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Female Growth and Offspring Quality over Successive Spawnings in a Caridean Shrimp *Neocaridina davidi* (Decapoda, Atyidae) with Direct Development

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Abstract. This study analyzed the quality of recently spawned eggs and of juveniles over five and six consecutive spawns, respectively, in a caridean shrimp *Neocaridina davidi* with direct development. The potential energetic antagonism between reproduction and somatic growth was also evaluated. The number of eggs per spawn per female was highest in the first spawn, while the number of recently hatched juveniles per spawn per female declined in the sixth spawn. Lower lipid concentration and energy content were detected in eggs of the fourth and fifth spawns, which may indicate for the first time a decrease in maternal provisioning as a result of multiple spawning in a decapod with direct development. This result had no effect on the size of eggs or of recently hatched juveniles, nor on the growth performance of juveniles during a 30-day period following hatching. Lipids were the most abundant biochemical component of eggs, followed by proteins and glycogen; the relative proportion of each component was probably related to embryonic development type. Egg volume was unsuitable as an indicator of nutrient content, as no correlation was found between these variables. The physiological costs of reproduction were evident from the lower energy content of females that reproduced *versus* females that remained virgin. The lower body weight of the reproductive females at the end of the experiment showed that allocation of resources to reproduction occurred at the expense of somatic growth. To our knowledge, this is the first empirical demonstration of a decapod with direct development.

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Introduction

In many decapod crustaceans, high energy costs are associated with ovarian maturation due to the increase in biosynthetic work that supports the lecithotrophic strategy of the embryos and pre-feeding larval stages (Sasaki *et al*., 1986; Gardner, 2001; Rosa *et al*., 2003; Rosa and Nunes, 2003). The accumulation of nutrients in the maturing ovary may rely on the mobilization of reserves from other tissues, especially the hepatopancreas (Teshima *et al*., 1988; Castille and Lawrence, 1989; Millamena and Pascual, 1990; Palacios *et al*., 2000; Yu *et al*., 2007). Multiple spawning can alter the physiological state of females, leading to their reproductive exhaustion as a result of insufficient time for storage of reserves in the hepatopancreas and their transport to the ovary (Primavera, 1985; Harrison, 1990). Reproductive exhaustion may cause a lower gonadosomatic index, disorganization of the hepatopancreatic structure, and lower levels of total proteins, lipids, and cholesterol in the hemolymph and hepatopancreas (Lumare, 1979; Cheng *et al*., 2000; Vazquez-Boucard *et al*., 2004; Yu *et al*., 2007). Such physiological alterations, in turn, may have an effect on offspring quality, as widely demonstrated in marine decapods with indirect development. The concept of offspring quality refers to "egg quality" and "larval or juvenile quality." The former is defined as the ability of an egg to be fertilized and undergo development (Holcomb *et al*., 2004), while the latter refers to the physiological condition and performance of the larvae or juveniles. Both parameters may be addressed by analyzing biochemical, morphological, and production variables. The biochemical composition of eggs is a particularly useful indicator of offspring quality,

as it influences the success of embryonic and larval development (Holland, 1978; Fraser, 1989; Hernández-Herrera *et al*., 2001; Palacios *et al*., 2001).

Some variables related to offspring quality, such as fecundity, egg biomass and hatchability, size, and starvation resistance of larvae, as well as development period and survival from zoeal stages I to II, decrease over consecutive spawns in marine crabs (Kobayashi, 2001; Ji *et al*., 2006; Nan *et al*., 2006; Andrés *et al*., 2010; Wu *et al*., 2010a; Verísimo *et al*., 2011) and penaeid shrimps (Beard and Wickins, 1980; Emmerson, 1980; Hansford and Marsden, 1995; Marsden *et al*., 1997; Wouters *et al*., 1999; Coman and Crocos, 2003). Other variables, such as the lipid and protein content of eggs, increase as a consequence of multiple spawning in the marine shrimp *Litopenaeus vannamei* Boone, 1931 (Arcos *et al*., 2003).

To our knowledge, no study has ever addressed the effect of multiple spawning on the offspring quality of freshwater decapods with direct development. Maternal provisioning is very important in this group, since large energy reserves must be provided for the developing embryo (Herring, 1974). This need, together with the fact that there is no larval stage, makes the relationship between offspring quality and maternal influence strongest and most direct (Marshall and Keough, 2008). Moreover, the energy costs associated with reproduction, including ovarian maturation, copulation, spawning, and brooding, may be high in this group. According to the Principle of Allocation proposed by Cody (1966), organisms have limited resource budgets and, therefore, allocation of resources to reproduction occurs at the expense of somatic growth or survival. This phenomenon applies to most malacostracan Crustacea, which begin reproduction before somatic growth is completed (Hartnoll, 1985), similar to apterygote insects and myriapods. Although the notion of an antagonistic relationship between both physiological processes is widely accepted, it has rarely been explored empirically. Few studies of decapod crustaceans have related reproduction to the molt cycle or inferred an energetic antagonism between them (Wickins and Beard, 1974; Hartnoll, 1985; Nelson, 1991; Kosuge, 1993; Barki *et al*., 1997). However, no study has compared, under controlled laboratory conditions, the growth performance of females of similar ages in the presence and absence of reproduction.

In this study, we tested the hypothesis that offspring quality declines with successive spawning, given the potentially antagonistic relationship between reproduction and somatic growth in a freshwater caridean shrimp with direct development. We studied the red cherry shrimp *Neocaridina davidi* (Bouvier, 1904), a species native to China (Cai, 1996), because it is an ideal model in which to test this hypothesis. The females are multiple spawners, and the incubation period is short (Tropea *et al*., 2015), allowing for analysis of several consecutive spawns in a relatively short period of time.

Materials and Methods

Experimental specimens

The female and male shrimps used in the present study were obtained from reproductive stock provided by Acuamanus Aquarium, Buenos Aires, Argentina. Individuals were maintained in dechlorinated tap water (pH 7.5, hardness 80 mg l^{-1}, as $CaCO_3$ equivalents) under continuous aeration and at a constant temperature of 27 ± 1 °C. The photoperiod was 14L:10D. Java moss (*Vesicularia* sp.) was provided for shelter. Shrimps were fed daily *ad libitum* balanced, commercially available food for tropical fish (Tetracolor, Tetra GmbH, Melle, Germany), with the following approximate composition: minimum crude protein 47.5%, minimum crude fat 6.5%, maximum crude fiber 2.0%, maximum moisture 6.0%, minimum phosphorus 1.5%, and minimum ascorbic acid 100 mg kg^{-1}. Water was replaced completely once a week. After hatching, juveniles were observed periodically. To ensure virginity at the start of the experiment, females were separated from males when their ovaries became visible through the cephalothorax. All of the shrimps studied were of similar ages to avoid a possible effect of age on spawning performance, as previously observed in the tiger prawn *Penaeus semisulcatus* De Haan, 1844 (Coman and Crocos, 2003).

Experimental design

One hundred sixty virgin females were haphazardly selected and separated into three groups: individuals of Group A (120 females) were used to analyze the quality of eggs in successive spawnings (first, second, third, fourth, and fifth spawns); in Group B (20 females), female growth and the quality of juveniles were evaluated in successive spawnings (first through sixth spawns); and in Group C (20 females), female growth in the absence of reproduction was analyzed. The shrimps were maintained under the same conditions of water quality, temperature, photoperiod, and feeding, as described above, for the entire experimental period, which lasted 210 days.

Egg quality (Group A)

Six virgin females (initial weight ± SE: 40.7 ± 1.4 mg) were placed with six males (initial weight ± SE: 31.2 ± 0.9 mg) in a plastic aquarium measuring 18 × 12.5 × 12 cm (533 shrimps/m^2). Each aquarium was a replicate, and a total of 20 replicates were used. Individuals were visually inspected once daily to determine the presence of ovigerous females. Immediately after being spawned, all of the eggs were gently removed from each female and counted. (In our

study, the number of eggs per spawn per female is considered *realized fecundity*.) The eggs were weighed (wet weight; precision: 0.1 mg); *individual egg weight* was calculated by dividing the sample weight by the number of eggs. The eggs were then measured along the major and minor axes stereomicroscopically. *Egg volume* was determined using the formula for an ellipsoid $4/3 \times \pi \times r_1 \times r_2 \times r_2$, where r_1 and r_2 are the radii of the major and minor axes, respectively (Clarke, 1993). A mean value of egg weight and volume was calculated for all of the broods obtained in each aquarium. Finally, the eggs were stored at –70°C for biochemical analysis. Females were weighed after the eggs were detached from their pleopods. This entire procedure was performed for a total of five successive spawnings.

