UNIVERSIDADE ESTADUAL PAULISTA
INSTITUTO DE BIOCIÊNCIAS

GESLAINE RAFAELA LEMOS GONÇALVES

ECOLOGIA POPULACIONAL DE *Libinia ferreirae* (BRACHYURA: MAJOIDEA) NO LITORAL SUDESTE DO BRASIL

DISSERTAÇÃO DE MESTRADO

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Palavras-chave: Ciclo de vida; Crescimento; Dinâmica populacional; Epizoísmo; Hábitos alimentares.

NEBECC
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"Sou biólogo e viajo pela savana de meu país. Nessa região encontro gente que não sabe ler livros. Mas que sabe ler o mundo. Nesse universo de outros saberes, sou eu o analfabeto"
Mia Couto

"O saber a gente aprende com os mestres e com os livros.
A sabedoria, se aprende é com a vida e com os humildes"
Cora Coralina

"Nos alicerces do inconciente se encontram todas essas bençãos que, lentamente, assomam à consiência e se tornam patrimonio da lucidez, fazendo o ser compreender que nem tudo quanto pode fazer, deve-o; da mesma forma que nem tudo quanto deve, pode; conseguindo a sabedoria de fazer somente o que deve e pode, como membro consiente que age de acordo com a harmonia cósmica"
Joanna de Ângelis

"Na vida, não vale tanto o que temos, nem tanto importa o que somos. Vale o que realizamos com aquilo que possuímos e, acima de tudo, importa o que fazemos de nós"

Chico Xavier

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SUMÁRIO

Considerações iniciais

Ana K Camargo - 2014

O presente estudo foi desenvolvido no Brasil em uma região marinha com características subtropicais, com grande diversidade de espécies, desempenhando papeis cruciais para os processos ecológicos. Infortunadamente estes locais são muito impactados, tendo seus recursos explorados indiscriminadamente através de métodos de captura nada seletivos, a exemplo a pesca de arrasto voltada a captura de camarões com valor comercial.

Mesmo que o esforço da pesca seja dirigido a uma espécie-alvo (ou grupo de espécies), sempre haverá a captura de outras espécies, chamadas espécies acessórias, denominadas "bycatch" (fauna acompanhante), que podem ser devolvidos ao mar (vivos ou mortos), por falta de interesse econômico e/ou tecnológico, denominando-se como rejeição ou descarte (Alverson et al., 1994; Graça Lopes et al., 2002). A técnica de pescaria que apresenta maior descarte é aquela que utilizam redes de arrasto, devido à baixa seletividade que estes apetrechos contêm (Broadhurst & Kennelly, 1996). Desta forma, este tipo de captura pode representar um risco potencial ao equilíbrio ambiental (Alverson et al., 1994).

No sul brasileiro, a fauna acompanhante deste tipo de pesca é composta por estágios tanto juvenis quanto adultos, representados por várias espécies, como: 7 de cnidários, 22 de moluscos, 42 de crustáceos, 11 de equinodermos e 134 de peixes; onde ultrapassam a captura da espécie de valor comercial (Branco et al., 2015). A pesca mundial de crustáceos representa cerca de 30% de toda a captura (Smith & Addison, 2003), sendo uma das atividades mais importantes de alguns países (Tully, 2003). No entanto, inexistem estudos sobre a ecologia e biologia de muitas espécies que são capturadas nessa fauna acompanhante, como é o caso do caranguejo aranha *Libinia ferreirae* Brito Capello, 1871.

No litoral sudeste do estado de São Paulo, Brasil, estão as regiões de Cananéia, Iguape e Ilha Comprida, inserido dentro do sistema estuarino de Cananéia, Iguape e lagoa Paranaguá. Estas áreas são reconhecidas nacional e internacionalmente como o terceiro ecossistema mais produtivo do Atlântico Sul, por possuírem características ambientais bem

conservadas (Mendonça et al., 2010). Em 1993, o bioma Mata Atlântica desta área foi considerado como Reserva da Biosfera (UNESCO, 2005); em 1999, foi nomeado Património Mundial Natural pela sua importância para a investigação científica e para a preservação dos valores humanos e conhecimentos tradicionais com base em padrões de desenvolvimento sustentável (UNESCO, 1999). No entanto, nestes sistemas há uma intensa actividade pesqueira, especialmente artesanal, que compreende mais de 3.000 pescadores (Mendonça & Katsuragawa, 2001).

Segundo Cergole et al. (2005), a pesca no Brasil está sobre explorada ou em declínio, devido a políticas claras que não limitam a alta pressão ambiental causada pela pesca. Moss em 1982 já tratava que o interesse econômico se sobressai sobre o nível das populações usadas como recurso, ocasionando uma sobrecarga de populações que muitas vezes tem sua plasticidade modificada ou até mesmo não conseguem ter uma recuperação de suas populações, declinando a ponto de se extinguirem em muitos locais (Ghalambor et al., 2007).

Segundo Mendonça et al. (2010), para se atingir um quadro de pesca sustentável é necessário desenvolver instrumentos de controle e regulamentação, contendo monitoramento das atividades de pesca, e orientar quanto a processos de tomada de decisões que implementem regras para manter um nível mínimo dos recursos que garantam a sobrevivência das espécies e assim a atividade pesqueira. Os atuais regulamentos da pesca consideram principalmente os aspectos técnicos e econômicos de manutenção ambiental e desenvolvimento econômico (Pezzoli 1997). Entre as técnicas de gestão mais utilizados em todo o mundo é co-gestão, definida por Jentoft et al. (1998) como "o processo colaborativo e participativo de tomada de decisões regulatórias entre os representantes dos grupos de utilizadores, agências governamentais e institutos de pesquisa", onde o envolvimento dos pescadores na elaboração das políticas ajuda a obter maior eficiência na exploração dos

recursos pesqueiros. A elaboração de leis que apenas proíbem a pesca dificilmente promove a gestão, sendo que tal medida não é o suficiente (Moraes, 2004).

Segundo Alverson et al. (1994), algumas opções emergenciais para reduzir o impacto da pesca de arrasto seria: redução do esforço de pesca, programas de incentivo aos pescadores, e transferir a responsabilidade da redução do "bycatch" movendo a responsabilidade pela redução das capturas acessórias em nível de embarcação. Outras técnicas que poderiam ser aplicadas para reduzir esta captura incluem: utilização de redes mais seletivas, a pesca como geradora de desenvolvimento para a comunidade, tentar tirar vantagens do comportamento diferenciado de cada espécie e restringir determinado tempo por área.

As proteções das áreas de pesca ainda não são suficientes, considerando a grande extensão da costeira brasileira. Apesar de inúmeras leis existentes e várias áreas protegidas no Brasil, a conservação da biodiversidade marinha ainda é insuficiente e inadequada (Branco et al., 2015).

Dentro dos crustáceos Pleocyemata estão inseridos os Brachyura que incluem siris e caranguejos que podem se distribuir desde de praias até grandes profundidades marinhas, assim como água doce e salobra. Poucos caranguejos têm interesse comercial, com destaque para o gênero *Callinectes*. Os Pleocyemata podem incubar embriões nos pleópodes e as larvas eclodem em uma fase posterior da larva náuplio, inserido nesta subordem está a superfamília Majoidea. Os Pleocyemata se encontram na ordem Decapoda Latreille, 1802, subclasse Eumalacostraca Grobben, 1892 e subfilo Crustacea Brünnich, 1772 (Martin & Davis, 2001).

O caranguejo aranha *L. ferreirae* deste estudo está inserida na família Epialtidae dentro da Superfamília Majoidea. A ecologia dos caranguejos Majoidea é diferenciada dos demais Brachyura por apresentarem dois tipos de muda: a pré-puberal e terminal/puberal. A

primeira antecede a muda terminal e pode iniciar da maturidade gonadal, e a segunda é caracterizada pela chegada à idade adulta com a maturidade gonadal e morfométrica e o fim do crescimento dos indivíduos. Adicionalmente, as fêmeas podem copular em intermuda (carapaça em estado de alta rigidez), armazenar espermatozoides por determinados períodos de tempo, e apresentar desovas múltiplas, podendo ainda copular mesmo que apresentando massa de ovos fecundados aderida ao abdômen (González-Gurriarán et al., 1998; Jones & Hartnoll, 1997; Hartnoll 1963).

Libinia ferreirae apresenta amplitude de distribuição restrita ao Atlântico Ocidental, ocorrendo da Venezuela ao Brasil (do Pará até Santa Catarina), desde a região costeira até a profundidade máxima de 35 m, tendo preferência por fundos lamosos (Melo 1996). Esta espécie apresenta relação de simbiose com a medusa *Lychnorhiza lucerna* Haeckel, 1880 durante sua fase juvenil, e com algas, esponjas, cnidários e outros grupos de animais durante sua fase adulta (Winter & Masunari 2006), o que possivelmente contribuiu para seu sucesso evolutivo, ao permitir que estes animais se locomovam com uma menor taxa de predação por um amplo espaço, além de proporcionar uma maior distribuição das espécies sésseis e sedentárias que se associam a este caranguejo (Nogueira Jr. & Haddad 2005; Hultgren & Stachowicz 2011).

