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Embryo brooding and its effect on feeding in the bivalve *Gaimardia bahamondei* Osorio & Arnaud, 1984

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Abstract

Gaimardia bahamondei is a small gonochoric bivalve which lives attached to subtidal algae. Females brood their embryos in the suprabranchial region of the pallial cavity, in close proximity to the gill filaments. We found no significant difference in clearance rate between males (non-brooders) and females (brooders), regardless of the numbers of embryos in the brood, suggesting that the presence of embryos does not interfere with particle capture by the brooding female. Embryos did not ingest microalgae, indicating that they do not compete with the female for food during incubation. These observations contrast with published data on other brooding bivalves in which particle retention by the adult is reduced during brooding, and the embryos may capture particles suspended in the pallial cavity. These differences among bivalve taxa in the effects of brooding on physiological processes in the female are attributable to distinct morphological adaptations of the gill for brooding.

Keywords: Brooding, Bivalve, Clearance rate, Embryos

Background

Brooding of embryos by aquatic invertebrates often constrains behavioural and physiological processes in both adults and offspring [48]. In bivalves, the brooding habit is usually associated with low fecundity and large eggs [27] and the embryos are confined within the pallial cavity of the brooding adult, usually in the branchial region, e.g. *Neogaimardia finlayi* [32], *Nutricola tantilla* (=*Transennella tantilla*) [24, 25], *Kingiella chilenica* [17], *Sphaerium striatinum* [5], *Gaimardia trapezina* [22], *Adacnarca nitens* [21], *Mysella charcoti* and *M. narchii* [38], *Neolepton salmoneum* [33]. In some species, the embryos can move freely within the female's mantle cavity during brooding [29, 34]. The available evidence suggests that in many cases the female does not undergo anatomical modifications for embryo maintenance (e.g. *Ostrea chilensis*, [10]. In other species, however, the embryos remain immobile during brooding, in which case they are maintained on the surface of the gill, either individually anchored to the gill filaments by the embryonic byssus or attached to the gill by means of mucous masses or specialised structures which prevent premature release of the embryos from the brooding chamber [3, 16, 17, 19, 28, 32, 35, 36, 39]. In some bivalve species gill structure becomes extensively modified during the incubation period in such a way that the water tubes in the inner demibranchs are temporarily transformed into marsupia in which the embryos are retained (e.g. *Anodonta cataracta*, [45].

Incubation of embryos in the bivalve mantle cavity may interfere with the filtration and ventilation activity of the gill of the brooding female, with consequences for physiology and energy acquisition. These include mechanical inhibition of particle retention and hence ingestion [50], reduction of water transport through the marsupial gill [45, 46], interception by the embryos of some of the particles removed from suspension by the brooder [8, 10], alterations in metabolic costs due to embryo ventilation [7], cleaning of the embryos through manipulation by the labial palps of the brooding female [10, 29], or direct transfer of nutritional substances to the embryo

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[30, 31, 42, 43, 45, 51]. A reduction in clearance rate has been recorded in brooding individuals of the marine bivalve *Ostrea chilensis* [9] and the freshwater bivalve *Pyganodon cataracta* [47], associated with the presence of embryos in the pallial cavity of the brooding female. A similar feeding process has been postulated by Mackie [26] for the brooded embryos of some freshwater bivalves (Sphaeriidae).

