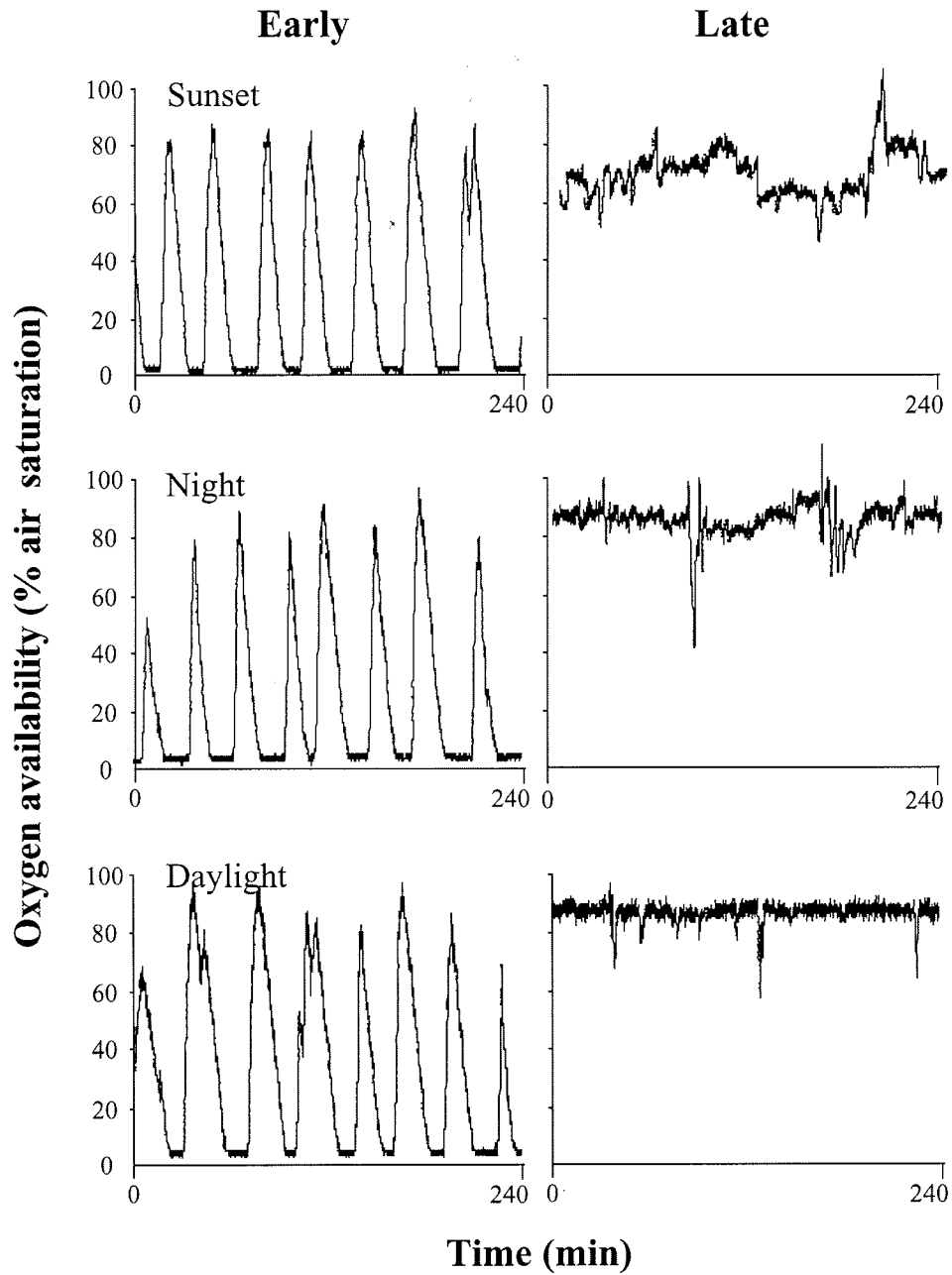


Fig. 3. Typical patterns of oxygen availability in the center of the egg mass of *H. plana* in females carrying early and late embryos, for three 4-h periods of the day: sunset (top panel), night (middle panel) and daylight (lower panel).

experiment (ANOVA;  $F=10.9$ ,  $df=1$ , 32;  $p=0.0024$ ). The interaction term was not significant (ANOVA;  $F=0.73$ ;  $df=1$ , 32;  $p=0.41$ ), which suggests that both brooding and nonbrooding females decreased the rate of food intake from the beginning towards the end of the experiment.

