Functional Ecology 2002 **16**, 241–251

Active brood care in *Cancer setosus* (Crustacea: Decapoda): the relationship between female behaviour, embryo oxygen consumption and the cost of brooding

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Summary

1. Previous studies have shown that oxygen is limiting in embryo masses of marine invertebrates. It has been suggested that several behaviours found in brooding females of brachyuran crabs are used to ventilate and provide oxygen to the embryo masses.

2. The relationship between female brooding behaviour, oxygen consumption of embryos and oxygen provision to the brood mass for embryos at different developmental stages was studied, using the marine crab *Cancer setosus*. The changes in oxygen consumption of brooding females associated with changes in oxygen provision to the brood were also estimated.

3. Brooding females of *C. setosus* behaved differently from non-brooding females. Abdominal flapping was associated with an increase in oxygen availability in the centre of the brood mass; the frequency of abdominal flapping increased with embryonic development, as oxygen demand of crab embryos increased. Oxygen consumption of brooding females also increased throughout embryonic development. The difference in oxygen consumption between brooding and non-brooding females was used as an indicator of the cost of oxygen provision (brooding).

4. These results provide the first evidence – among crabs and other marine invertebrates – of a direct link between active brood care and oxygen provision. It is possible that parental care in marine invertebrates is strongly linked to oxygen provision, since oxygen limitation has been reported for several brooding taxa. The simple physiological constraint of oxygen provision in marine invertebrates may have important ecological and evolutionary consequences.

Key-words: Embryo mass, metabolic cost, oxygen provision, parental care *Functional Ecology* (2002) **16**, 241–251

Introduction

The degree of parental care varies widely among marine invertebrate taxa, and even within a taxon (Clutton-Brock 1991; Thiel 1999). Among marine crustaceans, some groups (e.g. euphausiids, penaeids) do not provide any kind of parental care, shedding fertilized eggs into the pelagic environment where development of embryos and larvae occur (Mauchline & Fisher 1969; Omori 1974). In other groups (amphipods, isopods), females carry their offspring in brood pouches and parental care can extend even after hatching (Dick, Faloon & Elwood 1998; Thiel 1999). However, many marine crustaceans exhibit indirect development (e.g. Decapoda); only the embryos are protected by the females (but see Diesel 1989, 1992 for exceptions). In crustaceans and other marine invertebrates several benefits of protecting embryos have been suggested, among them protection of eggs and juveniles from predators or adverse abiotic conditions (Chaffee & Strathmann 1984; Strathmann 1985; Thiel 1999) and physiological provision (Morritt & Spicer 1998). However, packing large numbers of embryos constraints oxygen diffusion to the centre of the embryo mass (e.g. Crisp 1959; Perron & Corpuz 1982; Chaffee & Strathmann 1984; Pechenik 1986; Strathmann & Strathmann 1995). The problem of oxygen limitation seems particularly critical among brachyuran crabs, which produce compact, spherical masses of embryos that frequently exceed 3-4 cm in diameter. These embryo masses are larger than the theoretical 1 mmlimiting thickness that allows sufficient oxygen supply by diffusion (Prosser 1973) and oxygen limitation occurs in the centre (Naylor et al. 1999; Fernández, Bock & Pörtner 2000). Oxygen limitation in embryo

†Author to whom correspondence should be addressed. E-mail: mfernand@genes.bio.puc.cl 242 J. A. Baeza & M. Fernández masses is not exclusive to marine crabs (e.g. Chaffe & Strathmann 1984; Cohen & Strathmann 1996), nor is female's involvement in helping oxygen diffusion (Lee & Strathmann 1998).

Among marine crabs, oxygen availability in the centre of the embryo mass seems to change throughout development, probably in response to embryo oxygen demands (Naylor et al. 1999). Early stage embryos of Cancer pagurus consume less oxygen per unit of mass (Naylor et al. 1999; Fernández et al. 2000) and, in females carrying early embryos, oxygen availability in the centre of the embryo mass varies between 0 and 100% air saturation, exhibiting a cyclic pattern (Fernández et al. 2000). Late stage embryos require more oxygen, and oxygen availability in the centre of the late embryo masses the few times it was measured was high (Naylor et al. 1999). This evidence suggests that in marine crabs, oxygen availability in embryo masses might be achieved through specific female behaviours that may change in type or frequency throughout embryonic development.

If oxygen is provided by females, female behaviour may be a critical factor during embryonic development, as oxygen limitation has been shown to influence survival, growth rate and size at hatching of larvae and embryos of several marine invertebrates (Palumbi & Johnson 1982; Chaffe & Strathmann 1984; Strathmann & Strathmann 1995). Furthermore, since oxygen demand of late embryos decreases dramatically at low oxygen partial pressures (Naylor et al. 1999; Fernández et al. 2000), the effect of oxygen limitation could be stronger during the late embryonic developmental stages when oxygen demand increases (Naylor et al. 1999; Fernández et al. 2000). Previous studies have detected some behaviours exclusive to brooding female crabs, such as abdominal flapping, pleopod beating and reverse gill ventilation, which have been suggested as mechanisms used by brooding females to ventilate and provide oxygen to their embryo masses (Wheatly 1981; de Vries, Rittschof & Forward 1991; Naylor & Taylor 1999; Naylor et al. 1999; Fernández et al. 2000). However, to date no attempt has been made to directly relate each behaviour with actual oxygen provision.

