Density and gender segregation effects in the culture of the caridean ornamental red cherry shrimp Neocaridina davidi Bouvier, 1904 (Caridea: Atyidae)

Nicolás D. Vazquez1,2, Karine Delevati-Colpo3, Daniela E. Sganga1,2 and Laura S. López-Greco1,2

1Laboratorio de Biología de la Reproducción y el Crecimiento de Crustáceos, Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria C1428EGA, Buenos Aires, Argentina; 2Consejo Nacional de Investigaciones Científicas y Técnicas-Instituto de Biodiversidad y Biología Experimental y Aplicada, Universidad de Buenos Aires, Ciudad Universitaria C1428EGA, Buenos Aires, Argentina; and 3Instituto de Limnología Dr. Raúl A. Ringuelet, Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad Nacional de La Plata, La Plata, 1900, Buenos Aires, Argentina

Correspondence: L.S. López-Greco e-mail: laura@bg.fcen.uba.ar

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ABSTRACT

The effect of density on growth, sex ratio, survival, and biochemical composition of the red cherry shrimp, Neocaridina davidi Bouvier, 1904, was studied to determine optimum rearing conditions in this ornamental species. It was tested whether gender segregation affected growth and survival of the species. To test the effect of density (Experiment 1), hatched juvenile shrimp were kept at three different densities: 2.5, 5, and 10 individuals l–1 (D2.5, D5 and D10, respectively). To test the effect of gender segregation (Experiment 2), 30-day juveniles were reared in three conditions: culture with only females, culture with only males, and mixed culture (females: males 1:1) at 5 individuals l –1 density. Experiments lasted 90 days, and shrimp were weighted either every 30 days (Experiment 1) or 15 days (Experiment 2). At day 90, females kept at D2.5 weighted 45% more than females stocked at D10 (P < 0.05), whereas females from D5 did not differ from those of other densities (P > 0.05). Males at D2.5 weighted 29% more than D5 and D10 (P < 0.05). Survival was high and unaffected by treatment. Sexual differentiation did not differ among treatments. Females from D2.5 had the lowest lipid and protein content, which would occur if they had a higher spawning. Males from D2.5 had higher content of proteins, probably due to their larger size. Gender segregation had no effect over growth and survival; females grew up to a larger size than males both in monosex and mixed culture. It was shown that given to their non-aggressive behavior, N. davidi is tolerant to a high-density condition, which makes it feasible as an ornamental species.

Key Words: aquaculture, biochemical composition, growth, rearing conditions, survival

INTRODUCTION

Keeping of aquarium species has become very popular around the word and nowadays is becoming increasingly attractive. Ornamental organisms support a multi-million-dollar global industry that provides marine and freshwater aquarist with an assortment of over 1,400 species of invertebrates, vertebrates, plants, and algae (Baeza & Behringer, 2017). In contrast to the ornamental fish trade, decapod crustaceans such as shrimps, crayfishes, and crabs are relatively new to the pet trade. Nevertheless, the popularity of ornamental shrimps in freshwater aquaria has rapidly increased in recent years and may represent an entry pathway for emerging pathogens (see Patoka et al., 2016).
may have ecologically important consequences, including its rapid
dispersion and its negative impact on populations (see Weber &
Traunspurger, 2016).

Recent studies have addressed several aspects of the reproduc-
tion and development of N. davidi (Tropea et al., 2015; Tropea &
Lopez-Greco, 2013; Sganga et al., 2016), including nutritional vul-
nérability of juveniles (Pantaleão et al., 2015a, b). Regardless of
its popularity, basic knowledge of the biology and optimal rear-
ing conditions of N. davidi is lacking. It is particularly important
to understand its requirements for culture to succeed. Some of
the parameters that have been considered are density (Palma et al.,
2009), diet (Calado et al., 2005b), vulnerability to disease (Boudad-
Reantaso et al., 2005; Boudad-Reantaso & Subasinghe, 2008), gen-
der segregation (Kunda et al., 2009), pH (Chen & Chen, 2003),
salinity (Wasielezky et al., 2003), substrate (Tidwell et al., 1998), and
temperature (Thomas et al., 2000) among others.

