Original Article

Nauplius

Crustacean Society

e-ISSN 2358-2936 www.scielo.br/nau www.crustacea.org.br

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Submitted 24 October 2021 Accepted 13 October 2022 Published 22 September 2023

DOI 10.1590/2358-2936e2023018

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Nauplius, 31: e2023018

Shelter preference and variation in the daily activity pattern of the ornamental shrimp *Neocaridina davidi* (Caridea: Atyidae)

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ABSTRACT

Neocaridina davidi (Bouvier, 1904) is a shrimp commonly used in aquaculture throughout the world. It is an exotic species in several places. We investigated the preference of *N. davidi* for four types of shelter (rock, *Vesicularia* sp., *Egeria* sp., and wood) and its nictemeral activity variation. Individuals were divided into three experimental groups: males, non-ovigerous females, and ovigerous females. They were placed in tanks with the four shelter types and observed every 3 hours for 72 hours. *Neocaridina davidi* presented cryptic behavior with 82.1 \pm 8.69% of individuals sheltering per observation. Sheltering was higher during the daytime (88.8 \pm 5.54%) than at nighttime (75.4 \pm 5.53%). *Vesicularia* sp. was the most used shelter followed by wood. The least used shelter type was a rock. Swimming and walking were more frequently observed at nighttime, especially for ovigerous females. We have demonstrated the importance of providing shelter for the maintenance of *N. davidi* in tanks as well as its higher activity at nighttime.

KEYWORDS

Aquarium trade, aquaculture, behavioral experiments, circadian rhythm, red cherry.

INTRODUCTION

The shrimp *Neocaridina davidi* (Bouvier, 1904) belongs to the family Atyidae and is native to China, Korean Peninsula, Taiwan, and Vietnam. It is broadly used in the aquarium trade throughout the world and is an exotic species in several locations outside its native distribution range (Cai, 1996; Liang, 2004; Wowor et al., 2004; Klotz et al., 2013; Mitsugi and Suzuki, 2018; Jablońska et al., 2018; Schoolman and Arndt, 2018). Its relatively small size and noticeable coloration are among the most attractive attributes related to its use by aquarists (Pantaleão et al., 2015).

Neocaridina davidi has been studied in recent years regarding its basic biology and improvement of culture conditions. There has been a special focus on post-embryonic development, effects of starvation, presence of biofilm and population density on growth and reproduction (Pantaleão et al., 2015; 2017; Wlodarczyk et al., 2017; 2019). Recently Tomas et al. (2020) have demonstrated the influence of color substrate on coloration of female individuals. No studies have yet focused on daily activity rhythm.

Shelters are valuable resources for crustaceans as they act as a defense against predators, lower the risk of cannibalism, and attract sexual partners (Marshall et al., 2005; Edwards and Herberholtz, 2005). Shelter type can influence survivorship and harvested biomass of cultivated species (Jones and Ruscoe, 2001). Shelters with higher structural complexity tend to be more attractive to shrimps as they provided more individualized spaces for animals to hide (Park et al., 2015). Decapod crustaceans are sensitive to stress (Elwood et al., 2009; Elwood, 2012; Magee and Elwood, 2013; Fossat et al., 2014), so creating a more pleasant environment by providing shelter could improve living conditions.

Shrimp species show a variation in their activity pattern throughout the day, being more active at night. This pattern has been reported for caridean species such as *Palaemon xiphias* Risso, 1818 (Guerao, 1995), *Palaemon serratus* (Pennant, 1777) (Guerao and Ribera, 1996), *Processa edulis* (Risso, 1816), and *Palaemon adspersus* Rathke, 1837 (Guerrao and Abelló, 1996), as well as for Dendrobranchiata species such as *Penaeus monodon* Fabricius, 1798, *Penaeus semisulcatus* De Haan, 1844 (Moller and Jones, 1975), Solenocera membranacea (Risso, 1816) (see Aguzzi et al., 2006), and Artemesia longinaris Spence Bate, 1888 (see Carvalho-Batista et al., 2012). Understanding the circadian rhythm of a cultivated species can improve its management by adjusting the feeding time to when animals are more active (Santos et al., 2016). Furthermore as N. davidi is widely utilized in the aquarium trade individuals of this species can be exposed to artificial light constantly. Artificial light at night can alter the behavior of some species of decapod crustaceans (Fisher et al., 2020), and the disruption of circadian cycle by the exposure to artificial light promote physiological alterations and increase the mortality rate (Fanjul-Moles et al., 1998; Farhadi and Jensen, 2016). So, the knowledge of their daily rhythm and how the species behaves each hour of the day is also essential to provide optimal conditions to these animals.

