

Large mothers, but not large fathers, influence offspring number in a caridean shrimp

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Abstract: The relationship between parental mass and female reproductive output, as well as offspring quality, was studied in the red cherry shrimp (*Neocaridina davidi* (Bouvier, 1904)) under controlled laboratory conditions. Adult males and females of the same age were paired combining different shrimp masses. The number of hatched juveniles from large females was higher than that from small ones, but no influence of paternal mass was detected on this variable. Both the mass of newly hatched juveniles and their growth increment during a 60-day period were similar for all parental masses. Shrimps reached sexual maturity at the end of the growth period in all treatments, and their biochemical reserves (glycogen, lipid, and protein concentrations) were not associated with maternal and paternal masses. However, lipid concentration was higher in female offspring than in male offspring. The present results show that, unlike maternal mass, paternal mass had no effect on female reproductive output and offspring quality, suggesting that the contribution of males to offspring development was adequate regardless of male size.

Key words: *Neocaridina davidi*, red cherry shrimp, offspring quality, parental size, maternal mass, paternal mass.

Résumé : La relation entre la masse des parents et l'efficacité de la reproduction des femelles, ainsi que la qualité de la progéniture, a été examinée chez la crevette cerise *Neocaridina davidi* (Bouvier, 1904) dans des conditions contrôlées en laboratoire. Des mâles et femelles adultes du même âge ont été accouplés en combinant des individus de différentes masses. Le nombre de juvéniles éclos issus de grosses femelles est plus élevé que celui de petites femelles, mais aucune influence de la masse paternelle sur cette variable n'est décelée. La masse des juvéniles nouvellement éclos et leur croissance durant une période de 60 jours sont semblables pour toutes les masses parentales. Les crevettes ont atteint la maturité sexuelle à la fin de la période de croissance pour tous les traitements, et leurs réserves biochimiques (concentrations de glycogène, de lipides et de protéines) ne sont pas associées à la masse de la mère ou du père. La concentration de lipides est toutefois plus élevée chez la progéniture femelle que chez la progéniture mâle. Les résultats démontrent que, contrairement à la masse de la mère, la masse du père n'a pas d'effet sur l'efficacité de la reproduction des femelles et la qualité de la progéniture, ce qui indiquerait que la contribution des mâles au développement de la progéniture est adéquate, quelle que soit leur taille. [Traduit par la Rédaction]

Mots-clés : *Neocaridina davidi*, crevette cerise, qualité de la progéniture, taille du parent, masse de la mère, masse du père.

Introduction

When the availability of food resources is fixed, individuals differentially allocate them among growth, somatic maintenance, and reproduction. Therefore, the amount of resources allocated to reproduction is limited, and there are many ways of partitioning them into offspring production. In this context, a trade-off between number and size of progeny has been reported for invertebrate species, with mothers producing either a large number of small progeny or a small number of large progeny (for revision see Fox and Czesak 2000 and Ramirez Llodra 2002). Smith and Fretwell's (1974) classic theory predicts that, within a given environment, all females produce offspring of the same size, but mothers with access to a greater amount of resources should deliver more offspring. Egg size and number might also be determined by physiological or morphological constraints such as adult size, ovary structure, or oogenic patterns, especially in brooding invertebrates (Fox and Czesak 2000; Ramirez Llodra 2002). In crustaceans generally and in caridean shrimps specifi-

cally, large females produce more eggs per clutch than small females. For example, positive correlations between female size and fecundity have been found in the Alvinocarididae *Mirocaris fortunata* (Martin and Christiansen, 1995) (Ramirez Llodra et al. 2000); in the Atyidae *Neocaridina denticulata denticulata* (De Haan, 1844) (Oh et al. 2003); in the Campylonotidae *Campylonotus vagans* Spence Bate, 1888 (Thatje et al. 2004); in the Crangonidae *Notocrangon antarcticus* (Pfeffer, 1887); in the Hippolytidae *Eualus gaimardii gaimardii* (H. Milne-Edwards, 1837) and *Chorismus antarcticus* (Pfeffer, 1887); in the Nematocarcinidae *Nematocarcinus lanceopes* Spence Bate, 1888 (Clarke 1993); in the Oplophoridae *AcanthePHYRA pelagica* (Risso, 1816), *AcanthePHYRA purpurea* A. Milne-Edwards, 1881, and *AcanthePHYRA kingsleyi* Spence Bate, 1888 (Ramirez Llodra 2000); in the Palaemonidae crossbanded grass shrimp (*Palaemon northropi* (Rankin, 1898)), American grass shrimp (*Palaemon pandaliformis* (Stimpson, 1871)), cinnamon river shrimp (*Macrobrachium acanthurus* (Wiegmann, 1836)), and bristled river shrimp (*Macrobrachium olfersii* (Wiegmann, 1836)) (Anger and Moreira 1998); and in the Pasiphaeidae