A continua captura do caranguejo *L. ferreirae* na pesca de arrasto, nos instigou sobre como seria a ecologia desta espécie, principalmente por ser uma espécie com uma plasticidade durante suas fases de vida, já que as mesmas possuem distintos hábitos simbióticos durante seu desenvolvimento, acarretando uma dependência de outras espécies para sua sobrevivência, enquanto gera uma distinta repartição de nichos entre jovens e adultos desta espécie. Desta maneira durante a execução do projeto Biota FAPESP (processo #2010/50188-8) está dissertação foi idealizada com o objetivo principal conhecer ao máximo

possível a ecologia do caranguejo aranha *L. ferreirae*, principalmente investigando sua biologia reprodutiva, relações simbióticas e hábitos alimentares.

O estudo sobre a ecologia do caranguejo aranha *L. ferreirae* foi iniciado em 2012. Para uma melhor compreensão e embasamento sobre o tema, está dissertação foi dividida em 5 capítulos, porém, os três primeiros foram encaminhados para publicação e, portanto, estão colocados em anexo.

O anexo I "Symbiotic relationship between the crab *Libinia ferreirae* and the jellyfish *Lychnorhiza lucerna"* foi submetido na revista The Biological Bulletin. No anexo II estão inseridos os capítulos já publicados como: "Morphometric and gonad maturity of the spider crab *Libinia ferreirae* Brito Capello, 1871 (Decapoda: Majoidea: Epialtidae) on the south-eastern Brazilian coast" - Journal of the Marine Biological Association of the United Kingdom, e "Decapod crustacean associations with scyphozoan jellyfish (Rhizostomeae: Pelagiidae) in the Southeastern Brazilian coast" - Symbiosis.

O capítulo inicial está voltado ao ciclo de vida do caranguejo aranha *L. ferreirae,* onde o mesmo se encontra em inglês no formato da revista que será submetido. Capítulo 1 – "Reproductive ecology of spider crab *Libinia ferreirae* (Brachyura: Majoidea): ontogenetic shifts in habitat use" na revista Invertebrate Reproduction and Development. Os 2º capítulo trata-se do "Hábito alimentar do caranguejo aranha *Libinia ferreirae* (Decapoda, Majoidea) durante sua fase de simbionte e de vida livre, na região litorânea do Sudeste Brasileiro". Maiores detalhes sobre cada temática serão abordados nos capítulos e anexos.

Referências

Alverson DL, Freeberg MH, Pope JG & Murawski JA (1994) A global assessment of fisheries bycatch and discards. FAO Fish Tech Pap, 339: 1–233

Branco JO, Freitas Júnior F, & Christoffersen ML (2015) Bycatch fauna of seabob shrimp trawl fisheries from Santa Catarina State, southern Brazil. Bio Neotrop, 15(2): 1–14

Broadhurst MK & E Kennelly SJ (1996) Effects of the circumference of codends and a new design of squaremesh panel in reducing unwanted by-catch in the New South Wales oceanic prawn-trawl fishery, Australia. Fish Res, 27: 203–214

Cergole MC, Ávila-Da-Silva AO & Rossi-Wongtschowski CLDB (2005) Análise das principais pescarias comerciais da região Sudeste-Sul do Brasil: dinâmica populacional das espécies em explotação. Instituto Oceanográfico, USP, São Paulo

Ghalambor C, McKay J, Carroll S & Reznick D (2007) Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Funct Ecol, 21: 394–407

González-Gurriarán E, Fernández L, Freire J & Muiño R (1998) Mating and role of seminal receptacles in the reproductive biology of the spider crab *Maja squinado* (Decapoda, Majidae). J Exp Mar Biol Ecol, 220(2): 269–285

Graça-Lopes R., Tomás ARG, Tutui SLS, Severino-Rodrigues E, & Puzzi A (2002) Fauna acompanhante da pesca camaroeira no litoral do estado de São Paulo, Brasil. Bol Inst Pesca de São Paulo, 28(2): 173–188

Hartnoll RG (1963) The biology of Manx spider crabs. Proc Zool Sot London, 141: 423-496

Hultgren K & Stachowicz J (2011) Camouflage in decorator crabs. In Stevens, M. & S. Merilaita (eds), Animal Camouflage: Mechanisms and Function. Cambridge University Press, Cambridge: 214–238

Jentoft S, Mccay B, & Wilson DC (1998) Social theory and fisheries co-management. Mar Policy, 22(4-5): 423–436

Jones DR & Hartnoll, RG (1997) Mate selection and mating behaviour in spider crabs. Estuar Coast Shelf S, 44(2): 185-193

Martin JW & Davis GE (2001) An updated classification of the recent Crustacea. Science Series, Natural History Museum of Los Angeles, 39;1-124

Mendonça JT & Katsuragawa M (2001) Caracterização da pesca artesanal no complexo estuarino lagunar de Cananéia-Iguape, Estado de São Paulo, Brasil (1995-1996). Acta Scientiarum, 23(2): 535–547

Mendonça JT, Verani JR & Nordi N (2010) Evaluation and management of blue crab *Callinectes sapidus* (Rathbun, 1896) (Decapoda - Portunidae) fishery in the Estuary of Cananéia, Iguape and Ilha Comprida, São Paulo, Brazil. Braz J Biol, 70:37–45

Moraes MBR (2004) Área de Proteção Ambiental – APA como agência de desenvolvimento sustentável: APA Cananéia – Iguape – Peruíbe / SP. São Paulo: Annablume; FAPESP

Pezzoli K (1997) Sustainable development: a transdisciplinary overview of the literature. J Environ Plan Manage, 40(5):549–74

Smith MT & Addison JT (2003) Methods for stock assessment of crustacean fisheries. Fish Res, 65(1-3): 231–256

Tully O (2003) Crustacean fisheries. Fish Res, 65(1-3): 1–2

UNESCO (1999) World Heritage Nomination - IUCN Technical Evaluation Atlantic Forests (southeast) (Brazil). UNESCO, 8 p

UNESCO (2005) World Network Of Biosphere Reserves – SC/EES – June 2005. The MAB Program. UNESCO, 19 p

Winter VC & Masunari S (2006) Macroepizoismo em *Libinia ferreirae* (Crustacea, Brachyura, Majidae). Iheringia Ser Zool, 96(2): 135–140

Cápitulo I
Reproductive ecology of spider crab *Libinia ferreirae* (Brachyura: Majoidea): ontogenetic shifts in habitat use

Abstract: The spider crab *Libinia ferreirae* is a poorly studied species, even being an important component of the trophic web in its habitat, and presenting symbiotic relationships with several organisms as well. The aim of this research is to describe the population parameters (reproductive periodicity, juvenile recruitment and ecological distribution) of this species, for both benthic and pelagic (symbiotic) life stages. Sampling was carried out in the Cananéia region (São Paulo state, Brazil) from June 2012 through May 2014, using a shrimp fishing boat outfitted with double-rig nets. In total, 921 crabs were collected, 564 adults (benthic) and 357 juveniles (pelagic), living in symbiosis to medusas of *Lychnorhiza lucerna*. The abundance of ovigerous (carrying embryos) females showed a positive association to salinity and grain size values (CANONICA, $r=0.44$; $p=0.000052$). The presence of such ovigerous females throughout the whole sampling period characterizes a continuous reproduction pattern, once they presented a positive relation to symbiotic juveniles at time lag "+2" (Cross-correlation, $p<0.05$). It can also evidence an effective spawning, since two months after reproductive peaks it is possible to observe peaks in juvenile recruitment. The periods with increased primary productivity (high in chlorophyll concentration) also preceded periods of juvenile recruitment, in agreement to the match-mismatch theory. Therefore, we propose that this species presents an adjustment in the spawning periods to the ones with higher primary productivity, in which the planktotrophic larval stages will be provided with a high source of food, increasing its subsequent larval success. This study showed an ecological strategy of habitat segregation among juvenile and adult individuals, thus avoiding the competition for food resources.

Keywords: association, distribution, sediment, effective spawning, juvenile recruitment, epibiont

Introduction

The spider crab *Libinia ferreirae* Brito Capello 1871 distributes itself exclusively in the Western Atlantic Ocean (Venezuela to Brazil), from coastal areas up to depths of 35m, mainly in muddy bottoms (Melo 1996).

Libinia ferreirae shows symbiotic relations during its whole life. Megalopa larvae and juveniles associate themselves to medusae of Scyphozoa, what possibly contributes to its juvenile recruitment success, allowing these animals to move with a lower predatory rate through a wide space (Gonçalves et al. 2016a). When adults, they can present several epibionts (e.g. algae, sponges and cnidarians), what provides an expansion in the amplitude of these sessile organisms distribution, as well as protection to the crabs (camouflage) (Nogueira Jr. & Haddad 2005; Hultgren & Stachowicz 2011).

Once it is a part of the shrimp fisheries bycatch, *L. ferreirae* undergo through heavy fishing pressures both in its juvenile (=immature) and adult stages. As juveniles when fishing gear capture their hosting medusae, and as adults because they share the same habitat as the target shrimp species. This is especially true for ovigerous females, who migrate to shallower waters prior to spawning, where they find favorable environmental features (high rates on temperatures and food resource) (Graça Lopes *et al*., 2002; Nogueira Jr. & Haddad 2005; Schroeder et al., 2014; Branco et al. 2015).

The Majoidea crabs (differently from other Brachyura) show two unique kinds of molt: the pre-pubertal molt (which can characterize the onset of gonadal maturity) and the terminal molt, when they reach both gonadal and morphometric maturities, and cease the somatic growth (Hartnoll, 1982; Gonçalves *et al*., 2016b). It turns their reproductive biology different from the others, once females can copulate during the intermolt period (hard carapace condition), store spermatozoids, and show multiple spawning, presenting even the capability of copulate with an egg mass adhered to the pleopods (Hartnoll 1963; Jones & Hartnoll, 1997; González-Gurriarán *et al*., 1998).

