

# Ontogenetic niche specialization of the spider crab *Libinia ferreirae* associated with the medusa *Lychnorhiza lucerna*

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## Abstract

Symbiotic relationships in marine environments are not fixed and can change throughout the animal's life. This study investigated the ontogeny of symbiosis of the spider crab *Libinia ferreirae* with the host medusa *Lychnorhiza lucerna*. We described the type of relationship, the temporal correlation among species, and food habits. More than 50% of the sampled crabs were symbionts, most in early life stages. The highest number of crabs found in a single medusa was 11. Symbiosis was observed throughout most of the year but was more evident in warm periods. The crab has many benefits in this relationship with a medusa. One is the use of food resources captured by the medusa, primarily copepods. Because the crab steals the medusa's food, it is a kleptoparasitic relationship. There is a niche partition between symbiont and the free-living crabs as they occupy different habitats and use nonoverlapping food resources. Previous research reported that symbiosis first developed during the crab's last larval phase (megalopa) when crab and medusa are in the same habitat. Observation of the crab's behavior shows that symbiosis occurs when the crab can grab to the medusa when the host touches the sea bottom. The crab also took advantage of water currents, releasing itself from the substrate and then drifting toward the medusa. The symbiotic relationship that crabs have with the medusa provides them with a nursery, food resources, shelter, dispersion, and decreased competition with free-living adult crabs, all essential for the crab's survival.

**Keywords:** behavior, development, kleptoparasitism, megalopa, niche segregation

Scyphomedusae are involved in many symbiotic relationships with different species. One example is the medusa *Lychnorhiza lucerna* Haeckel, 1880 from the Southwestern Atlantic Ocean (Schiariti et al. 2008). This medusa has received an especial attention due to its abundance, commercial importance for the pharmaceutical industry, and symbiotic ecological relationship with invertebrates and fish (Morandini et al. 2005; Schiariti et al. 2014; Gonçalves et al. 2016). Medusae are relatively large (e.g., *L. lucerna* umbrella diameter [UD] ≥ 38 mm) and therefore considered a morphologically complex refuge, serving as an important floating nursery, habitat/environment, and means of transport for several symbiotic species that depend on this relationship (Morandini et al. 2005; Sal Moyano et al. 2012; Gonçalves et al. 2016). Some studies have shown that this medusa maintains a symbiotic relationship with invertebrates such as the caridean shrimps *Periclimenes pavaei* Chace, 1969 and *Leander paulensis* Ortmann, 1897 (Martinelli-Filho et al. 2008; Gonçalves et al. 2016; Baeza et al. 2017; Moraes et al. 2017), brachyuran crabs *Libinia ferreirae* Brito Capello, 1871, and *Libinia spinosa* Guérin, 1832 (Nogueira and Haddad 2005; Sal Moyano et al. 2012; Gonçalves et al. 2016, 2017a). The relationship with these invertebrates is described as commensalism, a relationship

in which the symbionts are protected and nourished by the medusa. Disadvantages to the medusa in this relationship remain unclear as well as its influence on the symbionts' lives. Knowing more about the symbiotic interaction could explain how the relationship occurs, benefits or damages to the symbiont and host, and whether the relationship is obligatory or facultative.

Symbiosis is an essential strategy for survival that can directly or indirectly affect the host or the symbiont (Peacock 2011). Symbionts can be generalists or specialists, facultative, or obligatory (Leung and Poulin 2008; Martin and Schwab 2012, 2013). According to Leung and Poulin (2008), interactions are usually mutualistic (reciprocal benefits), commensal (symbiont has benefits with no cost to the host), or parasitic (symbiont uses the host as a resource, resulting in harm to the host). Kleptoparasitism (when symbiont steals food from the host) is an interesting symbiotic relationship in which the symbiont takes advantage of the host by feeding on the prey captured by the host (Masuda et al. 2008; Ingram et al. 2017; González-Ortegón et al., 2021). Kleptoparasites do not injure the hosts in directly way other than through loss of nourishment (González-Ortegón et al., 2021), that is, is a form of intra-specific competition (Iyengar 2008). Nevertheless,

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symbiotic interactions are not fixed and may change throughout the life cycle of an organism as well as through evolutionary time (Towanda and Thuesen 2006).

In Brazil, a common symbiotic relationship observed is between the medusa *L. lucerna* and a spider crab of the genus *Libinia* Leach, 1815. *Libinia ferreirae* maintains a distinctive behavior during the juvenile phase compared to other benthic crabs associated with a medusa, probably at the last larval phase and during the initial juvenile stages (Gonçalves et al. 2017a, 2020a, 2020b, 2021). Crabs leave the medusa when they are reaching the adult phase, probably because the medusa dies or is too small for the crab (Sal Moyano et al. 2012; Gonçalves et al. 2017a). Afterward, crabs will move to the marine benthic habitat where they change from symbiotic behavior and become a host for innumerable epibionts, particularly anemones. The epibionts bring protection to the crab as camouflage (Winter and Masunari 2006; Gonçalves et al. 2017a). This strategy is especially observed in ovigerous females to protect the embryos, lowering the risk of predation (Nogueira et al., 2006; Winter and Masunari 2006).

There is evidence that the medusa greatly affects the life of *L. ferreirae*, for example, the niche partition between a symbiont that uses resources available via the host and a free-living crab that uses benthic resources (Gonçalves et al. 2020a). Anger et al. (1989) suggest that the medusa is a specific substrate for the last larval phase (megalopa) of the *L. ferreirae* necessary for metamorphosis to juvenile, proposing that without this medusa substrate, the megalopae dies. The importance of this relationship has been supported by studies that found megalopae associated with medusa (Gonçalves et al., 2017a, 2020b) and by juvenile crabs with 15 mm of carapace width (CW) being only observed in association with medusa and never found alone in the benthos (Gonçalves et al., 2017a, 2020a, 2020b). Gonçalves et al. (2020b) found differences in the abundance and occurrence of symbionts and free-living crabs among seasons and sites when studying *Libinia* spp. populations in different regions. The authors proposed that the medusa host is responsible for maintaining the genetic connection among the species in different regions (Gonçalves et al. 2020b). The fact that medusa are a means of transport that takes their symbionts to different regions has become a matter of concern, because exotic species may be using this relationship to occupy new habitats, as shown by the scyphomedusa *Catostylus tagi* (Haeckel, 1869) (Rhizostomeae) and the nonindigenous spider crab *Pyromaia tuberculata* (Lockington, 1877) by Martins et al. (2020). Therefore, preventive monitoring needs to be carried out for early detection and warning of bioinvasions.