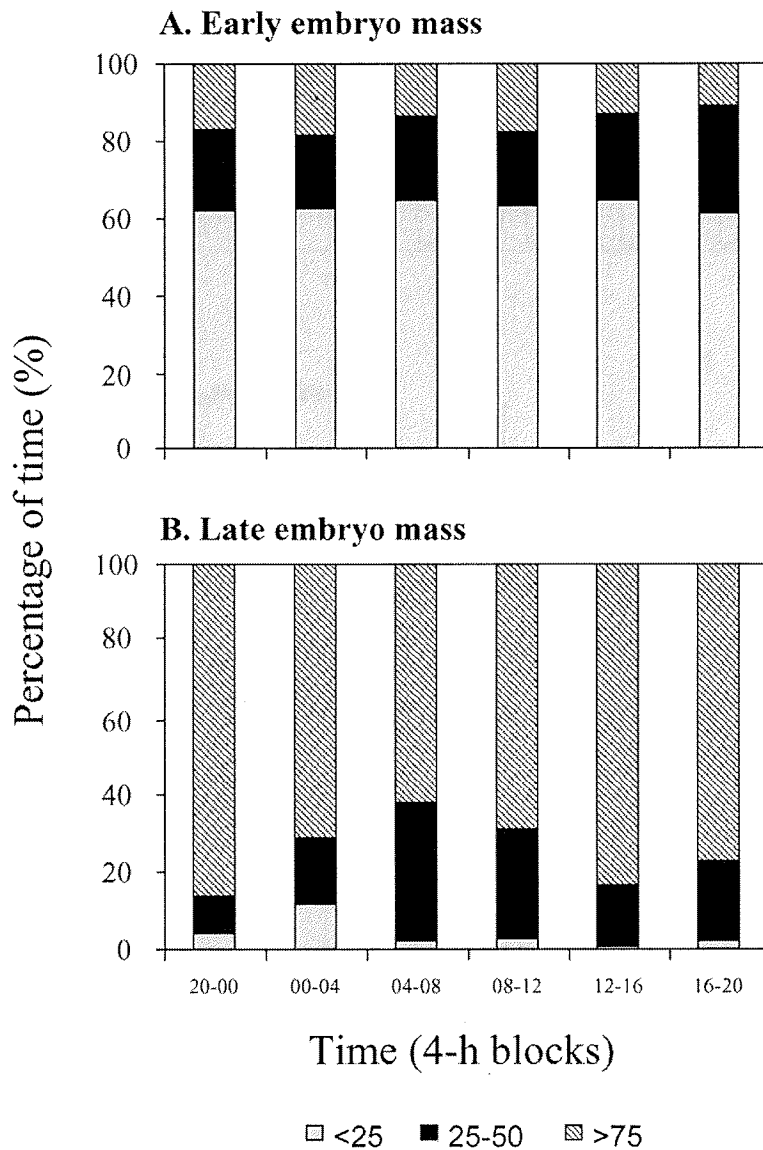


Fig. 4. Mean percentage of time that the early (A) and late (B) embryos were exposed to low (<39.7 mm Hg), intermediate (39.7–79.4 mm Hg) and high  $PO_2$  levels (>79.4 mm Hg) during the six different time blocks (4 h each).