The period and length of the reproductive activities in crustaceans are strongly influenced by changes in environmental factors. The chlorophyll content (phytoplanktonic productivity), for instance, is higher in periods with higher temperature, increasing the density of food resources, and consequently, enhancing the zooplankton's abundance. Phytoplankton is the main primary producer in coastal environments, responsible for the onset of mass and energy flow along these trophic webs (Thorson 1950; Vega-Peres 1993). Therefore, it also contributes in fertilizing such areas, directly supporting herbivorous taxa, and indirectly, animals included in the higher levels of the trophic web, which include economically important species (Dring 1992). Besides the food supply, water temperature can also directly influence reproduction. Continuous reproductive patterns are commonly observed in species inhabiting tropical regions (constant temperatures along the year). On the other hand, seasonal reproductive patterns are generally observed to species from higher latitudes, influenced by different environmental conditions (periodic or not). Thus, populations adjust their life cycle to periods with more favorable conditions to their development and survival (Sastry 1983).

However, the reproductive success of crustaceans can be evaluated by the occurrence of "effective spawning". Such term refers to situations where right after a peak in abundance of reproductive individuals, it is possible to observe a peak in the number of juvenile individuals. Thus it is possible to evidence that reproductive individuals observed in a given period generated descendants observed in the subsequent months, as proposed by Crocos & Van der Velde (1995).

According to Varisco & Vinuesa (2011) and Castilho et al. (2008a), obtaining knowledge concerning the reproductive biology of certain species is fundamental to understand the relations among the reproductive and recruitment periods, as well as spawning processes and how peculiar environmental conditions can influence such parameters. The aim of this study was to describe population biology of *L. ferreirae* in the region of Cananéia, São Paulo state (southeastern Brazil), addressing its reproductive periodicity, juvenile recruitment,

sex ratio and ecological distribution. Additionally, we tested the influence of the environmental factors (bottom water salinity and temperature, chlorophyll content and sediment features) over different life stages of the crabs (pelagic juveniles living in symbiosis with medusae, as well as benthic adults).

Material and methods

Biological sampling

Biological sampling were performed monthly, along seven sampling stations by 30-minutes trawling in each station. Trawls were conducted in the Estuarine-Lagoon System of Cananéia-Iguape and its adjacent oceanic area (figure 1), using a shrimp fishing boat outfitted with double-rig nets. Benthic crabs were sampled from July 2012 through May 2014, while individuals living in symbiosis to *Lychnorhiza lucerna* Haeckel, 1880 were sampled from February 2013 through May 2014.

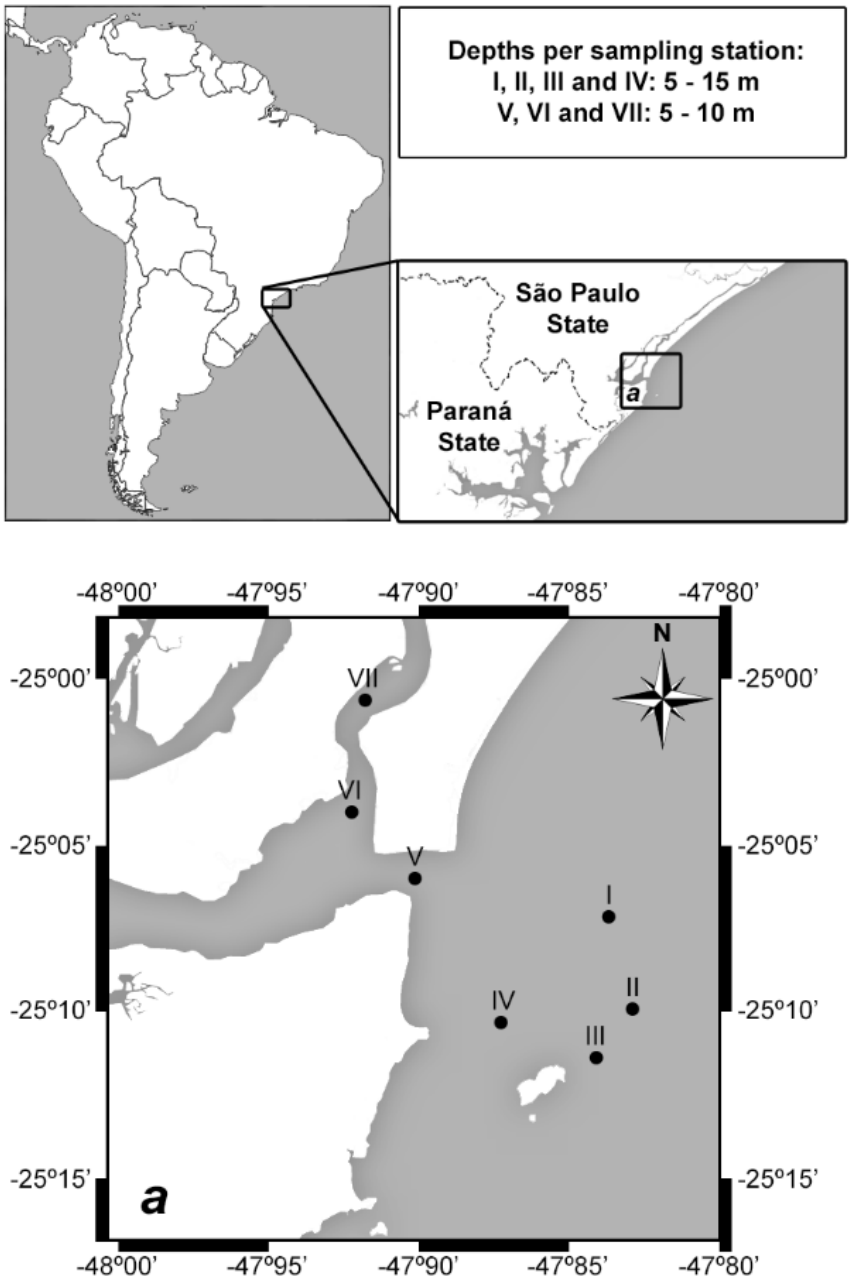


Figure 1. Sampling stations in the Cananéia region, São Paulo state, Brazil, where crabs were sampled.

Crabs were identified (Melo 1996; Pohle et al. 1999; Tavares & Santana 2012) and specimens were sexed using as criteria the shape of the abdomen (thin shape for males and oval shape for females) and the number of pleopods (2 pairs for males and 4 pairs for females) (Ingle 1977; Almeida et al. 2013). Then, animals were measured to the carapace width (CW) (largest linear distance between the lateral body boarders excluding spines and tubercles), using a digital caliper (to the nearest 0.01mm). When animals presented CW smaller than 5

mm, we used a stereomicroscope (Zeiss® Stemi SV6) equipped with image-capturing system (Zeiss® Stemi 2000-C) to assess such measurements.

The abundances for each demographic class (see below) were plotted in graphs of geographical distribution according to their percentages using the software Surfer (version 8.0, Golden Software, California).

Reproductive aspects

Individuals were analyzed for the adherence of their abdomen to the cephalothoraxic sternite, from which they were classified as juveniles (unable to reproduction) (abdomen sealed to sternite by a cementing substance) (Bolla & Negreiros-Fransozo 2015). On the other hand, adults (able to reproduction) were considered the ones with unsealed abdomen, with no cementing substances (Haefner 1990; Guinot & Bouchard 1998). All crabs were submitted to the macroscopical analysis of the gonads and determination of the stages of gonadal development based on the shape, color and size of ovaries, testicles and vase deferens (Choy 1988; Abelló 1989; Gonçalves et al. 2016a).

Besides juveniles, two adult (able to reproduction) gonadal stages were considered for females: spent (SP) (thin and whitish-colored ovaries) and reproductive (RE) (from thin and light orange-colored to thicker and dark orange colored ovaries). Likewise, two adult (able to reproduction) gonadal stages were characterized for males: spent (SP) (translucent and thin vas deferens) and reproductive (RE) (thicker and white colored vas deferens) (for more details, see Choy (1988) and Abelló (1989)). Females carrying embryos (fertilized eggs) adhered to the pleopods (ovigerous females – OF) were counted to posterior analyses.

Environmental characterization

Environmental factors related to the water column (temperature and salinity) were sampled monthly in each of the seven sampling stations throughout the whole studied period.

Additionally, water chlorophyll content was analyzed during the period of one year (January through December 2013). All of the referred measurements were performed using a multiparameters probe EUREKA® (Manta 2-4.0).

Sediment samples were obtained for each season of the year in all sampling stations using a Petersen grabber, and frozen until laboratorial analysis. Samples were dried (70º C for 72h), and from each sample, a subsample of 10g was ash-weighted in order to determine the organic matter content (OM) associated to the substrate. Additionally, 100g subsamples were used to determine the grain size at the Phi scale, according to the methodology proposed by Hakanson & Jansson (1983) and Tucker (1988), adopted by Castilho et al. (2008b).

Statistical analysis

In this study, data showed non-normal and non-homoscedastic distributions according to Shapiro-Wilk and Levene tests, respectively. Thus, differences in the spatio-temporal distribution of crabs were tested performing a Kruskal-Wallis test. Sex ratio was tested performing a chi-square test.