No studies on this symbiotic relationship have yet accounted for 1) the positive or negative effect when a crab is on the host, 2) the type of symbiosis (parasitic, commensalistic, mutualistic), 3) degree of dependency (facultative or obligatory), and 4) the crab's behavior during the relationship. Therefore, we describe the symbiotic relationship between *L. ferreirae* and *L. lucerna*, with the following main objectives: 1) to verify the temporal relationship between crab and medusa, analyzing the life cycle correlation between the 2 species; 2) to analyze the food habits of symbiont and free-living crabs, testing the niche partition theory; and 3) to comprovating the kleptoparasitism relationship that the symbiont crab has with the medusa, that was proposed by Gonçalves et al. (2021). The knowledge of these interactions will provide important

insights into how symbiont and host species coexist and how the type of symbiosis can influence the lives of both species.

## Materials and Methods

Collections of crabs and medusae with or without symbionts were conducted monthly from February 2013 to May 2014. Sampling was done in Cananéia and the adjacent oceanic area in the state of São Paulo, Brazil (25°05'S to 47°53'W), using a shrimp fishery boat equipped with double-rig nets (mesh size 20 mm and 15 mm in the cod end) (Castilho et al. 2012; Queirolo et al. 2016). Samples were collected from 7 sampling stations each month (see Gonçalves et al. 2017a). In March 2013, samples were only collected at 3 sampling stations due to adverse environmental conditions. We sorted the animals on the boat. Each medusa was examined for the presence of crabs in the following body parts: exumbrella, oral arms, oral pillars, gastric cavity, subgenital pouches, and gonads. We recorded the number of medusae without crabs and returned them to the environment. Medusae with associated crabs were carefully isolated in labeled plastic bags. Crabs without a host were placed in plastic bags. All the samples were stored in a thermal box with ice.

Crabs were identified based on relevant literature: for the megalopa stage, we used Pohle et al. (1999); for juveniles and adults, we used Melo (1996) and Tavares and Santana (2012). The identification of the medusa *L. lucerna* was confirmed using Morandini et al. (2005). Animals were carefully sorted by color group according to life habit, that is, 1) white in color, symbiont crabs in association with a medusa (Figure 1B) or 2) dark in color, free-living benthic crabs bearing symbiotic or fouling organisms on the carapace (Figure 1C).

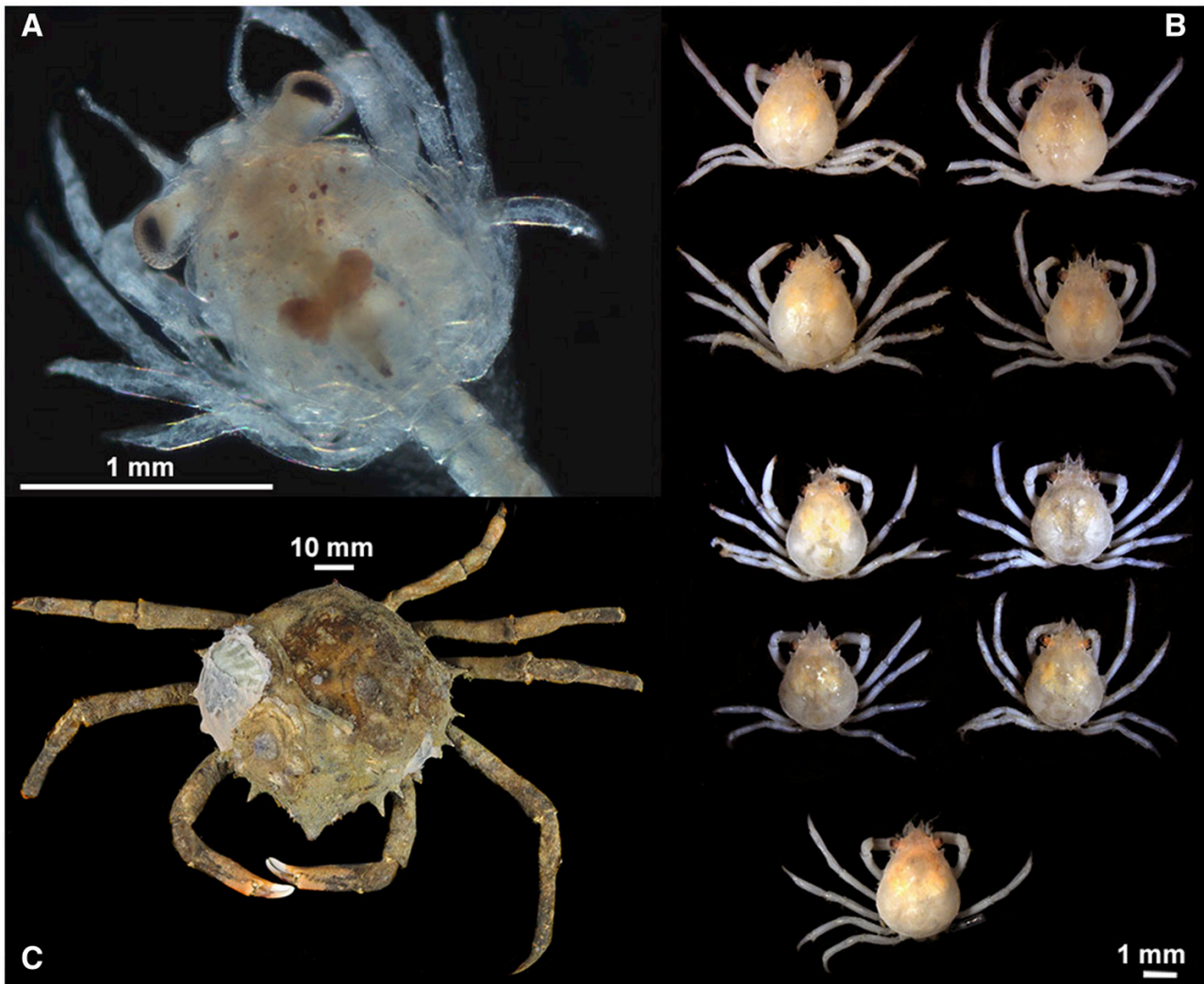
After removing the crabs, each medusa was transferred to a slightly inclined tray to remove the seawater excess. Each medusa's biomass was weighed (MWe) with an analytical balance with a precision of 0.01 g, and its UD = between 2 opposite rophalia was measured using a standard metric ruler. Only undamaged specimens were included in the study (Nogueira and Haddad 2005). UD was grouped into 50 mm size classes.