Gaimardia bahamondei [37] is a small, gonochoric marine bivalve which broods its embryos in the suprabranchial cavity of the female. Each embryo is attached to the abfrontal region of the branchial filament by means of a peduncle [13, 22]. The gill is homorhabdic and the inner demibranch is larger than the external one; embryos are attached to both [13]. During its lifetime, each individual exhibits a single, continuous and prolonged reproductive event, in the breeding season (austral spring-summer, [1], when all the gametes have been released, following the death of the adult [37]. The limited information on the species indicates great variability in the number of embryos brooded by similar-sized females, and the presence of three cohorts in different development stages within a single female at any given time [13] suggests that the release of juveniles may be more or less continuous and that *G. bahamondei* is a sequential brooder [6, 13, 37]. During the reproductive season, most females brood three cohorts of embryos on the gill simultaneously while maintaining three cohorts of oocytes under development in the gonad. It is not known how many cycles of oocyte production and embryo incubation occur during the single reproductive season. Each cohort of embryos is released from the suprabranchial cavity to the outside environment when it reaches the juvenile stage and is replaced by the next cohort from the gonad. Although the species has been considered to be semelparous [6, 37], it does not seem to conform strictly to this mode of reproduction, since at least 6 distinct cohorts of embryos are produced continuously by an individual brooder during the single reproductive event which occurs in its lifetime. However, other species of small bivalves with a similar reproductive strategy (production of more than one cohort of embryos in a single reproductive season) have been classified as semelparous (e.g. *Kingiella chilenica*, [17]; *Transennella tantilla*, [2]. This study examines the reproductive process, particularly brooding, and its implications for food acquisition in *G. bahamondei*. Specifically, we compare the clearance rates of individual brooders (females) with those of non-incubators (males) to test the hypothesis that the incubation of embryos on the gills interferes with the feeding process in brooding adults. We also investigate whether embryos at an advanced developmental stage can remove a proportion of the suspended particulate matter carried into the mantle cavity by the ventilation current, resulting in competition for food between the brooding female and the incubated embryo.

Methods

Collection and maintenance of specimens

Adult specimens of *Gaimardia bahamondei* [37] were obtained by SCUBA diving during January and February 2009 from approximately 8 m depth in Bahía de Corral, San Carlos (39°51′S; 73°27′W), southern Chile. The individuals were attached by their byssal threads to the red alga *Callophyllis variegata*. The seaweed was collected and transferred to the laboratory, where it was kept in tanks of seawater with constant aeration. Adult specimens of *G. bahamondei* were separated from the algae to which they were adhering and held for a maximum of 2 weeks in glass aquaria supplied with constantly aerated, running seawater. Sediment was removed from the aquaria frequently.

Prior to the experiments, the specimens were maintained for at least 2 days in aquaria with continuously aerated seawater (salinity 32 psu, temperature 13 °C) which had been passed through fibre-glass filters (ADVANTEC GC 50, nominal retention 0.5 µm, diameter 47 mm). The water was renewed daily. During this period, the animals were fed with the microalga *Isochrysis galbana* twice daily at a concentration of approximately 30,000 cells ml^{-1} [9, 49].

Specimens were classified as female or male according to the presence or absence of embryos, following Chaparro et al. [13], who determined histologically that all incubating individuals contain always embryos and developing eggs, whereas all non-incubating individuals >3 mm long contain only developing sperm and are therefore males.

Clearance rate

Clearance rate (CR) was measured in 174 specimens of *G. bahamondei* of shell length 3.47–7.96 mm, corresponding to adults with developing gonads and capable of brooding [13]. In each of the 17 experimental runs, 13 or 14 glass chambers (volume 250 ml) were used, of which 10–11 contained a single specimen and 3 were kept without animals, serving as controls for particle numbers. All chambers were kept under the same conditions as those in which the specimens had been previously maintained, including constant aeration to maintain algae in suspension. Cell counts in experimental chambers were corrected according to values from the controls [14].

The initial concentration of *I. galbana* in each experimental and control chamber was adjusted to 30,000 cells ml^{-1} (Beckman Coulter Z2 particle counter). To determine the reduction in particle concentration resulting

from clearance by the specimen, duplicate particle counts were made on water samples removed from the chambers every hour. No further samples were taken after the concentration of microalgae had been reduced by more than 40 % of the initial concentration.

Clearance rate (CR) was estimated following the method described by Coughlan [14] for chambers without food replacement and was expressed as L h^{-1} individual^{-1} and also per standard individual (5 mm shell length). Standardisation of CR was carried out using the exponent ($b = 0.23$) of the regression equation relating CR to shell length using data from all experimental individuals [15].

Immediately after each set of measurements, the experimental specimens were transferred to 70 % alcohol (one specimen per vial) for subsequent processing.

Determination of chlorophyll a in brooded embryos

Experiments were undertaken to determine whether the embryos were capable of removing and ingesting particles during the brooding period and thus whether they contributed to the CR of the mother–embryo complex. The specimens used in these experiments were not those used for CR determinations. Glass chambers (250 ml) were filled with filtered seawater (fibre-glass filters ADVANTEC CG 50, diameter 47 mm) and maintained with constant aeration at 13 °C and salinity 32 psu. One group of specimens ($n = 13$) was kept in individual chambers with *Isochrysis galbana* (30,000 cells ml^{-1}, fed group) and a second group ($n = 13$) in chambers with no addition of microalgae (unfed group). Since not all the individuals used were incubating females, there was variation in the number of cohorts obtained in each treatment in relation to the numbers of individuals used. Food was added (fed group only) every 8 h until the end of the experiment (24 h). The algal concentration and frequency of feeding were selected to correspond with standard hatchery practice [11, 20, 41].