We investigate the preference of *N. davidi* for shelter type as well as their daily activity variation and shelter use. We hypothesize that individuals will use shelters during most of their daytime cycle and will prefer shelters with high structural complexity. We also hypothesize that most locomotion will happen during the nighttime.

MATERIAL AND METHODS

Animal acquisition and culture

The specimens were commercially acquired and transported to the laboratory where they were kept in tanks. They were fed twice a day with the commercial fish food TetraColor (TETRA; Tetra GmbH, Herrenteich, Germany) with the following approximate composition: 47.5% minimum crude protein, 6.5% minimum crude fat, 2.0% maximum crude fiber, 6% max moisture, 1.5% minimum phosphorus, 100 mg.kg⁻¹ minimum ascorbic acid (vit C), 8000 mg.kg⁻¹ minimum omega 3-fatty acids. This diet was previously tested and has been considered adequate for culture of this species (Pantaleão et al., 2015).

Shrimps were placed in three tanks 24 hours before the beginning of the experiments for acclimation. Tanks measuring $44 \times 20 \times 27$ cm (length \times width \times height) with a 23-liter capacity were filled with filtered water and kept aerated with a pump, with constant light and temperature (23 °C). All material was cleaned between replicates; tanks and shelters were washed with treated running water to remove microorganisms that could have grown from previous experiments.

Sexing was based on macroscopic analysis of the following secondary sexual characters: presence (males) or absence (females) of the appendix masculina on the second pair of pleopods (Smith and Williams, 1980), and larger first pair of pleopods (males) (Pantaleão et al., 2017). Ovigerous females were those with embryos attached to the pleopods.

Experimental procedure

Shrimps were divided into three experimental groups: males, non-ovigerous females, and ovigerous females. Ten individuals from the same experimental group were placed together in each tank with four shelter types: 1) Java moss (*Vesicularia* sp.); 2) rock fragment; 3) Brazilian Waterweed (*Egeria* sp.); and 4) piece of wood. All shelters used in the experiments had a total surface area of approximately 250 cm² and were placed at least 5 cm apart (Fig. 1). Shelter areas were measured using the software ImageJ (Schneider et al., 2012). Shelter position was varied in the replicates to avoid placement effect (1 to 4) and preference for a specific side of the tank. Five consecutive replicates

were made for each shrimp experimental group (totaling 5 tanks and 50 shrimps per group). All shrimps used in the experiments had their carapace length (CL) measured with a digital caliper (0.01 mm), and we attempted to minimize the size variation inside the groups by choosing individuals with similar CL to compose the groups. The carapace length varied from 3.04 to 3.68 mm (3.41 \pm 0.28 mm) for males, 4.74 to 6.39 mm (5.44 \pm 0.49 mm) for non-ovigerous females, and from 5.1 to 6.39mm (5.74 \pm 0.64 mm) for ovigerous females.

Animals were kept at a 12/12-hour light/dark cycle throughout the experiment. A white fluorescent light bulb (15 Watts) was used for the daytime phase while a red incandescent light bulb (15 Watts) was used for the nighttime observations (Rodriguez and Naylor, 1972; Hindley, 1975; Scudder et al., 1981; Pontes, 2006). The room where the experiments were carried out is closed to prevent any light entry, so the only light available came from the light bulb. After acclimation, animals were observed for three consecutive days. Individual behaviors were evaluated by the focal animal method (adapted from Martin and Bateson, 1993). The number of animals in shelters was recorded every two minutes for 20 minutes throughout pre-established observation periods, totaling 11 records per observation period. We established eight observation periods in a 24-hour period (adapted



Figure 1. Representation of experimental tanks containing the four shelter types (*Egeria* sp., wood, Java moss, and rock) offered to *Neocaridina davidi* (Bouvier, 1904).

from Freire et al., 2011): four periods in at daytime (7am: 07:00 – 07:20am; 10am: 10:00 – 10:20am; 1pm: 1:00 – 1:20pm; 4pm: 4:00 – 4:20pm) and four at nighttime (7pm: 7:00 – 7:20pm; 10pm: 10:00 – 10:20pm; 1am: 1:00 – 1:20am; 4am: 4:00 – 4:20am). The number of individuals not using shelters and animals walking or swimming was also recorded during the observations to investigate the daily activity pattern for the species (Fig. 2). Animals were not fed throughout the experiment.