The relation between environmental factors (temperature, salinity, Phi and OM) and the abundance of adult females, ovigerous females, adult males and juvenile individuals was tested performing a Multivariate Analysis of Canonic Correlation (CANONICA). Such statistical analysis directly measures the strength of the relation between two sets of variables. The first set is given by the environmental conditions, and the second one, by the abundance of each group of individuals. The variables' values were transformed – log(1+x) – prior to the statistical analyses, according to Castilho et al. (2008b).

To assess the time lag between spawning and juvenile recruitment, the temporal distribution of ovigerous females was tested against the juveniles' one, performing temporal series analyses (cross-correlations). Such analyses cross the data in cause-effect intervals (lags) of three earlier and later months, where significant relations show different association

coefficients. Association coefficients equal to 1 were classified as perfect; between 0.99 and 0.7, strong; between 0.69 and 0.4, moderate; and among 0.4 and 0.1, weak (more details: Castilho et al. 2015). For all the statistical analyses performed in this study, we adopted a 5% significance level (Zar, 1999).

Results

Throughout the sampling period, we collected 564 benthic (non-associated) individuals, and 357 crabs living in association to *L. lucerna*. Peaks in abundance of benthic individuals were recorded in September 2012 (winter) and January 2014 (summer) (figure 2 A1), with significant differences in the distribution throughout the studied months (Kruskal-Wallis: H=37.1, p=0.02). The highest abundance of collected specimens was recorded in the sampling station I, which was statistically different from the others (Kruskal-Wallis: H=67.51, p=0.00, figure 2 - A2).

Crabs living in association to *L. lucerna* were more abundant in March 2014 (summer), statistically different from the other months (Kruskal-Wallis: H=38.1, p=0.0009) (figure 2 - B1). The highest abundance of such individuals was recorded in the sampling station VI (figure 2 – B2), even though no statistically significant difference has been observed among the sampling stations (Kruskal-Wallis: H=6.477, p=0.3719).

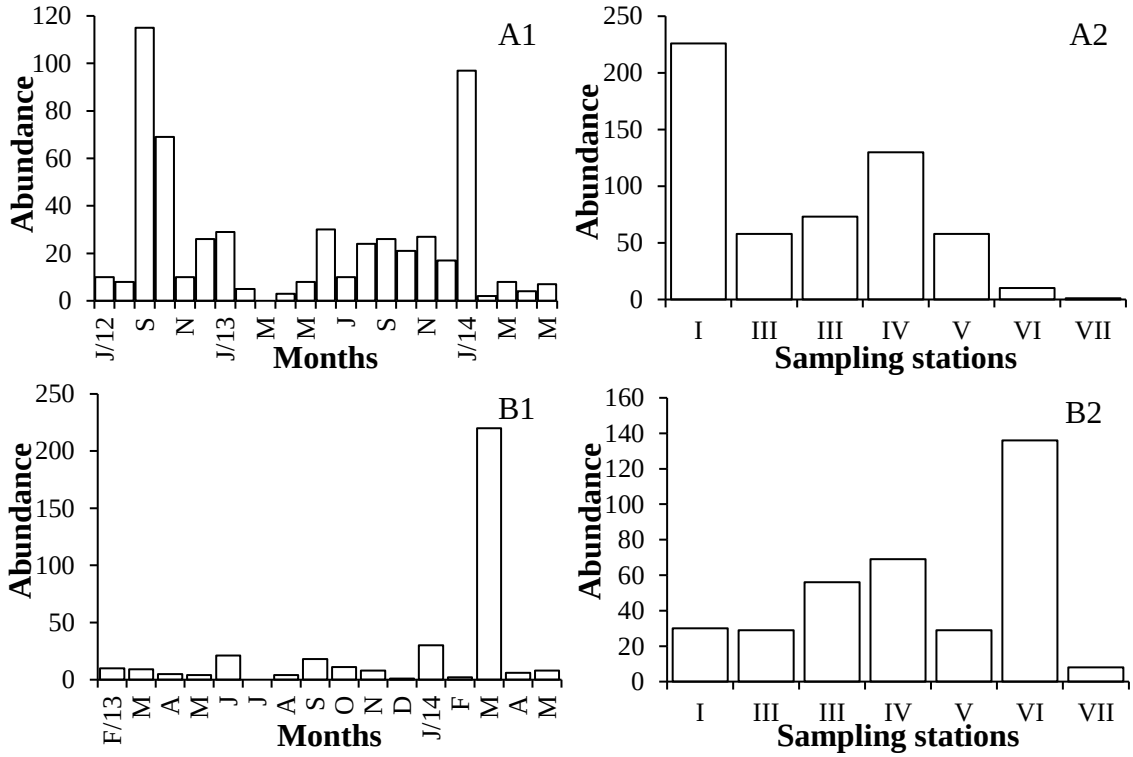


Figure 2. Monthly (A1 and B1) and spatial (A2 and B2) abundance of the spider crab *Libinia ferreirae* Brito Capello, 1871, in the Cananéia region, southern littoral from São Paulo state. A) Number of benthic crabs; B) number of individuals living in association to *Lychnorhiza lucerna* Haeckel, 1880.

The influence of environmental factors

We observed higher rates on the capture of symbiotic individuals at temperatures between 25 and 28º C and salinities between 30 and 34, while benthic crabs were sampled in higher abundance at temperatures between 28 and 31º C and salinities between 34 and 38. Benthic crabs were found in higher abundance at Phi values greater than 3 (mainly >4) and at 5-10% and 15-20% values of OM (figure 3).

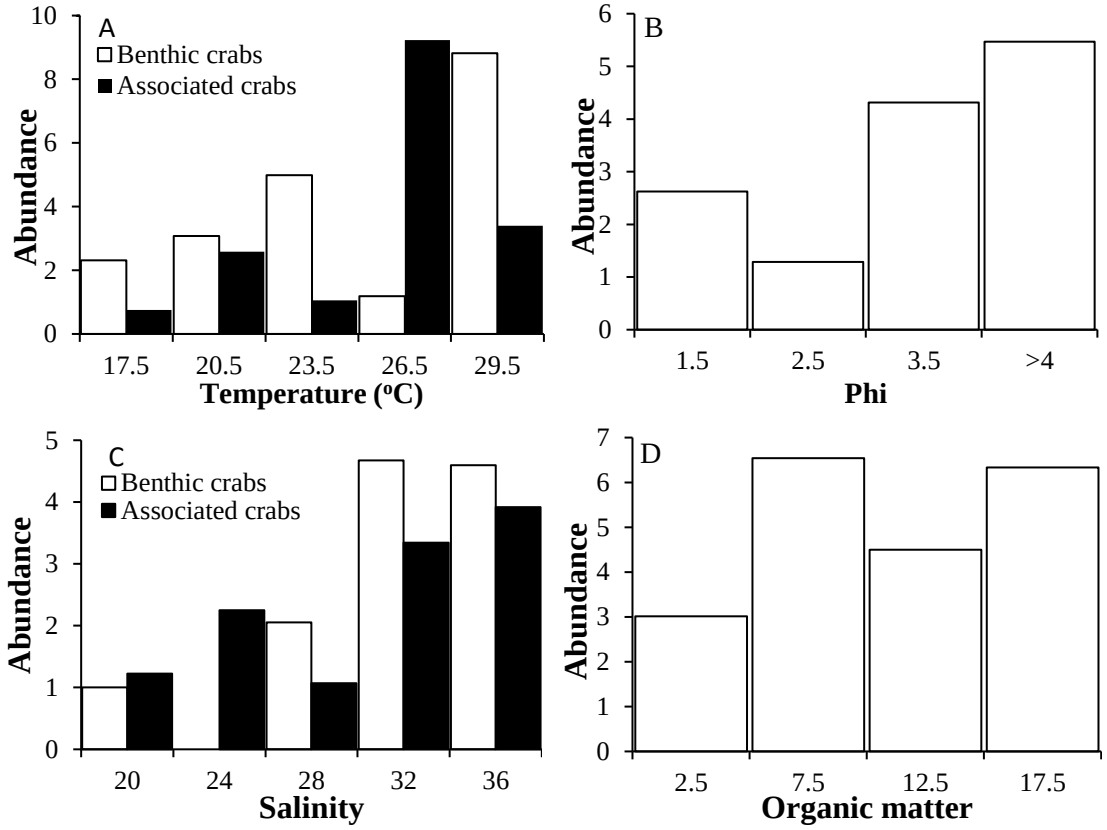


Figure 3. Variation in the mean abundance of the spider crab *Libinia ferreirae* Brito Capello, 1871. Abundance of benthic and symbiotic individuals in the Cananéia region, southern littoral from São Paulo state, from July 2012 through May 2014, showing the catch per unit effort (CPUE), regarding: A) bottom water temperature (º C), B) Phi, C) bottom water salinity, and D) organic matter content (%). The X axis' values represent the midpoint of classes, i.e.: temperature 17.5 ºC = 16 to 18.9 ºC, Phi 1.5 = 1 to 1.9, salinity 20 = 18 to 21.9, organic matter 2.5 = 0 to 4.9 %, and so on.

Environmental factors were tested against the abundance of the different studied demographic groups (adult females, ovigerous females, adult males and immature juveniles), which correlated themselves (CANONICA: r=0.44, p=0.000052), with a significant first canonic pair (r=0.30, p=0.048).

The canonical loads (types of correlation among the variables) and the canonical weights (magnitude of the relation between environmental factors and biological data) can be observed in Table 1.