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Systellaspis debilis (A. Milne-Edwards, 1881) and grooveback shrimp (*Parapasiphae sulcatifrons* S.I. Smith, 1884) (Ramirez Llodra 2000). The relationship between body size and fecundity might be related to the physical limit imposed on eggs by the brooding chamber, which constrains the number of embryos to be brooded (Ramirez Llodra 2002; Guay et al. 2011). Regarding male size, a positive correlation between this variable and ejaculate size has been reported in some decapod species, leading to sperm limitation in certain cases. This may have an even stronger influence on clutch size than female size (MacDiarmid and Sainte-Marie 2006; Butler et al. 2015).

Other traits are commonly used to evaluate offspring quality along with fecundity and egg size: morphological variables such as size, mass, or colour, biochemical composition, offspring behaviour, and survival after stress tests. The majority of these can be assessed not only in eggs, but also in newly hatched juveniles and in advanced stages of development (Racotta et al. 2003). Biochemical composition is particularly useful as a predictive criterion of offspring quality, in as much as lipids and proteins are energetic reserves and also play a structural role. Furthermore, carbohydrates might be involved in the synthesis of chitin, influencing offspring performance during the moult cycle (Calado 2008). To our knowledge, the effect of maternal and paternal sizes on these traits has been poorly studied in caridean shrimps.

The red cherry shrimp (*Neocaridina davidi* (Bouvier, 1904)) (family Atyidae) is a freshwater species with highly abbreviated development. Fertilized eggs are attached to female pleopods during the entire incubation period, which lasts approximately 15 days, depending on temperature (Tropea et al. 2015). The number of hatched juveniles in a single spawning event ranges from 14 to 80 (Tropea et al. 2015; D.E. Sganga, personal observation). Females spawn several times throughout a life span of 1.5 years under culture conditions (Barbier 2010; Marciano et al. 2018). Although the mating system has not been reported for the species, laboratory observations seem to indicate that it corresponds to the “pure search mating system” described by Correa and Thiel (2003). Males search actively for receptive females and leave them soon after sperm transfer at mating. Females are identified by their yellow or green ovaries, which become visible through the translucent carapace at an approximate mass of 40 mg (Tropea et al. 2015). This mass is achieved approximately 45 days after hatching, although the length of this period varies depending on culture density (Vazquez et al. 2017). Ovarian rematuration takes place a few days after hatching or even during egg incubation (Barbier 2010; Tropea and López Greco 2015).

Neocaridina davidi is one of the most popular freshwater ornamental shrimp species. The assessment of parental traits that influence offspring quality is important to produce high-quality brooders and consequently enhance the efficiency of *N. davidi* culture at a commercial scale. Hence, the aim of the present study was to evaluate the effect of parental size on offspring quality and female reproductive performance of *N. davidi*. The effect of parental size on offspring quality was assessed in both newly hatched juveniles and shrimps (females and males) after a 60-day growth period. We hypothesized that both maternal and paternal sizes affect female reproductive performance and offspring quality, and that this effect is stronger for maternal size.

Materials and methods

Experimental specimens

The reproductive stock was provided by a local supplier (Acuamanus Aquarium, Buenos Aires, Argentina) and maintained in dechlorinated tap water (pH 7.5, hardness 80 mg/L, as CaCO₃ equivalents) under continuous aeration and at a constant temperature of 27 ± 1 °C. The photoperiod was 14 h light : 10 h dark. A small portion of Java moss (species of the genus *Vesicularia* (C. Muell.) C. Muell.) was provided as substrate and also partially as food. Shrimps were fed daily ad libitum commercial balanced

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. Water was replaced completely once a week. Juveniles obtained from the reproductive stock were reared until sexual maturity. To guarantee the virginity of all individuals at the beginning of the experiment, juveniles were observed periodically and females were separated from males as soon as ovaries were noticed through the cephalothorax. After reaching sexual maturity and differentiating sexual characters (Sganga et al. 2016), same-aged males and females were weighed on a Mettler AE 160 balance (accuracy: 0.01 mg).