Density is one of the key parameters to take into account
to maximize the return of investment during culture. Overcrowding
leads to a decrease in growth rates (Liu et al., 2014; Golubev et al.,
2016), or an increase prevalence of diseases (Noga, 2010). Low
density would denote that the system has not reached its full
potential. Neither option is favorable for a producer of ornamen-
tal species, hence it is important to understand how density affects
breeding. In species with a pronounced sexual dimorphism such as
the caridean shrimp Macrobrachium rosenbergii (De Man, 1879),
gennder segregation (also known as “monoculture”) has proven to be
an effective measure to increase production and reduce the
negative effect of the reproductive-dominance hierarchy in males (Ra’Anan & Sagi, 1985; Mohanakumaran Nair et al., 2006).

This study aimed to investigate the effects of density and gender
segregation on the growth and survival of N. davidi. Also investi-
gated was how density affected the biochemical composition (total
glycogen, lipids, and protein content) for both sexes of this species.

MATERIALS AND METHODS

Broodstock maintenance and production of juveniles

The reproductive stock was obtained from a commercial supplier
(Acuamanus Aquarium, Buenos Aires, Argentina). Approximately
20 adult females and 20 adult males were randomly selected from
the stock and placed in an aquarium (0.33 × 0.25 × 0.19 m) con-
taining 8 l of water, and nearly 1.6 g of Vesicularia sp. (“Java moss”),
supplied as substrate and shelter. Once females became ovigerous,
they were isolated in smaller recipients (0.18 × 0.12 × 0.12 m) contain-
ing 2 l of water until juveniles hatched; newly hatched or
30-day old juveniles were assigned to the experimental trials.

All shrimp employed (including those from the stock) were kept
under the same rearing conditions: dechlorinated tap water (pH 7.5,
hardness 80 mg l⁻¹ as CaCO₃ equivalents), temperature was set at
27 ± 1 °C by the use of water heaters (100 W, precision 1 °C), photoperiod of 14 light:10 dark, and continuous aeration.
They were daily fed ad libitum with tropical-fish, balanced food
(Tetracolor®, Tetra, Melle, Germany), with an approximate com-
position of 47.5% minimum crude protein, 6.5% minimum crude
fat, 2.0% maximum crude fiber 6.0% maximum moisture, 1.5%
minimum phosphorus, and 100 mg kg⁻¹ minimum ascorbic acid.
These culture conditions were based on Tropea et al. (2015).

Effect of density

The effect of density (Experiment 1) was evaluated by applying
different stocking densities: 2.5, 5, and 10 shrimp l⁻¹ (herein-
after referred to as D₂₅, D₅, and D₁₀, respectively). Newly-hatched
juveniles were randomly assigned to a treatment and placed in
small recipients (experimental unit) each containing 2 l water and
0.7 g of Vesicularia sp. as substrate. The number of replicates per
density varied from 7 to 9. All individuals in each experimental
unit were weighted to 0.1 mg every 30 d after being hand dried
with paper napkins. Although size rather than weight is the usual
parameter, we used weight because of the small size of individuals
and the difficulties in accurately measuring the smallest juveniles.

The correlation between total length (TL), measured from the
tip of the rostrum to the posterior end of the telson and weight
was equation: weight = 0.019 TL² + 0.83, r² = 0.98 for females and
weight = 0.03 TL² + 0.91, r² = 0.99 for males (KDC and LSLG,
unpublished). Total grow-out period lasted 90 d, therefore individ-
ual shrimp were weighted at days 30, 60, and 90. Food was
provided ad libitum to avoid feeding constraints (4% of food of
the replicate’s total weight). The presence of ovigerous females
was also recorded, and offspring born during the experiment
were removed to prevent changes in density. Water was com-
pletely replaced once a week. Experimental animals were frozen at
–20 °C at the end of the experiment period for biochemical
analysis.

Total glycogen, lipid, and protein were determined spectropho-
tometrically in homogenates of all individuals from each replicate,
in agreement with the methods described by Folch et al. (1957),
Van Handel (1965), and Bradford (1976), respectively, and modi-
fied by Tropea et al. (2015) for N. davidi. In cases in which biomass
was not sufficient for testing, replicates were pooled. Females and
males were analyzed separately. Number of replicates per treatment
varied from four to six depending on the amount of biomass
available.

