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Reproductive output of the ornamental shrimp *Lysmata vittata* (Stimpson, 1860) (Decapoda: Caridea) in wild populations and under different maturation diets

Andréa Santos e Almeida[a], Samara de Paiva Barros-Alves[a,b], Gustavo Luis Hirose[a,b] and Douglas Fernandes Rodrigues Alves[a]

[a]Laboratório de Carcinologia, Universidade Federal de Sergipe, UFS, São Cristóvão, Brazil; [b]Group of Studies on Crustacean Biology, Ecology and Culture – NEBECC, Departamento de Zoologia, Universidade Estadual Paulista, UNESP, Botucatu, Brazil

ABSTRACT

Reproductive output parameters (fecundity and egg volume) of *Lysmata vittata* were examined in a population in northeastern Brazil. Effect of maturation diets on the reproductive output of these shrimp under laboratory conditions was evaluated. Reproductive output was estimated for 25 shrimp collected in the wild. Another 45 pairs of shrimp were used for diet experiments, 15 pairs per treatment (T1: industrialized food, T2: fresh food, T3: mixed diets). For wild population, mean fecundity and egg volume were differed between developmental stages of the eggs. Fecundity was significantly lower in the specimens subjected to T1 diet (267 ± 141 eggs) compared with shrimp from the wild population (393 ± 183 eggs). Egg volume was significantly lower in shrimp subjected to three diets tested compared with those from wild population. Egg loss during embryonic development in *L. vittata* may be caused by several factors (e.g. aborted development and maternal cannibalism). Fresh food proved to be important for improving reproductive output in *L. vittata* reared in culture. We emphasize the significance of improving nutritional value and palatability of diets to improve cultivation efficiency. *Lysmata vittata* can be used as a model organism for future studies aiming to improve the cultivation techniques for shrimp of the genus.

Abbreviations PSH: protandric simultaneous hermaphroditism; ARS: artificial refuge structures; CL: carapace length; EV: egg volume; L: largest diameter of the egg; S: smallest diameter of the egg; T1: industrialized pellets; T2: fresh ingredients; T3: mixed diets; ANCOVA: analysis of covariancr; SD: standard deviation.

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Caridea; diets; Lysmatidae; ornamental; reproductive output

Introduction

Shrimp from the genus *Lysmata* Risso, 1816 belong to the Lysmatidae family (*sensu* Christoffersen 1987) (Baeza 2013) and include 47 species that are widely distributed in tropical and subtropical regions worldwide (Anker et al. 2009; De Grave and Fransen 2011; Prakash and Baeza 2017; De Grave and Anker 2018). In recent decades, several studies have described the biology and ecology of shrimp from this genus, including: (1) the wide diversity of lifestyles, from species living in dense aggregations (e.g. *Lysmata seticaudata* (Risso 1816) (d'Udekem d'Acoz 2002) and *Lysmata californica* (Stimpson 1866) (Bauer and Newman 2004)) to those that live in breeding pairs in low densities (e.g. *Lysmata grabhami* (Gordon 1935)(Wirtz 1997) and *Lysmata amboinensis* (De Man 1888) (Fiedler 1998)); (2) the confirmation of protandric simultaneous hermaphroditism (PSH) in all species studied (Bauer 2006; Baeza et al. 2009; Baeza 2013) with an early male-phase; (3) a varied sociobiology related to the adoption of different mating systems (monogamous or promiscuous, Baeza 2010; Baeza et al. 2016) and other aspects of reproductive biology, such as brood size (number of eggs per brood) and frequency of spawning (interspawn interval in days) (Bauer 2005).

In addition to the ecological, social, and behavioral characteristics described above, shrimp from the *Lysmata* genus can be recognized by their unique colour pattern, as well as for the cleaning behaviour or the control of some aquarium pests (e.g. *Aiptasia* anemones) presented by some species, which may also have contributed to making these shrimp a target of the ornamental marine industry (Calado 2008). Among the 20 species of marine invertebrates that were most imported into the USA to supply the demand of the ornamental industry in 2008, 2009, and 2011, the *Lysmata* genus was the most highly represented (e.g. *Lysmata ankeri* Rhyne and Lin 2006; *Lysmata debelius* Bruce 1983; *L. amboinensis*) (Rhyne et al. 2017).

CONTACT Samara de Paiva Barros-Alves barros_samara@hotmail.com Laboratório de Carcinologia, Universidade Federal de Sergipe, UFS, Av. Marechal Rondon, s/n, São Cristóvão, Sergipe 49100-000, Brazil