Immediately after the experimental period, each individual was opened in order to identify brooding females, which were dissected to separate the embryos from the gill filaments. Embryos from cohorts 2 and 3, the most advanced developmentally (shell present), were removed for examination under a stereomicroscope and counted (for cohort details see next section). Those from cohort 1 were not used, since development was not sufficiently advanced to permit feeding on exogenous particles. For each experimental female, all embryos of cohort 2 were collected on one 24 mm diameter fibre-glass filter, which was placed in an aluminium foil envelope and immediately frozen to await analysis of chlorophyll *a*. The procedure was repeated for embryos of cohort 3.

Chlorophyll *a* was determined fluorometrically [44]. Filters loaded with embryos were placed in 90 % acetone at 4 °C for 18 h in darkness to extract the chloropigments. Fluorescence values were obtained from a Turner Designs TD 700 fluorometer and compared with a calibration curve. The amount of chlorophyll *a* present in each embryo was calculated separately for cohorts 2 and 3 in each experimental treatment (fed and unfed).

Processing the specimens used in the clearance rate (CR) experiments

Once CR had been quantified, each individual used in the experiments was photographed under a stereomicroscope. The shell length of each specimen was obtained from the photographs with image processing software Image-Pro plus 5.0 and a graduated rule. The valves were then separated under the stereomicroscope, allowing brooding and non-brooding specimens to be distinguished (all brooding individuals >3 mm were females and all non-brooding individuals >3 mm were males [13]. In each of the 99 females, the embryos (sensu [40]) were separated from the body mass of the adult. The embryos from each female were classified by cohort according to their level of development.

Since the capacity of the embryos to use exogenous particles as a food source may vary according to stage of development and since the CR of a brooding female may depend on the distribution of embryos among developmental stages, it was necessary to characterise embryos of different stages. Embryos could be differentiated according to shell colour, presence or absence of valve ornamentation, presence or absence of shell, and shell hardness [13]. These characteristics allowed for separation of the embryos into three categories: cohort 1 (early development: shell absent; colourless; smooth surface); cohort 2 (intermediate development: shell present but poorly calcified and sculpture weakly defined; reddish tinge); cohort 3 (advanced development: well-calcified, hard shell with defined sculpture). For each female, the number of embryos in each of the 3 cohorts was counted. The anteroposterior valve length was used as an indicator of size for the embryos with shells, whereas the diameter of the embryos was used for cohort 1 (shell not yet developed). For determination of dry weight of the embryos, all individuals from individual cohorts were retained under gentle vacuum on fibre-glass filters (Advantec CG 50, diameter 24 mm) which had previously been washed with distilled water, dried at 60 °C for 48 h and then weighed. Each loaded filter was washed quickly with distilled water to remove the seawater salts, then folded, placed in an aluminium foil envelope, dried at 60 °C for 48 h, cooled in a desiccator and weighed (all weighings

±0.00001 g). Dry weight of the embryos of each cohort was obtained by difference.

After removal of embryos, the flesh of each adult was extracted from the shell and placed on a pre-weighed aluminium dish, which was immediately dried at 60 °C for 48 h, cooled in a desiccator and weighed. Total brooded biomass (TBB) was defined as the sum of the dry masses of all embryo cohorts brooded simultaneously by each female.

Statistical analyses

A forward stepwise multiple linear regression was used to identify those variables that accounted for most of the variation observed in CR for both brooders (females) and non-brooders (males). In females, the independent variables included in the CR analysis were shell length and dry flesh weight of the adult, number of brooded embryos, TBB and total dry biomass (adult flesh + embryos). For males, only adult shell length and dry flesh weight were included in the regression analysis.

Non-standardised CR values were compared between males and females using ANCOVA. Those factors with major significance in the linear regression analysis were used as covariables.