If female crabs engage in specific brooding behaviours, directed to provide oxygen to embryos, there may be associated costs. However, brooding behaviours and their contribution to investment in reproduction have been virtually ignored among marine invertebrates, in sharp contrast to studies of fish (Jones & Reynolds 1999), terrestrial vertebrates (amphibians: McDiarmid 1978; reptiles: Gans 1988; birds: Lack 1968) and terrestrial invertebrates (insects: Wilson 1971). Only in rare cases have costs associated with brooding been estimated (cost of producing extra-embryonic material: Hughes & Roberts 1980; Perron 1981; Perron & Corpuz 1982; Lee & Strathmann 1998; cost of oxygen provision in crabs during early development of the embryos: Naylor, Taylor & Bennett 1997; Fernández et al. 2000). If female brooding behaviour in brachy-

© 2002 British Ecological Society, *Functional Ecology*, **16**, 241–251 uran crabs is associated with oxygen provision, both brooding behaviour and metabolic cost should change throughout embryonic development.

The goal of this work was to assess the relationship between female brooding behaviour, oxygen consumption of embryos and oxygen provision to the brood mass for embryos at different developmental stages in the marine crab, Cancer setosus (Molina 1782). The metabolic cost associated with changes in oxygen provision was also estimated. We studied: (1) female crab brooding behaviour throughout embryonic development; (2) the relationship between each brooding behaviour identified and oxygen availability in the embryo mass; (3) oxygen demand of the developing embryos at different stages and oxygen partial pressures; and (4) the oxygen consumption of brooding females associated with each level of oxygen provision to the embryos. In this paper we refer to brooding in a narrow sense, since we evaluated only the changes in female behaviour in relation to oxygen provision, and the costs associated with those changes. We are aware that brooding has other benefits and costs.

Methods

Females of Cancer setosus were collected by local fishermen between July 1999 and April 2000, at several localities in central Chile, between El Quisco (33°23' S, 71°42' W) and San Antonio (33°36' S, 71°38' W). Experimental animals were transported immediately after collection to the laboratory, and maintained in 500-1 tanks with constant aeration and flowing sea water at approximately 14 °C (average water temperature at the sampling sites; average salinity 34.9 psu). In the holding tanks, crabs were feed ad libitum on fresh mussels (Choromytilus chorus). The carapace width (CW) and body wet mass (BW) of 49 brooding females used for experiments varied between 95.2 and $140.7 \text{ mm} (\text{mean} \pm \text{SD} = 120.25 \pm 10.52)$ and between 160 and 450 g (mean \pm SD = 299.9 \pm 69.1), respectively. The estimated wet mass of embryo masses of a subsample of 39 brooding females varied between 40 and $90.81 \text{ g} \text{ (mean} \pm \text{SD} = 69.54 \pm 15.88)$. CW and BW of non-brooding (or control) females (n = 10)ranged between 95.2 and 132.3 mm (mean \pm SD $= 115.56 \pm 13.49$) and between 160 and 370 g (mean \pm SD = 280 \pm 74.5), respectively.

When females were selected for experiments, embryo masses were inspected under a stereomicroscope and the developmental stage of the embryos was classified according to Vargas (1995). The following stages were determined and used as treatments in the experiments described below: stage I (embryos with uniformly distributed yolk, absence of cleavage and eyes, until c. 10 days of development); stage II (embryos with cleavage and yolk reduced to not less than 75% of embryo volume, c. 25 days of development); stage III (embryo with visible but not developed eyes, and presence of pigments, c. 45 days of development); and stage IV (embryo with Active brood care and brooding costs in a marine crab well-developed eyes, pumping heart and appendages, *c*. 60 days of development, just before hatching).