Table 3

Results of two-way ANOVAs conducted to test for the effect of the optic fiber on female behavior (abdominal flapping and locomotor activity) of *H. plana* with different developmental stages (early and late embryos) for the two experimental conditions (with and without optic fiber)

Factor	F-ratio	df	p
<i>(A) Abdominal flapping (natural log transformation)</i>			
Stage of development of the embryos	29.6	1	0.0001
Experimental condition	3.98	1	0.063
Interaction	0.31	1	0.59
Error		16	
<i>(B) Locomotor activity (data were not transformed)</i>			
Stage of development of the embryos	1.098	1	0.3113
Experimental condition	0.001	1	0.971
Interaction	0.17	1	0.691
Error		15	

### 6.3. Daily patterns of oxygen availability

Oxygen availability in the center of the embryo mass varied with the stage of development, however, the patterns of oxygen availability observed in both embryo stages did not show any daily pattern (Fig. 3). Fig. 3 shows one female in each case, however, these results were similar in all studied individuals (the results considering all individuals are shown in Fig. 4). No differences among the different time blocks of the day analyzed were found in the mean percentage of time that the embryos were exposed to low and intermediate  $PO_2$  (<39.7 and 39.7–79.4 mm Hg) but significant differences were detected between early and late stages (Fig. 4; Table 2A). Early stage embryos spent more time at low  $PO_2$  (<39.7 mm Hg) and at intermediate levels of  $PO_2$  (39.7–79.4 mm Hg) than late stages ( $p < 0.05$ ; Table 2B). No effect of the time of day was found on the mean percentage of time that the embryos spent at high  $PO_2$  among different treatments (4-h time blocks during 24 h; Table 2C), but again embryo stage did affect the response variable (Fig. 4; Table 2C). In this case, late stage embryos were exposed for longer time periods at high  $PO_2$  than early embryos.

The use of the optic fibers to measure oxygen availability in the center of the embryo mass did not affect female brooding behavior (abdominal flapping) neither during early nor during late development (Table 3). The percentage of time spent with locomotor activity was not affected either by the presence of the optic fiber, and did not vary with the stage of development (Table 3).

## 7. Discussion

Contrasting patterns in the percentage of occurrence of brooding and nonbrooding behaviors were found throughout the day and also throughout development. While locomotor activity showed a daily pattern which was stronger during early development and smoother in females carrying late eggs, abdominal flapping did not exhibit a daily

pattern. The percentage of occurrence of both behaviors increased throughout development. Locomotor activity peaked during the night, remained unrelated to the level of oxygenation in the embryo mass, and exhibited a clear increase between females carrying early and late embryos. In contrast, abdominal flapping seems to be the behavior which supplies oxygen to the embryos, supporting evidences from other crab species (Baeza and Fernández, 2002). Furthermore, the lack of a daily pattern in oxygen availability in the center of the embryo mass is consistent with the lack of a daily pattern in abdominal flapping. These findings emphasize that oxygen is a crucial factor during early development, relevant for the normal rate of development of the embryos (Strathmann and Strathmann, 1995; Cohen and Strathmann, 1996; Lee and Strathmann, 1998). In consequence, females permanently provide oxygen to the brood despite daily cycles in other behaviors and activity levels.

The daily cycle of the nonbrooding behavior is consistent with other reports for marine invertebrates (Zann, 1973; Pino et al., 1994), including Brachyuran crabs (*C. maenas*, *Sesarma reticulatum*, *Sesarma sinereum*, *Cancer pagurus*; Brown et al., 1954; Arudpragasam and Naylor, 1964; Palmer, 1973, 1995; Naylor et al., 1997). The increase in the locomotor activity during the night may likely help to avoid predators or to enhance prey encounter probabilities. An interesting observation was the increase in locomotor activity between nonbrooding and brooding females. The level of locomotor activity clearly depended on whether animals were carrying embryos and also on embryo age, with increasing levels of locomotor activity from nonbrooding females to those with early and then those with late eggs. In a parallel study, we showed that the oxygen demand of the adult animals also increased in that order, according to increasing costs of brooding (Fernandez et al., 2000; Brante et al., submitted for publication). At first sight, this rise in oxygen demand may have stimulated an increase in foraging activity, visible as a rise in spontaneous activity. Furthermore, the clear diurnal rhythm found in nonbrooding females and in those with early eggs was diminished in those specimens with late embryos. They exhibited some locomotor activity even during the day. However, food uptake rate is not higher in brooding compared to nonbrooding females, indicating that this behavior does not only serve the purpose of foraging. Furthermore, food intake is lower in females carrying late embryos. In this context, observations in other crustaceans like *C. maenas* (cf. Wheatly, 1981) or *C. pagurus* (cf. Naylor et al., 1999a) suggest that locomotor activity and associated behavior may support the purpose of egg ventilation. However, a direct link between this behavior and oxygen availability in the embryo mass was not detected in our study and has not yet been reported for other crab species. We think that enhanced locomotor activity could cause a global water exchange around the animal and egg mass and, thereby, likely contributes to avoid the build-up of oxygen depleted water layers around the egg mass, especially during late development when embryo oxygen demand is highest. However, in natural habitats current may have a stronger effect than adult locomotion. The progressive loss of diurnal rhythmicity in locomotor activity emphasizes the role of continuous ventilation in embryo development and, thereby, also supports these conclusions.

The observation that maxilliped movements are associated with reduced levels of embryo oxygenation coincides with the observation that during these periods the frequency of abdominal flapping is reduced. Recently, Baeza and Fernández (2002)

suggested that this behavior may help females detect oxygen conditions in the embryo mass, considering the high concentration of chemosensory setae in the mouth region (Ache, 1982). These setae are probably involved in the detection of active chemical compounds of the surrounding environment (Rittschof, 1989). The use of maxillipeds to detect oxygen conditions in the embryo mass, generating water currents to the branchial chamber where chemosensory receptors are found, was also suggested (Naylor et al., 1999b).

The positive relationship between abdominal flapping and oxygen availability suggests that embryo development depends largely on the active behavior of female crabs. The constant pattern of oxygen availability and abdominal flapping during the day, when other behaviors show daily patterns, suggests that female crabs need to be full time mothers, providing oxygen all day long to assure development of the embryos. This contradicts previous reports suggesting that ventilation occurs during the night (Naylor et al., 1997). Daily patterns of oxygen availability in embryo masses of marine invertebrates have been already reported (Cohen and Strathmann, 1996), but only for brooding species which exhibit passive ways to help oxygen diffusion into the egg mass (e.g., gel). The difference between these species and Brachyuran crabs is the role of female behavior in supplying oxygen to the embryos. We think that abdominal flapping is the single, most important female behavior to supply oxygen to the embryos, especially those located in the central part of the egg mass. In accordance with previous work, the level of abdominal flapping increases with progressive embryo development and oxygen demand, such that late embryos experience longer periods at high oxygen tensions in accordance with their elevated oxygen demand. The increase in the frequency of abdominal flapping and the lack of diurnal rhythm emphasizes the role of continuous oxygen supply in embryonic development. The increase in metabolic demands, which may be associated to the increase in brooding activities throughout development (Baeza and Fernández, 2002), are not met by a higher rate of food intake. Furthermore, the increase in locomotor activity during late development, even during the day, suggests that the costs of brooding are not compensated immediately but met upon reserves. It is important to emphasize that food intake may be lower in nature than under experimental conditions, since searching time may affect food intake rate. This implies that assignation of energy to eggs may be regulated by females, considering the total energy available and the substantial amount of energy required to ventilate the eggs. Thus, investment in eggs among Brachyuran crabs may not only be determined by space available for yolk accumulation (Hines, 1982), but also by energy saved for the brooding season. This suggestion needs further investigation.