One-way ANOVAs were performed to identify differences between females (brooders) and males (non-brooders) in standardised CR, shell length and dry weight of soft tissue, and also to compare the chlorophyll *a* concentration between embryos from fed and unfed treatments. Dry tissue weights from males and females were compared by one-way ANOVA ($n = 171$ specimens). We were able to obtain this information for only 171 of the original 174 specimens.

A one-way ANOVA followed by a post hoc Tukey test was used to detect differences among cohorts (C1-C3) in shell length, dry weight of soft tissue and number of embryos present. All females were included in these analyses ($n = 99$). A logarithmic transformation was applied to shell length values to comply with the requirements of ANOVA. Each female was assigned to one of three groups according to the relative number of embryos brooded in each cohort: (FC1) females in which cohort 1 contained the most embryos, (FC2) females in which cohort 2 was dominant and (FC3) females in which cohort 3 dominated. This categorisation of females was made because CR of any given female may be influenced by the proportions of embryos at each stage being brooded simultaneously.

Similar analyses (one-way ANOVA with a post hoc Tukey test) were employed to determine differences among FC1, FC2 and FC3 in embryo size, TBB, total biomass (combined biomass of female and brooded embryos) and total number of brooded embryos.

Results

Shell length and dry weight of soft tissue

No significant differences were observed between females (brooders) and males (non-brooders) in shell length (ANOVA: $F_{1,172} = 0.868$, $P = 0.352$) or dry weight of soft tissue (ANOVA: $F_{1,169} = 2.927$, $P = 0.088$). The range in shell length of the specimens used in this study was 3.47–6.54 mm for females (brooders) and 3.77–7.96 mm for males (non-brooders). No significant differences were found in shell length among the 3 groups of females (FC1, FC2, FC3) (ANOVA: $F_{2,96} = 1.666$, $P = 0.194$).

Clearance rate

Shell length alone accounted for 30 % of the variation in CR in females and in males (multiple regression). Inclusion of total biomass (dry mass of soft tissue of the female plus dry mass of the embryos) in the analysis increased the variation explained by only 4 % (females only, Table 1).

ANCOVA with shell length as covariate showed no significant difference in CR between females (brooders) and males (non-brooders) ($F_{1,171} = 2.566$, $P = 0.111$, Fig. 1), and confirmed the effect of shell length on CR ($F_{1,171} = 75.262$, $P < 0.001$). Females (brooders) exhibited a CR of 0.032 ± 0.015 L h^{-1} ind^{-1} (mean ± SD), a shell length of 5.16 ± 0.68 mm and dry flesh weight of 2.31 ± 1.85 mg, while the corresponding values for males (non-brooders) were 0.036 ± 0.018 L h^{-1} ind^{-1}, 5.26 ± 0.81 mm and 2.72 ± 1.84 mg, respectively.

A comparison of CR in individuals of standard shell length 5 mm showed no significant difference between females (brooders) and males (non-brooders) (mean values 0.029 ± 0.012 L h^{-1} mm^{-1} and 0.031 ± SD 0.014 L h^{-1} mm^{-1} respectively) (ANOVA: $F_{1,172} = 0.389$, $P = 0.533$, Fig. 2).

No significant differences were found in standardised CR among the three groups of females (FC1, FC2, FC3) (ANOVA: $F_{2,96} = 0.702$, $P = 0.187$). Values for standardised CR were 0.0324 ± 0.009 (mean ± SD),

Table 1 *Gaimardia bahamondei*: Multiple regression for clearance rate in brooding females and in males

Variable	Step	Multiple *R*	Multiple R^2	R^2 change	*P*
Female					
Shell length (mm)	1	0.54	0.30	0.30	0.0001
Total biomass (mg)	2	0.59	0.34	0.04	0.009
Male					
Shell length (mm)	1	0.55	0.30	0.30	0.0001

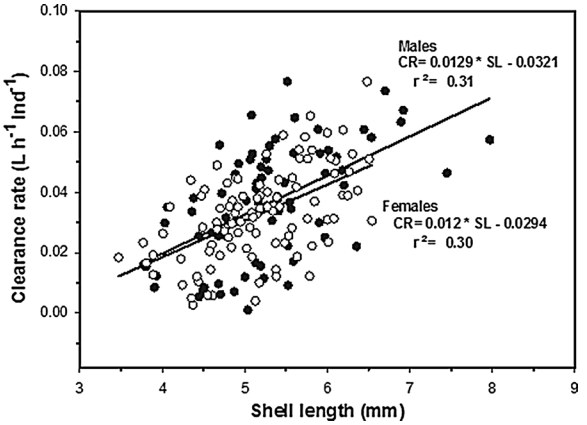

Fig. 1 *Gaimardia bahamondei*. Relationship between clearance rate and shell length. Males ($n = 75$, *full circles*), brooding females ($n = 99$, *empty circles*)