Experimental design

One virgin female and one virgin male were placed in a plastic aquarium (18 cm × 12.5 cm × 12 cm) containing 2 L of dechlorinated tap water and maintained under the same conditions of water quality, temperature, photoperiod, and feeding as described above ($n = 40$). Same-aged brooders were paired considering all possible mass combinations. The aquaria were visually inspected once a day to determine the presence of ovigerous females. The spawning and hatching dates were recorded for each of them. Both dates were used to calculate the duration of the incubation period of each brood. The number of newly hatched juveniles (juveniles I: JI) was counted as a measure of actual fecundity (number of newly hatched juveniles per brood per female). A random sample of 10 JI from each brood was weighed (accuracy: 0.01 mg) and mean JI mass was calculated by dividing total sample mass by the number of JI (initial mass). Even though the first posthatching stage of *N. davidi* juveniles cannot be sexed, Pantaleão et al. (2017) demonstrated that male and female sizes are similar in the first stages of development. Hence, we assumed that female and male initial masses were equal. Another sample of 15 JI per brood were randomly selected and placed in an aquarium (18 cm × 12.5 cm × 12 cm) under the same experimental conditions as described above to evaluate their growth during a 60-day period. After this time span, individuals were sexed and weighed (final mass; accuracy: 0.01 mg), and a mean final mass was calculated for males and females separately. Growth increment was also calculated for males and females separately as follows: $GI = 100 \times [(W_f - W_i)/W_i]$, where W_f is the mean final mass and W_i is the mean initial mass. Sixty-day-old shrimps were then sacrificed after being cold-anaesthetized at -20 °C for 15 min and then stored at -20 °C for biochemical determinations. Males and females were analysed separately.

Biochemical analyses

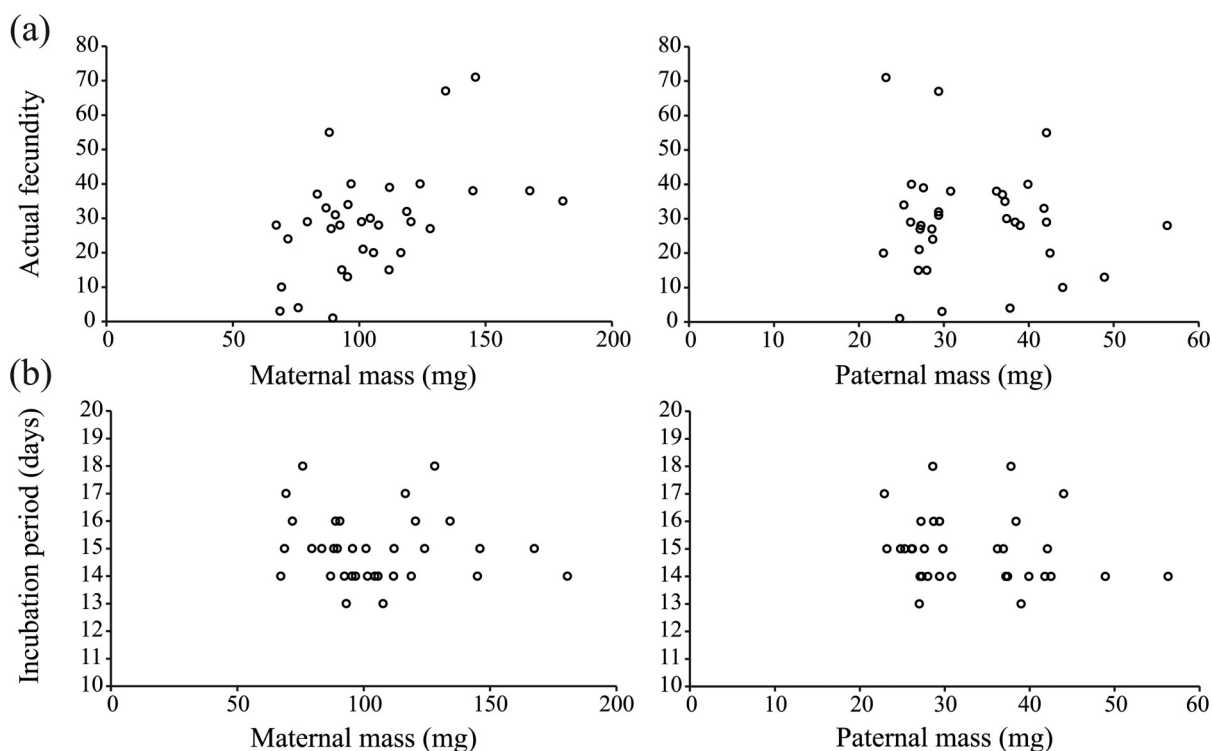
Protein, lipid, and glycogen contents in 60-day-old males and females were determined spectrophotometrically, according to the methods described by Bradford (1976), Folch et al. (1951, 1957), and Van Handel (1965), respectively, and modified for the species by Tropea et al. (2015). For protein determination, samples weighing 20–100 mg were homogenized in 4:1 volume:mass of 50 mmol/L Tris-HCl buffer and pH 7.5, and centrifuged at 10 000 g in a refrigerated centrifuge (4 °C) for 30 min. Total protein content was measured in the supernatant using the Coomassie blue dye method. Absorbance was read at 595 nm and compared with bovine serum albumin as the standard (concentration: 1 mg/mL). To determine lipid content, samples weighing 50–200 mg were homogenized in 20:1 volume:mass of a mixture of chloroform and methanol (2:1, v/v), then mixed with 0.9% NaCl and centrifuged at 2000 r/min to separate the lipid fraction. Total lipids were quantified by the sulfo-phospho-vanillin method. Extra virgin olive oil diluted in absolute ethanol (concentration: 1 mg/mL) was used as the standard and absorbance was read at 530 nm. For glycogen determination, samples weighing 30–100 mg were boiled for 1 h with 4:1 volume:mass of 30% KOH. After cooling, glycogen was