Crabs were weighed (crab mass = CWe) on an analytical balance (precision of 0.001 g) and the CW was measured (in millimeter). Crabs  $\geq 15$  mm were measured using a digital calliper (0.01 mm precision), whereas  $\leq 14.9$  mm were measured under an optic microscope (Zeiss®, Axioskop 2 plus) and stereomicroscope (Zeiss®, Stemi SV6), equipped with a digital imaging system (Zeiss®, Stemi 2000-C; precision = 0.001 mm).

Crabs were classified as female or male based on the shape of the abdomen and the number of pleopods. Juvenile-adult were classified according to morphological and gonad maturity following Gonçalves et al. (2017b).

## Crab stomach contents

We analyzed the stomach contents of *L. ferreirae* sampled from February 2013 to May 2014. The crabs were classified into the following 4 demographic categories: AS—adult symbiont; JS—juvenile symbiont; AF—adult free-living; JF—juvenile free-living. Stomachs were removed and fixed in 70% alcohol and observed with either a light microscope (Zeiss® Axioskop 2 plus) or stereomicroscope (Zeiss® Stemi SV6), equipped with a digital imaging system (Zeiss Stemi 2000-C; precision = 0.001 mm). The identification of food items was



**Figure 1.** Development stage of the crab *L. ferreirae*. (A) Megalopa (zooplanktonic stage); (B) Juvenile individuals ( $N = 9$ , median  $2.69 \pm 0.13$  mm CW) found associated with a single medusa specimen (167 mm UD) (notice the whitish coloration of the juvenile crab); (C) A benthic adult crab not associated with a medusa (notice the brownish coloration and the presence of epibionts on the carapace).

performed according to Mariscal (1974), Barros et al. (2008), and Gonçalves et al. (2020a).

We used a modified version of the quantitative scoring procedure from Williams (1981) and Mantelatto and Christofolletti (2001) to analyze the food items in the crab's stomach. We evaluated the relative contribution of each food category (in relation to the total volume of each stomach) assigning a classification score from 1 to 10: 1 = contribution of 0–10% of the stomach's volume, 2 = contribution of 10–20% of the stomach's volume, 3 = contribution of 20–30% of the stomach's volume, and so on.

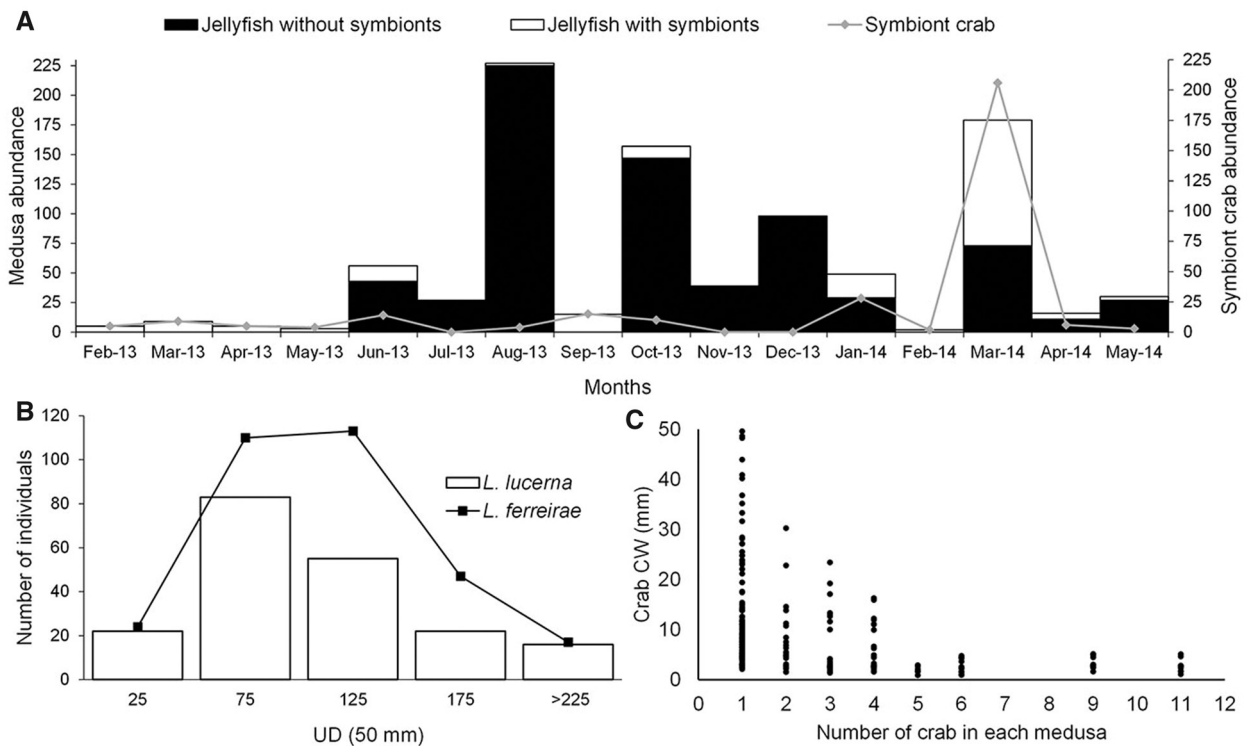
### Data analyses

For the correlation analysis of the medusa–crab relationship, we used 311 crabs that were on or inside the body of the medusa. We did not include 46 symbiont crabs that were unattached after the net was placed on the deck of the boat. As they dissociated during collection, we were unable to identify the medusa they were associated with. Regardless of attachment, we identified them as symbionts due to their white color. Correlations in morphometric relationships of medusa with a single crab were tested as follows: 1) crab CW

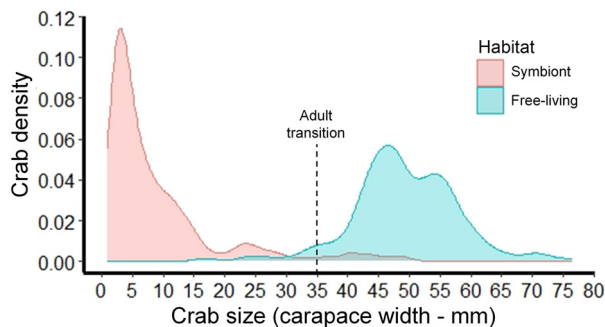
with medusae UD, and 2) CWe with MWe. A Mann–Whitney U test was used to test if there were size differences between crabs associated with medusae and free-living benthic crabs. The wet biomass of the medusa was subtracted from the total biomass (medusa + symbionts crabs) to obtain the overall crab biomass. The biomass of associated crabs carried by a medusa was represented as the ratio between the overall crab biomass and that of the medusa host. Statistical analyses were carried out using R software, package “cars” (R Core Team 2017).