Monoculture versus mixed culture

Gender segregation was evaluated by applying three rearing con-
ditions: mixed culture, monosex culture with only females and
monosex culture with only males (Experiment 2). For this purpose,
recently hatched juveniles were placed in 8 l aquaria for 30 days
or until they reached the proper size in which secondary sexual
characters became evident, so that sexes could be easily deter-
mined under a stereomicroscope. Specimens were sexed based on
the morphology of the first and second male pleopods (Barbier,
2010; Pantaleão et al., 2013b). Individuals within 10 and 15 mg
(mean wet weight of 12.5 ± 2.5 mg) were selected for the experi-
ment. For the mixed culture, females and males were set at an
equal ratio, whereas each replicate contained either all females
or all males for the monosex culture. Juveniles were placed in
2 l aquaria, and randomly assigned to the treatments. Stocking
density was 5 individual l⁻¹ (with five replicates per treatment).
Individual shrimp were wet weighted every 15 days, and the total
growth period was 90 days. The presence of ovigerous females
was also recorded, and offspring born during the experiment
were removed to prevent changes in density and treatment.

Statistical analysis

The weight of shrimp over time was registered for both experi-
ments. Because shrimp could not be individualized throughout the
experiments, the mean weight of individuals from each experi-
mental unit (replicate) was used as a response variable, and com-
pared within each essay by using a general linear mixed model
with a repeated measures design. The replicate was included as
a random effect, and the fixed effect factors were density (for Experiment 1, effect of density), gender and treatment (for Experiment 2, monosex culture), and time (for both). Variance
was modeled using varExp (Zuur et al., 2010) to correct for the
violation of the homogeneity of variance assumption (assessed by
graphical tools according to Zuur et al., 2010), and an autore-
gressive correlation of order 1 as a correlation structure (see Zuur
et al., 2009).

For Experiment 1, the final weight of males was analyzed with a
general linear mixed model, considering density as a fixed effect
factor, and replicate as a random effect. Final weight of females
RESULTS

Effect of density

Shrimp stocked at D$_{2.5}$ and D$_{5}$ attained a larger weight than those from D$_{10}$ (Fig. 1). At 30 days, individuals from all densities grew uniformly, but individuals from D$_{2.5}$ and D$_{5}$ presented a higher weight than D$_{10}$ (density:time; df = 4; F = 5.978; P = 0.0004) at 60 days. This tendency prevailed until the end of the experiment (90 days) (Fig. 1, Table 1), when females from D$_{2.5}$ weighted 45% more than males from D$_{10}$ (df = 2, F = 9.03, P = 0.0015) (Fig. 2). Females from D$_{5}$ did not differ from the other densities. Ovigerous females were substantially larger (81.55 ± 3.02 mg) than non-ovigerous ones (74.81 ± 2.74 mg) regardless of density (df = 1, F = 8.27, P = 0.0051), mean values displayed therefore correspond to a global mean weight. Females became ovigerous after a two-month growth period and at all densities (data not shown). D$_{2.5}$ males had the lowest concentration of lipids, whereas D$_{5}$ males had the highest protein content, whereas the remaining groups had the lowest content (Table 4).

Table 1. Parameter estimates (regression coefficients) of fixed factors from the repeated measures multiple regression testing the effect of density (D$_{2.5}$, D$_{5}$, and D$_{10}$ individuals l$^{-1}$) on the growth of Neocaridina davidi over time. Parameter estimates were calculated as contrasts with the intercept: “D$_{10}$” and “Day 30”. The level of significance was set at 0.05.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Estimates</th>
<th>Standard Error</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>18.10</td>
<td>1.72</td>
<td>10.54</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>D$_{2.5}$</td>
<td>3.65</td>
<td>2.4</td>
<td>1.51</td>
<td>0.1356</td>
</tr>
<tr>
<td>D$_{5}$</td>
<td>6.76</td>
<td>2.40</td>
<td>2.82</td>
<td>0.0064</td>
</tr>
<tr>
<td>Day 60</td>
<td>18.19</td>
<td>1.78</td>
<td>10.25</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Day 90</td>
<td>29.84</td>
<td>2.54</td>
<td>11.74</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>D$_{2.5}$ x Day 60</td>
<td>12.00</td>
<td>2.72</td>
<td>4.41</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>D$_{5}$ x Day 60</td>
<td>6.52</td>
<td>2.59</td>
<td>2.52</td>
<td>0.0144</td>
</tr>
<tr>
<td>D$_{10}$ x Day 90</td>
<td>16.38</td>
<td>4.02</td>
<td>4.08</td>
<td>0.0001</td>
</tr>
<tr>
<td>D$_{2.5}$ x Day 90</td>
<td>7.74</td>
<td>3.73</td>
<td>2.07</td>
<td>0.0423</td>
</tr>
</tbody>
</table>