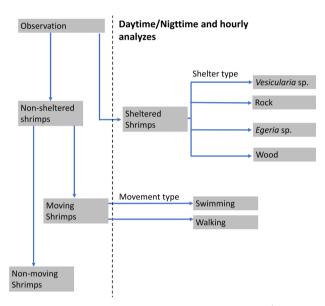


Figure 2. Recorded behaviors of *Neocaridina davidi* (Bouvier, 1904) during the observation periods.

Characterization of shelters and degree of complexity

The degree of complexity of each shelter was defined according to its architecture (Beukers and Jones, 1998), for example, if the structure of a shelter allows a shrimp to enter its interior, the degree of complexity of that specific shelter is considered high. And if the structure of a shelter is simple, with little branching and making it impossible for the shrimp to hide completely, the degree of complexity of this shelter is considered low.

Among the four shelters used in the present study, the ones with the highest degree of complexity were wood and Java moss, while *Egeria* sp. and rock were the shelters with the least complexity. Both the wood, and especially the Java moss, had openings that allowed the shrimp to enter their interior, either partially (wood) or fully (Java moss). The branches of Java moss float in the water column, creating numerous small openings that the shrimps could access while remaining hidden within the moss. The wood fragments had small cracks allowing a limited number of shrimps to hide. On the other hand, *Egeria* sp. and the rocks have a simpler architecture, not having many branches or crevices where these shrimps could enter and be hidden, so they were considered shelters with low structural complexity.

Beyond the difference in complexity level the shelters were chosen due to their popularity with aquarists and because they can be easily found and used by shrimp keepers. Plants of the genus *Egeria* are very common in Brazil and very popular in aquarium trade (Negrisoli et al., 2003; Lorenzi, 2008), wood and rock are used by many aquarists to ornament tanks. Moreover, Java moss has already been used as a shelter in experiments with *N. davidi* (Tomas et al., 2020).

Data analyses

The number of animals presenting each behavior was converted to frequency within the tank. The Wilcoxson test was used to compare shelter use frequency (regardless of type) between daytime and nighttime. The same test was used to analyze the frequency of moving individuals between daytime and nighttime. To assess the difference between shelter type usage and to compare the frequency of moving individuals during observation times we used the Friedman test, followed by the Student-Newman-Keuls test. A significance interval of 95% was used for all tests (Zar, 1999) for both general and group-related (male, non-ovigerous female, ovigerous female) results.

RESULTS

Shelter use

Neocaridina davidi presented highly cryptic behavior. In all observations, most individuals were using the shelters, with an average of $82.1 \pm 8.69\%$ sheltered individuals. The frequency of individuals using shelters was significantly higher during the daytime ($88.79 \pm 5.54\%$) than at nighttime ($75.44 \pm 5.53\%$) (Wilcoxson, Z = -24.7, P < 0.001). Ovigerous females presented more pronounced cryptic behavior, with an average of $88.81\% \pm 11.32\%$ of individuals sheltered per observation while the average was $78.27 \pm 9.90\%$ for non-ovigerous females and $74.71\% \pm 13.17\%$ for males. All experimental groups sheltered more during the daytime, particularly ovigerous females (Wilcoxson, Z = -16, P < 0.001) (Tab. 1).

In all experimental groups, 7 am was the interval with the highest percentage of sheltered individuals. The proportion of sheltered individuals fluctuated throughout the daytime. There was a significant reduction in shelter use during the nighttime (Wilcoxson, P > 0.01) (Tab. 1, Fig. 3).

least used shelter was rock fragment, with an average of 2.47 \pm 1.60% of individuals per observation, and it was also the shelter least used by all experimental groups (Fig. 4). The shelter most frequently used by males and ovigerous females was Java moss, with 31.01 \pm 24.44 and 57.02 \pm 22.01% of individuals per observation, respectively. For non-ovigerous females, wood was the most frequently used shelter type (35.70 \pm 17.73%) although there was no significant difference between the use of this shelter type and Java moss for this class (35.38 \pm 21.52; Friedman test, P > 0.05).