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To supply the demand of the marine ornamental industry, the *Lysmata* shrimp are collected from their natural environments, mainly coral reefs in tropical and subtropical regions of the world, e.g. in the USA, *Lysmata boggessi* Rhyne and Lin 2006 is widely exploited and is the most marketed peppermint shrimp worldwide according to Baeza and Behringer (2017); in India, *L. debelius* and *L. amboinensis* are among the top 10 invertebrates in the ornamental trade (Prakash et al. 2017); and in Brazil, the cleaner shrimp *L. grabhami* and peppermint shrimp *Lysmata wurdemanni* complex (Rhyne and Lin 2006) are captured for this proposal (Gasparini et al. 2005). In view of the growing demand for *Lysmata* shrimp in the marine ornamental trade (Gasparini et al. 2005; Prakash et al. 2017; Rhyne et al. 2017), the maintenance of natural stocks depends on the development of management and conservation measures. One essential measure to be taken is to collect information on reproductive biology and other aspects of the natural populations of the target species. Such information can be used to implement management strategies, such as setting minimum and maximum size limits, establishing species-based quotas, and establishing closed seasons for the shrimp. Other important measure is to assess the issues that contribute to the development of techniques for captive breeding of the shrimp. This could be important to use as a strategy to reduce capture and other possible impacts that may be caused by the decrease in the natural population and, consequently, by the services provided by the shrimp in the reef environment (Rosa et al. 2014). Studies about reproductive parameters, like fecundity, fertility, and egg size are meaningful to add information about life history traits and conditions of reproductive strategies and, furthermore, to evaluate potential for commercial scale cultivation and stock size of a natural population (Valenti et al. 1989; Bertini and Baeza 2014).

Importantly, improving the crustacean maturation diet (diets based on nutrient requirements for a successful maturation and spawning of the broodstock) may enhance reproductive performance and, consequently, improve the efficiency in cultivation (Lin and Shi 2002). According to Calado (2008), to replenish nutrient reserves that are necessary for the gonadal maturation, some nutrients are indispensable for a successful broodstock maturation in captivity, like: lipids, to be used as a source for metabolic energy, energy storage and to supply essential fatty acids (Xu et al. 1994; Cavalli et al. 2001); proteins, to provide essential and non-essential amino acids; and other important nutrients like carbohydrates, vitamins, minerals and carotenoids. The maturation diet given to the reproductive specimens is of major importance for appropriate captive breeding; several reproductive parameters (e.g. fecundity, egg fertilization, egg size and number of larvae originating from the same brood) can be affected by the quality of the food given to these specimens in the cultivation systems (see Lin et al. 2002; Coman et al. 2007; Tziouveli et al. 2011). Thus, in *Lysmata* species, as they are commonly classified as protandric simultaneous hermaphrodites and can act as both male and female (Bauer 2005), a good diet may be required to restore the energy used for oogenesis and spermatogenesis (Calado et al. 2009).

In this study, *Lysmata vittata* (Stimpson 1860) was used as a model organism to understand the effects of different maturation diets on the reproductive output of ornamental shrimp, given to the breeding specimens in captivity. The red-striped *L. vittata* shrimp are commonly found in the Pacific and Indian Oceans in subtropical and tropical waters (Marin et al. 2012a, 2012b). In the southwestern Atlantic, this shrimp is known as to be an exotic species (Soledade et al. 2013). In its natural environment, *L. vittata* can be found freely or in dense aggregations, in rock crevices, sponges, or coral reefs, but mainly in estuarine regions (Marin et al. 2012a, 2012b; Soledade et al. 2013). The reproductive biology of *L. vittata* is still poorly understood. Soledade et al. (2013) showed morphological evidence (internal and external) indicating that the sexual system of *L. vittata* is PSH. Thus, the aim of this study was to evaluate two different individual-level reproductive output parameters (fecundity and egg volume) in brooding *L. vittata* hermaphrodites obtained from a wild population in northeastern Brazil. We also evaluated the effects of different maturation diets on the reproductive output of these ornamental shrimp under laboratory conditions.

Material and methods

Sampling and laboratory procedures

L. vittata specimens (Figure 1(a)) were collected in the estuary region of the Vaza-Barris River, Sergipe state, northeast Brazil (11°05′59″S, 37°08′59″W) (Figure 1(b)). To collect the animals, we built nine artificial refuge structures (ARS). Each structure consisted of a plastic cylinder (15 cm diameter × 30 cm length) with a mesh size of 1 cm^2, filled with flexible plastic tubes (1.8–2.4 cm diameter × 8 cm length) (Figure 1(c)). Free diving performance was used to install and remove the ARS onto pilasters of a pier, between 3 m and 5 m deep. After around 15 days, each ARS was