Juvenile quality and female growth (Group B)

One virgin "reproductive" female (initial weight ± SE: 59.6 ± 1.8 mg) was placed with two males (initial weight ± SE: 37.5 ± 1.8 mg) in a plastic aquarium measuring 18 × 12.5 × 12 cm (133 shrimps/m^2). Each aquarium was a replicate; a total of 20 replicates were used. The aquaria were visually searched daily for ovigerous females. Spawning and hatching dates were recorded for each female to calculate the incubation period. Immediately after hatching, juveniles I (JI) were counted. (In our study, the number of JI per spawn per female is referred to as *actual fecundity*.) A sample of 10 JI was taken from each brood, and the cephalothorax length of each juvenile was measured stereomicroscopically from the tip of the rostrum to the posterior end of the cephalothorax. A mean value of this variable was calculated for the sample. The sample was then weighed (wet weight; precision: 0.1 mg), and individual juvenile weight was calculated by dividing the sample weight by the number of JI. This entire procedure was performed for a total of six successive spawnings. The interspawning interval was calculated as the number of days between consecutive spawning events for females that spawned six or more times. Female molting was recorded during the experiment; *molting frequency* was calculated as the ratio between the total number of molts and the experimental period. At the end of the experiment, females were sacrificed after being cold-anesthetized at −20°C for 15 min, weighed (precision: 0.1 mg), and stored at −70 °C for biochemical analysis. *Weight increment per molt* was calculated as the ratio between final body weight minus initial body weight and the total number of molts.

Ten broods from each spawn order were haphazardly selected. A sample of 10 JI was taken from each brood to analyze juvenile survival and growth during a 30-d period following hatching. Each sample was maintained in a plastic aquarium measuring 18 × 12.5 × 12 cm (444 shrimps/m^2). Juveniles were fed Tetracolor daily *ad libitum*, and water was replaced completely once a week. At the end of the 30-day period, juveniles were sacrificed after being cold-anesthetized at −20°C for 15 min. Their body weight and cephalothorax length were recorded, and they were then stored at −70°C for biochemical analysis.

Growth in the absence of reproduction (Group C)

To maintain females at the same density as Group B females, but at the same time to prevent them from reproducing, one "non-reproductive" female (initial weight ± SE: 60.8 ± 1.8 mg) was placed with two smaller females (initial weight ± SE: 36.2 ± 1.3 mg) in a plastic aquarium measuring 18 × 12.5 × 12 cm (133 shrimps/m^2). Each aquarium was a replicate (n = 20). During the experiment, the total number of molts was recorded for each female. At the end of the experiment, shrimps were sacrificed, weighed, and stored exactly as described for Group B females.

Biochemical analysis

Protein, lipid, and glycogen concentrations (expressed in μg/mg) were determined spectrophotometrically in homogenates of eggs, 30-d-old juveniles, and adult females, according to the methods described by Bradford (1976), Folch *et al.* (1957), and Van Handel (1965), respectively. Because of the high mass of eggs to analyze, the 20 original replicates of each treatment (*i.e.,* spawn order) were haphazardly divided into 5 groups of 4 original replicates per group, and used as samples, To analyze the 30-d-old juveniles, 5 replicates of each treatment were haphazardly selected. The biochemical composition of adult females was determined by selecting 10 individuals from Group B and 10 from Group C. In all cases, calculations were performed on a wet weight basis.

To determine protein concentrations, samples weighing 20–100 mg were homogenized in 4:1 volume:weight of 50 mmol l^{-1} Tris-HCl buffer and pH 7.5, then centrifuged at 10,000 × g for 30 min in a refrigerated centrifuge (4 °C). Total proteins were estimated in the supernatant by the Coomassie blue dye method, with bovine serum albumin as standard. Absorbance was read at 595 nm. For lipid determination, samples weighing 30–180 mg were homogenized in 20:1 volume:weight of a mixture of methanol and chloroform (2:1, v/v), then mixed and centrifuged with 0.9% NaCl to separate the lipid fraction. Total lipids were quantified by the sulfophosphovanillin method, for which extra virgin olive oil (Indalo Clásico; initial concentration: 1 g/ml) was diluted with absolute ethanol (1:1000 dilution to give a final concentration of 1 mg/ml) as standard. Absorbance was read at 530 nm. To calculate glycogen concentration, samples weighing 20–90 mg were boiled with 4:1 volume:weight of KOH 30% for 1 h. After cooling, glycogen was precipitated with the addition of 75 μl of saturated

Na_2SO_4 and 1875 μl of absolute ethanol, and centrifuged at 2000 $\times$ *g* for 10 min. The precipitate was then dissolved in 250 μl of distilled water, and glycogen was measured by the anthrone-reagent method. Rabbit liver (Fluka; Sigma-Aldrich Corp., St. Louis, MO) was used as standard, and absorbance was read at 620 nm.

The total energy content of each egg, juvenile, and female sample was calculated from the biochemical composition using the following conversion factors: 39.31 J/mg for lipids, 23.42 J/mg for proteins, and 17.14 J/mg for carbohydrates (Winberg, 1971).

Statistical analyses

Repeated measures ANOVA was used to assess changes in duration of the incubation period and interspawning interval, egg quality (number of eggs per spawn, egg wet weight, volume, biochemical composition, and total energy content), and juvenile quality (number of JI per spawn; cephalothorax length and weight of JI and 30-d-old juveniles; survival, biochemical composition, and total energy content of 30-d-old juveniles) among successive spawns. This analysis was performed through mixed models, using "spawn order" as the repeated measure factor (with five levels for egg quality and six levels for juvenile quality) and "female" as the random factor. "Female weight" was used as a covariate for analysis of the variables related to egg quality and JI quality. The post-hoc Tukey test was applied when significant differences were found. Final body weight, molting frequency, weight increment per molt, biochemical composition, and total energy content of adult females from Groups B ("reproductive" females) and C ("non-reproductive" females) were analyzed by one-way ANOVA. Survival was compared with the Fisher exact test.

The results per treatment are presented as means $\pm$ SE. All tests were carried out at the 95% significance level with INFOSTAT version 2014 software (Infostat Group, FCA-UNC, Córdoba, Argentina) (Sokal and Rohlf, 1995; Gómez *et al.*, 2012).

Results

Egg quality

Of 120 females studied, 3.3% never spawned during the experimental period; 10% spawned once; 15%, twice; 12.5%, three times; 24.2%, four times; and 35%, five or more times. The average weight of Group A females increased from 64 mg at the onset of the experiment to 99 mg at the end of the experiment. Consequently, females that spawned for the first, second, third, fourth, and fifth time weighed 50–75 mg, 60–90 mg, 70–100 mg, 75–105 mg, and 80–110 mg, respectively. The relationship between realized fecundity and female weight is shown in Figure 1A

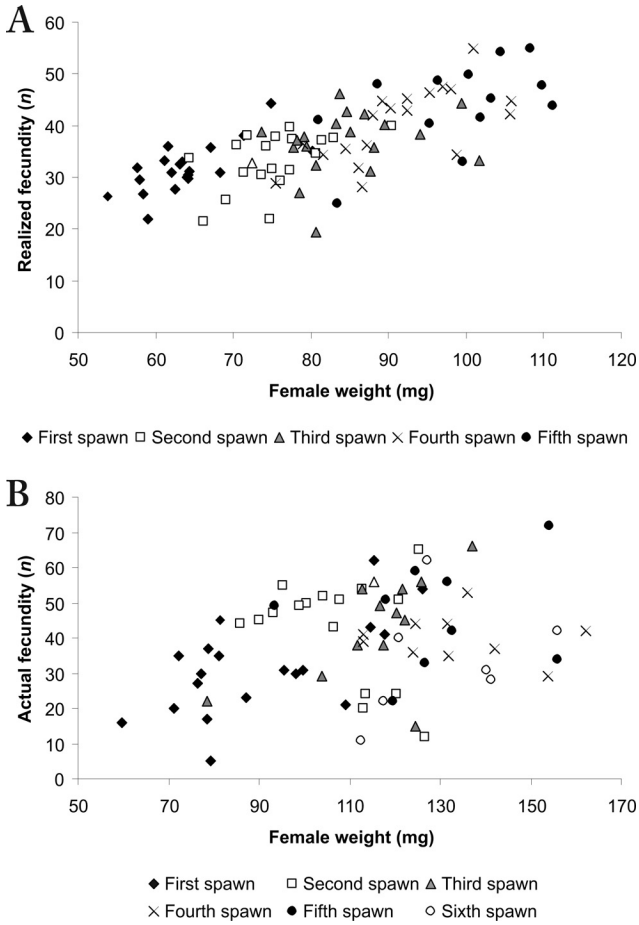

Figure 1. Relationship between realized fecundity (*i.e.,* number of eggs per spawn per female) and female weight (A), and between actual fecundity (*i.e.,* number of newly hatched juveniles (juveniles I) per spawn per female) and female weight (B) for each spawn order of the freshwater shrimp *Neocaridina davidi*. After eggs were spawned, they were removed and counted immediately; juveniles I were counted immediately after they were hatched. A significant statistical relationship was found only between realized fecundity and female weight ($P = 0.000$).