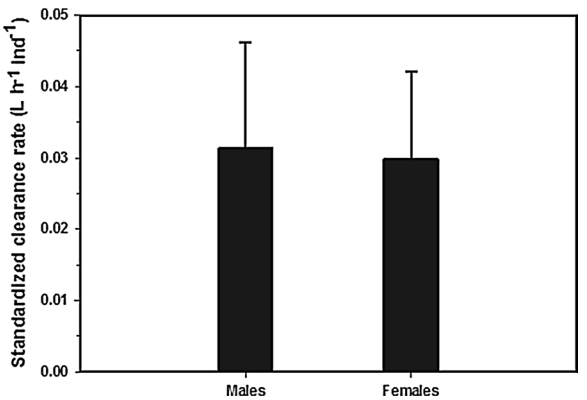

Fig. 2 *Gaimardia bahamondei*. Clearance rate (mean ± SD) for males ($n = 75$) and females ($n = 99$). Values standardised for an individual of shell length 5 mm

0.0281 ± 0.010 and 0.0287 ± 0.013 L h^{-1} for FC1, FC2 and FC3, respectively (Fig. 3).

Chlorophyll *a* in brooded embryos

From the 13 fed adults, all were brooding females. From those, we were able to collect 13 cohort C3 and 4 cohort C2. On the other hand, from the 13 unfed adults, only 11 were brooding females, and we were able to collect 11 cohort C3 and 3 cohort C2. No significant differences in the chlorophyll *a* content of embryos were observed between fed and unfed females, either for cohort C2 (ANOVA: $F_{1,5} = 1.258$, $P = 0.312$) or C3 (ANOVA: $F_{1,22} = 1.882$, $P = 0.183$), nor between embryos of C2 and C3 in the fed group (ANOVA: $F_{1,15} = 0.582$, $P = 0.457$).

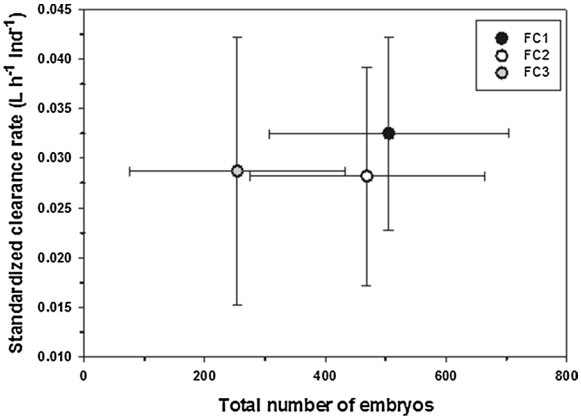

Fig. 3 *Gaimardia bahamondei*. Relationship between clearance rate (mean ± SE) and mean number of embryos brooded for each female cohort FC1 ($n = 18$), FC2 ($n = 18$), FC3 ($n = 63$). Values standardised for an individual of shell length 5 mm. FC1: females for which C1 embryos were most numerous; FC2: females for which C2 embryos were most numerous; FC3: females for which C3 embryos were most numerous

Number and biomass of embryos; shell length and biomass of brooding females

The number of embryos incubated by a female varied between 16 and 943 (range in shell length of females: 3.47–6.54 mm). Significant differences were identified in the mean size of the embryos among the three cohorts present in each female (ANOVA: $F_{2,294} = 1246{,}38$, $P < 0.001$, Fig. 4), C1 (the most recent) having smaller embryos than C3 (the most advanced) and C2