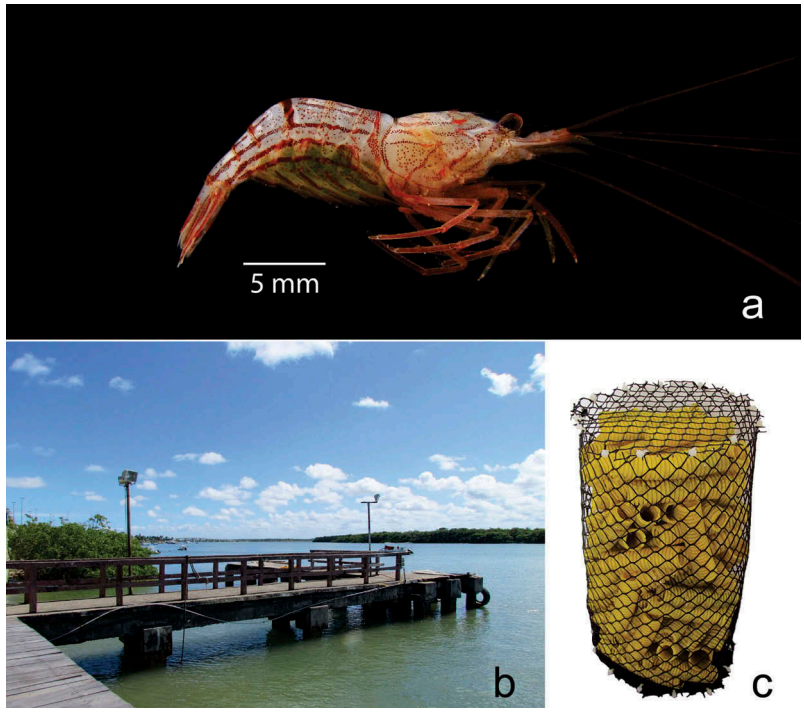

Figure 1. (a) Lateral view of a *Lysmata vittata* ovigerous hermaphrodite (Stimpson, 1860); (b) sampling site at the Vaza-Barris estuary, Brazil; (c) artificial refuge structures (ARS) installed on the pilasters of a pier and used to capture the shrimp.

recovered from the local. Each ARS was placed into a net (500 µm mesh size), untied, and immediately taken to the pier, where the shrimp were manually removed. The collected shrimp were individually placed into plastic bags with water from the collection site (200 mL). Shrimp were transported alive in thermal boxes to the laboratory. The sampling procedure was carried out five times between March and May 2016.

Shrimp were randomly separated into two groups: (1) to study the reproductive output of *L. vittata* in the wild and (2) to evaluate the effects of different maturation diets on the reproductive output of *L. vittata* under experimental conditions. For the laboratory experiments, we only used those specimens that were morphologically intact and that appeared to be healthy (i.e. usual coloration and swimming).

Reproductive output in L. vittata *in the wild*

A total of 25 specimens of *L. vittata* from the wild population that were carrying eggs were analyzed. In the laboratory, the specimens were sacrificed and preserved in ethanol (70%). The carapace length (CL) (distance from the posterior-orbital margin to the posterior margin of the carapace) was measured with a vernier caliper (accuracy 0.01 mm). Reproductive parameters for each shrimp from the wild population were analyzed: (1) the developmental stages of the eggs, (2) fecundity, and (3) egg volume.

Eggs carried by hermaphrodite shrimp were classified according to three different categories (Wehrtmann and Lardies 1999; Hayd and Anger 2013): stage I, rounded eggs with yolk uniformly distributed, no eye pigments visible; stage II, ovoid eggs, embryos with eye pigments elongated; and stage III, ovoid eggs, with well-developed eyes and free pleon. All eggs adhered to the pleopods of each shrimp were removed, and the total number of eggs were counted under a stereomicroscope (Leica M205 C). Egg volume of an egg sample (N = 30) was also calculated for each shrimp, following the same criteria using in the previous studies on other decapod (e.g. Clarke 1993; Lin and Shi 2002). Thus, we measured the largest (L) and smallest (S) diameter of each egg. The eggs were measured using the Leica Application Suite software. Egg volume (EV) was calculated using the formula: $EV = 1/6(LS^2\pi)$ (Turner and Lawrence 1979).

Effects of different maturation diets on the reproductive output of L. vittata *under experimental conditions*

Maintenance of shrimp in the laboratory

Other 90 specimens of *L. vittata* were sampled to evaluate the effects of different maturation diets on the reproductive output under experimental conditions. The shrimp used in the experiment were kept in plastic

rearing tanks with a 1 mm mesh size (12 × 7 × 12 cm) in six aquaria (45 × 20 × 30 cm) interconnected by a filtration system with recirculated water (for details about the recirculated water system, see Calado et al. 2007). Experiments were conducted with water at a temperature of 26 ± 1°C, a salinity of 30 ± 1, and a photoperiod of 12 h light and 12 h dark. The water used in the experiment was prepared using freshwater purified by a reverse osmosis/DI unit and synthetic sea salt suitable for marine aquaria (Red Sea®). Salinity and temperature of water were recorded daily.

Experimental design

In this experiment, we selected three different diets (industrialized, fresh, or mixed) commonly used in small laboratory cultures of ornamental shrimp (Lin et al. 2002; Calado et al. 2009; Gregati et al. 2010) to determine: (1) which diet to use in laboratory cultures of *L. vittata* shrimp to obtain a better reproductive output; and (2) whether the reproductive output is similar or greater than that in the wild populations with any of these diets. Thus, in this experiment, three sets of shrimp were fed with three different diets, with the cultivation conditions described above maintained between treatments. After selection for use in the experiment (as described above), the shrimp (6.43 ± 0.80 mmCL) were distributed among the treatments and, after a day of acclimatization to the laboratory conditions, the shrimp were fed with the diets. The experiment lasted approximately 120 days.