FEMALE BROOD CARE BEHAVIOUR AND OXYGEN AVAILABILITY IN THE EMBRYO MASS

To test whether ovigerous females of C. setosus provide oxygen to their brooded embryos, female behaviour and oxygen partial pressure in the embryo mass were recorded simultaneously under laboratory conditions, and the relationship between oxygen availability and female behaviour was analysed. Females with embryos at different developmental stages (I to IV, n = 4 per stage) were placed individually in a 16-1 transparent plastic tank $(25 \times 25 \times 25 \text{ cm}^3)$ filled with a 2-cm layer of shell hash, large rocks and aerated sea water (14 °C). The behaviour of each female was videotaped continuously over a 24-h period using a Sony (time-lapse) video recorder and a Pelco vigilance camera, starting 2 h after the crab was introduced to the tank. The day and night cycle (12:12 h) during videotaping was simulated with white and red light, respectively, using an automatic switch system. However, we analysed female behaviour only during the night (between 2000 and 0800 h), to avoid potential effects of the presence of people in the laboratory during daytime. Behaviour of ovigerous females during the experimental period was classified and quantified as events or states, according to their relative extent. Behaviours classified as events occurred as discrete acts of relatively short duration (less than 1 min, e.g. abdominal flapping) while behaviours of relatively long duration (more than 1 min, e.g. standing, pereiopod probing) were considered as states (sensu Martin & Bateson 1986). The frequency of occurrence of each behavioural event (n events per hour) and the proportion of time spent in each behavioural state (% time per hour) were recorded during two time blocks of 1 h each, for females carrying embryos at each different developmental stage. Time blocks were randomly selected when females were facing frontally or diagonally to the camera, and their behaviour could be recorded. Also, non-brooding females (n = 4) were videotaped and their behaviour was recorded and measured as described above, to identify brooding and non-brooding behaviours.

To test whether behaviours related to brood care varied during embryonic development, the frequency or percentage of time that each behaviour was performed per unit of time (1 h) was compared among brooding females carrying embryos at different developmental stages using a one-way ANOVA. The behaviour of non-brooding females was included in these ANOVAS only when the events or states analysed were performed at least once by these females. Whenever, a specific event or state was not observed in each one of the four non-brooding females, the behaviour was not considered part of the behavioural repertoire of these females, and was not included in the ANOVA analysis. Data were square root or natural log-transformed in

© 2002 British Ecological Society, *Functional Ecology*, **16**, 241–251 order to meet the assumptions of the ANOVA model, and a Newman–Keuls test was conducted for *a posteriori* comparisons (Zar 1996).

To assess whether female behaviour affected oxygen concentration in the centre of their brood mass, the relationship was examined between the duration of specific brooding behaviours and the differences in oxygen availability (% air saturation) in the brood mass, recorded immediately before and right after each specific (target) behaviour was performed. To exclude the confounding effect of other behaviours coupled in time to the target behaviour, only those behaviours that occurred in isolation, in females carrying embryos of stages I and II were considered in the analysis. Females carrying late embryos (stages III and IV) were not used to analyse the relationship between oxygen availability and behaviour since these females seldom performed behaviours isolated from each other. When videotaping the behaviour of ovigerous females, oxygen availability (% air saturation) in the centre of the brood masses was monitored continuously with a PreSens microoptode (Precision Sensing GmbH, Regensburg, Germany). Microoptodes are oxygen microsensors designed for high-resolution (temporal and spatial) measurements of dissolved oxygen (Klimant et al. 1997; Holst et al. 1997). The microoptodes do not consume oxygen. A microoptode (optic fibre) with a tip diameter of approximately 100 µm was used to monitor oxygen availability. This fibre was placed in the centre of the embryo mass by drilling a small hole through the six abdominal segment of each brooding female. A small tube of variable length (depending on embryo mass diameter) was inserted into this hole and glued to the abdomen using cyanoacrilate and dental wax. The tip of the microoptode was placed in the centre of the brood mass after passing it through the tube, and the fibre was glued in place. The microoptode was calibrated (0% air saturation: solution saturated with Na₂SO₃; 100% air saturation: aerated water) at 14 °C before fixing it to each female's abdomen. Oxygen partial pressure (% air saturation) was recorded on a computer every 5 s during the 24-h experiment. Correlations between the studied variables were examined by the Pearson product-moment coefficient or Kendall rank correlation coefficient depending on the homoscedasticity of the contrasted data sets (Zar 1996).

To assess the influence of the optic fibre on the behaviour of brooding females, the frequency or duration of each behaviour performed by brooding females without optic fibre carrying early (stage I) and late (stage IV) embryos, was recorded and measured as above (n = 4 per stage). The frequency or duration of each observed behaviour between females with and without optic fibres was compared by a *t*-test (Zar 1996). Females carrying early and late stage embryos were examined separately. When homoscedasticity between the contrasted data set was not possible to achieve, a Mann–Whitney *U*-test was conducted to detect significant differences between control and 244 J. A. Baeza & M. Fernández brooding females in the frequency or duration of specific behaviours.