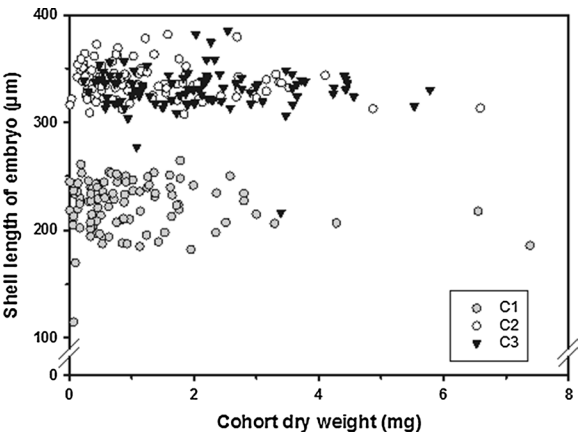

Fig. 4 *Gaimardia bahamondei*. Cohort dry weight and their mean shell length (C2 and C3) or diameter (C1) for offsprings brooded by females used in the CR experiments. *Each dot* represent the embryos belonging to each particular cohort brooded by a single female. Cohort 1 ($n = 99$), cohort 2 ($n = 99$) and cohort 3 ($n = 99$)

(intermediate) ($P < 0.001$). There was no significant difference in shell length between C2 and C3 embryos ($P = 0.182$). Significant differences were observed among cohorts in total dry weight of embryos (ANOVA: $F_{2,294} = 3.888$, $P = 0.021$, Fig. 4), specifically between C2 and C3 ($P = 0.043$) and C1 and C3 ($P = 0.040$) (Table 2). There was also a significant difference among cohorts in the mean number of embryos per cohort per female (ANOVA: $F_{2,294} = 6.003$, $P = 0.002$). This was recorded between C2 and C3 ($P = 0.027$) and between C1 and C3 ($P = 0.002$) (Table 2). In 64 % of the 99 females analysed, C3 contained the greatest number of embryos, whereas C2 dominated in 19.7 % of the females and C1 in the remaining 16.3 %.

The mean dry weight of a brooding female was 2.31 mg ± SD 1.85, while TBB was 4.21 ± 2.01 mg (mean ± SD).

Significant differences were observed in mean total biomass (dry flesh weight of females + dry weight of embryos) among females in conditions FC1, FC2 and FC3 (ANOVA: $F_{2,96} = 12.831$, $P < 0.0001$). Specifically, FC1 differed significantly from FC3 ($P < 0.0001$) and FC1 from FC2 ($P < 0.0001$). The mean total biomass (female soft tissues plus embryos) for FC1 was higher than for FC2 or FC3 (Table 3). However, TBB (mass of all embryos present) did not differ among female cohorts FC1, FC2 and FC3 (ANOVA: $F_{2,96} = 0.398$, $P = 0.67$) (Table 3).

Significant differences were recorded among females with different brooding condition in the mean number of embryos brooded per female (Table 3; ANOVA: $F_{2,96} = 16.345$, $P < 0.0001$). Specifically, FC2 differed from FC3 ($P < 0.0001$) and FC1 from FC3 ($P < 0.0001$). In FC1 and FC2, the mean number of brooded embryos was almost double that in FC3.

Table 2 *Gaimardia bahamondei*: Number, total dry weight and shell length (mean ± SD) for embryos in cohort 1 (early development), cohort 2 (intermediate development) and cohort 3 (advanced development) from all brooding females ($n = 99$)

Cohort	Number of embryos (embryos cohort^{-1})	Total dry weight (mg cohort^{-1})	Shell length (µm)
C1	95 ± 106 (a)	1.06 ± 1.18 (a)	223 ± 23 (a)
C2	105 ± 83 (a)	1.29 ± 1.15 (a)	337 ± 14 (b)
C3	139 ± 85 (b)	2.06 ± 1.25 (b)	332 ± 14 (b)

Different letters in parentheses indicate significant differences among means

Discussion

Females of *G. bahamondei* incubate their embryos in the suprabranchial cavity, attached to the abfrontal region of the branchial filaments and facing the water flow from the infrabranchial to the suprabranchial region. Our data showed no difference in clearance rate (CR) between females (brooding individuals) and males (non-brooding), regardless of the number of embryos brooded or the relative proportions of brooded embryos in each of the three phases identified (early, intermediate and advanced), refuting our hypothesis that the presence of embryos on the gill affects the process of feeding in *Gaimardia bahamondei*. Although they did not measure feeding rate, Benavides and Cancino [6] found no effects of brooding condition on the rates of oxygen uptake and ammonia excretion in *G. bahamondei*, observations which are consistent with our own.