Table 1. Canonical loads and weights based on the analysis of canonical correlation between environmental factors and the abundance of demographic groups crabs (adult females, ovigerous females, adult males and juvenile individuals) of the spider crab *Libinia ferreirae* Brito Capello, 1871, sampled from July 2012 through May 2014 in the Cananéia region, southern littoral from São Paulo state, Brazil.

Environmental variables	**Canonical load**	**Canonical weight**
Bottom temperature (º C)	0.23	0.27
Salinity	-0.86	-0.76
Phi	-0.66	-0.34
OM	-0.46	-0.13
Biological data		
Adult females	-0.76	0.19
Ovigerous females	-0.85	-1.49
Adult males	-0.84	-0.15
Juvenile individuals	-0.32	0.83

The environmental factors with greater canonical loads (>0.5) were salinity (-0.86) and Phi (-0.65), even though only salinity showed a high canonical weight (-0.75). Ovigerous females was the only one among the demographic groups to show both high canonical load (-0.85) and weight (-1.49). Thus, it is possible to infer that the variation in salinity ruled the abundance of ovigerous females (the number of ovigerous females tended to increase as salinity increases).

Reproductive biology

From 921 sampled individuals, 788 were identified to its sex, from which 473 were females (51%) and 313, males (34%), 135 individuals could not be sexed due to their small size (CW <4mm) and absence of evident secondary sexual characters.

Benthic crabs showed an average sex ratio of 1:1.7 (M:F), from which males represented 37% of the population, evidencing the difference of abundance among sexes (Chi-square, $p<0.00$). Such characteristic repeated itself considering the whole population (benthic+symbiotic abundances), with a 1:1.5 (M:F) sex ratio (Chi-square, $p<0.00$). This relation was also observed to the symbiotic individuals, however, in a smaller scale (1:1.2) (M:F), showing no significant difference among the proportion of sexes (Chi-square, $p>0.05$).

The size of juvenile males varied from 4.2 to 44.3 mm CW, while adult males' size varied from 30 to 78 mm CW. The smallest reproductive males were observed in the mean size class of 31.5 mm CW (from 30 to 33 mm CW), evidencing the onset of physiological maturity. All male bigger than the mean class of 46.5 mm CW (from 45 to 48 mm CW) was physiologically adults (figure 4).

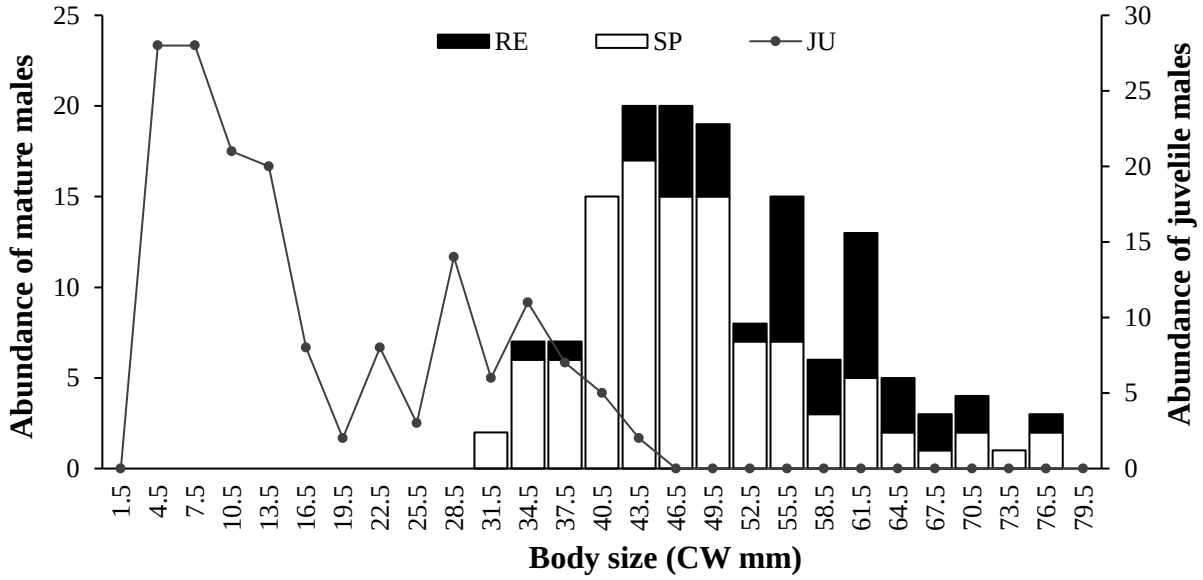


Figure 4. *Libinia ferreirae* Brito Capello, 1871. Distribution of males' demographic groups (RE: reproductive; SP: spent; JU: juvenile) in size classes, for individuals sampled in the Cananéia region, southern littoral from São Paulo state, from July 2012 through May 2014. The X axis' values represent the midpoint of size classes, i.e.: 1.5 mm = 0 to 3 mm, and so on.

The size of juvenile females varied from 4.23 to 44.44 mm CW, while for adult females, the size variation was from 30.2 to 71.6 mm CW. The highest abundance of individuals was observed in the mean size class of 46.5 mm CW (from 45 to 48 mm), class in which we observed the highest abundance of ovigerous females as well. The largest ovigerous female showed 71.64 mm CW (figure 5).

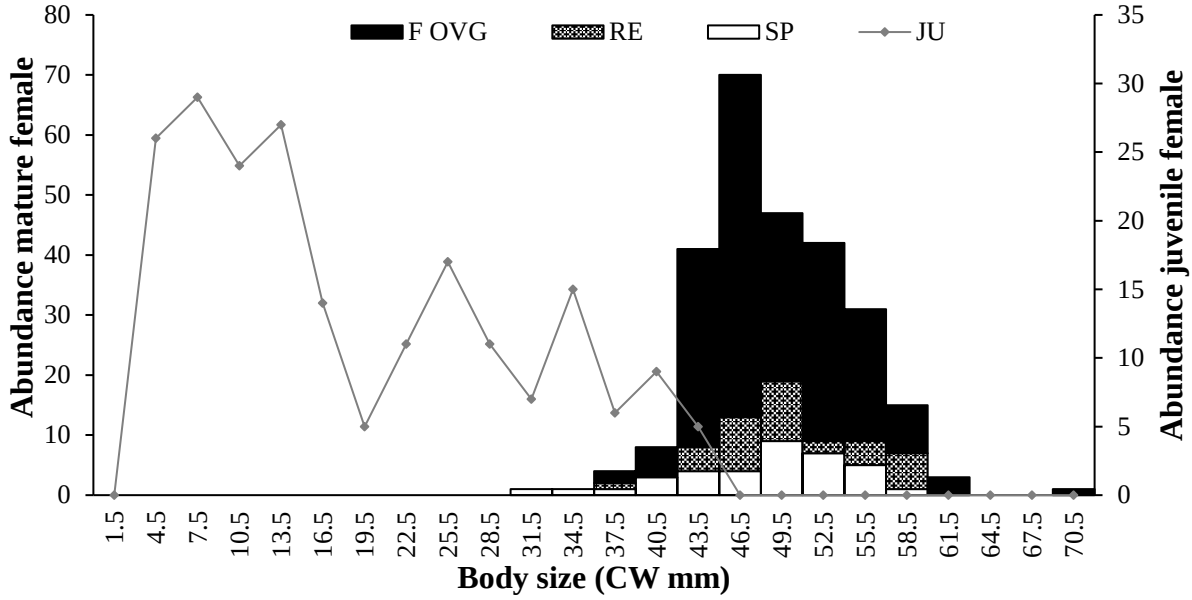


Figure 5. *Libinia ferreirae* Brito Capello, 1871. Distribution of females' demographic groups (OVG: ovigerous female; RE: reproductive SP: spent; JU: juvenile) in size classes, for individuals sampled in the Cananéia region, southern littoral from São Paulo state, from July 2012 through May 2014. The X axis' values represent the midpoint of size classes, i.e.: 1.5 mm = 0 to 3 mm, and so on.

The abundance of ovigerous females was observed to be continuous, with peaks in January, June, August, September and November 2013, and January 2014, when we observed the greatest number of individuals recorded to the studied months (figure 6). Around 85% of the sampled ovigerous females presented developing or developed gonads, ready for the next spawning. Reproductive males did not show a clear pattern of abundance along the studied period, with a low number of sampled crabs (42 individuals).

Juvenile individuals showed two abundance peaks, recorded in September 2012 and March 2014, periods preceded by peaks in the abundance of ovigerous females (figure 5). Correlating the abundance of ovigerous females and juvenile individuals, we could observe a 2-month lag correlation (2 months after peaks in abundance of ovigerous females, we observed increases in the number of juvenile individuals (Cross-correlation: lag= 2, $p<0.05$, r= 0.96).