for each spawn order. Fecundity increased significantly with weight increase (repeated measures ANCOVA, F = 32.05, $P = 0.000$), regardless of spawn order. After controlling for the effect of female weight, realized fecundity was higher (repeated measures ANCOVA, $F = 13.22$, $P = 0.000$; Tukey test, $P < 0.05$) in the first spawn than in the second to fifth broods (Fig. 2A). The temporal order of spawns had no effect on egg volume (repeated measures ANCOVA, $F = 0.29$, $P = 0.881$) or egg weight (repeated measures ANCOVA, $F = 0.56$, $P = 0.692$); mean values were 2.45 $\pm$ 0.01 mm^3 and 285.22 $\pm$ 1.73 μg, respectively (Fig. 2B, C). However, lipid concentration and total energy content were highest in the eggs from the first, second, and third spawns, and lowest in eggs from the fourth and fifth spawns (repeated measures ANCOVA, $F = 15.00$, $P = 0.000$ for lipid concentration; $F = 14.04$, $P = 0.000$ for total energy

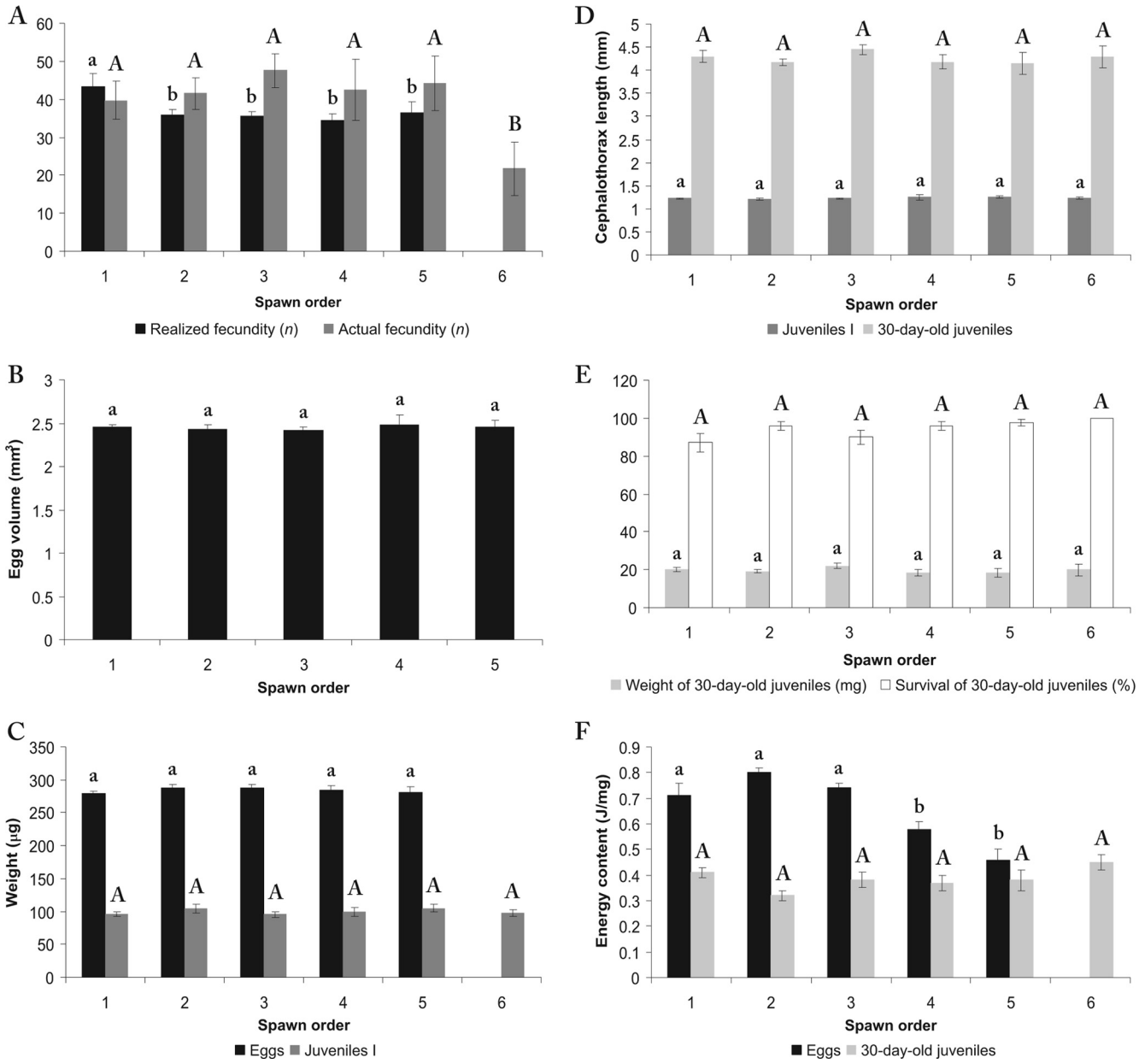

Figure 2. Quality of eggs and of juveniles of the freshwater shrimp *Neocaridina davidi* on successive spawnings. Realized and actual fecundities (A), egg volume (B), wet weight of eggs and newly hatched juveniles (juveniles I) (C), cephalothorax length of juveniles I and 30-d-old juveniles (D), weight and survival of 30-d-old juveniles (E), and total energy content of eggs and 30-d-old juveniles (F) were compared among five or six consecutive spawns. Upper- and lowercase letters (a, A, b, B) indicate statistically significant differences among consecutive spawns for each variable. ($P < 0.05$).

content; Tukey test, $P < 0.05$). Protein concentration was similar among successive spawnings (repeated measures ANCOVA, $F = 2.35$, $P = 0.118$), reaching a mean value of 6.49 ± 0.35 μg/mg. However, glycogen concentration was significantly lower in the second and fifth spawns than in the first, third, and fourth spawns (repeated measures ANCOVA, $F = 6.18$, $P = 0.007$; Tukey test, $P < 0.05$) (Fig. 2F; Fig. 3).

Juvenile quality

Of 20 females, one (5%) female never spawned during the experimental period, three (15%) spawned once, two (10%) spawned twice, two (10%) spawned three times, three (15%) spawned four times, two (10%) spawned five times, and seven (35%) spawned six or more times. The percentage of berried females successfully hatched was

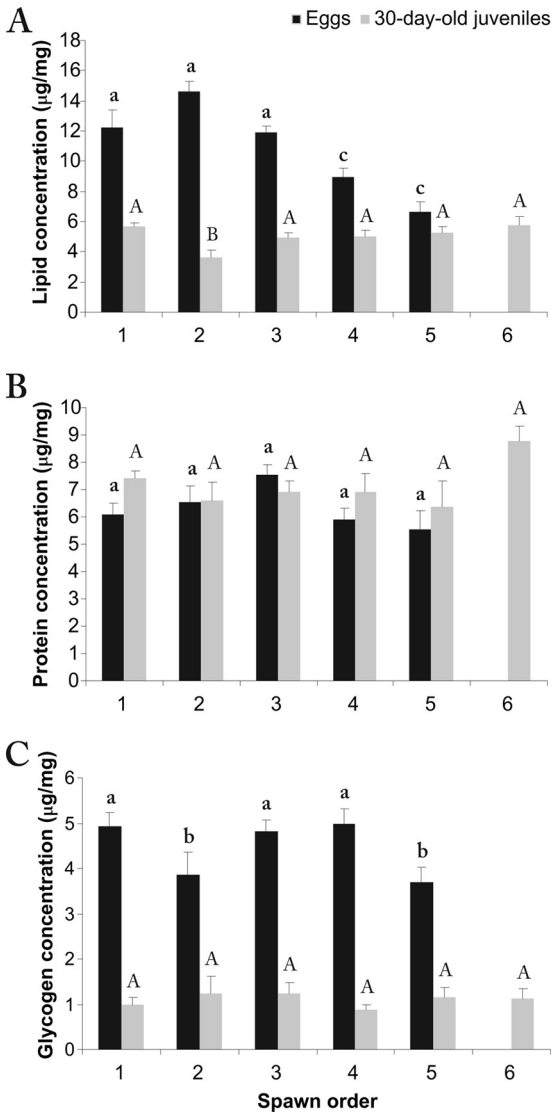

Figure 3. Biochemical composition of eggs and 30-d-old juveniles of the freshwater shrimp *Neocaridina davidi*. Lipid (A), protein (B), and glycogen (C) concentrations (mean ± SE) were measured in recently spawned eggs and in 30-d-old juveniles. Upper- and lowercase letters (a, A, b, B, c) indicate statistically significant differences among consecutive spawns for eggs and juveniles, respectively. In both cases, the protein concentration was similar among treatments, as was the glycogen concentration of juveniles. Lipid concentration was lowest in eggs from the fourth and fifth spawns and in 30-d-old juveniles from the second spawn, while the glycogen concentration of eggs was lowest in the second and fifth spawns.