OXYGEN CONSUMPTION OF DEVELOPING EMBRYOS

To test whether oxygen consumption of embryos varied throughout embryonic development, and with oxygen partial pressure, oxygen depletion (between 100% and 0-5% air saturation, under constant temperature, 14 °C) was recorded for embryos at different developmental stages (I to IV). For this purpose, small numbers of embryos were removed from brooding females, also maintained at 14 °C, and were immediately placed on a fine grid in a double wall, closed microchamber, filled with 2 ml of stirred, filtered $(0.2 \,\mu\text{m})$ sea water and added antibiotic (20 μ l with 5 mg ml^{-1} of penicillin and 5 mg ml^{-1} of amoxicillin). After calibration (see above), the rate of oxygen depletion was recorded by Eschweiler electrodes (oxygen monitor M 200 and electrode 2000-100 (MT-1-AC), Eschweiler GmbH, Kiel, Germany) connected to a chart recorder (Kipp & Zonen, BV, Delft, The Netherlands). During measurements, a stir bar under the grid was used to mix water and homogenize oxygen partial pressure inside the chamber. The grid allows avoidance of direct contact between the stir bar and the embryos, which could produce some disturbance and also oxygen depletion (near the embryos when unstirred; Naylor et al. 1999). At least six measurements were conducted for each developmental stage. At the end of the experimental time, embryos were weighed (wet mass) and oxygen consumption per unit of time and mass was estimated for each developmental stage, at each level of oxygen saturation (0-10%), 11-25%, 26-50%, 51-75%, 76-100%). The total time that embryos were maintained in the microchamber ranged between 6 and 24 h - depending on embryonic developmental stage - and embryos were subjected for at least 30 min to each level of oxygen saturation. We tested for the effect of embryos' developmental stage and oxygen concentration (% air saturation) on oxygen consumption of the embryos, using a two-way ANOVA. Data were root-root transformed in order to meet the assumptions of the ANOVA model (Zar 1996).

THE METABOLIC COST OF BROOD CARE

To test whether female behaviour associated with oxygen provision (brood care) represented an energetic cost for the brooding females, oxygen consumption of non-brooding (embryos removed at least 48 h before females were used for experiments) and brooding females carrying embryos at different developmental stages (I to IV) was measured (six females of each category were used). It was assumed that differences in the metabolic rate between brooding and non-brooding females was the cost of oxygen provision (brooding). Females were individually introduced into a closed

© 2002 British Ecological Society, *Functional Ecology*, **16**, 241–251 respiration chamber (11 l), which was placed in a large reservoir tank (80 1, $67 \times 40 \times 30$ cm³) with sea water at 14 °C. After calibration, the rate of oxygen depletion (between 100% and 70% air saturation) was monitored continuously with oxygen electrodes connected to a chart recorder (see above). The oxygen electrode was placed into a holder located inside the large reservoir tank. Water flowed from inside the respiration chamber to the electrodes through a 3.5-mm C-FLEX tubes connected to a Masterflex 7524-05 peristaltic pump (flow rate 30 ml min⁻¹) (Cole-Parmer Instrument Co., www.masterflex.com). In order to estimate oxygen consumption of brooding females, the consumption of their brooded embryos was subtracted. When possible, experimental females and their embryo masses were weighed (71% of the cases, 17 out of 24 brooding females) and the consumption of the embryo mass was obtained as the product of the mass of the embryo mass and the rate of oxygen consumption of embryos per gram at the same developmental stage. When direct estimates of female wet mass and embryo wet mass were not available, the expected mass of the brood mass of a similarly sized female, with embryos at the same developmental stage, was calculated using the following relationships between female CW and embryo wet mass:

- Stage I: log embryo wet mass = 2⋅5834(log female CW) - 3⋅6052, r² = 0⋅75, t-test: t₍₄₇₎ = 11⋅81, P < 0⋅05;
- Stage II: log embryo wet mass = 2·7922(log female CW) − 4·01, r² = 0·74, t-test: t₍₁₆₎ = 6·76, P < 0·05;
- Stage III: log embryo wet mass = 2·6109(log female CW) − 3·5906, r² = 0·76, t-test: t₍₄₁₎ = 11·28, P < 0·05;
- Stage IV: log embryo wet mass = 2·3526(log female CW) − 2·999, r² = 0·84, t-test: t₍₃₂₎ = 13·06, P < 0·05.

Similarly, female wet mass was estimated from the log–log relationship between female size and mass (log female wet mass = $2.88(\log \text{ female CW}) - 3.5499; r^2 = 0.93, t$ -test: $t_{(125)} = 40.67, P < 0.05)$. Then, the consumption of the embryo mass was obtained as the product of the expected mass of the embryo mass and the rate of oxygen consumption of embryos per gram at the same developmental stage. Oxygen consumption per unit of time and wet mass of non-brooding and brooding females (carrying embryos at each of the four different developmental stages) was compared using a one-way ANOVA.

Results

FEMALE BEHAVIOUR AND OXYGEN CONCENTRATION WITHIN THE EMBRYO MASS

Five behaviours were observed and recorded in control or brooding females of *Cancer setosus*. Two of these behaviours were considered as events (i.e. abdominal flapping, chela probing), while the remaining three behaviours were classified as states (i.e. standing, maxilliped beating, pereiopod probing). Four out of Active brood care and brooding costs in a marine crab the five behaviours recorded for *C. setosus* were found to be exclusive to brooding females. Only maxilliped beating was also detected in non-brooding females. The recorded behaviours are described below, ordered according to the sequence in which they were most commonly performed by the brooding females.