Three diet treatments were tested: (1) Treatment 1 (T1, industrialized food) was industrialized pellets (Thera New Life Spectrum®); (2) Treatment 2 (T2, fresh food) was a processed diet based on the following fresh-frozen ingredients: octopus (*Octopus* sp.), cuttlefish (*Loligo* sp.), shrimp (*Farfantepenaeus* sp.), and mussels (*Perna perna*), in equal proportions. This treatment was tested because maturation diets consisting of two or more fresh ingredients showed better reproductive output in several *Penaeus* species than diets with a single component (Chamberlain and Lawrence 1981; Bray et al. 1990); (3) Treatment 3 (T3, mixed diet) was both industrialized pellets and the fresh food. All diets were provided *ad libitum*, twice per day to the shrimp in each treatment group. Remaining food was removed before a new portion was provided.

In this experiment, each treatment group consisted of 15 pairs of shrimp (each pair of shrimp representing a replica) totaling 45 pairs of shrimp, i.e. 90 *L. vittata* individuals were used throughout the experiment. We standardized the social structure in the 45 replicas, i.e. a pair of shrimp by replica, due to the hermaphrodite nature of the *Lysmata* genus and because the social structure of the culture can affect the brood size (number of eggs per brood) (Bauer 2005). Thus, all replicas in this experiment were composed of one male-phase shrimp (individuals of the smallest size class and without ovarian development) and one female-phase shrimp (individuals carrying eggs in development).

All female-phase shrimp sampled were carrying eggs in development, but under different conditions of ovarian development (rudimentary, in the development and developed). To eliminate the effect of the nutrients obtained in the wild by each shrimp, we discarded the first spawning that occurred in the laboratory, and only considered the second spawning for analysis. Thus, we were able to guarantee that the gonadal development and, consequently, the second spawning was generated under each different diet treatments provided in the laboratory. The male-phase shrimp of each replica fertilized the female-phase shrimp by copulation during the experiment. Copulation of *L. vittata* under such cultivation conditions was considered viable by observations verified prior to this study.

Specimens in the female-phase were observed daily, and the presence or absence of eggs adhered to the pleopods was noted. Shrimp were sacrificed after the second spawning in the laboratory and preserved in ethanol (70%). The CL of the shrimp was measured with a vernier caliper (accuracy 0.01 mm). Thereafter, fecundity and egg volume were verified for each shrimp (as described above) in each treatment group and for shrimp from the wild population. The wild population group (control) used to compared with other treatments (T1, T2, and T3) consisted of specimens obtained in the wild carrying eggs only in stage I or II of development, since the loss of eggs was recorded for shrimp with eggs in the stage III (see results, Reproductive output in *L. vittata* in the wild).

Biochemical analysis of diets

Industrialized pellets and fresh food were analyzed to estimate the biochemical composition of the diets given to the breeding shrimp during the experiment. For both diets, analyses were performed in triplicate. The moisture content was determined by oven drying at 70°C for 48 h. Crude protein and total lipid content were determined from the freeze-dried material, using the method by Silva and Queiroz (2009). Ash was determined as the residue after muffle furnace ignition at 600°C for 4 h.

Statistical analysis

Statistical analyses were performed after checking that all data satisfied the assumptions (homogeneity of

variance and distribution of residuals) (Zar 2010). First, to verify whether fecundity increased in relation to the body size (CL) of the hermaphrodites from the wild population, the data were log-transformed and analyzed by simple linear regression (log y = log a + b log x, where a is the y-intercept and b is the allometric constant).

An analysis of covariance (ANCOVA) was conducted to test whether the different individual-level reproductive output parameters (fecundity and egg volume) of hermaphrodites from the wild population differed by egg stage (eggs with embryo development in stages I, II, or III, as the independent factor) and hermaphrodite body size (CL, as covariate) (Zar 2010). We conducted multiple comparisons of means (between different egg stages) using the Tukey test (Zar 2010), where the main effects were significant at $P < 0.05$.

Lastly, to determine whether any diet affected reproductive output, we compared the mean values of the reproductive parameters between different groups of hermaphrodites (each treatment group and a group of shrimp from the wild population). We used ANCOVA to compare individual-level reproductive output parameters (fecundity and egg volume) by hermaphrodite group (T1, T2, and T3, and a group of shrimp from the wild population, as the independent factor) and hermaphrodite body size (CL, as covariate) (Zar 2010). We conducted multiple comparisons of means (between egg stages) using the Tukey test (Zar 2010), where the main effects were significant at $P < 0.05$.

Results

Reproductive output in L. vittata *in the wild*

A total of 25 *L. vittata* hermaphrodites carrying eggs were collected, of which eight were in stage I, eight were in the stage II, and nine were in the stage III. The body size (CL) of shrimp carrying eggs varied from 5.11 to 7.51 mm. The slope of the regression relationship between CL and fecundity did not differ significantly ($F = 2.70$; $P = 0.11$), indicating that fecundity did not increase linearly with shrimp body size (Figure 2).