Table 1. Comparison of most optimal models for actual fecundity, duration of the incubation period, Juvenile I (JI) mass at hatching, and growth increment of 60-day-old red cherry shrimps (*Neocaridina davidi*).

Response variable	Explanatory variables	R^2/D^2	AIC
Actual fecundity	Maternal mass*	0.153	—
Duration of the incubation period	Maternal mass + paternal mass	0.672	163.915
	Paternal mass	0.384	161.996
	Maternal mass	0.248	160.105
JI mass at hatching	Maternal mass + paternal mass	0.040	-62.638
	Paternal mass	0.040	-64.638
	Maternal mass	0.007	-64.126
Growth increment	Offspring sex*	0.200	667.997

Note: AIC, Akaike's information criterion. *, $P < 0.05$.

Fig. 1. Reproductive performance of female red cherry shrimps (*Neocaridina davidi*). The association between maternal and paternal masses and the variables actual fecundity (number of newly hatched juveniles per brood per female) (a) and incubation period (b) was studied ($n = 34$).



precipitated through the addition of 75 μL of saturated Na_2SO_4 and 1875 μL of absolute ethanol, and then centrifuged at 2000 r/min for 10 min. The precipitate was dissolved in 250 μL of distilled water and glycogen was measured by the anthrone-reagent method. Glycogen from rabbit liver (Fluka; Sigma-Aldrich Corp., St. Louis, Missouri, USA) was used as the standard (concentration: 1 mg/mL) and absorbance was read at 620 nm.

Statistical analyses

The protocol described by Zuur et al. (2010) was used for data exploration. A backward stepwise multiple regression procedure was performed using linear models (LM) to analyse the association between the covariates "maternal mass" and "paternal mass" and the following response variables: actual fecundity, JI mass, biochemical composition (protein, lipid, and glycogen contents) of 60-day-old shrimps, and growth increment of 60-day-old shrimps. For the duration of the incubation period, a generalized linear model (GLM) with Poisson distribution and log link function was used. As atypical observations were detected during data exploration of actual fecundity, a robust linear regression was performed with a tuning constant equal to 3.44 for 85% efficiency (Maronna