similar and near 100% in all spawn orders (first, 100%; second, 88.9%; third, 100%; fourth, 100%; fifth, 90%; and sixth or more, 100%). Duration of the incubation period was also similar among successive spawnings (repeated measures ANOVA, $F = 0.15$, $P = 0.979$), and ranged from 14 to 16 d. The interspawning interval tended to increase with spawn order, but did not reach statistical significance (repeated measures ANOVA, $F = 1.37$, $P = 0.275$; Fig. 4).

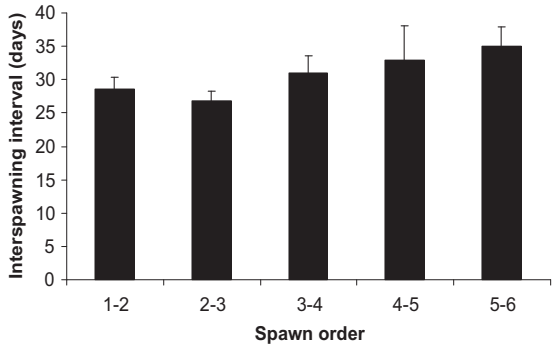

Figure 4. Interspawning interval between consecutive spawns of *Neocaridina davidi* females that spawned six or more times. Although no statistical significance was found, the interval tended to increase between the last spawns.

Qualitative observations showed that the rate of ovarian rematuration varied among females; in some cases, this process was nearly completed during the incubation period, or several days after hatching in other cases.

The average weight of Group B females increased from 59 mg to 140 mg throughout the experiment. Individuals that spawned for the first, second, and third times weighed 60–100 mg, 90–120 mg, and 110–130 mg, respectively; those that spawned for the fourth, fifth, and sixth times weighed 120–160 mg. The relationship between actual fecundity and female weight is shown in Fig. 1B for each spawn order. Actual fecundity was independent of female weight in all spawn orders (repeated measures ANCOVA, $F = 0.70$, $P = 0.407$), and was lowest in the sixth spawn *versus* the first to fifth broods (repeated measures ANCOVA, $F = 4.48$, $P = 0.002$; Tukey test, $P < 0.05$) (Fig. 2A). Multiple spawning had no effect on cephalothorax length (repeated measures ANCOVA, $F = 0.98$, $P = 0.734$) or weight (repeated measures ANCOVA, $F = 0.71$, $P = 0.618$) of JI. Multiple spawning also had no effect on the performance of juveniles, as reflected by their similar cephalothorax lengths (repeated measures ANOVA, $F = 1.74$, $P = 0.152$), body weights (repeated measures ANOVA, $F = 1.13$, $P = 0.362$), and survival (repeated measures ANOVA, $F = 1.85$, $P = 0.130$) at the end of a 30-d period following hatching (Fig. 2C–E). Lipid concentration was lowest in 30-d-old juveniles from the second spawn (repeated measures ANOVA, $F = 4.41$, $P = 0.009$; Tukey test, $P < 0.05$), while glycogen concentration was similar among spawns (repeated measures ANOVA, $F = 0.63$, $P = 0.676$), with a mean value of 1.10 ± 0.06 μg/mg. Both protein concentration and total energy content tended to be higher in 30-d-old juveniles from the sixth spawn, although this finding was not statistically significant (repeated measures ANOVA, $F = 2.71$, $P = 0.052$ for protein concentration; $F = 2.95$, $P = 0.058$ for total energy content) (Fig. 2F, Fig. 3).

Table 1

Growth performance and biochemical composition of females of Neocaridina davidi *that did and those that did not reproduce over a 210-d period**

Variable	Experimental groups	
	Reproductive females	Non-reproductive females
Initial weight (mg)	60.77 ± 1.78 (20)[a]	59.23 ± 1.81 (20)[a]
Final weight (mg)	165.06 ± 6.09 (16)[a]	140.93 ± 5.16 (10)[b]
Lipid concentration (μg/mg)	10.44 ± 0.58 (10)[a]	6.04 ± 1.08 (10)[b]
Protein concentration (μg/mg)	7.48 ± 0.35 (10)[a]	5.41 ± 0.35 (10)[b]
Glycogen concentration (μg/mg)	2.40 ± 0.22 (10)[a]	2.37 ± 0.24 (10)[a]
Energy content (J/mg)	0.64 ± 0.03 (10)[a]	0.41 ± 0.04 (10)[b]
Molting frequency (molts/mo, *n*)	2.25 ± 0.12 (16)[a]	1.84 ± 0.08 (10)[b]
Weight increment per molt (mg)	10.65 ± 1.33 (16)[a]	11.15 ± 1.08 (10)[a]
Survival (%)	80.00[a]	50.00[a]

* Values in parentheses are the number of replicates used to calculate each variable.

[a,b] Significant diffferences in means.

Female growth

Body weight (ANOVA, $F = 6.03$, $P = 0.022$) and molting frequency (ANOVA, $F = 8.23$, $P = 0.008$) of "reproductive" females were significantly lower than in the "non-reproductive" females at the end of the experimental period. However, weight increment per molt did not differ between the two groups (ANOVA, $F = 0.09$, $P = 0.772$) (Table 1; Fig. 5). Lipid (ANOVA, $F = 13.46$, $P = 0.002$) and protein (ANOVA, $F = 17.49$, $P = 0.001$) concentrations were significantly lower in "reproductive" females, which resulted in a 35% lower energy content (ANOVA, $F = 23.87$, $P = 0.000$) (Table 1). A trend of higher survival among "non-reproductive" females was observed, but was not statistically significant (Fisher exact test, $P = 0.253$; Table 1).

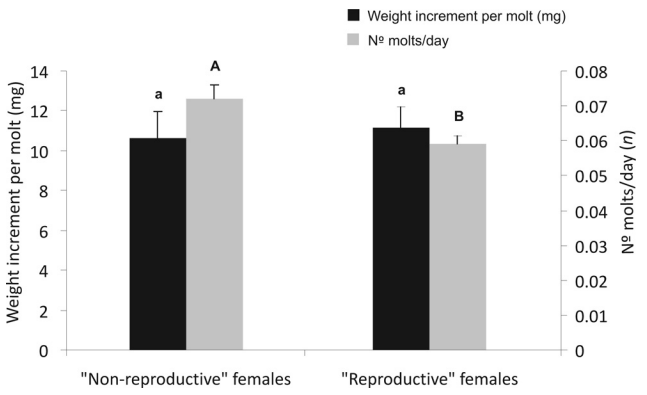

Figure 5. Molting frequency and weight increment per molt for *Neocaridina davidi* females over a 210-d period. Letters (a, A, B) indicate statistically significant differences ($P < 0.05$) between females that reproduced ("reproductive") and those that did not reproduce ("non-reproductive"). Molting frequency was lower in the reproductive females, while the weight increment per molt was similar in both groups of females.

Discussion

This study is, to our knowledge, the first to analyze the offspring quality of a freshwater shrimp with direct development over several consecutive spawns, under controlled conditions, and while ensuring similar physiological and nutritional states of all of the female spawners. Our results showed that fecundity varied with spawn order, and multiple spawning affected the biochemical composition and energy content of eggs, but not the size of eggs or JI, or the growth performance of juveniles.

The effect of multiple spawning on offspring quality is inconsistent among decapod crustaceans; the related variables decline, increase, or undergo no change, depending on the species. For example, egg hatchability decreased from the first to the second spawn in the swimming crab *Portunus trituberculatus* (Miers, 1876) (Wu *et al*. 2010a), but was similar over consecutive spawnings in the white shrimp *Litopenaeus vannamei* (Palacios and Racotta, 2003). Moreover, some variables, such as egg fertilization rate and number of nauplii per spawn, showed different patterns of variation in consecutive spawns, even within the same species (Emmerson, 1980; Browdy and Samocha, 1985; Ottogalli *et al*., 1988; Bray *et al*., 1990). In some cases, the inconsistent, or apparently contradictory, results reported by these authors may have been due to differences in age or the nutritional state of the spawners, or in the experimental conditions under which they were maintained. These factors also may explain the differences observed in offspring quality from consecutive spawns. In this sense, Wu *et al*. (2010a) suggested that the higher quality of the first broods of *P. trituberculatus* was due to a better broodstock diet and longer periods of ovarian maturation and egg incubation as a result of lower water temperature. Similarly, Verísimo *et al*. (2011) proposed that the lower quality of the first spawns of the spider crab *Maja brachydactyla* Balss, 1922 was related to the fact that they occurred in winter, when environmental conditions were unfavorable and females had not yet built up sufficient reserves in their bodies to invest in reproduction.

In our study, realized fecundity was highest in the first spawn, and remained constant and lower in the successive spawns. This result partially contradicts Arcos *et al.* (2003), who observed no differences in the number of eggs per spawn between the first and successive broods of *L. vannamei* under controlled conditions. Wu *et al.* (2010a) also found no differences between the first and second broods of *P. trituberculatus* in this variable, while Verísimo *et al*. (2011) reported lower fecundity in the first spawns of *M. brachydactyla*. However, the inclusion of strong components of environmental variation in these studies makes it

difficult to compare these results with our own. As was reported for penaeid (Hansford and Marsden, 1995; Peixoto *et al*., 2004) and caridean shrimps (Anger and Moreira, 1998; Thatje *et al*., 2004; Lara and Wehrtmann, 2009; Echeverría-Sáenz and Wehrtmann, 2011), realized fecundity increased with female weight, a relationship that was independent of spawn order. The increased space available for yolk accumulation in the body cavity of larger females may explain this positive relationship, as proposed by Hines (1982) for brachyuran crabs. Unlike realized fecundity, the number of JI per spawn was not related to female weight, a result that coincided with what was reported in the same species (Tropea *et al.*, 2015). Larger females apparently spawn more eggs than smaller females, but hatch the same number of JI, perhaps as a consequence of higher egg loss during the incubation period. Although loss of a certain percentage of eggs is a common feature in caridean shrimp species (Balasundaram and Pandian, 1982; Corey and Reid, 1991; Brillon *et al.*, 2005), the relationship between egg loss and female size is a less studied phenomenon. Greater egg loss in the first spawn may have led to similar actual fecundity between the first and the second to fifth spawns in spite of the different realized fecundity. This factor also may have led to the decline of actual fecundity noted in the sixth spawn compared with the other five spawns.

With respect to egg size, both the wet weight and volume of *Neocaridina davidi* eggs were similar over consecutive spawns. Clarke *et al*. (1991) and Clarke (1993) found a correlation between egg volume and the amount of nutrients stored in the yolk of caridean shrimps. They stated that the differences in egg volume between females represented real differences in their investment per embryo. However—and contrary to what was expected—egg volume did not reflect nutrient content in the present study; eggs with the same volume had similar levels of proteins and glycogen, but different levels of lipids (eggs from the first spawn *vs.* those from the fifth spawn). Palacios *et al*. (1999) also failed to find a correlation between egg diameter and biochemical composition in the white shrimp *Litopenaeus vannamei*. It is unlikely that the accumulation of carotenoid pigments, a minor component of yolk, had compensated for the lipid decrease in the present study (leading to the same egg volume); the color of the eggs was yellow or green in all cases, and did not appear to change over successive spawnings. In addition, carotenoid levels decrease toward the end of the spawning period in some species (Palacios *et al*., 1999). It seems that in *N. davidi*, egg size (in volume and wet weight) is a fixed feature defined by other factors, such as post-spawning hydration, along with nutrient composition. Therefore, egg dry mass or organic content, rather than egg volume, should be used as a measure of maternal investment (McEdward and Carson, 1987).

Interestingly, total lipid levels and, consequently, total energy content of *Neocaridina davidi* eggs decreased in the fourth and fifth spawns. Since the eggs were removed from females immediately after being spawned, their biochemical composition was still not modified by embryo consumption of yolk components. For this reason, egg biochemical composition may be used as a direct measure of maternal investment on yolk synthesis. Only two studies, both in the marine shrimp *Litopenaeus vannamei*, have evaluated the effect of multiple spawning on egg biochemical composition. Both studies showed a similar transfer of reserves to the eggs (Palacios and Racotta, 2003), or even an increased transfer (Arcos *et al*., 2003) over successive spawns. In contrast, our results showed that *N. davidi* females could not accumulate the same levels of lipids in the rematuring ovary. This finding may be the first to show a decrease in maternal provisioning over consecutive spawnings in a freshwater shrimp with direct development. Nan *et al*. (2006) observed lower egg production during ovarian rematuration in the Chinese mitten crab *Eriocheir sinensis* H. Milne-Edwards, 1853. These researchers suggested that the nutritional supply was insufficient to support maturation of all of the oocytes, with some oocytes being reabsorbed. However, in *N. davidi*, the lower transfer of lipids to the oocytes during ovarian rematuration did not lead to lower egg production, as evidenced by a similar realized fecundity in the fourth and fifth spawns *versus* the second and third spawns.

One surprising result of our study was the higher proportion of lipids than proteins contained in *N. davidi* eggs. This relative proportion contradicts the general trend reported by many authors for egg biochemical composition of decapod crustaceans with indirect development (Holland, 1978; Chu and Ovsianico-Koulikowsky, 1994; Petersen and Anger, 1997; Lemos and Phan, 2001; Palacios *et al*., 2001; Roustaian *et al*., 2001; Arcos *et al*., 2003; García-Guerrero, 2009; Wu *et al*., 2010b). The yolk molecules are mainly comprised of proteins, free amino acids, lipids, and carbohydrates (Sasaki *et al*., 1986). Proteins have a fundamental role as structural components of the embryonic tissue and, under certain conditions, also serve as fuel (Holland, 1978; Clarke *et al*., 1990; Gardner, 2001; García-Guerrero *et al*., 2003). Lipids, on the other hand, are the most important source of metabolic energy for the developing embryo, and they, too, are essential structural components of cell membranes (Herring, 1974; Holland, 1978; Amsler and George, 1984; Xu *et al*., 1994; Mourente *et al*., 1995; Petersen and Anger, 1997; Nates and McKenney, Jr., 2000; Roustaian *et al.*, 2001). In decapods with abbreviated development, the embryo hatches at a more advanced stage of development as a result of longer embryogenesis. This requires increased lipid reserves in the eggs, as reflected by the enhanced C:N ratio (*i.e.,* indicator of the lipid:protein ratio) in freshwater and terrestrial crab species (Anger, 1995). In line with this study, Nates and McKenney, Jr. (2000) found large amounts of lipids in the eggs of *Lepidophthalmus louisianensis*

(Schmitt, 1935), a ghost shrimp with abbreviated development. However, the egg biochemical composition reported by García-Guerrero *et al*. (2003) in the crayfish *Cherax quadricarinatus* (von Martens, 1898), a species with direct development, resembled that of other species with indirect development. Therefore, additional comparisons are necessary to determine whether the relative proportion of lipids and proteins found in the eggs of *Neocaridina davidi* is related to embryonic development type, or is a particular feature of the genus *Neocaridina* and/or the family Atyidae.

Even though lipid levels and total energy content of the eggs decreased as a consequence of multiple spawning, the incubation period—which reflects the rate of embryo development—and the sizes of the recently hatched juveniles (JI) were similar and independent of spawn order. In view of these results, it seems that JI size does not depend entirely on the lipid content of eggs, or that levels of the fatty acids that ensure normal embryonic development were not affected in the fourth and fifth spawns. These fatty acids may include eicosapentaenoic (EPA) and docosahexaenoic (DHA) among the long-chain, poly-unsaturated fatty acids (PUFA); palmitic (16:0) and stearic (18:0) saturated fatty acids (SFA); and/or the palmitoleic (16:1n-7) and vaccenic (18:1n-7) monounsaturated fatty acids (MUFA), which are some of the most abundant components of eggs from crabs (Figueiredo and Narciso, 2008; Wu *et al*., 2010b), lobsters (Rosa *et al*., 2003, 2005), and caridean shrimps (Wehrtmann and Graeve, 1998; Morais *et al*., 2002). It is also possible that egg proteins, which did not differ in successive spawnings, were used as fuel in a higher proportion by the embryos from the last (fourth and fifth) spawns, compensating for the lower availability of energy from lipids. Future studies analyzing the variation in biochemical composition of eggs from consecutive spawns throughout embryonic development are necessary to understand what is being consumed and what determines the size of recently hatched juveniles.

Unlike the effect of multiple spawning on survival to zoeal stages I and II reported in *Penaeus monodon* Fabricius, 1798 (Hansford and Marsden, 1995) and *Portunus trituberculatus* (Wu *et al*., 2010a), in our study, juvenile survival and growth over a 30-d period following hatching were similar under normal feeding conditions. These results may indicate that the decrease in maternal provisioning that was noted over consecutive spawns had no effect on the performance of advanced juvenile stages. The exposure of recently hatched juveniles to stressful conditions may be useful to determine if their quality diminishes only when resource availability is restricted, as proposed by Moland *et al*. (2010). Protein was the major biochemical component of juveniles, followed by lipids and glycogen, a common trend in decapod larvae and postlarvae (Pandian, 1970; Capuzzo and Lancaster, 1979; Anger *et al*., 1983; Mourente *et al*., 1995; Roustaian *et al*., 2001). Protein levels of 30-d-old juveniles were higher than in eggs, which may be a consequence of the accumulation of internal stores and organic matter taken from external food sources for the expression of new tissues (Anger, 2001). However, lipid levels decreased from recently spawned eggs to 30-d-old juveniles, which suggests that lipids were used as the major metabolic source of energy for somatic growth, as demonstrated by Roustaian *et al.* (2001) in the caridean prawn *Macrobrachium rosenbergii* (de Man, 1879).

On the other hand, *Neocaridina davidi* females differed in their spawning performance; some females spawned six or more times while others spawned only once. The reason for this phenomenon, which has been observed in penaeid shrimps (Arcos *et al*., 2003; Palacios and Racotta, 2003), is unclear. Age, origin, and rearing conditions were the same for all of the study females before and throughout the experiment. Spawning capacity may be determined by other factors, such as the quantity of gonad-inhibiting hormone (GIH) produced by the sinus gland, or the sensitivity level of GIH receptors (Palacios and Racotta, 2003). Different efficiencies in the assimilation, storage, and mobilization of nutrients may also account for the varying ability of females to go through successive periods of ovarian maturation and spawning. The accumulation of biochemical components, especially lipids, in the maturing ovary has been reported in several penaeid shrimps, with nutrients likely being transported from other tissues, particularly from the hepatopancreas (Millamena and Pascual, 1990; Mohamed and Diwan, 1992; Palacios *et al*., 2000). When food supply is insufficient to provide the large amount of energy needed for ovarian rematuration, total lipid content of the hepatopancreas may decrease, as observed in some penaied shrimps and crabs after consecutive spawns (Cheng *et al*., 2000; Vazquez-Boucard *et al*., 2004). One consequence of reproduction, especially in species with abbreviated development, is that egg-laying imposes a great drain on lipid reserves (Herring, 1973). In our study, this physiological cost was evident from the lower lipid and protein concentrations seen in the "reproductive" females *versus* females that remained virgin over the entire experiment. Moreover, the energy allocated to reproduction was clearly at the expense of somatic growth, since the "reproductive" females weighed less than the virgin ones at the end of the experiment. Our results suggest that the greater final weight seen in the virgin females was a consequence of higher molting frequency rather than higher weight increment per molt. The energetic antagonism between reproduction and growth has been inferred in previous studies that reported a lengthening, or inhibition, of the molting cycle by ovarian maturation or spawning in some decapod species, such as the crayfish *Cherax quadricarinatus*, the ocypodid crab *Macrophthalmus boteltobagoe* Sakai, 1939, and the freshwater prawn *Macrobrachium rosenbergii* (Wickins and Beard, 1974; Kosuge, 1993; Barki *et al*., 1997). In this

context, the present study may be the first to demonstrate empirically the negative relationship between reproductive and somatic investments in a caridean shrimp with direct development.

In summary, fecundity varied with spawn order; and the biochemical composition of eggs of *Neocaridina davidi* was affected by multiple spawning, with apparently no effect on the size or weight of eggs and JI, or on the growth performance of juveniles. Multiple spawning led to physiological deterioration of female spawners, as reflected by their lower body weight, lipid and protein concentrations, and survival in comparison to the non-reproductive females over the entire experimental period. These results offer a clear demonstration of the energetic antagonism between reproduction and somatic growth.

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Literature Cited

Amsler M. O., and R. Y. George. 1984. Seasonal variation in the biochemical composition of the embryos of *Callinectes sapidus* Rathbun. *J. Crustac. Biol.* **4:** 546–553.

Andrés, M., A. Estévez, C. G. Simeó, and G. Rotllant. 2010. Annual variation in the biochemical composition of newly hatched larvae of *Maja brachydactyla* in captivity. *Aquaculture* **310:** 99–105.

Anger, K. 1995. The conquest of freshwater and land by marine crabs: adaptations in life-history patterns and larval bioenergetics. *J. Exp. Mar. Biol. Ecol.* **193:** 119–145.

Anger, K. 2001. *The Biology of Decapod Crustacean Larvae,* Crustacean Issues 14. A. A. Balkema, Lisse, The Netherlands. 420 p.

Anger, K., and G. S. Moreira. 1998. Morphometric and reproductive traits of tropical caridean shrimps. *J. Crustac. Biol.* **18:** 823–838.

Anger, K., N. Laasch, C. Püschel, and F. Shorn. 1983. Changes in biomass and chemical composition of spider crab (*Hyas araneus*) larvae reared in the laboratory. *Mar. Ecol. Prog. Ser.* **12:** 91–101.

Arcos, F. G., A. M. Ibarra, E. Palacios, C. Vazquez-Boucard, and I. S. Racotta. 2003. Feasible predictive criteria for reproductive performance of white shrimp *Litopenaeus vannamei:* egg quality and female physiological condition. *Aquaculture* **228:** 335–349.

Balasundaram, C., and T. J. Pandian. 1982. Egg loss during incubation in *Macrobrachium nobilii* (Henderson & Mathai). *J. Exp. Mar. Biol. Ecol.* **59:** 289–299.

Barki, A., T. Levi, G. Hulata, and I. Karplus. 1997. Annual cycle of spawning and molting in the red-claw crayfish, *Cherax quadricarinatus,* under laboratory conditions. *Aquaculture* **157:** 239–249.

Beard, T. W., and J. F. Wickins. 1980. Breeding of *Penaeus monodon* Fabricius in laboratory recirculation systems. *Aquaculture* **20:** 79–89.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72:** 248–254.

Bray, W. A., A. L. Lawrence, and L. J. Lester. 1990. Reproduction of eyestalk-ablated *Penaeus stylirostris* fed various levels of total dietary lipids. *J. World Aquacult. Soc.* **21:** 41–52.

Brillon, S., Y. Lambert, and J. Dodson. 2005. Egg survival, embryonic development, and larval characteristics of northern shrimp (*Pandalus borealis*) females subject to different temperature and feeding conditions. *Mar. Biol.* **147:** 895–911.

Browdy, C. L., and T. M. Samocha. 1985. The effect of eyestalk ablation on spawning, molting and mating of *Penaeus semisulcatus* de Haan. *Aquaculture* **49:** 19–29.

Cai, Y. 1996. A revision of the genus *Neocaridina* (Crustacea: Decapoda: Atyidae). *Acta Zootaxon. Sin.* **21:** 129–160.

Capuzzo, J. M., and B. A. Lancaster. 1979. Some physiological and biochemical considerations of larval development in the American lobster, *Homarus americanus* Milne Edwards. *J. Exp. Mar. Biol. Ecol.* **40:** 53–62.

Castille, F. L., and L. A. Lawrence. 1989. Relationship between maturation and biochemical composition of the gonads and digestive glands of the shrimps *Penaeus aztecus* Ives and *Penaeus setiferus* (L.). *J. Crustac. Biol.* **9:** 202–211.

Cheng, Y. X., N. S. Du, and W. Lai. 2000. The lipid accumulations during the stages of the ovarian fast maturation and their effect on the spawning of *Eriocheir sinensis. Journal of Fisheries of China* **24:** 113–119.

Chu, K. H., and N. N. Ovsianico-Koulikowsky. 1994. Ontogenetic changes in metabolic activity and biochemical composition in the shrimp, *Metapenaeus ensis. J. Exp. Mar. Biol. Ecol.* **183:** 11–26.

Clarke, A. 1993. Egg size and egg composition in polar shrimps (Caridea: Decapoda). *J. Exp. Mar. Biol. Ecol.* **168:** 189–203.

Clarke, A., J. H. Brown, and L. J. Holmes. 1990. The biochemical composition of eggs from *Macrobrachium rosenbergii* in relation to embryonic development. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **6:** 505–511.

Clarke, A., C. C. E. Hopkins, and E. M. Nilssen. 1991. Egg size and reproductive output in the deep-water prawn *Pandalus borealis* Kröyer, 1838. *Funct. Ecol.* **5:** 724–130.

Cody, M. L. 1966. A general theory of clutch size. *Evolution* **20:** 174–184.

Coman, G. J., and P. J. Crocos. 2003. Effect of age on the consecutive spawning of ablated *Penaeus semisulcatus* broodstock. *Aquaculture* **219:** 445–456.

Corey, S., and D. M. Reid. 1991. Comparative fecundity of decapod crustaceans: I. The fecundity of thirty-three species of nine families of caridean shrimp. *Crustaceana* **60:** 270–294.

Echeverría-Sáenz, S., and I. S. Wehrtmann. 2011. Egg production of the commercially exploited deepwater shrimp, *Heterocarpus vicarius* (Decapoda: Pandalidae), Pacific Costa Rica. *J. Crustac. Biol.* **31:** 434–440.

Emmerson, W. D. 1980. Induced maturation of prawn *Penaeus indicus. Mar. Ecol. Prog. Ser.* **2:** 121–131.

Figueiredo, J., and L. Narciso. 2008. Egg volume, energy content and fatty acid profile of *Maja brachydactyla* (Crustacea: Brachyura: Majidae) during embryogenesis. *J. Mar. Biol. Assoc. UK* **88:** 1401–1405.

Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226:** 497–509.

Fraser, A. J. 1989. Triacylglycerol content as a condition index for fish, bivalve, and crustacean larvae. *Can. J. Fish. Aquat. Sci.* **46:** 1868–1873.

García-Guerrero, M. 2009. Proximate biochemical variations in eggs of the prawn *Macrobrachium americanum* (Bate, 1869) during its embryonic development. *Aquac. Res.* **40:** 575–581.

García-Guerrero, M., I. S. Racotta, and H. Villarreal. 2003. Variation in lipid, protein, and carbohydrate content during the embryonic

development of the crayfish *Cherax quadricarinatus* (Decapoda: Parastacidae). *J. Crustac. Biol.* **23:** 1–6.

Gardner, C. 2001. Composition of eggs in relation to embryonic development and female size in giant crabs *Pseudicarcinus gigas* (Lamarck). *Mar. Freshw. Res.* **52:** 333–338.

Gómez, S., V. Torres, Y. García, and J. A. Navarro. 2012. Statistical procedures most used in the analysis of measures repeated in time in the agricultural sector. *Cuban J. Agric. Sci.* **46:** 1–7.

Hansford, S. W., and G. E. Marsden. 1995. Temporal variation in egg and larval productivity of eyestalk ablated spawners of the prawn *Penaeus monodon* from Cook Bay, Australia. *J. World Aquacult. Soc.* **26:** 396–405.

Harrison, K. E. 1990. The role of nutrition in maturation, reproduction and embryonic development of decapod crustaceans: a review. *J. Shellfish Res.* **9:** 1–28.

Hartnoll, R. G. 1985. Growth, sexual maturity and reproductive output. Pp. 101–128 in *Factors in Adult Growth,* Crustacean Issues 3. A. M. Wenner, ed. A. A. Balkema, Rotterdam.

Hernández-Herrera, R., C. I. Perez-Rostro, F. Arcos, J. L. Ramírez, A. M. Ibarra, E. Palacios, and I. S. Racotta. 2001. Predictive criteria of shrimp larval quality: an experimental approach. Pp: 242–245 in *Larvi 2001: 3rd Fish Shellfish Larviculture Symposium,* Ghent, Belgium, September 3–6, 2001. European Aquaculture Society, Oostende, Belgium.

Herring, P. J. 1973. Depth distribution of the carotenoid pigments and lipids of some oceanic animals. 2. Decapod crustaceans. *J. Mar. Biol. Assoc. UK* **53:** 539–562.

Herring, P. J. 1974. Size, density and lipid content of some decapod eggs. *Deep-Sea Res.* **21:** 91–94.

Hines, A. H. 1982. Allometric constraints and variables of reproductive effort in brachyuran crabs. *Mar. Biol.* **69:** 309–320.

Holcomb, M., J. G. Cloud, J. Woolsey, and R. L. Ingermann. 2004. Oxygen consumption in unfertilized salmonid eggs: an indicator of egg quality? *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **138:** 349–354.

Holland, D. L. 1978. Lipid reserves and energy metabolism in the larvae of benthic marine invertebrates. Pp. 85–123 in *Biochemical and Biophysical Perspectives in Marine Biology,* D. C. Malins and J. R. Sargent, eds. Academic Press, London.

Ji, Y., X. G. Wu, Y. X. Cheng, M. Wille, and P. Sorgeloos. 2006. Effects of different diets on reproductive performance and HUFA composition of Chinese mitten crab (*Eriocheir sinensi*s) broodstock during second spawning. *Journal of Fishery Sciences of China* **13:** 92–99.

Kobayashi, S. 2001. Fecundity of the Japanese mitten crab *Eriocheir japonica* (de Hann). *Benthos Res.* **56:** 1–7.

Kosuge, T. 1993. Molting and breeding cycles of the rock-dwelling ocypodid crab *Macrophthalmus roteltobagoe* (Sakai, 1939) (Decapoda, Brachyura). *Crustaceana* **64:** 56–65.

Lara, L. R., and I. S. Wehrtmann. 2009. Reproductive biology of the freshwater shrimp *Macrobrachium carcinus* (L.) (Decapoda: Palaemonidae) from Costa Rica, Central America. *J. Crustac. Biol.* **29:** 343–349.

Lemos, D., and V. N. Phan. 2001. Energy partitioning into growth, respiration, excretion and exuvia during larval development of the shrimp *Farfantepenaeus paulensis. Aquaculture* **199:** 131–143.

Lumare, F. 1979. Reproduction of *Penaeus kerathurus* using eyestalk ablation. *Aquaculture* **18:** 203–214.

Marsden, G. E., J. J. McGuren, S. W. Hansford, and M. J. Burke. 1997. A moist artificial diet for prawn broodstock: its effect on the variable reproductive performance of wild caught *Penaeus monodon. Aquaculture* **149:** 145–156.

Marshall, D. J., and M. J. Keough. 2008. The evolutionary ecology of offspring size in marine invertebrates. *Adv. Mar. Biol.* **53:** 1–60.

McEdward, L. R., and S. F. Carson. 1987. Variation in egg organic content and its relationship with egg size in the starfish *Solaster stimpsoni. Mar. Ecol. Prog. Ser.* **37:** 159–169.

Millamena, O. M., and F. P. Pascual. 1990. Tissue lipid content and fatty acid composition of *Penaeus monodon* Fabricius broodstock from the wild. *J. World Aquacult. Soc.* **21:** 116–121.

Mohamed, K. S., and A. D. Diwan. 1992. Biochemical changes in different tissues during yolk synthesis in marine prawn *Penaeus indicus* H. Milne Edwards. *Indian J. Mar. Sci.* **21:** 30–34.

Moland, E., E. Moland Olsen, and N. C. Stenseth. 2010. Maternal influences on offspring size variation and viability in wild European lobster (*Homarus gammarus*). *Mar. Ecol. Prog. Ser.* **400:** 165–173.

Morais, S., L. Narciso, R. Calado, M. L. Nunes, and R. Rosa. 2002. Lipid dynamics during the embryonic development of *Plesionika martia martia* (Decapoda; Pandalidae), *Palaemon serratus* and *P. elegans* (Decapoda; Palaemonidae): relation to metabolic consumption. *Mar. Ecol. Prog. Ser.* **242:** 195–204.

Mourente, G., A. Medina, S. González, and A. Rodríguez. 1995. Variations in lipid content and nutritional status during the larval development of the marine shrimp *Penaeus keraturus. Aquaculture* **130:** 187–199.

Nan, T. Z., Y. X. Cheng, X. G. Wu, J. Q. Wang, L. J. Wang, and B. I. Liu. 2006. Comparison on the first and second berried crab on embryo and larval quality (Z1) of *Eriocheir sinensis. Journal of Shanghai Fisheries University* **15:** 41–46.

Nates, S. F., and C. L. McKenney, Jr. 2000. Ontogenetic changes in biochemical composition during larval and early postlarval development of *Lepidophthalmus louisianensis,* a ghost shrimp with abbreviated development. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **127:** 459–468.

Nelson, K. 1991. Scheduling of reproduction in relation to molting and growth in malacostracan crustaceans. Pp. 77–113 in *Crustean Egg Production,* Crustacean Issues 7. A. M. Wenner and A. Kuris, eds. A. A. Balkema, Rotterdam.

Ottogalli, L., C. Galine, and D. Goxe. 1988. Reproduction in captivity of *Penaeus stylirostris* in New Caledonia. *J. Aquacult. Trop.* **3:** 111–125.

Palacios, E., and I. S. Racotta. 2003. Effect of number of spawns on the resulting spawn quality of 1-year-old pond-reared *Penaeus vannamei* (Boone) broodstock. *Aquac. Res.* **34:** 427–435.

Palacios, E., C. I. Perez-Rostro, J. L. Ramirez, A. M. Ibarra, and I. S. Racotta. 1999. Reproductive exhaustion in shrimp (*Penaeus vannamei*) reflected in larval biochemical composition, survival and growth. *Aquaculture* **171:** 309–321.

Palacios, E., A. M. Ibarra, and I. S. Racotta. 2000. Tissue biochemical composition in relation to multiple spawning in wild and pond-reared *Penaeus vannamei* broodstock. *Aquaculture* **185:** 353–371.

Palacios, E., I. S. Racotta, H. Heras, Y. Marty, J. Moal, and J. F. Samain. 2001. Relation between lipid and fatty acid composition of eggs and larval survival in white pacific shrimp (*Penaeus vannamei,* Boone, 1931). *Aquac. Int.* **9:** 531–543.

Pandian, T. J. 1970. Ecophysiological studies on the developing eggs and embryos of the European lobster *Homarus gammarus. Mar. Biol.* **5:** 154–167.

Peixoto, S., R. O. Cavalli, W. Wasielesky, F. D'Incao, D. Krummenauer, and Â. M. Milach. 2004. Effects of age and size on reproductive performance of captive *Farfantepenaeus paulensis* broodstock. *Aquaculture* **238:** 173–182.

Petersen, S., and K. Anger. 1997. Chemical and physiological changes during the embryonic development of the spider crab, *Hyas araneus* L. (Decapoda: Majidae). *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **117:** 299–306.

Primavera, J. H. 1985. A review of maturation and reproduction in closed thelycum penaeids. Pp. 47–64 in *Proceedings of the First*

International Conference on the Culture of Penaeid Prawns/Shrimps, 4–7 December 1984, Iloilo City, Philippines, Y. Taki, J. H. Primavera, and J.A. Llobrera, eds. Aquaculture Department, Asian Fisheries Development Center, Iloilo City, Philippines.

Rosa, R., and M. L. Nunes. 2003. Tissue biochemical composition in relation to the reproductive cycle of deep-sea decapod *Aristeus antennatus* in the south Portuguese coast. *J. Mar. Biol. Assoc. UK* **83:** 963–970.

Rosa, R., S. Morais, R. Calado, L. Narciso, and M. L. Nunes. 2003. Biochemical changes during the embryonic development of Norway lobster, *Nephrops norvegicus. Aquaculture* **221:** 507–522.

Rosa, R., R. Calado, A. M. Andrade, L. Narciso, and M. L. Nunes. 2005. Changes in amino acids and lipids during embryogenesis of European lobster, *Homarus gammarus* (Crustacea: Decapoda). *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **140:** 241–249.

Roustaian, P., M. S. Kamarudin, H. B. Omar, C. R. Saad, and M. H. Ahmad. 2001. Biochemical changes in freshwater prawn *Macrobrachium rosenbergii* during larval development. *J. World Aquacult. Soc.* **32:** 53–59.

Sasaki, G. C., J. M. Capuzzo, and P. Biesiot. 1986. Nutritional and bioenergetic considerations in the development of the American lobster *Homarus americanus. Can. J. Fish. Aquat. Sci.* **43:** 2311–2319.

Sokal, R. R., and F. J. Rohlf. 1995. *Biometry. The Principles and Practice of Statistics in Biological Research,* 3rd ed. W. H. Freeman, New York.

Teshima, S. I., A. Kanazawa, S. Koshio, and K. Horinouchi. 1988. Lipid metabolism in destalked prawn *Penaeus japonicus:* induced maturation and accumulation of lipids in the ovaries. *Nippon Suisan Gakkaishi* **54:** 1115–1122.

Thatje, S., G. A. Lovrich, and K. Anger. 2004. Egg production, hatching rates, and abbreviated larval development of *Campylonotus vagans* Bate, 1888 (Crustacea: Decapoda: Caridea), in subantarctic waters. *J. Exp. Mar. Biol. Ecol.* **301:** 15–27.

Tropea, C., L. Stumpf, and L. S. López Greco. 2015. Effect of temperature on biochemical composition, growth and reproduction of the ornamental red cherry shrimp *Neocaridina heteropoda heteropoda* (Decapoda, Caridea). *PLoS One* **10:** e0119468. doi:10.1371/journal.pone.0119468.

Van Handel, E. 1965. Estimation of glycogen in small amount of tissue. *Anal. Biochem.* **11:** 256–265.

Vazquez-Boucard, C. G., J. Patrois, and H. J. Ceccaldi. 2004. Exhaustion of lipid reserves in the hepatopancreas of *Fenneropenaeus indicus* broodstock in relation to successive spawnings. *Aquaculture* **236:** 523–537.

Verísimo, P., C. Bernárdez, E. González-Gurriarán, J. Freire, R. Muiño, and L. Fernández. 2011. Changes between consecutive broods in the fecundity of the spider crab, *Maja brachydactyla. ICES J. Mar. Sci.* **68:** 472–478.

Wehrtmann, I. S., and M. Graeve. 1998. Lipid composition and utilization in developing eggs of two tropical marine caridean shrimps (Decapoda: Caridea: Alpheidae, Palaemonidae). *Comp. Biochem. Physiol. D Biochem. Mol Biol.* **121:** 457–463.

Wickins, J. F., and T. W. Beard. 1974. Observations on the breeding and growth of the giant freshwater prawn *Macrobrachium rosenbergii* (de Man) in the laboratory. *Aquaculture* **3:** 159–174.

Winberg, G. G., ed. 1971. *Methods for the Estimation of Production of Aquatic Animals.* Academic Press, London.

Wouters, R., L. Gómez, P. Lavens, and J. Calderón. 1999. Feeding enriched *Artemia* biomass to *Penaeus vannamei* broodstock: its effect on reproductive performance and larval quality. *J. Shellfish Res.* **18:** 651–656.

Wu, X. G., Y. X. Cheng, C. S. Zeng, C. L. Wang, and Z. X. Cui. 2010a. Reproductive performance and offspring quality of the first and the second brood of female swimming crab, *Portunus trituberculatus. Aquaculture* **303:** 94–100.

Wu, X. G., Y. X. Cheng, C. S. Zeng, C. L. Wang, and X. Z. Yang. 2010b. Reproductive performance and offspring quality of wild-caught and pond-reared swimming crab *Portunus trituberculatus* broodstock. *Aquaculture* **301:** 78–84.

Xu, X. L., W. J. Ji, J. D. Castell, and R. K. Odor. 1994. Influence of dietary lipid sources on fecundity, egg hatchability and fatty acid composition of Chinese prawn (*Penaeus chinensis*) broodstock. *Aquaculture* **119:** 359–370.

Yu, Z. Y., X. G. Wu, G. L. Chang, Y. X. Cheng, Z. J. Liu, and X. Z. Yang. 2007. Changes in the main biochemical composition in ovaries and hepatopancreas of Chinese mitten crab, *Eriocheir sinensis* (H. Milne-Edwards) during the second ovarian development. *Acta Hydrobiol. Sin.* **31:** 45–52.