STANDING

Females raised their body extending their pereiopods, while maintaining one or both chelae firmly attached to the substrate. When in the standing position, the abdomen was gently lifted from the substrate; thus, water circulation near the embryo mass may be affected. In addition, this position may facilitate the movements of pereiopods and chelae, and the abdominal flapping. Usually, this standing position was the first behaviour of the sequence exhibited by brooding females.

ABDOMINAL FLAPPING

Females extended their abdomen backwards and forwards, either strongly or smoothly, rhythmically beating their embryo mass. The abdomen was extended either completely or slightly, and flapping would take place once or several times. Females flapped their abdomen when standing, and pleopods were also observed to beat in such instances, helping to shake the entire embryo mass.

PEREIOPOD PROBING

Females repeatedly introduced the dactyls of the pereiopods into the embryo mass. Pereiopods were observed to pierce the mass slightly or they were deeply inserted into the brood mass. Frequently, females directed their pereiopods to their own mouth region after probing embryos. Females used either a single pereiopod, several, or even all of them when probing their embryos. Although, this behaviour was frequently conducted after abdominal flapping, it was also observed prior to abdominal flapping.

CHELA PROBING

Females used one of their two chelae to pierce the embryo mass. Generally, the smaller chela was used to poke embryos and several times this appendage was also directed to the female mouthparts after embryos were pierced. Chela probing was not observed in females with embryos at early developmental stages (stage I), in contrast to the other behaviours which were observed in brooding females, regardless of embryo developmental stage.

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MAXILLIPED BEATING

Maxillipeds were vigorously beaten during abdominal flapping, but most frequently before or after abdominal

flapping. This pattern was consistent in brooding females, regardless of the developmental stage of the embryos they carried. Non-brooding females also beat the maxillipeds.

As pointed out above, the behaviours performed by brooding females followed specific sequences, especially in females incubating early stage embryos (Fig. 1a). Females sitting on the bottom of the tanks first began to beat their maxillipeds, then raised themselves abruptly when extending their pereiopods (standing position), flapped several times (abdominal flapping) and occasionally probed their embryos with one or several pereiopods while still raised. Females then beat their maxillipeds and returned to the resting position, sitting again on the bottom of the tanks. Pereiopod probing and maxilliped beating were also observed in females resting at the bottom of the tanks (Fig. 1a). The sequence described above was less noticeable in females carrying late embryos, since they remained in a standing position almost all the time and flapped their abdomen frequently (Fig. 1c,d; see below). Furthermore, the behavioural sequence often resulted in overlap between maxilliped beating, pereiopod probing and chela probing in females carrying embryos at stages II, III and IV (Fig. 1b,c,d). Summarizing, brooding females exhibited four behaviours that followed a specific sequence at the beginning of the brooding period. However, the sequence was less evident in females carrying late stage embryos.

CHANGES IN BEHAVIOUR IN RELATION TO EMBRYONIC DEVELOPMENT

The developmental stage of the embryos incubated by females affected the frequency or duration of some of the behaviours described above. The frequency of abdominal flapping and the total time spent in a standing position varied significantly among females incubating embryos at different developmental stages (one-way ANOVA: $F_{3,12} = 82.73$, P < 0.001 for flapping, and $F_{3,12} = 12.29$, P < 0.001 for standing; Fig. 2). Brooding females with embryos at late developmental stages (stage IV) exhibited an almost 10-fold increase in abdominal flapping in comparison with females incubating early embryos (stages I and II; a posteriori Newman–Keuls: P < 0.005; Fig. 2). Females carrying late embryos (stages III and IV) spent almost half of their time standing in contrast to less than 20% registered for females carrying early embryos (stages I and II; a posteriori Newman–Keuls: P < 0.05). In contrast, no significant differences were detected in the frequency of chela probing (one-way ANOVA: $F_{2,9}$ = 1.06, NS), and in the duration of pereiopod probing (one-way ANOVA: $F_{3,12} = 1.3$, NS) and maxilliped beating (one-way ANOVA: $F_{4,15} = 1.00$, NS) among females carrying embryos at different developmental stages. Also, the time that females spent beating their maxillipeds did not differ significantly between non-brooding and brooding females, regardless of the stage of

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Fig. 1. Oxygen availability (% air saturation) measured in the centre of the brood mass (left side of each quadrant) and timing of behaviours recorded simultaneously for a single brooding female of *Cancer setosus* (right side of each quadrant) over a period of 20 min in the laboratory. Each quadrant shows oxygen availability and behaviour of females carrying embryos at each developmental stage (stages I to IV), from the beginning of the observations (top) to the end (bottom). Female behaviour and oxygen availability were recorded during the night (between 2000 and 0800 h). Behaviours are Mx = maxilliped beating; St = standing; Flp = abdominal flapping; Prp = pereiopod probing; Ch = chela probing. Bars represent behavioural states while arrows represent behavioural events.