The crab food web network interaction was obtained from the analysis of 339 crabs from 4 demographic categories AS, JS, and AF. We found 13 food item types in these animals. To determine the modularity level in this weighted bipartite network, we used the QuanBiMo algorithm (Dormann and Strauss 2014). This method implements an assimilated annealing Monte-Carlo approach to find the best division of species into modules. A maximum of  $10^{10}$  Markov Chain Monte Carlo steps with a tolerance level of  $10^{-10}$  was used in 339 interactions, retaining the iterations with the highest likelihood value as the optimal modular configuration. We tested whether the network was significantly more modular





**Figure 2.** Symbiotic relationship between symbiont crab and medusa host. (A) Abundance of *L. lucerna* medusa without symbionts and with *L. ferreirae* crab symbionts, in monthly collection from February 2013 to May 2014; (B) Relationship between the number of symbiont crabs ( $N = 311$ ) in the host and the medusa's UD ( $N = 128$ ). X-axis values are the size class midpoint; (C) Number of symbiont crab by CW (mm) according to association abundance (1–11 crabs per medusa) in each medusa host.



**Figure 3.** Crab density related to the size of CW of symbiont and free-living *L. ferreirae* crabs. The dotted line indicates the initial size transition to adult (around 35 mm CW), according to Gonçalves et al. (2017b).

than null networks by running the same algorithm in 1,000 random matrices. The significance of modularity was tested for 339 iterations by comparing the empirical vs. the null modularity indices using a  $z$ -score test (Dormann and Strauss 2014). After testing the modularity of our network, we determined the number of modules and identified the group of each module. Modularity analyses were performed using R software, packages “bipartite” and “vegan” (Dormann et al. 2009; R Core Team 2017).

Statistical analyses were performed using the balanced dataset ( $n = 339$  sequences per sample). Community scale multivariate analyses including nonmetric multidimensional scaling (NMDS) were performed in R (R Development Core Team, 2017) using the statistical package “Vegan” (Oksanen et al. 2009). The model used “food items” as a random factor nested within the fixed factor “demographic categories (AS,

JS, AF, JF).” The NMDS analysis is a nonparametric ordination-based method for reducing the complexity of ecological community data and identifying meaningful relationships among communities. The distance-based permutational multivariate analysis of variance (PERMANOVA) was used to test the difference of food items in the structure of diet among age and habitat for each demographic category (AS, JS, AF, and JF). After that, the specialization of the interaction network of the group of crabs (AS, JS, AF, and JF) vs. food items was tested through the specialization index ( $H2'$ ). Based on Shannon's entropy, we inferred how generalist or specialized the interaction network was.

## Results

We collected a total of 654 crabs (357 medusae symbiont and 297 free-living) in different lifecycle stages including megalopae (Figure 1A), juveniles (Figure 1B), and adults (Figure 1C). A total of 916 medusae of *L. lucerna* were sampled, from which 198 individuals (UD average  $\pm$  standard deviation [SD] =  $108.12 \pm 54.41$  mm, range 24–328 mm) contained 311 associated crabs (98% juveniles with white color, Figure 1B). Crabs were mostly found inside the medusae's oral pillars and gastric cavity, whereas few were found in the medusae's exumbrella and oral arms.

There were 3 peaks of medusae abundance within the study. The highest medusa abundance was seen in August (winter) followed by October (spring) and March (summer). The highest number of symbiont crabs was recorded in a summer month (March 2014) (Figure 2A). In this month, the number of symbiont crabs was higher than the number of hosts carrying symbionts. Crabs were more abundant in median-sized medusae (75–175 mm) UD (Figure 2B).

We found an abundance from 0 to 11 crabs (average 2.72 mm of CW) associated with a single medusa (Figure 2A, C). We recorded 3 medusae carrying 9 crabs. Figure 1B shows 9 crabs with similar size (average  $2.69 \pm 0.13$  mm CW) in the same medusa (167 mm UD). One medusa host (UD 159 mm) was carrying 11 crabs with an average size of CW 1.59 mm (SD  $\pm 1.53$  mm). This was the highest number of symbionts found in a single host. Eight medusae hosted 5–11 crabs, totaling 60 crabs (average of  $2.49 \pm 1.89$  mm CW). Symbiont size was inversely correlated to the number of symbionts per host (Linear Regression,  $r^2 = 0.29$ ;  $df = 308$ ;  $F = 128.6$ ;  $P = 3.9E-25$ ), that is, crabs who occurred as singletons were larger than crabs occurring in counts of  $\geq 2$  (Figure 2C). One symbiont crab carried the anemone *Carinactis dolosa* Riemann-Zurneck, 1975. Symbiotic crabs ranging from CW 0.89 mm (megalopa) to 49.59 mm (average of  $7.85 \pm 8.41$  mm) were significantly smaller than free-living benthic crabs that ranged from 16.35 mm to 76.37 mm (average  $49.27 \pm 8.67$  mm) (Mann–Whitney U-test,  $U = 1214.0$ ,  $n1 = 297$ ,  $n2 = 357$ ;  $P < 0.05$ , Figure 3). A total of 88.75% of symbiotic crabs measured under 15 mm CW. The smallest juvenile crab specimen associated with a medusa had 1.38 mm CW. Three female (40.17–48.25 mm CW) and 4 male (35.16–49.59 mm CW)