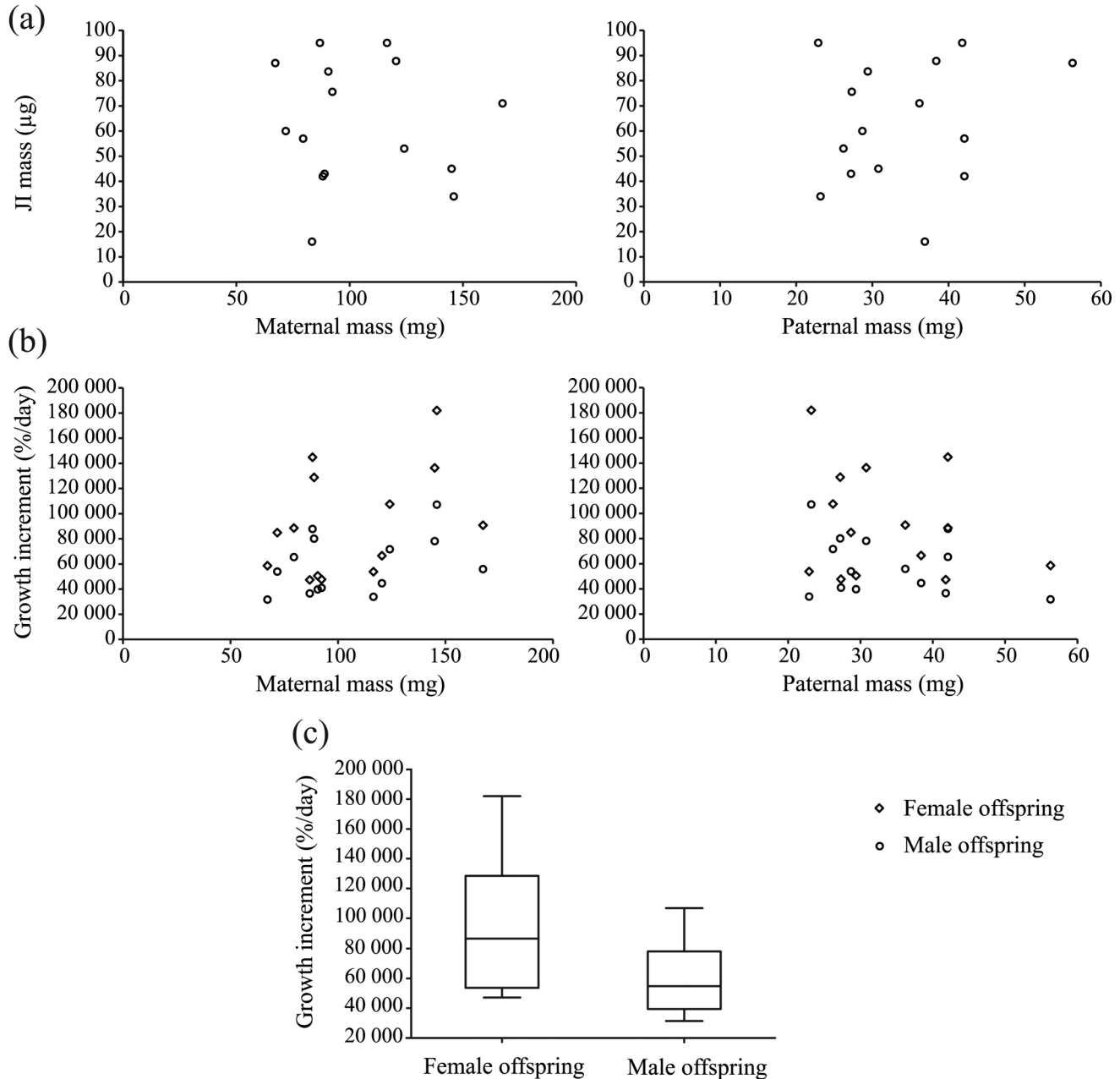
et al. 2006). In all models, maternal and paternal masses were included as fixed factors, together with their interactions. Offspring sex was also included as a categorical variable when analysing biochemical composition and growth increment of 60-day-old shrimps. An information-theoretic approach was followed to find the optimal set of covariates for each model (Burnham and Anderson 2002). Statistical analyses were performed using lme4 and robustbase packages (Bates et al. 2015; Maechler et al. 2016) for the R software (R Core Team 2016). The α level of statistical significance was set at 0.05. The Bonferroni correction was used (Dunn 1961) to account for multiple testing when analysing protein, lipid, and glycogen contents, and those comparisons for which $P < 0.05/3 = 0.0167$ were considered significant. All results were expressed as mean value ± 1.96 times the standard error, which corresponds to the 95% confidence interval (Wasserman 2004).

Results

Female reproductive performance

Eighty-five percent of the initial 40 couples spawned during the experimental period.

Fig. 2. Offspring quality in red cherry shrimps (*Neocaridina davidi*). Initial mass (*a*) was recorded in newly hatched juveniles, whereas growth increment (*b*) was recorded at the end of a 60-day growth period ($n = 15$ and 28 , respectively). The association of these variables with maternal and paternal masses was assessed including the categorical variable offspring sex for growth increment. Significant statistical differences were found between sexes (*c*).



Actual fecundity, with 29.15 ± 5.19 newly hatched juveniles per brood per female, was associated with maternal mass but not with paternal mass ($t = 2.162$, $P = 0.038$) (Table 1, Fig. 1a). The duration of the incubation period, on the other hand, was not associated with maternal mass ($z = -0.285$, $P = 0.776$) and paternal mass ($z = -0.393$, $P = 0.694$), and averaged 14.97 ± 0.42 days (Table 1, Fig. 1b).

Offspring quality

No association was found between JI mass and maternal mass and between JI mass and paternal mass. The mean value of this response variable was $63.00 \pm 8.53 \mu\text{g}$ (Table 1, Fig. 2a). Shrimps reached sexual maturity during the 60-day growth period following hatching, with their final mass averaging 53.82 ± 4.10 mg for female offspring and 35.25 ± 1.80 mg for male offspring. No can-

nibalism was detected in any aquarium, while ovigerous females were observed in all aquaria. Maternal and paternal masses were not associated with juvenile growth increment during the 60-day period, but growth increment was 55.74% higher in female offspring than in male offspring ($t = -2.548$, $P = 0.0171$) (Table 1, Figs. 2b, 2c). Protein, glycogen, and lipid concentrations in 60-day-old female and male offspring were not associated with maternal and paternal masses (Table 2, Figs. 3a, 3b, 3c). However, lipid concentration was 34.89% higher in female offspring than in male offspring ($t = -3.172$, $P = 0.003$) (Fig. 3d).

Discussion

In this study, we evaluated the effect of maternal and paternal sizes on offspring quality in the caridean shrimp *N. davidi*. Fecun-

Table 2. Comparison of most optimal models for the response variables protein, lipid, and glycogen concentrations in 60-day-old male and female red cherry shrimps (*Neocaridina davidi*).

Response variable	Explanatory variables	R ²	AIC
Protein concentration	Maternal mass + paternal mass + offspring sex	0.181	237.688
	Paternal mass + offspring sex	0.150	236.896
	Offspring sex	0.086	237.304
Lipid concentration	Offspring sex*	0.245	163.117
Glycogen concentration	Maternal mass + paternal mass + offspring sex	0.096	81.005
	Paternal mass + offspring sex	0.096	79.008
	Offspring sex	0.076	77.627

Note: AIC, Akaike's information criterion. *, $P < 0.0167$.

dity is one of the most widely used criteria to assess juvenile production (Racotta et al. 2003), with the present results showing a positive correlation between actual fecundity and maternal mass. These results differ from those obtained previously by Tropea and López Greco (2015) in the same species, who reported a statistically significant relationship between realized fecundity (i.e., number of eggs per spawn per female) and maternal mass, but not between actual fecundity and maternal mass. Even though larger females laid more eggs, we hypothesized that egg loss may increase with maternal mass, leading to an equal production of newly hatched juveniles. Notwithstanding that egg loss was not measured in our study, it is evident that any egg loss would have affected all females equally, regardless of their mass; consequently, the higher the realized fecundity, the higher the number of JI. Guay et al. (2011) suggested that realized fecundity and offspring size are conditioned by nonadaptive anatomical scaling constraints, as eggs and offspring must fit in female reproductive structures. These anatomical constraints may be particularly important in a species with direct development (Marshall and Keough 2007), such as *N. davidi*. Actual fecundity was also found to increase with maternal size in other freshwater species with direct development such as the Australian yabby (*Cherax destructor* Clark, 1936) (Austin 1998; Truong et al. 2002), the red-claw crayfish (*Cherax quadricarinatus* (von Martins, 1868)) (Tropea et al. 2012), and the spiny-cheek crayfish (*Orconectes limosus* (Rafinesque, 1817)) (Kozák et al. 2006).