### **Acknowledgements**

We thank our colleagues Antonio Baeza, Katherine Jenó, Rubén Soto, Patricio Manriquez, Evie Wieters and anonymous reviewers for their helpful comments. We also thank Iván Albornoz, Antonio Brante and Juan Carlos Castilla for their collaboration. We appreciate financial support from FONDAP (O and BM#3-Crustacean), the Volkswagen Foundation, and FONDAP-FONDECYT (Grant 1501-0001). We also thank the Deutscher Akademischer Austauschdienst (DAAD) and the von Humboldt Foundation. [SS]

## References

- Ache, B.W., 1982. Chemoreception and thermoreception. In: Bliss, D.E. (Ed.), *The Biology of Crustacea*, vol. 3. Academic Press, London, pp. 369–398.
- Arudpragasam, K., Naylor, E., 1964. Gill ventilation volumes, oxygen consumption and respiratory rhythms in *Carcinus maenas*. *J. Exp. Biol.* 41, 309–321.
- Babcock, R., Bull, G., Harrison, P., Heyward, J., Oliver, J., Wallace, C., Willis, B., 1986. Synchronous spawnings of 105 scleractinian coral species on the Great Barrier Reef. *Mar. Biol.* 90, 379–394.
- Baeza, A., Fernández, M., 2002. Active brood care in *Cancer setosus* (Crustacea: Decapoda): the relationship between female behaviour, embryo oxygen consumption, and the cost of brooding. *Funct. Ecol.* 16, 241–251.
- Brante, A., Fernández, M., Eckerle, L., Mark, F., Pörtner, H.O., Arntz, W. Reproductive investment in the crab, *cancer setosus*, in a latitudinal cline: egg production, egg losses and egg ventilation. *Mar. Ecol.: Prog. Ser.* (submitted for publication).
- Brown, F., Bennett, W., Webb, H., 1954. Daily and tidal rhythms of O<sub>2</sub>-consumption in fiddler crabs. *J. Cell. Comp. Physiol.* 44, 477–506.
- Cloudsley-Thompson, J., 1960. Adaptive functions of circadian rhythms. *Cold Spring Harbor Symp. Quant. Biol.* 25, 345–355.
- Cohen, C., Strathmann, R., 1996. Embryos at the edge of tolerance: effects of environment and structure of egg masses on supply of oxygen to embryos. *Biol. Bull.* 190, 8–15.
- Fernández, M., Bock, C., Pörtner, H., 2000. The cost of being a caring mother: the ignored factor in the reproduction of marine invertebrates. *Ecol. Lett.* 3, 487–494.
- Fernández, M., Pardo, M., Baeza, A., (in press) Female crab brooding behavior: is there any relationship between embryo oxygen demand and oxygen provision?
- Forward, R., 1987. Larval release rhythms of decapod crustaceans: an overview. *Bull. Mar. Sci.* 41, 165–176.
- Gifford, C., 1962. Some observations on the general biology of the land crab, *Cardisoma guanhumii* in South Florida. *Biol. Bull.* 123, 207–223.
- Hastings, J., Astrachan, L., Sweeney, B., 1961. A persistent daily rhythm in photosynthesis. *J. Gen. Physiol.* 45, 69–76.
- Hines, A., 1982. Allometric constraints and variables of reproductive effort in Brachyruan crabs. *Mar. Biol.* 69, 309–320.
- Homma, K., Hastings, J., 1989. Cell growth kinetics, division asymmetry and volume control at division in the marine dinoflagellate *Gonyaulax polyedra*: a model of circadian clock control of the cell cycle. *J. Cell Sci.* 92, 303–318.
- Lee, C., Strathmann, R., 1998. Scaling of gelatinous clutches: effects of sibling competition for oxygen on clutch size and parental investment per offspring. *Am. Nat.* 151, 293–300.
- Lessios, H., 1991. Presence and absence of monthly reproductive rhythms among eight Caribbean echinoids off the coast of Panama. *J. Exp. Mar. Biol. Ecol.* 153, 27–47.
- Maier, R., 1998. *Comparative Animal Behavior: An Evolutionary and Ecological Approach*. Allyn and Bacon, USA.
- Morgan, S., Christy, J., 1994. Plasticity, constraint, and optimality in reproductive timing. *Ecology* 75, 2185–2203.
- Morgan, S., Christy, J., 1995. Adaptive significance of the timing of larval release by crabs. *Am. Nat.* 145, 457–479.
- Naylor, J., Taylor, E., Bennett, D., 1997. The oxygen uptake of ovigerous edible crabs (*Cancer pagurus*) (L) and their eggs. *Mar. Freshw. Behav. Physiol.* 30, 29–40.
- Naylor, J.K., Taylor, E.W., Bennett, D.B., 1999a. Oxygen uptake of developing eggs of *Cancer pagurus* (Crustacea: Decapoda: Cancridae) and consequent behaviour of the ovigerous females. *J. Mar. Biol. Assoc. U.K.* 79, 305–315.
- Naylor, J.K., Taylor, E.W., Bennett, D.B., 1999b. Heart rate and gill ventilation in ovigerous and non-ovigerous edible crabs, *Cancer pagurus*: the effects of disturbance, substrate and starvation. *Mar. Freshw. Behav. Physiol.* 32, 129–145.
- Palmer, J., 1973. Tidal rhythms: the clock control of the rhythmic physiology of marine organisms. *Biol. Rev.* 48, 377–418.
- Palmer, J., 1995. *The Biological Rhythms and Clocks of Intertidal Animals*. Oxford Univ. Press, New York.