Activity pattern

Shelter type preference

The most frequently used shelter was Java moss, with an average of $41.14 \pm 16.27\%$ of individuals. The

The average frequency of moving animals during each observation was $4.80 \pm 4.53\%$. Of these, 3.19 ± 1.71 % were walking and $6.42 \pm 5.72\%$ were swimming. Males moved more frequently ($6.72 \pm 7.02\%$) than both non-ovigerous females ($4.08 \pm 5.68\%$) and

 Table 1. Percentage of individuals of Neocaridina davidi (Bouvier, 1904) using shelters (average ± standard deviation) during the daytime and nighttime.

| Activity | Category | Day (%) | Night (%) | Ζ | Р |
|---------------|-----------------------|------------------|-------------------|---------|---------|
| Shelter usage | General | 88.79 ± 5.54 | 75.44 ± 5.53 | -24.701 | < 0.001 |
| | Males | 82.55 ± 8.28 | 66.87 ± 12.59 | -15.306 | < 0.001 |
| | Non-ovigerous females | 83.58 ± 7.43 | 72.97 ± 9.31 | -12.726 | < 0.001 |
| | Ovigerous females | 96.04 ± 4.05 | 81.58 ± 11.94 | -16.007 | < 0.001 |

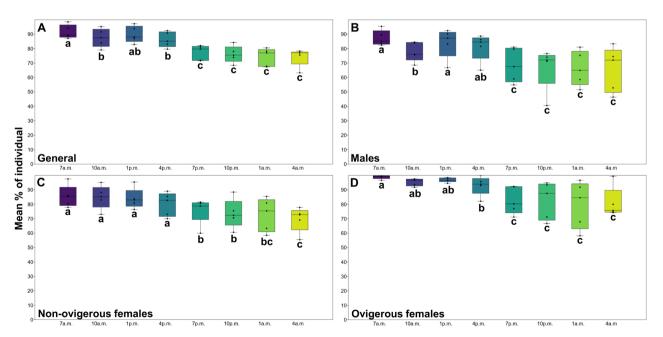


Figure 3. Frequency of individuals of *Neocaridina davidi* (Bouvier, 1904) using shelters at each observation time. The line inside the box indicates the median, box indicates the first and third quartiles and the whiskers indicate the minimum and maximum variation. **A**, General; **B**, males; **C**, non-ovigerous females; **D**, ovigerous females. Different letters indicate significant difference (P < 0.05).

ovigerous females ($3.80 \pm 5.82\%$). During the daytime, the general moving frequency was $2.13 \pm 1.33\%$. The majority of moving individuals were walking ($2.56 \pm 1.55\%$) while the average swimming frequency was $1.69\% \pm 0.92\%$. Most moving was recorded at nighttime. During this period, the general moving frequency was $7.49 \pm 5.01\%$ and there was an increase in both walking ($3.82 \pm 1.66\%$) and swimming ($11.15 \pm 4.52\%$) frequency. Males moved more frequently than the other experimental groups in both periods (daytime and nighttime) and ovigerous females presented a higher distinction in movement between periods (Tab. 2).

There was a difference in walking individuals between observation times (Friedman test, P < 0.05). Walking frequency was significantly lower at 7 am generally and for each class separately (Friedman test, P < 0.05). The remaining observation times varied slightly in walking frequency. The highest frequency values were recorded between 4 pm and 1 am. There is a clear separation of swimming individuals between periods. No significant difference was recorded during the daytime (7 am, 10 am, 1 pm, and 4 pm; Friedman

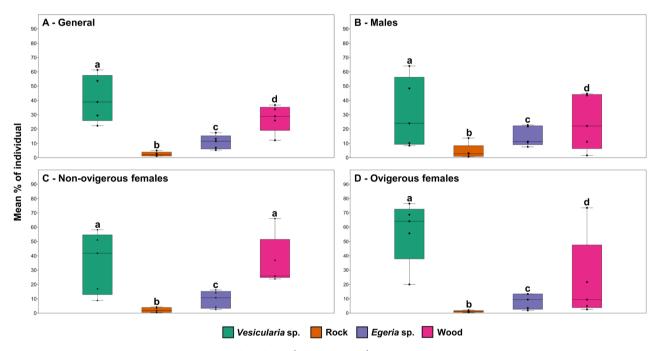


Figure 4. Frequency of individuals of *Neocaridina davidi* (Bouvier, 1904) using each shelter type. The line inside the box indicates the median, box indicates the first and third quartiles and whiskers indicate the minimum and maximum variation. **A**, General; **B**, males; **C**, non-ovigerous females; **D**, ovigerous females. Different letters indicate significant difference (*P* < 0.05).