Table 1. Results of *t*-tests conducted to test for differences in the behaviour of *Cancer setosus* brooding females with and without optic fibres carrying early (stage I) and late (stage IV) embryos. Independent *t*-tests were conducted separately for the mean frequency of occurrence of each event (i.e. abdominal flapping, chela probing) and for the mean percentage of time that each state (i.e. standing, pereiopod probing, maxilliped beating) was performed by brooding females with and without optic fibres. The *t* statistic, degrees of freedom (df) and *P*-value are shown for each comparison. A Mann–Whitney *U*-test was conducted to test for differences in the mean percentage of time that maxilliped beating was performed between females with and without optic fibres, since homoscedasticity between the contrasted data sets could not be achieved (*)

Behaviour	With microoptode	Without microoptode	t	df	Р
Stage I					
Standing	$6.38 (\pm 3.42)$	$6.50 (\pm 6.45)$	0.03	6	0.9738
Abdominal flapping	10.38 (± 3.66)	$10.00 (\pm 5.49)$	0.11	6	0.9132
Pereiopod probing	$13.10 (\pm 11.82)$	$4.52 (\pm 5.28)$	1.33	6	0.2333
Chela probing	_	_	_	_	_
Maxilliped beating*	11·36 (± 2·07)	9·50 (± 9·20)	5*	6	0.3865
Stage IV					
Standing	41·13 (±16·56)	50·63 (± 8·78)	1.01	6	0.3499
Abdominal flapping	$98.50 (\pm 21.34)$	84·00 (± 15·93)	1.09	6	0.3179
Pereiopod probing	$10.09 (\pm 7.60)$	19.69 (± 17.03)	1.03	6	0.3429
Chela probing	$2.25 (\pm 2.72)$	$2.25 (\pm 2.34)$	0.00	6	1
Maxilliped beating	22·49 (± 15·47)	21·26 (± 5·33)	0.12	6	0.8859

© 2002 British Ecological Society, *Functional Ecology*, **16**, 241–251 development of the brooded embryos (one-way ANOVA: $F_{4,15} = 1.00$, NS).

The optic fibre did not affect the behaviour of the brooding female crabs. No significant differences were

detected in the frequency or duration of the recorded behaviours between females with or without optic fibres, regardless of the stage of development of the brooded embryos (Table 1). 247

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Fig. 2. Behaviour of brooding and non-brooding females of *Cancer setosus* recorded under laboratory conditions. The frequency (*n* events per hour) and total percentage of time (% time in 1 h) that each event (discrete acts, < 1 min duration) and state (> 1 min duration) was performed by non-brooding (N.Br) and brooding females carrying embryos at each developmental stage is shown (n = 4 per stage). Vertical lines indicate standard deviation. Different letters beside the bars indicate significant differences at P < 0.05; N.O. = not observed.

BEHAVIOURS AND CHANGES IN OXYGEN AVAILABILITY

Visual inspection of the data revealed that certain behaviours exhibited by females appeared to be associated with changes in the oxygen availability in the centre of the brood mass (Fig. 1). In females carrying early embryos (stages I and II), pereiopod probing was usually performed when oxygen availability decreased

or was low (Fig. 1a,b). This association between oxygen availability and pereiopod probing seemed less evident for females carrying late embryos (stage IV) since oxygen availability in these brood masses remained constant over time (Fig. 1d). However, occasionally this pattern was observed in females carrying embryos at stage III, when exposed to low partial pressures (Fig. 1c). In contrast, standing and abdominal flapping tended to be associated with an increase in the percentage air saturation at the centre of the embryo mass. This was especially true for females carrying early embryos, but not for females carrying late stage embryos, because oxygen availability was usually high and almost never reached low levels. The lack of an apparent association between abdominal flapping and an increase in oxygen availability in females carrying late embryos was probably due to the high frequency at which this behaviour was performed (see above).

When the relationship was examined between the duration of a specific behaviour (σ_t) and the difference in oxygen partial pressure that occurred in the centre of the brood mass before and after each specific and isolated behaviour was performed (σ_{02}), a positive significant relationship was detected only for abdominal flapping (Pearson correlation coefficient: r = 0.83, n = 38, P < 0.0001; Fig. 3). In contrast, a negative relationship between σ_{02} and σ_t , was observed for maxilliped beating (Kendall rank correlation: $\tau = -0.35$, n = 33, P = 0.0044) and pereiopod probing (Kendall rank correlation: $\tau = -0.38$; n = 32, P = 0.0024; Fig. 3). The negative correlation found between the studied variables for maxilliped beating and pereiopod probing was because both behaviours were commonly performed before flapping (see above). Chela probing and standing were not analysed since they were always observed to be coupled to other brood care behaviours. Based on the pattern of female behaviour observed, and the correlation between duration of each behaviour and the difference in oxygen availability in the centre of the brood mass, oxygen was provided to the embryos mainly through abdominal flapping actively performed by ovigerous females. Not only the duration of this behaviour but also the frequency with which it was performed played a role in rapidly increasing oxygen provision to brooded embryos. In fact, the higher frequency at which abdominal flapping was performed by females carrying late embryos, and the overlap between different brooding behaviours precluded us from determining any association between oxygen availability and each abdominal flapping.

OXYGEN CONSUMPTION OF CRAB EMBRYOS

Oxygen consumption of crab embryos varied with developmental stage (two-way ANOVA: $F_{3,12} = 107.83$, P < 0.0001) and oxygen partial pressure (two-way ANOVA: $F_{4,12} = 72.89$, P < 0.0001). Oxygen consumption of embryos increased significantly throughout development (*a posteriori* Newman–Keuls: stage

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Fig. 3. Relationship between the duration of behaviours performed by brooding females (min or s) of Cancer setosus and changes in oxygen availability (σ_{02} ,% air saturation), occurring in the centre of the brood masses. Chela probing and standing position were not analysed since these behaviours were always observed coupled with other behaviours. The Kendall rank correlation coefficient is shown for maxilliped beating and pereiopod probing since contrasted data sets were not homoscedastic. A positive or negative correlation between the studied variables indicates an increase or decrease in the oxygen availability at the centre of the brood mass immediately after a specific behaviour was performed, respectively.





I < II < III < IV, P < 0.05 for all cases) and was significantly lower at 0-25% than at 26-100% air saturation (a posteriori Newman–Keuls: 0–10% = 11–25% < 26– 50% = 51-75% = 76-100%; *P* < 0.05). The interaction term was not significant (two-way ANOVA: $F_{1,12} = 0.75$, P = 0.704; Fig. 4).

THE METABOLIC COST OF BROODING

Oxygen consumption of C. setosus females varied with embryo developmental stage (one-way ANOVA: $F_{4,25} = 27.08$, P < 0.0001; Fig. 5). Females carrying late stage embryos (stages III and IV) consumed significantly more oxygen per unit of time and female wet



Fig. 5. Oxygen consumption of non-brooding females of Cancer setosus (N.Br) and brooding females carrying embryos at different developmental stages (stages I to IV). Vertical lines indicate one standard deviation. Different letters in the base of bars indicate significant differences at P < 0.05. Oxygen consumption of brooding females was calculated by subtracting oxygen consumption of the brood mass.

mass than non-brooding females, and females carrying embryos at early developmental stages (stage I and II; a posteriori Newman–Keuls: P < 0.05). Oxygen demand was also significantly different between females carrying embryos at stage III and IV (a poste*riori* Newman–Keuls: P < 0.05). Thus, the standard metabolic rate of females increased during incubation and females carrying late stage embryos doubled their metabolic rate in comparison with control females, or less active brooding females (stages I and II).

Discussion

Females of Cancer setosus exhibited several behaviours exclusive to brooding compared with non-brooding

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Active brood care and brooding costs in a marine crab females. We suggest that these are active brooding behaviours, some associated with an increase in oxygen availability in the centre of the brood mass, while others may be related to the detection of the oxygen conditions. The frequency of occurrence of brooding behaviours was related to levels of oxygen availability in the embryo mass, and may be a response to the increase in oxygen demand of developing embryos. Brooding behaviour and the changes in the frequency of those behaviours throughout embryonic development have important consequences for the levels of oxygen consumption of brooding females in relation to their standard metabolism. As far as we are aware, these results provide the first evidence among crabs and other marine invertebrates of (1) the association between female behaviour and oxygen provision; (2) a relationship between female behaviour and oxygen demand of the embryos; and (3) changes in brood care behaviour and costs of oxygen provision (brooding) throughout embryonic development. These results are particularly relevant, considering the role that oxygen might have played in the evolution of modes of development of marine invertebrates.

Oxygen availability in egg masses of several marine invertebrate species has been suggested to be an important factor affecting modes of development of marine invertebrates (Strathmann & Strathmann 1982; Chaffe & Strathmann 1984; Lee & Strathmann 1998). Oxygen limitation in embryo aggregations has been observed, as well as retarded development of inner embryos, emphasizing the importance of this factor (Chaffe & Strathmann 1984; Cohen & Strathmann 1996). Furthermore, it has been suggested that oxygen affects the shape of embryo masses, embryo density, investment in gel and deposition sites (Strathmann & Chaffe 1984; Strathmann & Strathmann 1995; Lee & Strathmann 1998). However, as of yet, there is no evidence of a direct link between the need to influence the supply of oxygen to embryos, female behaviour, and investment in oxygen provision. We think that parental care among marine invertebrates is strongly influenced by the need to provide oxygen to the embryos, although other forces and benefits may also have played a role.

Parental care has seldom been reported for marine invertebrates (but see Phillips 1971; Wheatly 1981), and this brings the question about the function it may have. In general, among marine invertebrates, parental care has been associated with protection of juveniles from predators or adverse abiotic conditions (e.g. Chaffee & Strathmann 1984; Strathmann 1985; Thiel 1999). Although female crabs may protect embryos from predators, and modify behaviour to protect embryos, it is clear from our study that ovigerous females of *C. setosus* directed brooding behaviours specifically to provide oxygen to the embryos. The behaviours performed by brooding females were not observed in non-ovigerous females and may be considered 'active brood care' related to

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oxygen provision, although not all behaviours could be linked to increases in oxygen availability in the embryo masses. We suggest that ovigerous female crabs provide oxygen to the brooded embryos by abdominal flapping, confirming early suggestions for several other marine crabs that the role of abdominal flapping is to ventilate the embryo masses (Wheatly 1981; de Vries et al. 1991; Naylor et al. 1999). Standing position probably improved the effect of flapping, since the abdomen was gently lifted from the substrate each time females exhibited this behaviour. In contrast to abdominal flapping, maxilliped beating, and pereiopod and chela probing were rarely observed to increase the oxygen availability in the centre of the brood masses. Among decapod crustaceans, the dactyl of pereiopods has been shown to bear setae capable to serve as mechanoreceptors or contact chemoreceptors (Ache 1982; Altner, Hatt & Altner 1983; Schmidt & Gnatzy 1984). A high concentration of chemosensory setae is also located on appendages in the mouth region (Ache 1982); these setae are probably involved in the detection of active chemical compounds of the surrounding environment (Rittschof 1992). Brooding females may display some behaviours (including gill ventilation, Naylor et al. 1999) to detect compounds produced by the embryos when subjected to low oxygen availability or low oxygen levels in the embryo mass and then respond, providing oxygen according to embryo requirements. Information gathering from the brood mass via pheromones has been previously reported for brooding female crabs hatching their embryos (Rittschof, Forward & Mott 1985; de Vries et al. 1991).

Since the frequency of abdominal flapping increases throughout the brooding period, an increase in the absolute amount of oxygen provided to the developing embryos occurs. Coupled with the increase in oxygen provision, the oxygen demand of crab embryos increased throughout development; oxygen consumption of late embryos doubled the consumption of early embryos. This suggests that brooding females are capable of modifying their behaviour, providing oxygen to the brood mass depending on embryo oxygen demand. In general, behavioural changes related to the requirements of offspring have been virtually ignored in marine invertebrates. Only two previous studies in brooding marine crustaceans (prawns, Phillips 1971; isopods, Dick et al. 1998) have shown that presumed behaviours related to oxygen provision increased throughout the development of the embryos. The increased amount of oxygen provided by brooding females throughout development probably serves to avoid problems related to oxygen limitation for the embryos (e.g. increased mortality, low development rate, small size at hatching; Chaffe & Strathmann 1984; Strathmann & Strathmann 1995).

Since brooding females of *C. setosus* behave according to embryo oxygen demands, reproductive investment in oxygen provision also changes throughout embryonic 250 J. A. Baeza & M. Fernández development. It was shown that there is an association between oxygen provision and metabolic cost for brooding females: the cost increased with the frequency at which oxygen was provided to the brooded embryos throughout their development. Thus, the cost of reproduction in parental females of *C. setosus* is not only related to the production of yolky eggs, but also to the active care of the brooded embryos. The reserves of females (carbohydrates, lipids, proteins) decline throughout extended brooding (Bosch & Slattery 1999), suggesting that there are costs associated with the brooding period.

This study of the reproductive behaviour of a brachyuran crab reinforces the ideas that (1) oxygen is a limiting factor in aggregated masses of marine invertebrates in general; (2) brooding can be achieved at large body size if females play a critical role providing oxygen to the embryos; and (3) there are costs associated with oxygen provision (brooding) in marine invertebrates that cannot be ignored. Furthermore, it was shown that oxygen may be such a critical factor that brooding behaviours are directed mostly to oxygen provision. We think that these findings can be extrapolated to other brachyuran crabs and to invertebrates in general, since oxygen limitation has been reported for several brooding taxa. Furthermore, we believe that more work along this line can elucidate the intricate problem of the evolution of modes of development in the sea.

Acknowledgements

We thank Luis Ebensperger, Matthew Lee, Patricio Manríquez, Paula Neill, Rubén Soto and two anonymous reviewers for their comments on earlier versions of this manuscript. We also thank Juan Carlos Castilla and Cristian Pacheco for their help, and our colleagues from ECIM, especially Iván Albornoz, for their collaboration in many different aspects of this work. We appreciate financial support from FONDAP (O & BM#3-Crustacean) and the Volkswagen Foundation.

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Received 16 February 2001; revised 15 August 2001; accepted 26 September 2001

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