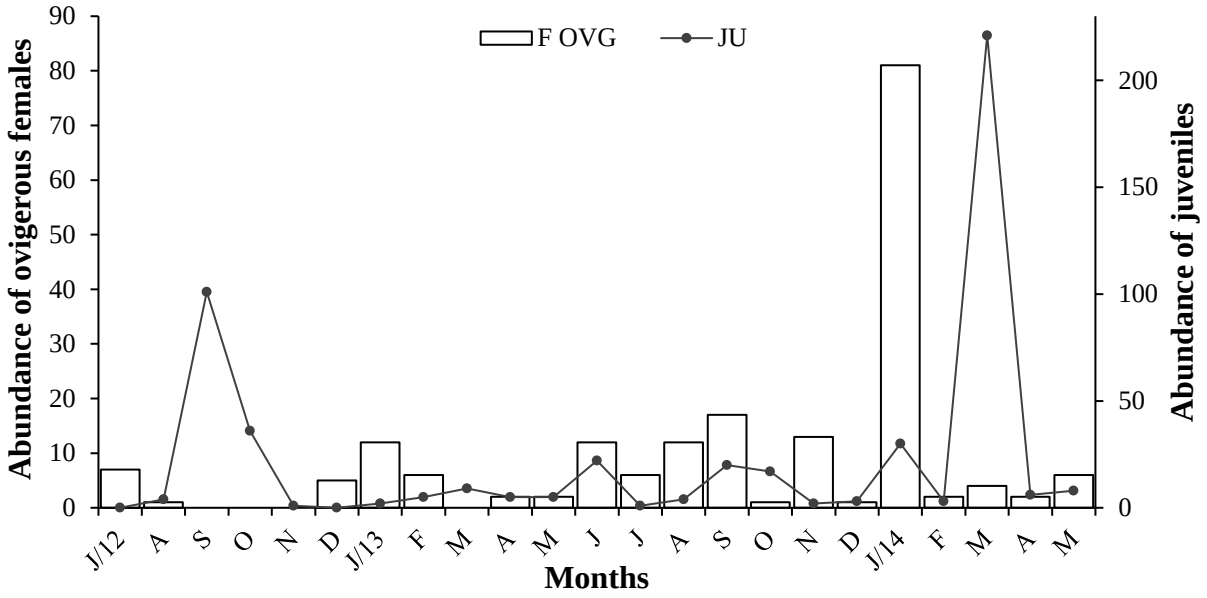


Figure 6. *Libinia ferreirae* Brito Capello, 1871. Monthly abundance of ovigerous females (F OVG) and juvenile (JU) individuals sampled in the Cananéia region, southern littoral from São Paulo state, from July 2012 through May 2014.

The highest abundance of ovigerous females was recorded in the sampling station I (figure 7A), while symbiotic individuals were more abundant in the sampling station VI (figure 7B). Additionally, the highest abundance of reproductive males was recorded at sampling station IV. In the estuarine region, only 11 benthic individuals were sampled (7 females and 4 males) (all of them in sampling station V).

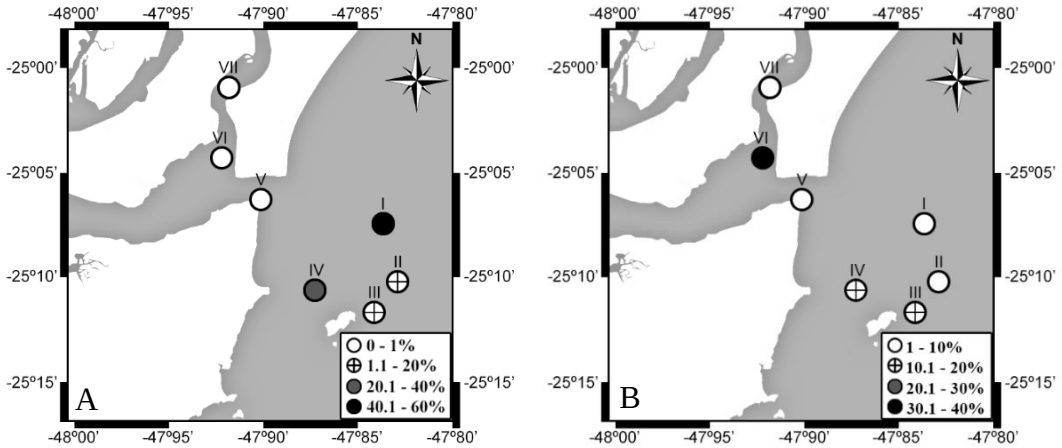


Figure 7. *Libinia ferreirae* Brito Capello, 1871. A) Spatial variation in the abundance of ovigerous females; B) spatial variation in the abundance of symbiotic juveniles, for individuals sampled in the Cananéia region, southern littoral from São Paulo state, from July 2012 through May 2014.

Peaks in the abundance of ovigerous females (January, June, September and November 2013) preceded the periods in which we recorded peaks of phytoplanktonic production (March, July, October and November 2013) (figure 8).

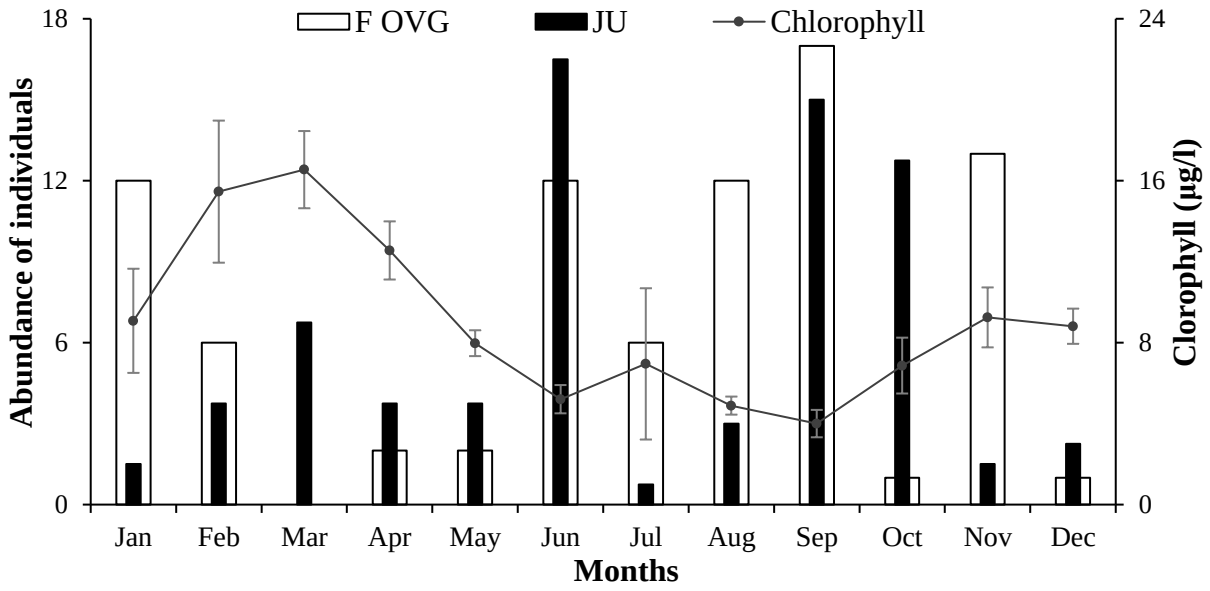


Figure 8**.** *Libinia ferreirae* Brito Capello, 1871. Abundance of ovigerous females and juveniles, and the bottom water chlorophyll concentration (µg/l), sampled in the Cananéia region, southern littoral from São Paulo state, from January through December 2013.

D**iscussion**

Spatiotemporal distribution

Our study evidences variations in spatiotemporal distribution of the spider crab population in the region of Canaéia, in agreement to results obtained to the congener species *Libinia spinosa* H. Milne Edwards, 1834 in the southeastern Brazilian littoral (Braga et al. 2007). We could evidence a clear niche partitioning due to the different habits of the species along their life cycle (symbiotic juveniles and benthic adults). Even though adults are free-living individuals, it is common to observe other living forms adhered to the dorsal portion of their carapace (Winter & Masunari 2006).

Carmona-Suárez (2003) pattern also observed for *Maja crispate* Risso, 1827, where one of the highest peaks in total abundance of benthic individuals was observed during winter,

however, the highest abundance of ovigerous females was observed during summer. Consequently, symbiotic (juvenile) crabs also presented higher abundance in summer, which is reasonable considering that in this period, there is a higher food supply and higher temperature levels which can accelerate larval hatching, shortening their larval stages (Anger 2001). Braga et al. (2007) also found greater number of juveniles of *L. spinosa* at summer and adults at winter, in the region of Caraguatatuba, São Paulo state.

The low abundance (or even the absence of individuals) in some months was also observed by O'Brien et al. (1999) studying *Libinia dubia* H. Milne Edwards, 1834 and *Libinia emarginata* Leach, 1815, which presented lower abundance of individuals in certain seasons of the year. The authors propose that crabs can get into a dormancy state or migrate to other areas in some periods, while De Goursey & Auster (1992) observed that *L. emarginata* actually migrates to deeper regions during winter, where individuals remain burrowed in the substrate until spring. Such statements can be applied to our study due to the low abundance of individuals during autumn and winter periods. However, studies including higher bathymetric amplitude are essential to confirm such migratory patterns.

Adult crabs were little representative in sampling stations inside the estuarine region, running right against the pattern observed for symbiotic individuals. This suggests that along their life cycle, juveniles and adults do not compete for environmental resources, since show different life habits and needs during their development and thus exhibit differential occupation in space and time.

The influence of environmental factors

The environment and its characteristics directly influenced the life habits of *L. ferreirae*. Environmental features such as water temperature, for instance, can alter their development, mainly concerning their growth rate (Hartnoll 1963; Petriella & Boschi 1997). The distribution pattern observed in this study allows us to infer that adult crabs are most

likely found in areas with higher salinity and temperature levels. Rontllant et al. (2014) studying *Maja brachydactyla* Balss, 1922 concluded that salinities higher than 38 can be a limiting factor to the species' life cycle, since it drastically decreases their larval survival. Such intolerance was also observed to congener species by Charmantier (1998), who attested that many Majoidea crabs as *L. emarginata* and *Chionoecetes opilio* (O. Fabricius, 1788) are weak regulators (or osmoconformers), a decisive condition to the establishment of many crustacean species.