- Pengelley, E., Asmundson, S., 1971. Annual Biological Clocks. Readings from Scientific American Animal Behavior, pp. 105–112. Selected and introduced by T. Eisner and E. Wilson.
- Pino, C., Oliva, D., Castilla, J., 1994. Ritmos de actividad en las lapas *Fissurella crassa* Lamark 1822 y *F. latimarginata* Sowerby 1835: efectos del ciclo de marea y fotoperiodo. Rev. Biol. Mar., Valparaíso 29, 89–99.
- Rao, K., 1954. Tidal rhythmicity of rate of water propulsion in *Mytilus*, and its modifiability by transplantation. Biol. Bull. 106, 353–359.
- Rathcke, B., Lacey, E., 1985. Phenological patterns of terrestrial plants. Ann. Rev. Ecol. Syst. 16, 179–214.
- Rittschof, D., Forward Jr., R.B., Simons, D.A., Reddy, P.A., Erickson, B.W., 1989. Peptide analogs of the mud crab pumping pheromones: structure–function studies. Chem. Senses 14, 137–148.
- Strathmann, R., Chaffee, C., 1984. Constraints on eggs masses: II. Effect of spacing, size, and number of eggs on ventilation of masses of embryos in jelly, adherents groups, or thin-walled capsules. J. Exp. Mar. Biol. Ecol. 84, 85–93.
- Strathmann, R., Strathmann, M., 1995. Oxygen supply and limits on aggregation of embryos. J. Mar. Biol. Assoc. U.K. 75, 413–428.
- Tauber, C., Tauber, M., 1981. Insect seasonal cycles: genetics and evolution. Ann. Rev. Ecol. Syst. 12, 281–308.
- Wheatly, M.G., 1981. The provision of oxygen to developing eggs by female shore crabs (*Carcinus maenas*). J. Mar. Biol. Assoc. U.K. 61, 117–128.
- Winfrey, A., 1982. Human body clocks and the timing of sleep. Nature 297, 23–27.
- Zann, L., 1973. Relationships between intertidal zonation and circatidal rhythmicity in littoral gastropods. Mar. Biol. 18, 243–250.
- Zar, J.H., 1996. Biostatistical Analysis. Prentice Hall, New Jersey.
- Zeng, Ch., Naylor, E., 1997. Rhythms of larval release in the shore crab *Carcinus maenas* (Decapoda: Brachyura). J. Mar. Biol. Assoc. U.K. 77, 451–461.