In other bivalves, however, the physiological processes of brooding individuals may be influenced by the presence of embryos. Tankersley and Dimock [46] recorded much lower mass-specific ventilation and oxygen consumption rates in brooding females of the freshwater mussel *Pyganodon cataracta* than in males, although no difference was observed in ventilation rate per individual. In a separate study on the same species, these authors observed lower CR and particle retention efficiency in females with gravid marsupia than in males and non-incubating females [47]. Beekey and Hornbach [5] investigated brood size and number of marsupial sacs in another freshwater bivalve, *Sphaerium striatinum*, and suggested that the embryos interfere with the feeding process in the brooding female. Chaparro and Thompson [9] observed several physiological changes as a result of brooding in the oyster *Ostrea chilensis*, particularly reductions in CR, ingestion rate and faecal production. Although no change was observed in oxygen uptake, and

Table 3 *Gaimardia bahamondei*: Shell length, clearance rate and brooding characteristics (mean ± SD; $n = 99$) for females grouped according to the proportion of brooded embryos per cohort (conditions FC1-FC3; see "Methods" section)

Condition	Shell length (mm)	Standardised CR (L h^{-1})	Total biomass (females + embryos) (mg)	Number of embryos female^{-1}	Brooded biomass (mg)
FC1	5.38 ± 0.64 (a)	0.032 ± 0.009 (a)	9.03 ± 2.10 (a)	487 ± 206 (a)	4.06 ± 1.75 (a)
FC2	5.10 ± 0.68 (a)	0.028 ± 0.010 (a)	5.74 ± 2.51 (b)	468 ± 194 (a)	4.38 ± 2.36 (a)
FC3	5.10 ± 0.71 (a)	0.028 ± 0.013 (a)	6.34 ± 2.38 (b)	253 ± 178 (b)	4.59 ± 2.18 (a)

Different letters in parentheses indicate significant differences among means

the brooding oyster partially compensated for reduced ingestion rate by increasing absorption efficiency, energy balance remained negative throughout the incubation period.

These differences among brooding bivalve species in the physiological responses to the presence of embryos must be attributable, at least in part, to the large variation in morphological adaptations for brooding observed in the group. In brooding females of the freshwater bivalve *Pyganodon cataracta*, and in other unionids, temporary secondary septa and thin membranes associated with the marsupia not only impede water circulation through the mantle cavity but also isolate the embryos from the surrounding medium [45]. Ctenidial swelling also inhibits water flow, further reducing particle retention efficiency. The reduced rates of ventilation, clearance and oxygen consumption observed in freshwater bivalves by various authors are consistent with the morphological adaptations of structures within the mantle cavity of the brooding female.

In the oyster *Ostrea chilensis*, however, the gill does not appear to be modified for brooding, and the embryos, which aggregate temporarily at the base of the labial palps, move freely in a sporadic but strong pallial countercurrent which transports them from the anterior to the posterior region [29]; the embryos then attach to the gill and return to the anterior region within the food grooves [10]. There are three possible ways in which the presence of the embryos may account for the observed decrease in CR of the brooding female oyster. First, it is possible that this countercurrent disrupts normal ventilation, although there is no direct evidence for this. Second, the presence of large numbers of embryos immediately adjacent to the faces of the lamellae is likely to reduce the number of suspended particles that reach the gill. Third, the embryos are not lecithotrophic and are able to remove particles suspended in the mantle cavity, thereby competing directly with the brooding adult for food resources [10]. Furthermore, embryos returning to the anterior region in the food grooves presumably impede the normal transport of mucus-bound food particles to the mouth of the adult, contributing to the observed reduction in ingestion rate [10]. Finally, manipulation of the embryos by the palps [10, 29] is likely to impede or interfere with particle sorting on the palp surfaces, thereby influencing ingestion rate.

The type of relationship between the embryo and the brooding female in *G. bahamondei* is unusual among bivalves. Each embryo is attached to the abfrontal margin of the gill by a peduncle. The embryos therefore lie in the suprabranchial cavity and do not interfere with the suspension-feeding activity of the brooding female, nor do they impede the transport of particles across the face of the lamella. This morphological adaptation in *G. bahamondei* explains our observation that CR is not reduced during brooding (Fig. 2). The faces of the lamellae and the food grooves are kept free of embryos, thereby minimising interference with the processes of feeding and digestion in the brooding female (contrast *O. chilensis*), and there is no requirement for the complex modification of the branchial filaments which is typically found in freshwater bivalves and which inevitably compromises feeding activity.