| Table 2. Frequency (average ± standard deviation) of moving individuals (walking or swimming), generally and by experimental |
|--|
| group, during the daytime and nighttime. |

| Activity | Category | Daytime (%) | Nighttime (%) | Ζ | Р |
|----------|-----------------------|-----------------|------------------|--------|---------|
| Walking | General | 2.56 ± 1.55 | 3.82 ± 1.66 | 5.555 | < 0.001 |
| | Males | 4.30 ± 2.74 | 5.41 ± 3.36 | 2.663 | 0.008 |
| | Non-ovigerous females | 2.27 ± 2.94 | 3.13 ± 2.26 | 2.328 | 0.020 |
| | Ovigerous females | 1.12 ± 1.68 | 3.02 ± 3.47 | 5.171 | < 0.001 |
| Swimming | General | 1.69 ± 0.92 | 11.15 ± 4.52 | 24.854 | < 0.001 |
| | Males | 2.57 ± 2.36 | 13.72 ± 9.73 | 15.321 | < 0.001 |
| | Non-ovigerous females | 1.52 ± 1.25 | 9.47 ± 8.78 | 13.870 | < 0.001 |
| | Ovigerous females | 0.76 ± 1.30 | 10.32 ± 7.84 | 14.799 | < 0.001 |

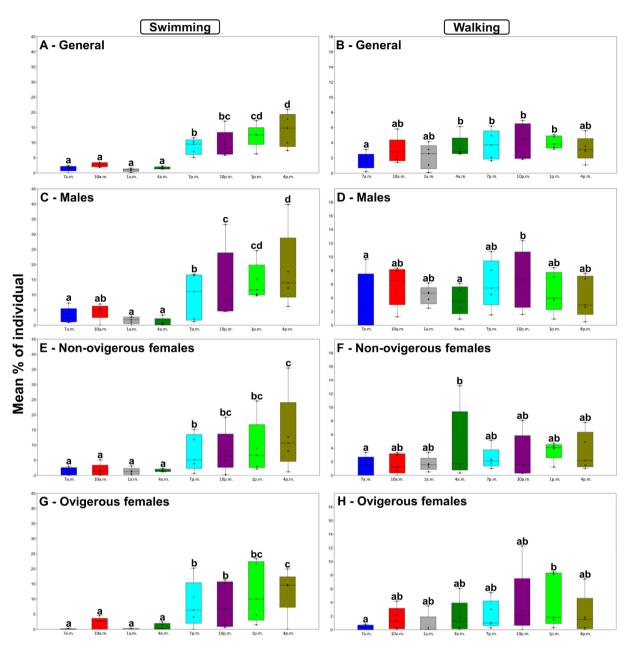


Figure 5. Frequency of individuals of *Neocaridina davidi* (Bouvier, 1904) swimming (left) and walking (right). **A** and **B**, General; **C** and **D**, males; **E** and **F**, non-ovigerous females; **G** and **H**, ovigerous females. Different letters indicate significant difference (*P* < 0.05).

test, P > 0.05), followed by a gradual increase of frequency during the nighttime, peaking at 4 am (Fig. 5).

DISCUSSION

We observed a highly cryptic behavior for *N. davidi* with a tendency of sheltering, especially during the daytime, confirming our initial hypothesis. As this species is frequently used in aquariums, providing

shelter sites seems an essential measure to improve the wellness of individuals. By sheltering, individuals can overcome the potential stress of excessive light in open spaces.

The analyzed shrimps were inactive in most observation times with most of these individuals in shelters. However, there was variation regarding sex and reproductive status in females. Several factors can influence the use of shelters, such as shelter quality, individual social position, and the presence of other individuals in the same tank. These factors have also been observed for the freshwater prawn *Macrobrachium australiense* Holthuis, 1950 (Lammers et al., 2009). The more pronounced cryptic behavior of ovigerous females can be related to the protection of the offspring, which is a natural strategy to ensure reproductive success, minimizing the number of eggs that could be lost or aborted due to exposure to predators and locomotion (Maciá and Robinson, 2009; Duarte and Flores, 2017; Pescinelli et al., 2017).