Among all *L. vittata* hermaphrodites collected from the wild population, fecundity varied from 74 to 662 with a mean (±SD) of 331 ± 178 eggs per shrimp. Fecundity in hermaphrodites carrying eggs in stage I varied from 74 to 570 with a mean (±SD) of 402 ± 173 eggs per shrimp. Fecundity in shrimp carrying eggs in stage II varied from 94 to 662 with a mean (±SD) of 384 ± 205 eggs per shrimp. In hermaphrodites carrying eggs in stage III, fecundity varied from 78 to 364 with a mean (±SD) of 220 ± 101 eggs per shrimp. ANCOVA detected an effect of egg stage (I, II, and III) on fecundity ($F = 4.98$; $df = 2, 21$; $P = 0.0169$). A

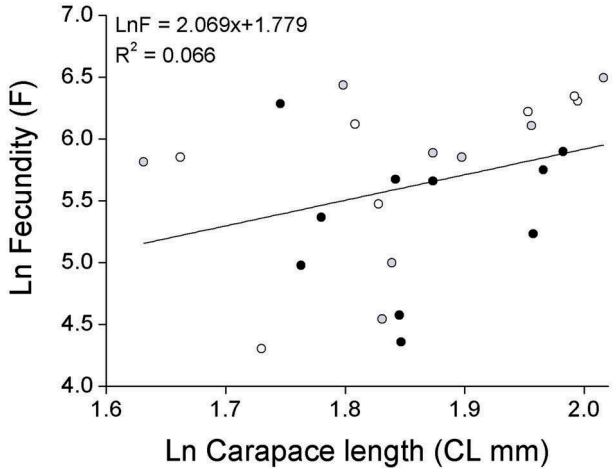

Figure 2. Fecundity in the *Lysmata vittata* shrimp from the wild population. The relationship between hermaphrodite body size (CL) and egg number. White, gray, and black dots represent hermaphrodites carrying eggs in stages I, II, and III, respectively. Linear regression equations obtained after log-log transformation of the data are shown.

posteriori test showed a significant difference in mean fecundity between stages I and III (Tukey test, $P = 0.043$) (Figure 3(a)). This suggests that hermaphrodites carry more eggs in stage I than in stage III, indicating a loss of eggs during embryo development. CL also affected in egg loss. Larger hermaphrodites carried more eggs than smaller hermaphrodites ($F = 6.81$; $df = 1, 21$; $P = 0.0163$). The interaction term of the ANCOVA was not significant ($F = 2.08$; $df = 1, 21$; $P = 0.1632$) (Figure 3(a)). This indicates that hermaphrodite size does not affect the quantity of eggs remaining in the late stages of development.

Egg volume in *L. vittata* varied from 0.020 to 0.126 with a mean (±SD) of 0.047 ± 0.015 mm^3. The mean egg volumes (±SD) in hermaphrodites carrying eggs in stages I, II, and III were 0.035 ± 0.006, 0.040 ± 0.006, and 0.063 ± 0.012 mm^3, respectively. ANCOVA detected an effect of egg stage (I, II, and III) on egg volume ($F = 22.76$; $df = 2, 21$; $P < 0.0001$). A posteriori test showed a significant difference in mean egg volume between stages I and III (Tukey test, $P < 0.001$) and between stages II and III (Tukey test, $P < 0.001$) (Figure 3(b)). This suggests that egg volume in stage III is significantly higher than in stages I and II. CL did not affect the egg volume ($F = 0.09$; $df = 1, 21$; $P = 0.7651$). The interaction term of the ANCOVA was significant ($F = 8.27$; $df = 1, 21$; $P = 0.009$) (Figure 3(b)). This indicates that hermaphrodite size does not affect the egg volume.

Effects of different maturation diets on the reproductive output of L. vittata *under experimental conditions*

The CL of hermaphrodites varied from 4.61 to 8.31 and fecundity varied from 74 to 662 (Table 1). ANCOVA

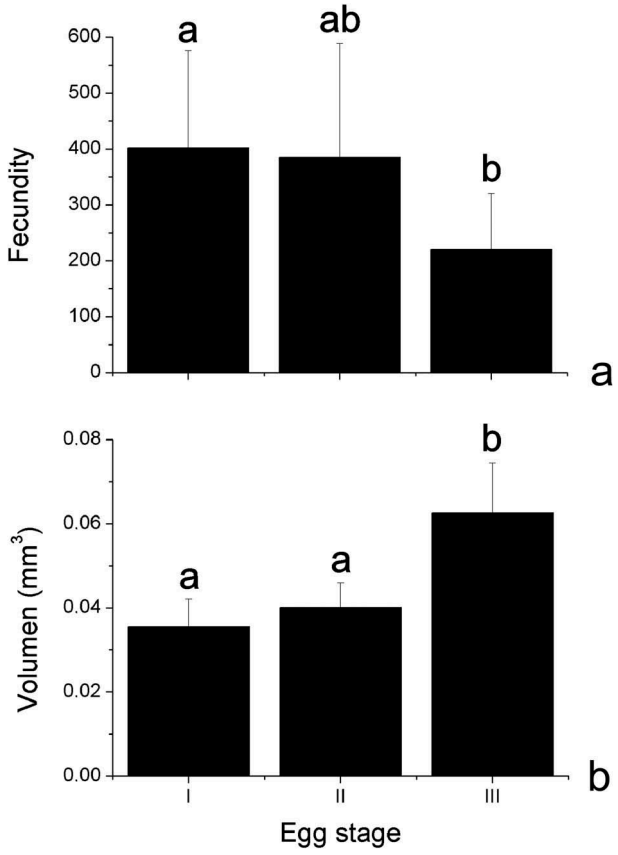

Figure 3. Reproductive output *Lysmata vittata* shrimp from the wild population. Mean (±SD) (vertical bars) of (a) fecundity and (b) egg volume in hermaphrodites carrying eggs in stages I, II, and III of development. Different letters indicate significant differences among treatments.

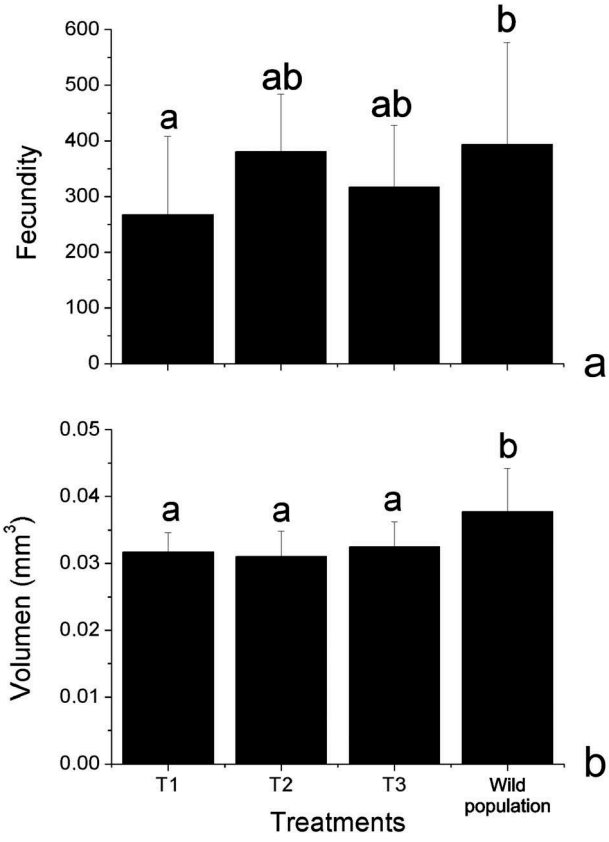

Figure 4. Reproductive output in the *Lysmata vittata* shrimp fed different maturation diets (T1, T2, and T3) and in the wild (control). Mean (±SD) (vertical bars) of (a) fecundity and (b) egg volume in hermaphrodites of the different treatments. Different letters indicate significant differences among treatments.

detected an effect of the group (T1, T2, T3, and wild population) on fecundity ($F = 3.27$; $df = 3, 56$; $P = 0.027$). A posteriori test showed a significant difference in mean fecundity between T1 and wild population groups (Tukey test, $P = 0.035$) (Figure 4(a)). This suggests that hermaphrodites carry more eggs in stage I than in stage III, indicating a loss of eggs during embryo development. CL also affected fecundity. Larger hermaphrodites carried more eggs than smaller hermaphrodites ($F = 13.79$; $df = 1, 56$; $P < 0.001$). The interaction term of the ANCOVA was not significant ($F = 1.47$; $df = 1, 56$; $P = 0.230$) (Figure 4(a)). This indicates that hermaphrodite size does not affect the loss of eggs in the late stages of development.

Table 1. Body size (CL mm), fecundity, and egg volume (mm^3) of the *Lysmata vittata* shrimp under effects of diferent maturation diets (T1, T2, and T3) and in the wild population.

	Body size (CL mm)	Fecundity	Egg volume (mm^3)
Group	Mean ± SD	Mean ± SD	Mean ± SD
T1	6.22 ± 0.91	267 ± 141	0.031 ± 0.003
T2	6.39 ± 0.63	380 ± 103	0.031 ± 0.004
T3	6.68 ± 0.83	317 ± 110	0.032 ± 0.004
Wild population	6.38 ± 0.74	393 ± 183	0.038 ± 0.006

Egg volume varied from 0.024 to 0.048 (Table 1). ANCOVA detected an effect of the group (T1, T2, T3, and wild population) on egg volume ($F = 7.77$; $df = 3, 56$; $P = 0.0001$). A posteriori test showed a significant difference in mean egg volume between T1 and the wild population (Tukey test, $P = 0.001$), T2 and the wild population (Tukey test, $P < 0.001$), and T3 and the wild population (Tukey test, $P = 0.008$) (Figure 4(b)). The egg volume of the shrimp from the wild population was significantly higher than in the shrimp treated with different diets during the experiment. CL did not affect the egg volume ($F = 3.77$; $df = 1, 56$; $P = 0.057$). The interaction term of the ANCOVA was significant ($F = 78.51$; $df = 1, 56$; $P < 0.0001$) (Figure 4(b)). This indicates that hermaphrodite size did not affect the egg volume during the experiment.

Biochemical composition of the diets

The percentage composition of the artificial and fresh food diets is shown in Table 2. The industrialized diet

Table 2. Proximate analyses (%) of the industrialized food (T1) and fresh food (T2) offered for the *Lysmata vittata* specimens during the experiment.

	Industrialized food (T1)				Fresh food (T2)			
	A	B	C	Mean ± SD	A	B	C	Mean ± SD
Protein	38.01	39.57	38.88	38.82 ± 0.78	78.99	78.93	78.68	78.86 ± 0.16
Lipid	15.29	14.07	12.64	14.00 ± 1.33	3.97	4.33	5.12	4.47 ± 0.59
Ash	95.01	94.63	94.77	94.80 ± 0.19	95.80	95.79	95.98	95.85 ± 0.10
Moist	5.5	8.41	4.76	6.22 ± 1.93	90.55	92.18	91.33	91.35 ± 0.81

contained a higher percentage of lipids than the fresh diet (Table 2). However, the fresh diet had a higher percentage of protein and moist (Table 2).

Discussion

In this study, some reproductive output parameters of *L. vittata* were evaluated. First, we evaluated the reproductive output in the wild population, like fecundity and egg volume, and their relation with *L. vittata* brooding. Also, we evaluated the effects of different maturation diets (industrialized pellets, fresh food, and a combination of both) on the reproductive output of shrimps reared in laboratory. From the results obtained, it was clear how the fecundity and egg volume were differed between developmental stages of the eggs, and how those same parameters were affected by the different diets offered. Those results are very relevant to contribute with improvements on the development of techniques for captive breeding of this ornamental species, showing that *L. vittata* is a good model organism to be used for reproduction studies aiming the genus *Lysmata*.

Reproductive output in L. vittata *in the wild*

Our results demonstrate that the fecundity of *L. vittata* is low (a maximum of 660 eggs in this study) compared with other species of the *Lysmata* genus (e.g. *L. boggessi, L. seticaudata*, and *L. wurdemanni*, that can incubate more than 1450, 1800, and 3000 eggs, respectively) (Calado and Narciso 2003; Bauer 2005; Baeza et al. 2014). The lower brood number in *L. vittata* may be a consequence of: (1) nutritional stress, since food quantity and quality can negatively affect the fecundity in Caridean shrimp (Calado and Narciso 2003; Bertini and Baeza 2014); (2) smaller body size of the *L. vittata* specimens that were incubating the eggs, compared with the larger body sizes of the other species mentioned above.

However, although *L. vittata* ovigerous hermaphrodites have a smaller body size than other congeners (*L. vittata*, CL between 5.1 and 7.5 mm [present study]; *L. boggessi*, CL between 6.0 and 10.4 mm [Baeza et al. 2014]), the egg volumes were also smaller (*L. vittata*, mean egg volume 0.047 ± 0.015 mm^3 [present study]; *L. boggessi*, mean of egg volume 0.086 ± 0.021 mm^3 in 'Out Front' and 0.102 ± 0.014 mm^3 in 'The Reef', i.e. localities from the shallow subtidal zone off the west central coast of Florida [Baeza et al. 2014]). These results are indicative that the smaller body size of *L. vittata* compared with other congeners is unlikely to be the only factor responsible for the low fecundity observed in this study.

Furthermore, fecundity can also be affected by local environmental conditions (Pandian 2016). Therefore, to better understand the reasons for the low fecundity observed for *L. vittata* in this study, further studies are needed to investigate the fecundity of this shrimp in other regions, such as the Atlantic Ocean (where this shrimp is considered an invasive species) (Soledade et al. 2013; Pachelle et al. 2016) and the Pacific Ocean (Ahyong 2010). Notably, fecundity has only been in investigated in a small proportion of species of the *Lysmata* genus, despite the ecological (Calado 2006; Rosa et al. 2014) and economic (Prakash et al. 2017; Rhyne et al. 2017) importance of these shrimp.

The variation in fecundity may also have been due to the natural social environment, where different individuals (in the male-phase or hermaphrodites in the female-phase) can act as males during intercourse. Bauer (2005) showed that the mating between an *L. wurdemanni* individual in the male-phase and one in the female-phase resulted in a greater fecundity in relation to the treatments, where the hermaphrodite can act as both male and female (two hermaphrodites in the female-phase together or several specimens in the male and female-phase together as in the natural environment). This difference in fecundity may be due to the energy used by the specimens in the female-phase in retaining male functions, both physically and behaviorally.

Our results also showed egg loss during embryogenesis since in the hermaphrodites carrying eggs with embryos at a more advanced stage of development, the number of eggs was smaller. Similarly, in crustaceans that incubate eggs that are adhered to their abdominal appendages (Pleocyemata order), it is common that a proportion of the fertilized eggs is lost

during the embryonic development (Kuris 1991; Pandian 2016). Brood loss in decapod crustaceans may be induced by several factors, including aborted development, mechanical loss due to abrasion, physiological stress, maternal cannibalism, embryo predation, and parasitism (Kuris 1991; Smith and Ritar 2005). These reasons, at least in part, account for the lack of correlation between body size and fecundity (egg number per shrimp) in this study. Thus, we suggest that the lack of correlation between body size and fecundity in *L. vittata* is largely affected by egg loss, which may occur in each individual at a different intensity. It is important to note that the population studied here inhabits an estuarine area, in which the abiotic factors (mainly temperature and salinity) vary throughout the day according to the tide. We propose that studies should be conducted to evaluate whether the variation in abiotic factors acts as a stress factor and contributes to the loss of eggs in *L. vittata*.