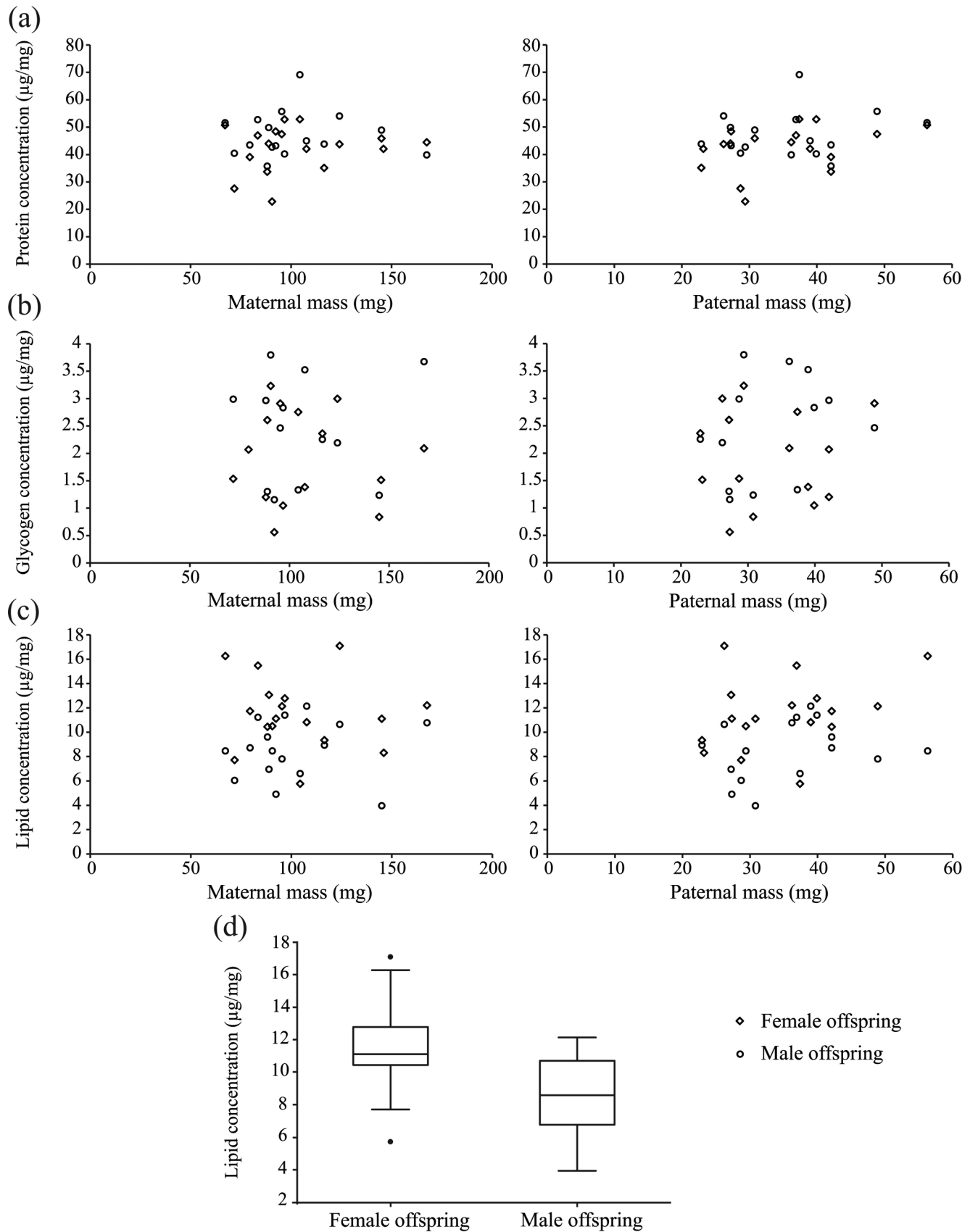
There are several examples of marine decapod crustaceans in which paternal mass has an effect on actual fecundity. In the coconut crab (*Birgus latro* (Linnaeus, 1767)) and the blue crab (*Callinectes sapidus* M.J. Rathbun, 1896), this is related to the spermatophore volume, the number of sperm per spermatophore, and the size of transferred ejaculates increase with paternal size (Jivoff 2003; Sato et al. 2008). Consistent with this, females of the king crab (*Paralithodes brevipes* (H. Milne-Edwards and Lucas, 1841)) showed significantly lower fertilization rates when mated with small males, as they were not able to increase ejaculate size with larger females. Female fecundity was therefore limited by the amount of sperm received from males (Sato et al. 2006). Similarly, clutch mass was found to depend on paternal and spermatophore sizes in the Caribbean spiny lobster (*Panulirus argus* (Latreille, 1804)) (Butler et al. 2015) and in the southern rock lobster (*Jasus edwardsii* (Hutton, 1875)) (MacDiarmid and Butler 1999). Unlike these species, the Japanese spiny lobster (*Panulirus japonicus* (Von Siebold, 1824)) showed a dependence of clutch size on maternal size, but not on paternal size, with similar fertilization rates for all parental sizes. This indicates that males were able to transfer an adequate amount of sperm independently of female size (Jinbo et al. 2017). The finding in the present study that actual fecundity was solely influenced by maternal mass suggests that the amount of sperm transferred by small males was sufficient for egg fertilization. This may be a result of the low number of eggs in freshwater decapods, such as *N. davidi*, compared with marine species. However, since realized fecundity was not measured in the present contribution, further studies are needed to assess whether