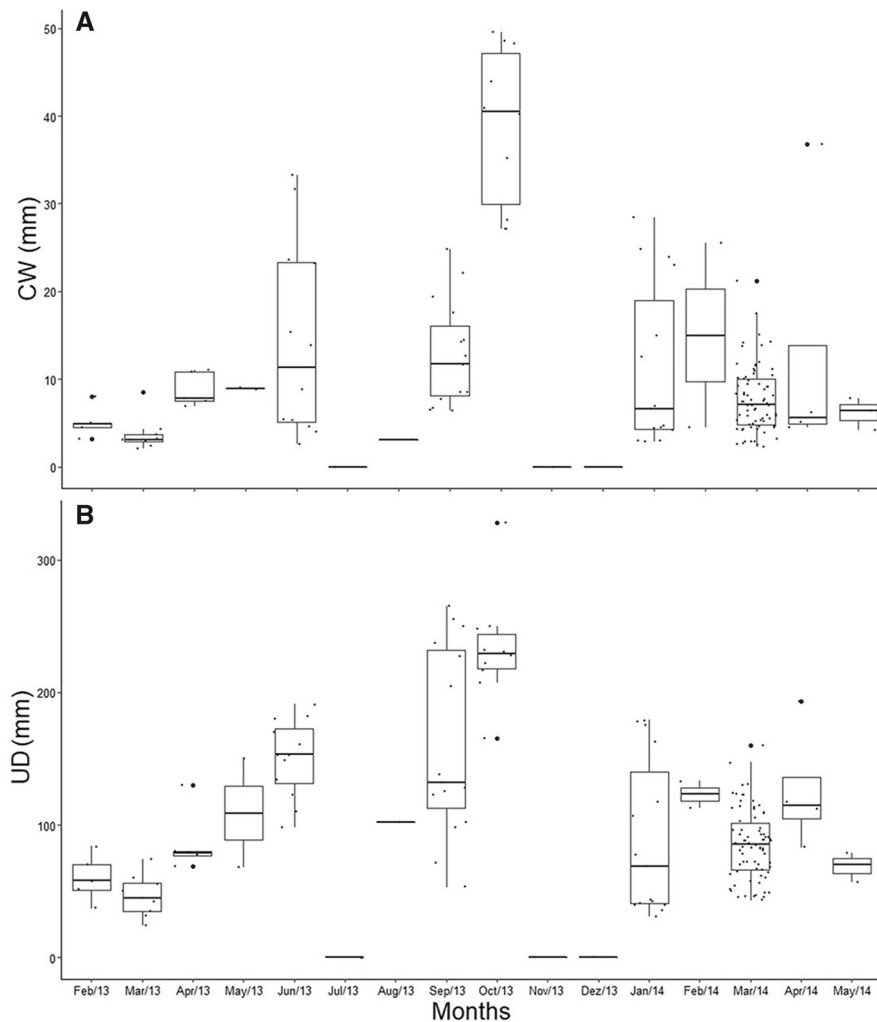
crabs with a size close to maturity were associated with medusa.

A positive correlation between medusa UD and the average CW of its associated crabs was found every month ( $r^2 = 0.76$ ;  $df = 11$ ;  $F = 35.3$ ;  $P < 0.001$ ). Thus, crabs tended to grow along with their medusa over time (Figure 4B). There was a relationship between crab CW and medusa UD (Figure 5A) ( $r^2 = 0.61$ ;  $df = 158$ ;  $F = 249.4$ ;  $P < 0.001$ ) as well as between crab's and the medusa's mass (Figure 5B) ( $r^2 = 0.66$ ;  $df = 158$ ;  $F = 309.9$ ;  $P < 0.001$ ).

The lowest relative crab mass (CWe) carried by a medusa host was 0.01%, and the highest was 14.4%, with an average of 1.57%. A total of 170 medusae carried symbiont crabs with a relative mass in relation to medusa's mass 3%. Only 27 medusae presented crabs with the relative mass in relation to medusa's mass  $> 3\%$ .

### Modularity and NMDS

The network of food interaction resulted from the analysis of 339 crabs' stomachs (data matrix). Less diverse food items were found for JS and JF than for the adults. Symbiont crabs, regardless of ontogenetic phase (AS and JS), exhibited the highest percentage of zooplankton as food, whereas the



**Figure 4.** Symbiont crab and medusa host monthly size. (A) Monthly mean range of CW of *L. ferreirae* crabs associated with the medusae *L. lucerna*. (B) Monthly mean range of the UD of the medusae *L. lucerna*. (Median: percentile 25% and 75%; Min–Max, minimum–maximum values).

free-living crabs (AF and JF) showed the highest percentage of benthic Crustacea as food. We found 12 types of food items and 1 nonfood item (plastic) in the stomachs (Figure 6).

We found modularity of 0.52 of the data matrix from the crabs AS, JS, AF, and JF with 13 different food items ( $P < 0.001$ ), indicating a difference in the consumption of certain types of food. The NMDS shows how groups are distributed in a multidimensional space in relation to the food items they consume (Figure 7). There were differences in the food preference of crabs according to habitat [medusa symbiont (S) and free-living (F)] (PERMANOVA, habitat:  $df = 1, F = 51.7549, P = 9.999e-05$ ). This indicates there is no food niche overlap between symbiont and free-living crabs. There was no significant difference between age and habitat vs. demographic category (PERMANOVA:  $df = 1, F = 0.55, P = 0.72$ , and  $df = 1, F = 1.22, P = 0.27$ , respectively). The network of the crab's

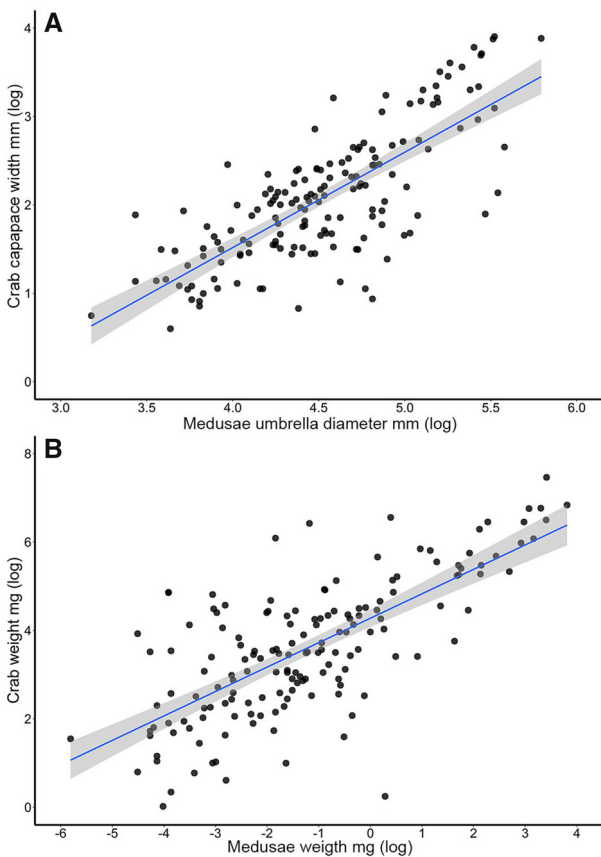
interactions with the food items showed a specialization value of  $H2' = 0.626 (P < 0.001)$ .

### Discussion

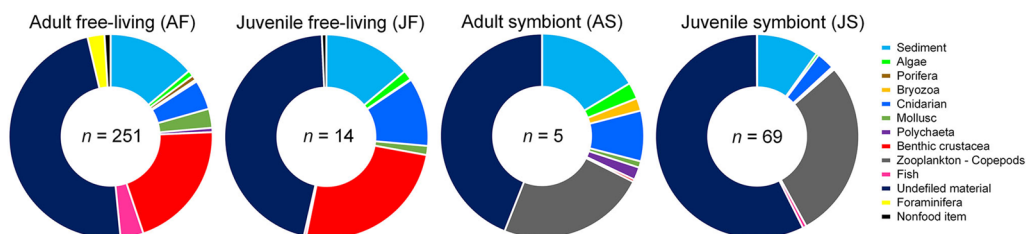
Overall, our study showed that crabs dwell in medusae mostly during the early life stages of megalopae and first-stage juveniles (Figure 3). The peak of association was during warm periods (Figure 2) when marine organisms invest more in reproduction, although symbiosis occurs all year round. This symbiosis results in synchronic growth of the crab and its medusa host, as indicated by the correlation between them (Figure 4), showing that the crab can choose a host and stay with it for a considerable period. Remaining in the same host increases the crab's survival, especially when the crab is more vulnerable due to the moulting process (Gonçalves et al. 2017b). The large abundance of juveniles found in a single medusa (Figure 2C) decreased as the crab's development stage and growth progressed. The host might no longer have enough space for numerous larger size crabs (>15 mm CW). The decline in abundance could also be due to the agonistic behavior of juvenile crabs toward each other (e.g., via confrontation, expulsion, and even death). The symbiotic relationship leads to niche partition because symbiont and free-living crabs live in different environments throughout their lives, not overlapping the food niche (Figure 7). The medusa host provides planktonic food available to the symbiont. Therefore, we propose that the symbiotic relationship in this study is kleptoparasitism—a relationship in which the crab steals food from its host.

In nature, a high number of associations likely occur when the *L. ferreirae* is in the megalopa phase (Anger et al. 1989; Gonçalves et al. 2020b), and this is supported in our study with the higher number of associated crabs that have a size between 1 and 3 mm CW (Figure 3). Crabs in the megalopa stage can easily find *L. lucerna* in the water column because they share the same environment (Carrizo et al. 2016). Anger et al. (1989) proposed that during the *L. ferreirae* transition from megalopa to juvenile phase, this species depends on the stimulus generated by the medusa host. This shows that the symbiotic relationship is essential for the development and survival of the crab with host protection.

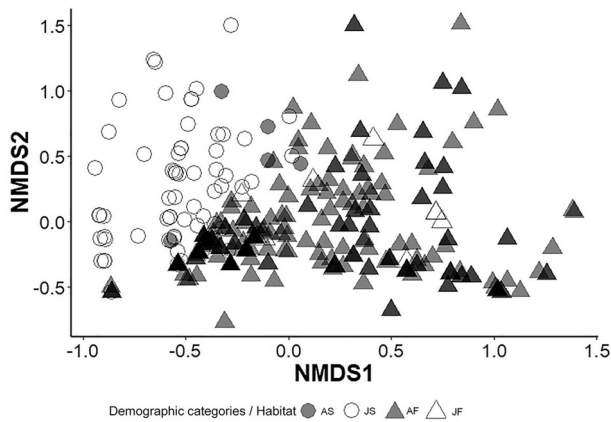
The higher abundance of *L. ferreirae* living with *L. lucerna* in our study seems to be related to the recruitment period of the crabs. This can be a reason why we found a higher abundance of symbiotic *L. ferreirae* than Nogueira and Haddad (2005), and of *L. spinosa* by Sal Moyano et al. (2012). During the recruitment periods, medusae hosted multiple crab symbionts (from 2 to 11; Figure 2C). The abundance peaked was recorded in March 2014 (Figure 2A), 2 months after Gonçalves et al. (2017a) documented a high abundance of ovigerous crabs in the benthos. Meanwhile, the lowest



**Figure 5.** *Libinia ferreirae* and *L. lucerna* correlation. (A) Relationship between UD and CW of associated crabs; (B) Relationship between the medusa's mass and the crab's mass.



**Figure 6.** Percentage of food items found in the stomachs of *L. ferreirae* crabs in distinct life habitats. *N* = Number of individuals.



**Figure 7.** Food habits of the crab *L. ferreirae* in distinct life habitats. These data were analyzed by NMDS. Circles—crabs associated with medusa, triangles—free-living crabs. Gray fill: adult crabs, white fill: juvenile crabs.

abundance was recorded in March 2013. This can be related to 2 facts. First, in January 2013, Gonçalves et al. (2017a) observed 25% of the ovigerous female number recorded for the same period of the previous year (January 2014). Second, fewer stations were sampled in March 2013 than in March 2014 due to adverse environmental conditions. Although symbiont crabs are found all year round, peaks were found during the warm periods (spring and summer). These peaks also coincide with food availability (plankton primary and secondary productivity), an important factor that affects reproduction (Thorson 1950; Mills 2001; Nagata et al. 2015). Synchronizing the reproductive cycle with this boom in food availability increases chances of survival. This strategy was previously showed for *L. ferreirae* and for *L. lucerna* (Gonçalves et al. 2017a, 2020b). According to Nagata et al. (2021), recruitment of *L. lucerna* is likely initiated in warmer months (December–February), further explaining the summer mass occurrence in South Brazil. Hence, both species (medusa and spider crab) have recruitment peaks at similar periods.

Although the correlation of both the size and mass between the symbiont and host was constant throughout the study (Figure 5), the overall size of the crabs and medusae was not. Medusa and crab size increased simultaneously around the beginning of the sampling period (Figure 4), followed by a sharp decrease of both species, and then a gradual increase again during the next months of study. We believe the sharp decrease in the size of both species could be due to medusa migration to other sites or the end of the life cycle/phase of the medusa host. After decrease, recruitment and growth of both species followed during the productive spring and summer months. We found that *L. ferreirae* lives in association with *L. lucerna* until the crab reaches or is close to reaching sexual maturity that happens between 8 and 11 months (Gonçalves et al. 2020c). Crabs leave the medusa when the inner spaces of the medusa are not sufficiently large for them to remain inside (Corrington 1927; Gutsell 1928; Nogueira and Haddad 2005). A study with *L. spinosa* and the medusa *L. lucerna* shows this symbiosis starts during the larval phase and can remain throughout the medusa's lifespan or as long as the cnidarian is able to carry the crabs, which depends on crab size (Sal Moyano et al. 2012). Then, crabs continue their life cycle in the benthic environment as free-living individuals (Gonçalves et al. 2017a, 2020b). Nonetheless, the life span of

the medusoid phase of *L. lucerna* should be further studied more deeply, as medusoid lifetime details are unknown.

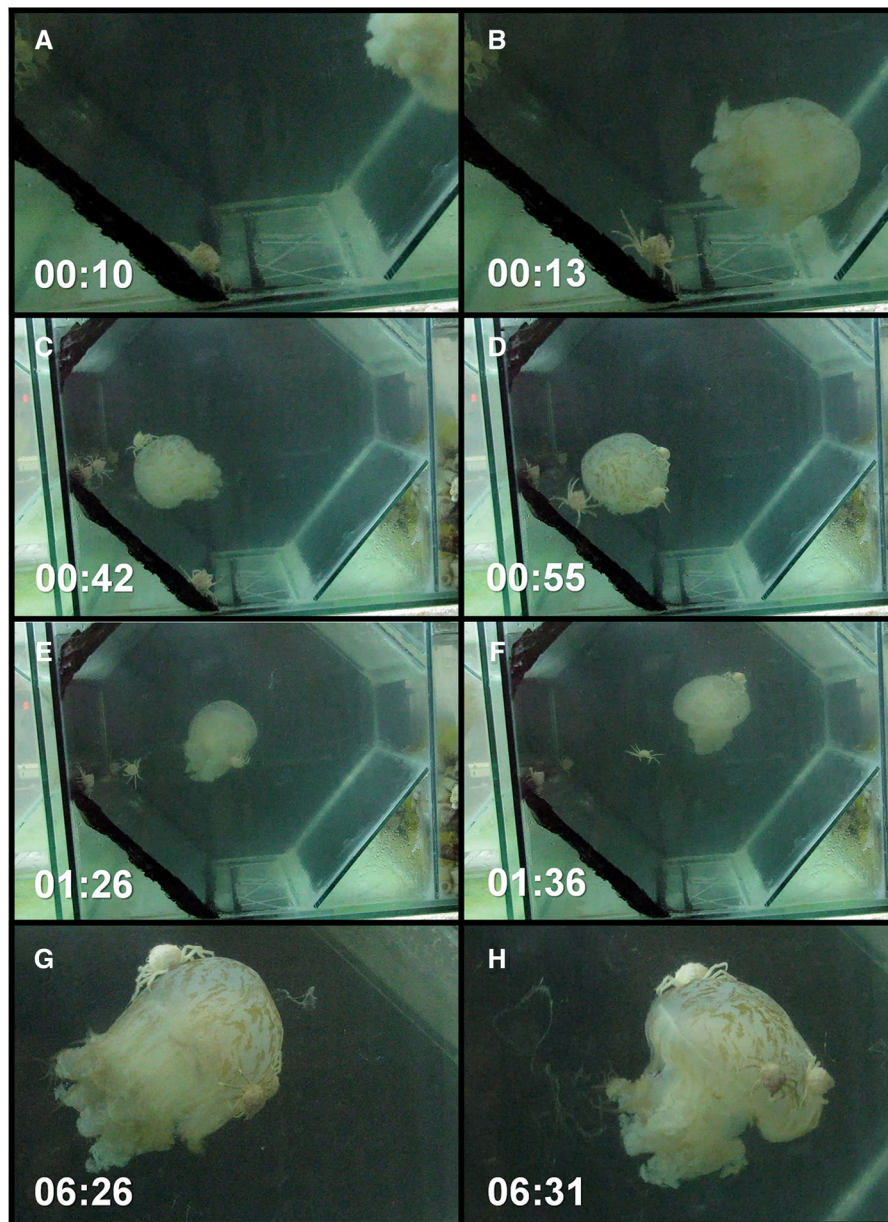
We observed animals in the laboratory as well as recorded videos (see Supplementary Material) to understand how this interaction takes place. It seems that symbiosis is established when the host approaches the symbionts (Figure 8B, D) because the juvenile crab rapidly recognizes the host. The crabs can associate with the medusae in 2 following ways: 1) by lifting up their chelipeds toward the medusa and grabbing the host (Figure 8B, D; Supplementary Video 1) and 2) by releasing themselves from the bottom, opening their pereopods and then drifting with the water current (only possible for juveniles  $\leq 15$  mm CW; Figure 8E, F; Supplementary Videos 2 and 3) until reaching the medusa for association (Supplementary Videos 2 and 3). After this, crabs move to the umbrella or oral arms. While observing a medusa with 5 symbiont crabs, we noticed that the host had difficulty moving, mucus was excessively produced, and the number of body pulsations increased (Figure 8H; Supplementary Video 3). Additionally, we recorded disputes and agonistic behaviors among symbiont crabs. The biggest crab (23 mm CW) associated with a medusa tried to expel other crabs from its host (Supplementary Videos 2 and 3), mutilating and killing 1 crab in the process (Figure 9). Crab fights damaged the umbrella of the host (tearing) during the confrontation. These laboratory observations are preliminary and should be repeated in an experimental level to account for different variables such as medusa size and number/size of crabs.

Although we did not evaluate the negative effects on the medusa during the association, the kleptoparasite symbiosis indicates, this negative effects occur, given that the crab consumes energy resources (Figure 6) captured by the host (Ingram et al., 2017; Gonçalves et al., 2020a, 2021). When carrying symbionts crabs close to maturity ( $>30$  mm CW), or in higher number the medusa will likely expend more energy by carrying more weight, as observed in the symbiosis laboratory observations where medusa increases mucus secretion and increased pulse rate (energy expenditure). However, Towanda and Thuesen (2006) found 1 medusa specimen (*Phacellophora camtschatica* Brandt, 1835) carrying 32 megalopae of *Cancer gracilis* (Dana, 1852) with 2% of the energetic cost to the host causing no apparent negative impact to the host. Thus, more studies need to be done to measure the negative effects for the medusa.

We observed that all crabs associated with medusae were pale or whitish in color, different from the color of free-living crabs. The whitish color could be the crab cryptic camouflage or adaptive coloration to be similar to the host (Bauer 1981; Stuart-Fox and Moussalli 2009; Stevens 2016), with the crab coloration blending into the background of the medusa's pale to whitish tissue. This makes the crab go unnoticed by its natural predators. On the other hand, it means being exposed to the medusa's predators. If dissociated in the benthos, this white coloration could even increase predation of the crab. However, studies about the camouflage strategies that this symbiont crab can have need to be done to clarify if it is able to have cryptic camouflage or adaptive coloration, or if the medusa nematocysts chemical could be responsible for the crab whitish color.