As for the activity behavior, we noticed that males are more active in comparison to females and ovigerous females, therefore, these individuals move more frequently in the tanks, even during daytime, but mainly at nighttime. This may be a natural reflection of the mating system called "pure search" (Sganga et al., 2016; Vazquez et al., 2017). In this system, males have a strategy to keep swimming in search of receptive females, when finding these females, these individuals copulate quickly and then the males go back to looking for other receptive females (Correa and Thiel, 2003; Bauer, 2004). Therefore, the activity pattern observed in this study may be a reflection of the natural behavior and associated with the type of sexual system for this species, with males moving more frequently due to the search for sexual partners.

Although it has been recently demonstrated that the Java moss Vesicularia sp. in tanks does not affect N. davidi survivorship (Tomas et al., 2020), this shelter type was the most frequently used by individuals. This higher frequency of use is probably related to its higher structural complexity, as it creates a three dimensional environment. Habitat complexity can determine organism distribution (Figueiredo et al., 2013; Gartner et al., 2013). High complexity habitats present higher food availability due to a larger surface for microalgae colonization and provide greater protection against predators (Chemello and Milazzo, 2002). In an experiment with artificial shelters, Park et al. (2015) observed a higher number of juvenile Pandalopsis japonica Balls, 1914 attached to the brushtype shelter and related this finding to the higher availability of independent spaces in relation to the other shelter types.

The only sex class in which Java moss was not the most frequently used shelter type was non-ovigerous females. Similar to *Macrobrachium brasiliense* (Heller, 1862) (Nogueira et al., 2018), this sex class used the wood shelters more frequently. According to the authors, wood favors biofilm formation while presenting gaps that can be used as hiding sites. The wood itself is a decaying matter source, which would favor biofilm formation on its own. Rock was the least used shelter type by all experimental groups, probably due to its three dimensional characteristics and lower structural complexity.

We observed a circadian rhythm regarding moving activity of N. davidi. Moving activity peaked at dawn, decreasing abruptly throughout the day. There was an increase in moving activity at 10 am and another decrease in frequency at the beginning of the afternoon. Several marine and freshwater caridean shrimps present higher activity during the night (Guerao, 1995; Guerao and Ribeira, 1996). This pattern has also been reported for Dendrobranchiata and other decapod groups, especially as a strategy of protection against visual predators, such as fish (Dall et al., 1990; Pontes, 2006; Ogburn et al., 2013; Trevisan et al., 2015). Furthermore, there was an increase in the swimming frequency of all experimental groups at nighttime. Such an increase was also observed in juveniles of Macrobrachium rosenbergii (de Man, 1879) (Scudder et al., 1981) and in females of Palaemon varians Leach, 1814 and P. longirostris H. Milne Edwards, 1837 (Finchan and Furlong, 1984).

We observed a cryptic behavior for *N. davidi* with a tendency of sheltering during the daytime, especially for females and even more for ovigerous ones. Java moss (*Vesicularia* sp.) was the most used shelter type and therefore recommended for microhabitat composition in tanks for aquarists. Finally, we have here demonstrated an increase in activity during nighttime and a shift in moving patterns between periods. While walking was predominant during the daytime, swimming was more frequent during the nighttime. This might be a reflection of natural behavior corresponding to the period of searching for food and sexual partners.

ACKNOWLEDGMENTS

We are grateful to all our laboratory colleagues and coworkers for their help during the experimental procedures.

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ADDITIONAL INFORMATION AND DECLARATIONS

Author Contributions

AC-B, CSN, and JAFP equally contributed to the design, execution, and analysis of the experimental results. All authors equally contributed to the writing and revision of the manuscript.

Consent for publication

All authors declare that they have reviewed the content of the manuscript and gave their consent to submit the document.

Competing interests

The authors declare that there are no conflicts of interest.

Data availability

The data that support the findings of this study are available from the corresponding author, AC-B, upon reasonable request.

Funding and grant disclosures

RCC thank the National Council for Scientific and Technological Development – CNPq (Research Scholarships PQ# 304368/2022-9) and São Paulo Research Foundation - FAPESP (Biota INTERCRUSTA 2018/13685-5).

Study association

This study was conducted at the Laboratório de Biologia de Camarões (LABCAM) and is part of the research focus of the group.

Study permits

This study was conducted under the permission (SISBIO n° 23012-3) issued by the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA).