Maternal control of calcium concentration in the larval nursery of the bromeliad crab, *Metopaulias depressus* (Grapsidae)

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SUMMARY

It has been proposed that parental care in invertebrates controls for physicochemical conditions. This suggests that the parent detects deviations from optimal conditions and responds by correcting the deviation for the benefit of the offspring. In the bromeliad crab, *Metopaulias depressus*, mothers accumulate snail shells (CaCO_3) in the leaf axil containing the larvae, which results in an increase in Ca^{2+} and pH, both beneficial to the young. I tested the hypotheses that mothers: (i) control for calcium levels and only collect shells when calcium is too low; (ii) always collect shells, independent of the calcium content of the nursery; (iii) collect shells if encountered, but increase their activity if the calcium content becomes critical. In a field experiment, I reduced the initial calcium concentration in the nurseries of one group and increased it in another group. In both groups mothers collected shells. However, they collected significantly more shell mass per day in the group with reduced levels than in the group with high levels. The results support hypothesis (iii) and unequivocally demonstrate maternal control of physicochemical conditions in the nursery. This is the first proof that crustacean mothers actively regulate abiotic conditions for their offspring.

1. INTRODUCTION

Parental care enhances offspring survival and development through protection from adverse physical conditions, predation and parasitism, and provides resources essential for development (reviewed in Clutton-Brock 1991). In invertebrates, parental care is commonly associated with stressful and dangerous abiotic and biotic environments or competition for resources (Wilson 1971; Eickwort 1981; Tallamy & Wood 1986). The benefits of parental care for offspring are documented in few invertebrate taxa, mostly insects. Preparation of nests or other nursery structures, defence against predators and provisioning the young with nutrients are among the more conspicuous parental activities; parental care related to physicochemical factors is less obvious and rarely documented. For example, females of the staphylinid beetle, *Bledius spectabilis*, prevent suffocation and reduce anoxia for eggs and larvae in intertidal burrows (Wyatt 1986), and for amphipods, Morritt & Spicer (1996) suggested maternal control of osmolality of the marsupial fluid for embryos.

It is frequently assumed that parental care controls for physicochemical stress exerted by factors such as temperature, anoxia, desiccation and salinity (see Clutton-Brock 1991). The term 'control' implies that the parent detects deviations from optimal conditions

for the offspring and that a regulatory response will then follow, correcting the deviation. But this is usually not demonstrated and alternative hypotheses to explain these behavioural patterns are not considered, e.g. incidental effects of the presence of the parent and of behaviour performed for the parent's own benefit, without entailing additional cost to the parent (see Trivers 1972). For example, wasps, bees and bumblebees regulate nest temperature by metabolic heat production, fanning and water evaporation (Gibo *et al.* 1974; Martin 1990; Heinrich 1984; Seeley 1985; Vogt 1986), but it is not clear whether benefits to the larvae are incidental and that this behaviour is for the parent's (or adult siblings) own benefit.

Calcium ions play a vital role in crustacean growth as they constitute a significant component of their exoskeleton (Greenaway 1985, 1993). Compared to marine species, freshwater and terrestrial crabs face a limited supply of calcium (Bliss 1968). To freshwater crustaceans, calcium and pH are critical factors limiting moulting (Malley 1980) and thus their distribution (Okland & Okland 1985).

In the Jamaican bromeliad crab, *Metopaulias depressus*, maternal care significantly increases survival and development of larvae and juveniles, and entails costs to the mother in slowing her growth (Diesel 1989, 1992a, unpublished data). This crab breeds in the rainwater-filled leaf axils of bromeliad plants in central

Jamaica. Leaf axils usually contain calcium ions in limited supply (median $4 \text{ mg Ca}^{2+} \text{ l}^{-1}$) and have fairly acid pH (median pH 4.8), both jeopardizing offspring survival and development (see Diesel 1992b; Diesel & Schuh 1993). Laboratory experiments showed that larvae and megalopae require a pH above 5.2 and about $10 \text{ mg Ca}^{2+} \text{ l}^{-1}$ (calculated for a mean size brood in a 240 ml nursery; see Diesel & Schuh 1993) to develop to the first juvenile stage. Larvae and megalopae take up calcium from the axil water and therefore depend on their mother for supplying the calcium necessary for moulting and growth.

Bromeliad crabs are restricted to limestone areas in Jamaica where calcium is not limited except in the rain-water pools in which they live. Females choose a large leaf axil to release their larvae. Within this nursery axil, larvae (here including the megalopa stage) develop and metamorphose in about 13 days to become juvenile crabs, which remain in the nursery for about two months, cared for by their mother (Diesel 1989; Diesel & Schuh 1993). Earlier studies and experiments have shown that (i) mother crabs accumulate snail shells (calcium carbonate; CaCO_3) only for the period of parental care and only in axils containing larvae or early juvenile stages (Diesel 1989, 1992b). For this, they depend on the availability of shells and snails (as prey) on the plant; (ii) when larvae were transferred to another axil on the plant, mothers switched from the nursery axil to the new axil containing the larvae, and continued shell collecting there (Diesel 1992b), showing that shell-collecting in the bromeliad crab is performed for the benefit of the offspring; (iii) if empty shells were provided (taped on to a leaf) on the plant, they were collected by the mother and deposited in the nursery (Diesel 1992b); (iv) experimentally adding shells into uninhabited axils increased the calcium content and pH of the axil water (Diesel 1992b). Although pH was influenced by humic acids and dissolved carbon dioxide in the water, there was a strong positive correlation between calcium content and pH ($r^2 = 0.62$, see Diesel 1992b). Even though maternal shell-collecting behaviour significantly increases calcium concentration and pH condition in the nursery, it has not been demonstrated that mothers sense conditions in the nursery and regulate the environment to fit the needs of their offspring. In a field experiment, I tested the following hypotheses: (i) mothers control for calcium content and only collect snail shells when it is too low; (ii) mothers do not control for calcium content and always collect and deposit available shells into the nursery, independent of the water condition of the nursery; (iii) mothers show a mix of both strategies: collecting shells if encountered (see ii), but increasing their activity if calcium content becomes critical (see i).

2. METHODS

Maternal response to different calcium and pH levels in the nursery axils was examined in 1993 and 1996 in Windsor (Trelawny), Jamaica. The complex and delicate ecosystem within the leaf axils of bromeliads lasts only a short time if brought into a laboratory and allows only limited physico-chemical manipulations. Attempts to breed bromeliad crabs

in captivity have failed, so the experiments could only be carried out in the field and were conducted on a remote hill site in the central Cockpit Country. Bromeliads with ovigerous females were located during the spring breeding season in April 1993 ($n = 12$) and 1996 ($n = 10$) and were in both years randomly assigned to two experimental groups. Experimental manipulations were carried out when larvae were released and the nursery axil established. I removed snail shells, if present, and about 30% of the axil water, and added the same amount as calcium-poor rain water ($\ll 1 \text{ mg Ca}^{2+} \text{ l}^{-1}$) in GROUP-N ($n = 6$ in 1993 and $n = 5$ in 1996) and as CaCO_3 -powder-enriched rain-water (about $50 \text{ mg Ca}^{2+} \text{ l}^{-1}$) in the GROUP-Ca ($n = 6$ in 1993 and $n = 5$ in 1996). The resulting calcium concentration and pH were measured 1–2 days later by titration (Merck: Aquamerck calcium kit 11110; accuracy: $2 \text{ mg Ca}^{2+} \text{ l}^{-1}$) and with a pH-meter (Hanna, pH-Check, accuracy: 0.02 pH units). Axil water manipulations for a group were the same in both years, but the duration of the experiment per axil ranged from 5–6 weeks in 1993 and 2–7 weeks in 1996.

All data satisfied assumptions for parametric statistics (ANOVA, *t*-test for dependent samples; see Sokal & Rohlf 1995), except data on shell mass per day (unequal variances), which were analysed using non-parametric Kruskal–Wallis ANOVA. Data are presented as mean $\pm$ standard error (s.e.) and were analysed with the program STATISTICA 5.0.

Two-way ANOVA with group and year (1993, 1996) as independent variables and pH, calcium and duration of experiment as dependent variables, revealed that the pH ($F_{1,18} = 0.03$, $p = 0.87$) and calcium ($F_{1,18} = 0.15$, $p = 0.70$) treatment at the start did not differ between years, and that the duration of the experiment did not differ between the groups ($F_{1,18} = 0.14$, $p = 0.71$; see table 1). The data obtained for groups in 1993 and 1996 were pooled for further analysis.

Following experimental manipulation at the start of the experiment, GROUP-N nurseries held significantly less calcium and had a lower pH than GROUP-Ca nurseries ($F_{1,18 \text{ Ca}} = 44.2$, $p < 0.001$, $F_{1,18 \text{ pH}} = 13.0$, $p = 0.002$; table 1). At the end of the experiment, all broods had developed into juvenile crabs, and for each nursery the calcium content and pH were measured and the snail shells that were deposited by the mother crab were removed and weighed in the laboratory. The behavioural response of a mother to the manipulation was determined as the amount of snail-shell material accumulated in the nursery divided by the time available for an individual mother to collect shells ($\text{mg}_{\text{shellmass}} \text{ d}^{-1}$). Snail shells are the most common resource for calcium in the habitat; other possible sources that may contribute to the calcium content in the nursery could not be assessed (for example, the skeletons of millipedes, which are a regular prey of crabs; Diesel 1989).

3. RESULTS

In both groups mothers collected shells during the course of the experiment. Mothers whose brood axils were exposed to a low calcium content (GROUP-N) at the start of the experiment, however, collected significantly more shell mass per day than mothers with high calcium content (GROUP-Ca) in their brood axils (Kruskal–Wallis ANOVA $H_{1,22 \text{ shellmass}} \text{ d}^{-1} = 5.6$, $p < 0.01$, one-tailed; see figure 1). The higher rate of shell collecting in GROUP-N resulted in a significant increase in calcium content and pH in the nursery axils (*t*-test for dependent samples, Ca^{2+} : $t = 2.6$, $p = 0.03$; pH: $t = 2.5$, $p = 0.03$), whereas in GROUP-Ca both levels did not change significantly (Ca^{2+} : $t = 1.0$, $p = 0.31$; pH: $t = 1.9$, $p = 0.19$).

Table 1. *Physicochemical conditions in nursery axils of the Jamaican bromeliad crab, Metopaulias depressus*

(Conditions in the nursery water in two experimental groups at the start and end of the experiment. In both groups snail shells and some 30% of the leaf axil water from the nursery were removed at start of the experiment and in GROUP-N refilled with calcium-poor rain water and in GROUP-Ca with calcium-enriched water. At the end of the experiment the amount of shell mass accumulated by mothers per day was analysed (mean $\pm$ s.e.). See text for details.)

test group	start		end		duration	
	pH	Ca ²⁺ (mg l ⁻¹)	pH	Ca ²⁺ (mg l ⁻¹)	(days)	<i>n</i>
GROUP-N	5.74 $\pm$ 0.2	7.8 $\pm$ 1.5	6.16 $\pm$ 0.2	17.3 $\pm$ 4.0	27.8 $\pm$ 4.6	11
GROUP-Ca	6.32 $\pm$ 0.2	31.8 $\pm$ 3.7	6.53 $\pm$ 0.1	29.2 $\pm$ 4.6	28.5 $\pm$ 4.7	11
<i>p</i>	<0.002		<0.002		n.s.	

Figure 1. Snail shell mass collected per day in nursery axils of the Jamaican bromeliad crab, *Metopaulias depressus*, calculated from mass accumulated at the end of the experiment. At the start of the experiment in GROUP-N ($n = 11$) nurseries calcium concentration was reduced and in GROUP-Ca ($n = 11$) it was increased (mean $\pm$ s.e.). See text for methods and analysis details.

4. DISCUSSION

This result demonstrates that mothers are sensitive to the physico-chemical conditions in the nursery water and collect more shells if conditions are unfavourable for developing larvae and young. Hypothesis (i), that mothers control for calcium content and only collect snail shells when it is too low, is not supported by the present study, because mothers of GROUP-Ca also collected some shells, although they were provided with more calcium (mean 31.8 mg Ca²⁺ l⁻¹) than usually occurs in nurseries (median 13.0 mg Ca²⁺ l⁻¹; see Diesel 1992b) and more than is required for development to the first juvenile stage (about 10 mg Ca²⁺ l⁻¹ for a mean nursery size and offspring number; see Diesel & Schuh 1993). Furthermore, the high calcium level at the end of the experiment shows that levels did not decline substantially or become critical during the course of the experiment, for example due to precipitation. These shells may either come from

snails the mothers have captured and transported to the nursery as food for the young and themselves, leaving the shells in the nursery (see Diesel 1989), or from shells that mothers found on the plant and carried to the nursery (see Diesel 1992b).

The present study also does not support hypothesis (ii) that mothers do not control for calcium content and always collect shells independently of the water condition of the nursery. Such behaviour would result in approximately equal shell masses in both groups.

The result of this study is consistent with hypothesis (iii) that mothers collect shells when encountered, and hence prevent a dip in calcium content of nursery water through dilution by heavy precipitation. Mothers obviously increased their shell-searching activities when calcium content became critical and thus indeed controlled physicochemical conditions in the nursery for their offspring.

This study on the bromeliad crab demonstrates a novel behavioural pattern within Crustacea: mothers gather external materials to regulate abiotic conditions in the nursery. Maternal control for calcium concentration in the nursery is particularly important for the development of nonfeeding larvae, as they take up calcium from the nursery water.

From the juvenile stage onwards, the crabs feed mainly on calcium-rich millipedes and may take up calcium through their diet rather than from the water (Diesel 1989; Diesel & Schuh 1993; see Hessen & Kristiansen 1991). When about two months old, the juveniles appear less sensitive to physico-chemical conditions of the axil water and disperse to other axils with less favourable conditions on the plant.

What cues do mothers use for their behavioural response? Mothers could perceive deviations in the nursery from optimal conditions directly by detecting calcium concentration or indirectly by using pH as a correlated indicator. In this experiment both factors were lower in the experimental groups at the start. Experiments in which I used phosphate buffers to alter calcium and pH independently failed because larvae did not survive the treatment ($n = 4$ nurseries). A further hypothesis is that the stimulus that promotes the shell-collecting behaviour in the mother crab comes from the larvae. Larvae under stress, caused by unfavourable

calcium and/or pH conditions, may provide chemical or behavioural signals that could stimulate the collecting behaviour of the mother. Chemically mediated stimulation of maternal behaviour by offspring occurs in crabs: De Vries *et al.* (1991) demonstrated that pheromones released by hatching eggs evoked stereotypic abdomen-pumping response in ovigerous marine and estuarine species. In the bromeliad crab, mothers do at least recognize the residence of the larvae on the plant, because mothers switched to the axil to which larvae had been experimentally transferred, and continued to care for them (for example feeding, shell-collecting; see Diesel 1992b). The question 'Which cues elicit maternal behaviour?' can only be answered by laboratory experiments, which are difficult, given the delicate ecological conditions within bromeliad leaf axils.

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