Effects of different maturation diets on the reproductive output of L. vittata *under experimental conditions*

Based on the assumption that diet can affect the reproductive output in ornamental shrimp (Lin and Shi 2002; Lin et al. 2002; Calado et al. 2009; Tziouveli et al. 2011), we evaluated the effect of broodstock diet in *L. vittata*, comparing the fecundity and egg volume in four different groups of shrimp (three groups of shrimp fed different diets in laboratory conditions, T1, T2, and T3, and a group shrimp from the wild population). Thus, we determined which diet (industrialized, fresh, or mixed) should be used in laboratory cultures of *L. vittata* shrimp to obtain a better reproductive output (greater fecundity and egg volume); and compared whether under any of these diets if reproductive output was similar or greater than that in the natural population.

Our experiment demonstrated that breeding shrimp fed with only a diet based on industrialized pellets (T1) had lower fecundities, in which the mean was significantly lower than that recorded for the shrimp of the wild population. Breeding shrimp that were fed diets containing fresh food (T2 and T3) also obtained lower mean fecundities when compared to shrimp of the wild population, however, these differences were not statistically significant. These results indicate that diets containing fresh food may be advantageous compared with diets containing only industrialized pellets. In this study, the diet that contained fresh food had a higher percentage of protein (mean of 78.86% (±0.16) for the fresh food diet and 38.82% (±0.78) for the industrialized pellets, see Table 2), which is an important macronutrient for animal growth and reproduction and has many functions, including as an energy source and as structural material for the cell membrane (Wang et al. 2014). However, the nutritional components responsible for these differences remain to be studied for ornamental shrimp, particularly in relation to the role of micronutrients (e.g. fatty and amino acids, since, for example, *n*-3 highly unsaturated fatty acids (HUFA) also has an important role in fecundity (Xu et al. 1994)). In this sense, it is known that mollusc tissue (cuttlefish and bivalves) have influence to play a significant role in metabolic and physiological functions in reproduction because is rich in unsaturated fatty acids, cholesterols, phospholipids, and amino acids (Wouters et al. 2001; Coman et al. 2007).

In this study, shrimp that were fed the mixed diet had intermediate fecundity (higher than that recorded in the T1 group, but lower than that in the T2 group). In the T3 diet, industrialized pellets and processed food made from fresh ingredients were given at the same time, but separately. Thus, shrimp were fed primarily processed food, and industrialized pellets were consumed secondarily. The apparent preference was not measured in this study, but it is known that for a diet to be effective, it is important that it has good acceptability and palatability in addition to its nutritional contents (Moorhead and Zeng 2010).

Regarding the egg volume, a parameter related to the energy reserves available for embryos (Anger and Schubart 2005), our results indicated that in all diet treatment groups, the breeding shrimp had lower egg volumes than those recorded for shrimp from the wild population. It is probable that none of the diets given under laboratory conditions were as varied as that available in the natural environment. Thus, the development of a nutritionally rich and balanced industrialized diet is a challenge. In the wild, adult shrimp eat a wide variety of microinvertebrates (gastropods, bivalves, crustaceans, and polychaetes) and plant material (Rothlisberg 1998). Moreover, shrimp maturation and reproduction are greatly affected by the environmental factors (Bray and Lawrence 1992; Ogle 1992). Therefore, we also suggest that other factors may negatively affect the ornamental shrimp reproduction in captivity, such as the social structure in which these shrimp live in the wild population; and stress conditions in which breeding shrimp are kept in culture.

In addition, Tziouveli et al. (2011) showed that *L. amboinensis* hermaphrodites fed diets based on industrialized pellets produced broods of a greater size with a higher egg mass compared with shrimp fed only natural fresh ingredients. This may be related to the lipid content of the pellet diet. The importance and effect of lipids on spawning and good reproductive

output have been demonstrated (Middleditch et al. 1980; Bray et al. 1990) and other nutrients present in the industrialized diet may complement and enrich maturation diets, such as certain fatty acids (e.g. *n*-3 HUFA) (Lytle et al. 1990). Moreover, there are a number of advantages of dry artificial diets over fresh food, e.g. reliable supply, reproducible and controlled quality, easy to use, improved stability in storage, reduced tank fouling, reduced risk of disease introduction, and easy delivery of chemotherapeutics, immunostimulants, and hormones (Harrison 1990, 1997). As a result, there has been a substantial investigation into the development of an efficient dry artificial diet for the growth and reproduction of cultivated shrimp. However, Wouters et al. (2001) highlighted that almost every attempt to completely replace fresh food with an artificial diet, resulted in a decrease in ovarian maturation, a reduced number of spawns, and an inferior egg quality. Moreover, some open questions remain, such as: what is the effect of the prolonged use of these diets on the reproductive output of the shrimp?

In conclusion, we have shown that *L. vittata* can be used as a model organism for future studies that aim to investigate and improve culture techniques for *Lysmata* shrimp. This is based on the rusticity and ease of maintenance of this species in a cultivation system. Thus, future advances that can generate adequate methods and techniques for *L. vittata* cultivation may result in the establishment of an efficient and profitable culture protocol.

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ORCID

Samara de Paiva Barros-Alves http://orcid.org/0000-0001-7216-3421
Douglas Fernandes Rodrigues Alves http://orcid.org/0000-0003-4363-6511

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