Figure 2. Female and male final mean body weight at the end of the growth period for Experiment 1 (90 days). Mean (± standard error) weight (mg) of adult Neocaridina davidi individuals cultured at different stocking densities (D$_{2.5}$, D$_{5}$, and D$_{10}$ individuals l$^{-1}$). Comparisons among densities were made in the same gender, and are shown in the superscripts, those in capital letters correspond to females, those in lower case to males. Treatments without a common superscript differ (P < 0.05).

Table 2. Effect of density on the survival of Neocaridina davidi at 90 days. The survival rates of shrimp are expressed as percentage and were estimated from the logistic regression coefficients after applying the inverse logit function to transform parameters to their natural scale. Treatments without a common superscript differ (P < 0.05).

<table>
<thead>
<tr>
<th>Density</th>
<th>Mean survival (%)</th>
<th>Confidence interval</th>
<th>Number of replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>D$_{10}$</td>
<td>92.50$^a$</td>
<td>(79.20 – 97.56)</td>
<td>8</td>
</tr>
<tr>
<td>D$_{5}$</td>
<td>93.33$^a$</td>
<td>(85.95 – 96.97)</td>
<td>9</td>
</tr>
<tr>
<td>D$_{2.5}$</td>
<td>87.14$^a$</td>
<td>(87.14 – 91.75)</td>
<td>7</td>
</tr>
</tbody>
</table>

was also analyzed with a general linear mixed model; however, the female condition (ovigerous versus non-ovigerous) was also regarded as a fixed factor. Residuals showed no patterns and adjusted for normality in both cases, there was therefore no need for transformation or further modeling. The biochemical composition of shrimp (glycogen, lipid, and protein contents) at the end of the growth period was compared through separate general linear models (with density, gender, and their interaction as fixed factors). In the case of lipid and protein content, generalized least squares (GLS) were employed to correct for heterogeneity in residuals using varPower as a function of variance (Zuur et al., 2010).

To evaluate the effect of density on sex ratio (number of females over total number of shrimps per replicate at final time), a generalized linear model (GLM) was used, with a binomial distribution and “logit” as link function. Survival rate (percentage of live shrimp at the end of the experimental period) was tested separately for both experiments also with a GLM (Zuur et al., 2010). The level of significance was set at 0.05. A posteriori comparisons were made using Tukey’s test in all cases. All analyses were carried out using R Studio version 3.3.1 (R Core Team, 2015).
Monoculture versus mixed culture

No differences were found among treatments at day 15. Females (both from mixed and monoculture treatments) had a larger mean weight than males ($P < 0.05$). This difference was maintained throughout the entire experiment. Monosex culture showed no disparity from mixed culture at any time (Fig. 3, Table 5). Survival rate was also unaffected by treatment and gender (Table 6). Ovigerous females were recorded at approximately 45 days after the start of the experience.

**DISCUSSION**

Achieving harvest size at a faster rate is one of the main goals of commercial aquaculture hence if survival, health, and growth are not compromised, the higher the stocking density, the higher the profits. Optimal stocking density will depend on the species although external factors such as temperature, water quality, or feeding, can affect this culture parameter (Baskerville-Bridges & Kling, 2000).

Adult mean weight (females and males) of *Neocaridina davidi* was significantly higher in those stocked at $D_{2.5}$ and $D_5$ in Experiment 1. Intraspecific competition occurs when two or more individuals from the same species compete for a limited resource such as food, shelter, space, or any other factor crucial for reproduction or survival. In *Macrobrachium amazonicum* (Heller, 1862) competition is known to heighten when such resources become insufficient (Moraes-Valenti et al., 2010). Because food availability was ad libitum and similar for all treatments, growth constraints were probably due to a lack of physical space. As density increases, space can become a limiting factor. This inhibition became evident in *N. davidi* after 30 days of growth.

Ovigerous females were larger than non-ovigerous females at all densities. In some gonochoristic species such as the caridean shrimp *Hippolyte williamsii* Schmitt, 1924, males were smaller than females, and also ovigerous females were larger than non-ovigerous females (Espinoza-Fuenzalida et al., 2008) as in our results. Females of *N. davidi* stocked at $D_{2.5}$ and $D_5$ were approximately 45% larger than those stocked at $D_{10}$ (Tropea & López-Greco, 2015) found a positive correlation between realized fecundity (number of eggs per spawn per female) and body weight in females of *N. davidi*.

**Table 3.** Sex ratio of *Neocaridina davidi* according to density at the end of the growth period of 90 days. Female odd ratio is estimated as the proportion of females ($\pi$) over the proportion of males ($1–\pi$). Treatments without a common superscript differ ($P < 0.05$).

<table>
<thead>
<tr>
<th>Density</th>
<th>Mean proportion of females (%)</th>
<th>Confidence Interval</th>
<th>Female odd ratio ($\pi/1–\pi$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_{2.5}$</td>
<td>40.5 ± 4.2*</td>
<td>(26.1 – 56.8)</td>
<td>0.682</td>
</tr>
<tr>
<td>$D_5$</td>
<td>54.8 ± 4.2*</td>
<td>(44.1 – 65.0)</td>
<td>1.211</td>
</tr>
<tr>
<td>$D_{10}$</td>
<td>44.3 ± 4.2*</td>
<td>(35.7 – 53.2)</td>
<td>0.794</td>
</tr>
<tr>
<td>Total</td>
<td>473 ± 4*</td>
<td>(41.1 – 60.9)</td>
<td>0.898</td>
</tr>
</tbody>
</table>

indicating that, as expected, larger females produce a larger number of eggs. Even though no relationship was found between actual fecundity (number of newly hatched juveniles per female) and female body size (Tropea & López-Greco, 2015; Tropea et al., 2015), larger individuals are often more colorful and conspicuous, therefore breeding larger females could be favorable for the producer. In contrast, males stocked at $D_{2.5}$ reached a greater mean weight, proving that low densities favored higher growth rates.

Sexual differentiation in *N. davidi* was not affected by stocking density and showed that sex ratio was slightly biased towards males. Sex ratio is usually inclined towards the gender with the least fertility values, according to mathematical models (Charnov & Bull, 1989). Bias among sexes and the strong sexual dimorphism may therefore be consequences of the asymmetrical energetic investment for females and males, given that the former expends more resources for the synthesis of gametes (see Charnov & Bull, 1989).

In species with a high degree of aggressiveness, such as the caridean shrimps *Macrobrachium rosenbergii* and *M. australiensis* Holthuis, 1950, males have hypertrophied cheliped that are used as weapons and actively compete for access to females. Dominant males often prevent smaller males from reproduction (Bauer, 2004). In contrast, *N. davidi* is a gregarious species lacking enlarged chelipeds, therefore direct competition for females is not likely to occur. Sexual size dimorphism in this species was also assessed by Sganga et al. (2016), who showed that not only adult females are larger than males, but also present morphometric differences in size of the carapace and rostrum than males. *Neocaridina davidi* has three characteristics that are consistent with a “pure search” mating system, as described by Wickler & Seibt (1981): populations with high densities of individuals, absence of cheliped weaponry, and sexual dimorphism in terms of size. Male mating success depends on their capacity to locate and copulate with females, and involves no courtship or agonist encounters between males (Correa & Thiel, 2003). Smaller males are more agile and therefore more efficient at finding females (Correa & Thiel, 2003; Rasch & Bauer, 2016). Another caridean, *Palaemon pugio* (Holthuis, 1949) presents a similar reproductive behavior as *N. davidi*, and females are also larger than males (Bauer & Abdalla, 2001). Both *N. davidi* and *P. pugio* are non-aggressive species and possess small chelipeds in both sexes. Reproductive competition occurs indirectly, a reason why larger size or chelipeds have not been selected through the course of their evolutionary history (see Bauer, 2004). Taking this information into account, it is not surprising that gender segregation (Experiment 2) had no effect over growth in both sexes in *N. davidi*.

Survival rate was also unaffected by density or gender segregation, reinforcing the “non-aggressive nature” of *N. davidi*. The morphology of this species provides no evidence of hierarchical structures (between or among genders) or aggressive behavior under rearing conditions, making it more tolerant to living at higher stocking densities. Studies on *Palaemon varians* (Leach 1814), showed a similar tendency, given that density had no effect over shrimp survival or larval size (Palma et al., 2009).

**Table 4.** Effect of density on the biochemical composition of *Neocaridina davidi* at the end of the experiment for the three densities tested ($D_{2.5}$, $D_5$, and $D_{10}$ individuals l$^{-1}$). Mean values are given ($±$ SE). Numbers between brackets represent the number of replicates. Treatments without a common superscript within a row are significantly different ($P < 0.05$).

<table>
<thead>
<tr>
<th>Gender</th>
<th>Density (individuals $l^{-1}$)</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$D_{2.5}$</td>
<td>$D_5$</td>
<td>$D_{10}$</td>
</tr>
<tr>
<td>Glycogen (ug/mg)</td>
<td>2.12 ± 0.25(5)</td>
<td>2.37 ± 0.23(6)</td>
<td>2.23 ± 0.26(4)</td>
</tr>
<tr>
<td>Lipid (ug/mg)</td>
<td>6.53 ± 1.49(5)</td>
<td>15.01 ± 4.11(6)</td>
<td>20.06 ± 6.03(6)</td>
</tr>
<tr>
<td>Protein (ug/mg)</td>
<td>41.92 ± 5.81(5)</td>
<td>146.95 ± 23.47(3)</td>
<td>112.19 ± 14.11(5)</td>
</tr>
</tbody>
</table>
Proteins were the most abundant biochemical components of *N. davidi*, followed by lipids and glycogen. These results are in agreement with previous studies on the species (Tropea et al., 2015), as well as for the embryological states of another decapod, the crayfish, *Cherax quadricarinatus* (Martens, 1868) (García-Guerrero et al., 2003), and the larval stages of other crustaceans (Racotta et al., 2003). Many studies have analyzed the effect of different parameters such as water quality, diet, and temperature on the biochemical composition of crustaceans (e.g., Dey et al., 1993; Anger, 1998; Rodríguez-González et al., 2006; Anger et al., 2009; Weiss et al., 2009; Cerón-Ortiz et al., 2015; Tropea et al., 2015), and other invertebrates (e.g., MerCEO et al., 1995; Dunstan et al., 1996; Fernandez, 1997), but to our knowledge, few have studied the effect of density (Montero et al., 1999; Khan, 1994; Calado et al., 2005a; Andrés et al., 2007). Glycogen levels were unaffected by gender or density in *N. davidi*. Females kept at D_{2.5} tended to have lower lipid and protein contents, although the difference was not statistically significant for the former. An analogous pattern was observed in the larvae of the brachyuran crab *Maja brachydactyla* Balss, 1922, where lipid and protein concentrations were higher in larvae reared at the highest density, with necrophagy suggested as a possible explanation (Andrés et al., 2007). Such a hypothesis, however, does not seem applicable for *N. davidi*. Triglyceride levels in the hemolymph of the brachyuran crab *Oxypleura quadrita* (Fabricius, 1787) showed a decrease during spring, which coincides with the beginning of the reproductive period when the gonad initiates gametogenesis (VinaGRE et al., 2007). Many studies have determined that oogenesis represents a major energetic expense for females since lipids are the primary source of nutrients for the developing embryo, especially in lecithotrophic species such as *N. davidi* (see Mourente & Rodríguez, 1991; Palacios et al., 2000). The results of our experiment could be a consequence of an increased reproductive frequency in females kept at the lowest density, although further research is needed to specifically address this point. Males at D_{2.5} were larger than those at other densities, so it is not surprising that they had the highest protein content, given that the decapod pleon consists mainly of muscle fibers.

Increasing the stocking density showed a negative effect on the growth rate of *N. davidi* although survival rate remained unaffected. It is therefore recommended that shrimp should be stocked at a density of 2.5 shrimp l^{-1} to obtain larger females and males. Moreover, gender segregation is not necessary for optimal grow-out.

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