On the other hand, it is supposed that the sampling of symbiotic juveniles in higher salinity level conditions is a consequence of the tide flows that carry their cnidarian hosts to such areas, where such salinity oscillations may occur.

Benthic crabs seem to show some preference by sediments composed mainly by silt+clay and very fine sand, as observed for *L. spinosa* in the Caraguatatuba region, São Paulo state (Braga et al. 2007). Therefore, the great abundance of individuals (especially ovigerous females) observed in the sampling station I (sediment mainly composed by very fine sand) can be associated to the burrowing behavior, observed in many Majoidean crabs. Winter & Masunari (2006) stated that *L. ferreirae* spends most of its time burrowed in the sand, when only epizoic associated individuals are visualized emerged from the sediment.

Ovigerous females are strongly influenced by the sediment's texture, since they show lower movement activities and thus burrow themselves in order to avoid predators. Additionally, females can use the nutrients found associated to the sediment as an alternative food source (Braga et al. 2007). Bas et al. (2007) reported that environmental food availability is a key factor in the egg production by females, once its scarcity can considerably reduce fecundity.

Reproductive biology

Ovigerous females were sampled in most of the studied months, and a great portion of them showed developing or even developed gonads, on the way to generate a new embryos mass. González-Gurriarán et al. (1998) reported the same pattern as well, in which as the incubation period of *M. squinado* advance, ovaries become mature again. According to the authors, females of this species can copulate when eggs are still attached to their pleopods, once they already show developed gonads and present the capability of store new spermatic masses. Thus females can show up to four successive spawning without copulate, as observed for *Chiocnocetes opilio* (Elner & Beninger 1995), in which females can present a new mass of eggs in approximately three days after larval hatching.

Our study demonstrates that *L. ferreirae* presents a continuous reproductive pattern as observed for *L. spinosa*, studied in a subtropical region as well (Braga et al. 2007). It is noteworthy that two months after a peak in ovigerous females, we observed a peak in the abundance of juveniles, characterizing a continuous spawning pattern (Crocos & Van der Velde 1995). The occurrence of juveniles was continuous, even though the highest recruitment peak was observed in summer 2014, when higher temperature levels were recorded.

An increasing tendency in the abundance of ovigerous females was observed as the chlorophyll levels (phytoplanktonic production) increase, in agreement to the proposed patterns for crustaceans that present planktotrophic larval stages. Once it is the basis of the food web, serving as food resource to uncountable species which present larval stages, it triggers several stimuli to their incubation of embryos and successive larval hatching (Starr et al. 1994; Elner & Beninger 1995). In the present study, we also observed that peaks in primary productivity were associated to high temperature periods, as proposed by Vega-Perez (1993). The adjustment of larval hatching to the oceanic productivity could justify the success in juvenile recruitment about two months after the peak in ovigerous females.

The onset of sexual maturity for both sexes agrees to the results obtained by Gonçalves et al. (2016b) and Sal Moyano et al. (2011). The amplitude in size of adult individuals was high, even though Majoidea crabs show a pre-pubertal and a terminal molt (Hartnoll 1978), which don't allow them to do susceptible growth molts when in the transition from juvenile to adult individuals. The size range was observed in other studies as well, such as the ones included in the genus *Chionoecetes* (Somerton 1981; Comeau & Conan 1992), which can be associated to differences in the age of sexual maturity or in growth rates (Gonzalez-Gurriarán et al. 1995). In majoidean crabs, the high variability in the size of the onset of sexual maturity is a common population phenomenon especially for males, once the terminal molt can confer them morphometric maturity, with a great size increment (Hartnoll 1963; Conan & Comeau 1986; Comeau & Conan 1992). According to Hartnoll et al. (1993) and Gonzalez-Gurriarán et al. (1995), spatiotemporal differences in environmental conditions (especially temperature and food availability) can influence individual growth rates as well, resulting in increases or decreases in the individuals' sizes.

The observed 1:1 (F:M) sex ratio in the early developmental stages compose a pattern in agreement to the proposed by Fisher (1930), since in these stages there are no agonistic behavior related to reproduction. However, when adults, it was possible to recognize a predominance of females in relation to males (sex ratio: 1:1.6) (M:F). Differently from the results here obtained, Gonzalez-Pisani (2011) observed a 1:1 (M:F) sexual proportion for adults of *L. spinosa*, as well as Degoursey & Auster (1992) to *L. emarginata*. The difference in the abundance of males and reproductive females observed in our study can be associated to the differential environmental occupation observed for each sex, or to the constant rivalry of territorial and⁄or reproductive natures, what could lead into a higher male mortality (Hartnoll 1969). Additionally, male can present a different distribution, including other regions that were not included in this study. According to Diesel (1986), male spider crabs are larger and much more errant than females. However, the low abundance of males should not

directly interfere in their reproductive success, once a single male can copulate uncountable females with no preparing or pre-copula energetic costs (e.g. protecting soft-carapace females, once females can copulate in intermolt stage) (Diesel 1991; Sainte-Marie & Lovrich 1994).

Conclusion

In our study, we could conclude that *L. ferreirae* completes its life cycle in the adjacent littoral from Cananéia since we sampled specimens of variable body sizes, live stages (i.e. juvenile, adult, reproductive) and living habits (symbiotic and free-living crabs). Individuals living in association to medusae seem to have no chance when are carried by their hosts to the estuarine environment, and thus, they must tolerate wide salinity and temperature variations. The sediment texture seems to be a direct influence to the burrowing activity of ovigerous females, which apparently present a higher reproductive activity in summer, when we could record an effective spawning.

This study highlights the importance in preserving marine environments, once *L. ferreirae* inhabits two different environments during its life cycle. It evidences the different habitat occupation among juveniles and adults, as well as the dependence of other living forms to complete their ontogeny. The peculiarity of each region is a key factor modeling its population dynamics. If there are no favorable environmental conditions to develop and reproduce, and additionally, there is a great pressure by the fishing activity in a given area, a population can face a collapse in its structure. The information here present can be used as base to the coastal marine resources management, as well as to bycatch species preservation, once they are affected by fishing activities during their life as well (e.g. *L. ferreirae*). Additionally, it is essential to conduct further studies focusing on community and behavioral aspects, ecological relations and the influence of such parameters over the local ecosystem.

References

Abelló, P., 1989. Reproduction and moulting in Liocarcinus depurator (Linnaeus, 1758) (Brachyura: Portunidae) in the northwestern Mediterranean. Scientia Marina, 53: 127–134.

Almeida, A. C., C. M. Hiyodo, V. J. Cobo, G. Bertini, V. Fransozo & G. M. Teixeira., 2013. Relative growth, sexual maturity, and breeding season of three species of the genus Persephona (Decapoda: Brachyura: Leucosiidae): a comparative study. Journal of the Marine Biological Association of the United Kingdom 93(6): 1581–1591.

Anger, K., 2001. The biology of Decapod Crustacean larvae. Crustacean Issues 14, Balkema Lisse.

Bas, C. C., E. D. Spivak & K. Anger, 2007. Seasonal and interpopulational variability in fecundity, egg size, and elemental composition (CHN) of eggs and larvae in a grapsoid crab, Chasmagnathus granulatus. Helgoland Marine Research 61: 225–237.

Bolla Jr. E. A. & M. L. Negreiros-Fransozo, 2016. Morphology of juvenile phase of Achelous spinimanus (Latreille, 1819) (Crustacea, Decapoda, Portunidae) reared in laboratory. Journal of the Marine Biological Association of the United Kingdom 3: 615–631.

Braga, A. A., A. Fransozo, G. Bertini & P. B. Fumis, 2007. Bathymetric distribution and recruitment of the spider crab Libinia spinosa H. Milne Edwards 1834 in the Ubatuba and Caraguatatuba regions, northern coast of São Paulo, Brazil (Crustacea, Brachyura, Majoidea, Pisidae). Senckenbergiana Biological 87(1): 7–16.

Branco, J. O., F. Freitas Jr. & M. L. Christoffersen, 2015. Bycatch fauna of seabob shrimp trawl fisheries from Santa Catarina State, southern Brazil. Biota Neotropica 15(2): 1–14.

Carmona-Suárez, C. A., 2003. Reproductive biology and relative growth in the spider crab Maja crispata (Crustacea, Brachyura, Majidae). Scientia Marina 67(1): 75–80.

Castilho, A. L., R. C. Costa, A. Fransozo & M. L. Negreiros-Fransozo, 2008a. Reproduction and recruitment of the South American red shrimp, Pleoticus muelleri (Crustacea:

Solenoceridae), from the southeastern coast of Brazil. Marine Biology Research 4: 361–368.

Castilho, A. L., M. R. Pie, A. Fransozo, A. P. Pinheiro & R.C. Costa, 2008b. The relationship between environmental variation and species abundance in shrimp community (Crustacea, Decapoda, Penaeoidea) in Southeastern Brazil. Journal of the Marine Biological Association of the United Kingdom 88: 119–123.

Castilho, A. L., R. T. Bauer, F. A. M. Freire, V. Fransozo, R. C. Costa, R. C. Grabowski & A. Fransozo, 2015. Lifespan and reproductive dynamics of the commercially important sea bob shrimp Xiphopenaeus kroyeri (Penaeoidea): synthesis of a 5-year study. J Crustacean Biology, 00(0): 1–11.