males adjust the amount of sperm that they transfer to females in relation to their size, allocating less sperm when mating with small females and thus reducing the energetic cost associated with reproduction. This behaviour has been previously observed in the freshwater crayfish *Austropotamobius italicus* (Faxon, 1914) (Rubolini et al. 2006).

Regarding juvenile quality, JI mass was not associated with paternal mass or maternal mass. Similarly, juvenile size is independent of maternal size in *C. quadricarinatus*, another freshwater decapod with direct development (Tropea et al. 2012). On the other hand, juvenile size was found to increase when females mated with large males in a decapod species (red swamp crayfish, *Procambarus clarkii* (Girard, 1852)) with a promiscuous mating system, similar to that of *N. davidi*. Females seem to adjust the quantity of deutoplasm stored in oocytes before spawning, and they may allocate more resources to broods sired by large males, which represent high-fitness offspring (Aquiloni and Gherardi 2008). It is important to note that the weak relationship or the lack of relationship between parental size and offspring size observed in some studies, including ours, may be the result of the constant and controlled laboratory conditions under which they were performed. Hence, field studies are required to corroborate whether the studied traits (e.g., paternal size) have a stronger influence on offspring quality in their natural environment (Marshall et al. 2010). Finally, we assessed the association between parental mass and offspring quality, by evaluating juvenile performance during a 60-day growth period. Paternal and maternal masses had no influence on growth increment or biochemical composition of juveniles. These results, together with the lack of association between JI mass and paternal mass, suggest that the primary reproductive investment of similar-sized females is unaffected by paternal size. Whether there is some kind of mate choice when females are given the option to mate with different-sized males remains to be determined. Interestingly, lipid concentration was higher in female offspring than in male offspring. During the maturation process, females incorporate lipids into their developing ovaries and store them in the oocytes (Rosa and Nunes 2003a, 2003b). The increased lipid levels in the ovaries of maturing females could account for the differences observed between male and female offspring by the end of the 60-day growth period.

In summary, the present study evaluated the effect of maternal and paternal sizes upon offspring quality and maternal reproductive performance in the freshwater shrimp *N. davidi*. Maternal mass was found to influence actual fecundity, but no effect of parental size was detected on juvenile size at hatching, growth, or biochemical composition. To our knowledge, this is the first study to assess the effects of both maternal and paternal sizes on offspring quality in this species. Several questions arise regarding male reproductive investment and future studies should address the possibility of male mate choice in relation to female size. The potential ability of males to allocate varying amounts of sperm when mating with different-sized females should also be evaluated in the future.

Fig. 3. Biochemical composition of male and female red cheery shrimps (*Neocaridina davidi*) after a 60-day growth period following hatching. The association between protein (a), glycogen (b), and lipid (c) concentrations ($n = 33, 33,$ and $28,$ respectively) with maternal and paternal masses were analysed for male and female offspring. Significant statistical differences in lipid concentration were found between sexes (c).



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