We suggest that the association of *L. ferreirae* and *L. lucerna* is kleptoparasitic, as the symbiont steals food captured by the host medusa, confirming the relationship that was proposed by Gonçalves et al. (2021). Thus, the relationship between



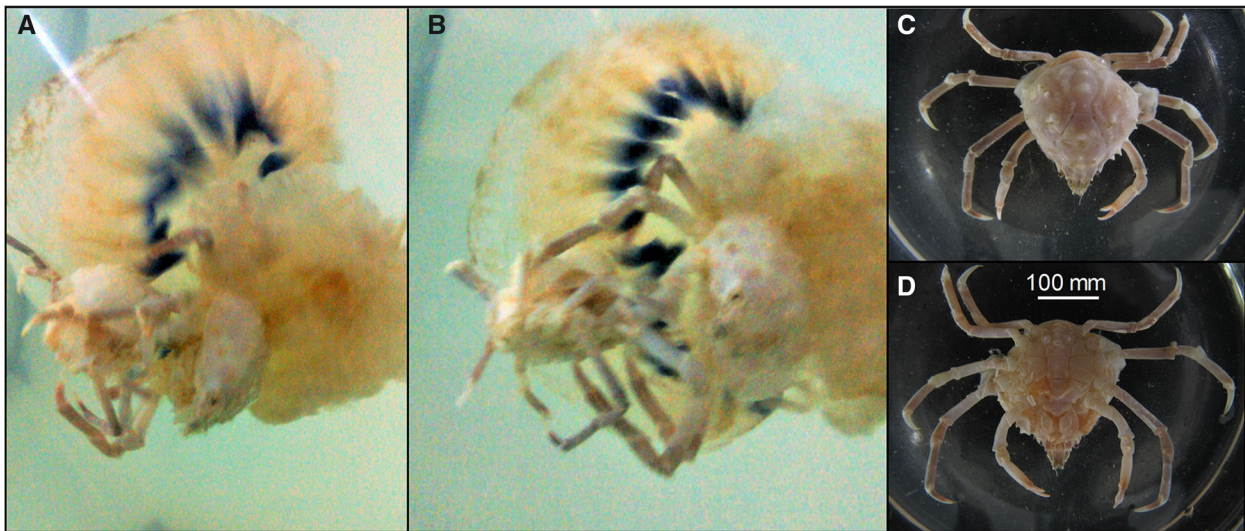


**Figure 8.** Sequence of the association behavior between the crab *L. ferreirae* and the medusa host *L. lucerna*. This association occurs approximately in the first minute after contact with the medusa. Time presented in hh:mm format. (A) Introduction of medusa into the aquarium with 5 crabs. (B) Crabs notice the host. (C) First crabs are associated after approximation to medusa. (D) 2 crabs associated and another crab trying to associate—notice the behavior from the crab that are not in association by lifting the chelipeds in the attempt to attachment to the medusa. (E) Crab start drifting. (F) 1 crab during the drifting behavior in the attempt to associate with the medusa host. (G) 1 crab associated with the medusa. (H) >1 crab associated with a medusa. More details in the [Supplementary Video 3](#).

the crab and medusa is not a commensalistic interaction as suggested for *L. ferreirae* by [Gonçalves et al. \(2017a\)](#) and *L. spinosa* by [Sal Moyano et al. \(2012\)](#). These authors observed nematocysts within the crabs' stomach contents, suggesting that juvenile crabs may ingest nematocysts accidentally during feeding when stealing prey or scavenging detritus from the oral arms of their hosts. [Gonçalves et al. \(2021\)](#) studied the symbiont crab food sources by stable isotopes confirm that the crab not prey the medusa tissue, this was ineligible food resource for the crab. As *L. ferreirae* is a generalist that feeds on what is available in the environment that they live ([Gonçalves et al., 2020a](#)), the habitat in which the crabs were found influenced the food preference ([Figure 7](#)). When a crab

associates with a medusa, their environment becomes one so the crab likely uses the medusa's prey as a food source ([Gonçalves et al., 2021](#)). This medusa species mostly feeds on Calanoid copepods, followed by cyclopid and poecilostomatoid copepods, and bivalve veligers ([Nagata and Morandini 2018](#)). We found a high percentage of copepods in the stomach of symbiont crabs ([Figure 6](#)). Still, the occurrence of Bryozoa in stomachs of some AS crabs and an anemone on the carapace of a JS indicate that the crabs could go to the benthos and later return to the association. We found that free-living crabs capture what is available in the environment, consuming a greater range of resources, with a preference for benthic crustaceans. Similar results were found by [Barros et](#)





**Figure 9.** Crab agonistic behavior during association with the medusa host *L. lucerna*. (A) 2 *L. ferreirae* crabs in the medusa's umbrella and tentacles during the agonistic behavior. (B) During agonistic behavior 1 crab opened the carapace of another, killing it in the process. (C) Dorsal view and (D) ventral view of the killed crab.

al. (2008) and Gonçalves et al. (2020a). Differences in life habits (symbiotic and free-living behaviors for instance) cause partition of resources between crabs. This reduces intraspecific resource competition and provides ecological segregation of the population (Gonçalves et al. 2020a, 2020b). The consumption of plastic and other nonfood items by free-living crabs showed that the pollution by different nonorganic materials has reached the benthos of the Cananéia region (Gonçalves et al. 2020a).

The ontogenetic niche specialization by the crab becomes clear due to the symbiosis. The crab-medusa symbiotic relationship reduces or even cancels the competition for food resources and the environment occupation across ontogeny. Although juvenile and adults of *L. ferreirae* do not seem to compete, symbiotic crabs may compete for resources among themselves. The symbiotic relationship proves to be beneficial for the crab in many ways: improving the distribution of the species, providing food resources, and bringing protection during the most vulnerable stages of development. Thus, we propose that the association is essential for the metamorphosis and survival of the crab, as proposed by Anger et al. (1989) and Gonçalves et al. (2017a, 2020a). It is an obligatory symbiosis during metamorphosis from megalopa to juvenile and facultative symbiosis during the transition from juvenile to adult stage.

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## Data Availability Statement

Crab behavioral videos and Table 1 are available in [Supplementary material](#). All the dataset used in this work is available in: Gonçalves, Geslaine Rafaela Lemos (2021), “Data base of *Libinia ferreirae* free-living and associated to the medusa, during the year 2013-2014,” Mendeley Data, V2, <http://dx.doi.org/10.17632/kdfyvr587.2>.

## Conflict of Interest Statement

The authors declare that they have no conflict of interest.

## Authors' Contribution

G.R.L.G. and A.L.C. originally formulated the idea; G.R.L.G., M.R.W., and A.L.C. conducted the fieldwork; G.R.L.G., M.R.W., M.L.N.F., and A.L.C. developed the methodology; G.R.L.G., F.A., and A.L.C. performed statistical analyses, G.R.L.G. wrote the main manuscript and all other authors provided editorial advice.

## Supplementary Material

“Supplementary material can be found at <https://academic.oup.com/cz>.”

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