Since there was no difference in chlorophyll *a* content between embryos from brooding *G. bahamondei* maintained on an algal diet and those from unfed brooding females, we can infer that the embryos do not feed on exogenous particles. Particulate matter that enters the pallial cavity and is retained on the gill is then available to the brooding female, which apparently does not compete with the embryos for suspended particulates. In other bivalve species, however, extravitelline feeding by brooded embryos has been observed, implying that they have the capacity to utilise particles drawn into the pallial cavity of the adult [10, 26]. The large size of the oocytes of *G. bahamondei* and the lack of evidence for microalgae ingestion by embryos indicate that development in this species is lecithotrophic. In the congeneric species *G. trapezina* there is a cell layer that envelops the entire oocyte and also gives rise to the peduncle which attaches the embryo to the gill tissue [22]. This layer remains in place throughout the entire incubation period and appears to form a barrier to the entry and ingestion of exogenous food particles. In other species of brooding microbivalves (Cyamioidea), the embryos are enclosed by a membrane which is attached to abfrontal region of the branchial filaments by means of a stalk peduncle [33, 39]. In the cyamiid *Cyamiocardium domaneschii* [39], the oocyte is covered by a thin cap of follicular tissue which persists throughout vitellogenesis, fertilisation and juvenile development, after which the membrane and peduncle disappear, leaving the juveniles moving freely within the suprabranchial cavity [39].

The higher total weight (brooded embryos biomass + female dry tissue weight) of FC1 females than FC2 and FC3 females in *G. bahamondei* is attributable to greater biomass of the adult body tissue in the FC1 group, because TBB does not differ among cohorts of females, notwithstanding the differences in embryo numbers. After the release of the older cohort, it appears that a new cohort replaces it, exploiting the space made available in the suprabranchial chamber. The TBB is approximately twice the biomass of the soft tissues of the brooding female, suggesting a high reproductive output and concomitant high energy costs to the female. This expensive process, together with the energy that the brooding

female *G. bahamondei* must consume to maintain a high CR, may explain, at least in part, why female biomass is lower in C2 and C3 females. Furthermore, it may be responsible for the high mortality observed in this species at the end of the reproductive period [37], probably a time when insufficient food is available to sustain further gamete production and development. A female may produce as many as six generations of juveniles during its lifetime, brooding up to 3 cohorts of embryos simultaneously in the suprabranchial cavity. High energy investment in reproduction has also been demonstrated in males, in which large numbers of spermatozoa have been observed adhering to the gills of both females and males at a location that coincides with the anchor point of the embryos to the gill filaments of the female [13]. At all times, gonads of all males contain numerous spermatozoa ready for release and other reproductive cells at less developed stages [13]. It is possible that males produce proportionately more sperm per unit body mass in *G. bahamondei* than in larger bivalve species, accounting for the relatively large volume of the body cavity occupied by the gonads.

Our observations suggest that both males and females maintain a continuously high production and evacuation of gametes, resulting in a considerable energy demand and accounting for the high CR observed in both sexes. The emphasis on reproduction in *G. bahamondei* is also reflected in the large size of the gonad relative to somatic tissue in both sexes [13]. Previous studies of *G. bahamondei* have shown a higher rate of oxygen consumption [6] than in other marine bivalves, e.g. *Crassostrea corteziensis* [18], *Acesta excavata* [23] and *Mytilus edulis* [4]. This high energy requirement in *G. bahamondei* probably accounts for the high CR observed in our study. Considering that less food is generally available for suspension feeders during winter in mid-latitudes [12], the high winter mortality observed in *G. bahamondei* [1] appears to be a consequence of the energy demand of a large investment in continuous gamete production which cannot be sustained during periods of food scarcity. Nevertheless, *Gaimardia bahamondei* is capable of a high reproductive output during the reproductive season, attributable in part to the high CR observed in both males and females of this species, which suggests that the presence of embryos in the suprabranchial cavity of the female does not impede particle capture by the gill.

Authors' contributions

PA participated in the design, carried out the experiments and wrote the first draft of the manuscript. LP drew up the experimental design and carried out the statistical analyses. OC also contributed to the experimental design and the interpretation and presentation of the data. RT contributed to discussions of experimental design and interpretation of results and assisted with the final draft. FP assisted with experiments and maintaining the animals. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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