Charmantier, G., 1998. Ontogeny of osmoregulation in crustaceans: a review. Invertebrate Reproduction Development 33: 177–190.

Choy, S. C., 1988. Reproductive biology of Liocarcinus puber and L. holsatus (Decapoda, Brachyura, Portu-nidae) from the Grower Peninsula, South Wales. Marine Ecology 9: 227–291.

Comeau, M. & G. Y. Conan, 1992. Morphometry and gonad maturity of male snow crab, Chionoecetes opilio. Canadian Journal of Fisheries and Aquatic Sciences 49: 2460–2468.

Conan, G. Y. & M. Comeau, 1986. Functional maturity and terminal molt of male snow crab, Chionoecetes opilio. Canadian Journal of Fisheries and Aquatic Sciences 43: 1710–1719.

Crocos, P. J. & T. D. Van Der Velde, 1995. Seasonal, spatial and interanual variability in the reproductive dynamics of the grooved tiger prawn Penaeus semisulcatus in Albatross bay, Gulf of Carpentaria, Australia: the concept of effective spawning. Marine Biology, 122: 557– 570.

DeGoursey, R. E. & P. J. Auster, 1992. A mating aggregation of the spider crab (Libinia emarginata). Journal of Northwest Atlantic Fishery Science, 13: 77–82.

Diesel, R., 1986. Optimal mate searching strategy in the symbiotic spider crab Inachus phalangium (Decapoda). Ethology 72: 311–328.

Diesel, R., 1991. Sperm competition and the evolution of mating behavior in Brachyura, with special reference to spider crabs (Decapoda: Majidae). In Bauer, R. G. & J. W. Martin (eds), Crustacean sexual biology, Columbia University Press, New York: 145–163.

Dring, M. J., 1992. The biology of marine plants. Cambridge University Press, Cambridge.

Elner, R. W. & P. G. Beninger, 1995. Multiple reproductive strategies in snow crab, Chionoecetes opilio: physiological pathways and bahavioral plasticity. Journal of Experimental Marine Biology and Ecology 193: 93–112.

Fisher, R. A., 1930. The genetical theory of natural selection. Clarendon Press, Oxford

Gonçalves, G. R. L., M. R. Wolf, R. C. Costa & A. L. Castilho, 2016a. Decapod crustacean associations with scyphozoan jellyfish (Rhizostomeae: Pelagiidae) in the Southeastern Brazilian coast. Symbiosis, 69: 193–198.

Gonçalves, G. R. L., E. B. Bolla Jr., M. L. Negreiros-Fransozo & A. L. Castilho, 2016b. Morphometric and gonadal maturity of the spider crab Libinia ferreirae Brito Capello, 1871 (Decapoda: Majoidea: Epialtidae) at Southeastern Brazilian coast. Journal of the Marine Biological Association of the United Kingdom doi:10.1017/S0025315416000370.

González-Gurriarán, E., L. Fernández, J. Freire & R. Muiño, 1998. Mating and role of seminal receptacles in the reproductive biology of the spider crab Maja squinado (Decapoda, Majidae). Journal of Experimental Marine Biology and Ecology 220(2): 269–285.

Gonzalez-Gurriaran, E., J. Freire, J. Parapar, M. P. Sampedro & M. Urcera, 1995. Growth at moult and moulting seasonality of the spider crab, Maja squinado (Herbst) (Decapoda:

Majidae) in experimental conditions: implications for juvenile life history. Journal of Experimental Marine Biology and Ecology 189: 183–203.

González-Pisani, X., 2011. Biología Reproductiva de los cangrejos araña Leurocyclus tuberculosus y Libinia spinosa (Brachyura, Majoidea) del norte de Patagonia. PhD Thesis, University of Buenos Aires, Argentina.

Graça-Lopes, R., A. R. G. Tomás, S. L. S. Tutui, E. Severino-Rodrigues & A. Puzzi, 2002. Fauna acompanhante da pesca camaroeira no litoral do Estado de São Paulo, Brasil. Boletim do Instituro de Pesca, São Paulo 28(2): 173–188.

Guinot, D. & J. M. Bouchard, 1998. Evolution of the abdominal holding systems of brachyuran crabs (Crustacea, Decapoda, Brachyura). Zoosystema 20: 613–694.

Haefner JR., J. A., 1990. Morphometry and size at maturity of Callinectes ornatus (Brachyura, Portunidae) in Bermuda. Bulletin of Marine Science 46: 274–286.

Hakanson, L. & M. Jansson, 1983. Principles of lake sedimentology. Springer-Verlag: New York, Berlin, Heidelberg, Tokyo.

Hartnoll, R. G., 1969. Mating in the brachyura. Crustaceana 16: 161–181.

Hartnoll, R. G., 1978. The determination of relative growth in Crustacea. Crustaceana 34: 281–293.

Hartnoll, R. G., 1982. Growth. In Bliss, D. E. (ed.), The Biology of Crustacea: embryology, morphology and genetics. Academic Press, New York: 111–196.

Hartnoll, R. G., 1963) The biology of Manx spider crabs. Proceedings of the zoological Society of London 141: 423–496.

Hartnoll, R. G., A. D. Bryant, & P. Gould, 1993. Size distribution in spider crab populations spatial and temporal variation. Journal Crustacean Biology 13(4): 647–655.

Hultgren, K. & J. Stachowicz, 2011. Camouflage in decorator crabs. In Stevens, M. & S. Merilaita (eds), Animal Camouflage: Mechanisms and Function. Cambridge University Press, Cambridge: 214–238

Ingle, R. W., 1977. The larval and post-larval development of the scorpion spider crab, Inachus dorsettensis (Pennant) (family: Majidae), reared in the laboratory. Bulletin of the British Museum (Natural History): Zoology 30: 329–348.

Jones DR & Hartnoll RG (1997) Mate selection and mating behaviour in spider crabs. Estuar Coast Shelf S, 44(2): 185–193.

Melo, G. A. S., 1996. Manual de identificação dos Brachyura (caranguejos e siris) do Litoral Brasileiro. São Paulo: Plêiade. FAPESP.

Nogueira Jr., M. & M. A. Haddad, 2005. Lychnorhiza lucerna (Scyphozoa, Rhizostomeae) and Libinia ferreirae Brito Capello (Brachyura, Majidae) association in southern Brasil. Revista Brasileira de Zoologia 22: 908–912.

O'Brien, S. B., M. Landau & K. W. Able, 1999. Sex ratios of two species of spider crabs, Libinia dubia H. Milne Edwards, 1834 and L. emarginata Leach, 1815: in the area of Great Bay, New Jersey. Crustaceana 72: 187–192.

Petriella, A. M. & E. E. Boschi, 1997. Crecimiento en crustáceos decápodos: Resultados de investigaciones realizadas en Argentina. Investigaciones Marinas 25: 135–157.

Pohle, G., F. L. Mantelatto, M. L. Negreiros-Fransozo & A. Fransozo, 1999. Larval Decapoda (Brachyura). In: Boltovskoy, D. (ed.), South Atlantic Zooplankton. Leiden, Backhuys Publisher: 1281–1351.

Rotllant, G. C. G. Simeó, G. Macià & A. Estévez, 2014. High environmental salinity reduces the reproductive potential of the spider crab Maja brachydactyla (Decapoda, Majidae). Marine Ecology 36(3): 469–505.

Sainte-Marie, B. & G. A. Lovrich, 1994. Delivery and storage of sperm at first mating of female Chionoecetes opilio (Brachyura, Majidae) in relation to size and morphometric maturity of male parent. Journal of Crustacean Biology 14: 508–521.

Sal Moyano, M. P., M. A. Gavio & M. D. Maggi, 2011. Morphometric and gonad maturity of the spider crab Libinia spinosa (Crustacea: Brachyura: Majoidea: Epialtidae) in

Argentina. Journal of the Marine Biological Association of the United Kingdom 91: 837–844.

Sastry, A. N., 1983. Ecological aspects of reproduction. In Vernberg, F. J. & W. B. Vernberg (eds). The Biology of Crustacea. Environmental Adaptation. Academic Press, New York: 179–270.

Schroeder, R., J. O. Branco, F. Freitas Jr. & C. Resgalla Jr., 2014. Preliminary assessment of the jellyfish bycatch captured off southern and southeastern Brazil. Latin American Journal of Aquatic Research 42: 289–300.

Somerton, D. A., 1981. Regional variation in the size of maturity of two species of tanner crab (Chionoecetes bairdi and C. opilio) in the eastern Bering Sea, and its use in defining management subareas. Canadian Journal of Fisheries and Aquatic Sciences 38: 163–174.

Starr, M., J. C. Theriault, G. Y. Conan, M. Comeau & G. Robichaud, 1994. Larval release in a sub-euphotic zone invertebrate triggered by sinking phytoplankton particles. Journal of Plankton Research 16: 1137–1147.

Tavares, M., W. Santana, 2012. On the morphological differentiation between Libinia spinosa and L. ferreirae (Crustacea: Brachyura: Majoidea: Epialtidae). Zoologia (Curitiba) 29: 577–588.

Thorson, G., 1950. Reproductive and larval ecology of marine bottom invertebrates. Zoological Museum, University of Copenhagen.

Tucker M., 1988. Techniques in sedimentology. Oxford: Blackwell.

Varisco, M. & J. Vinuesa, 2011. Reproductive biology of the spider crab Leucippa pentagona (Decapoda: Epialtidae), in Bustamante Bay, Argentina. Revista de Investigaciones Marinas 39(3): 471–480.

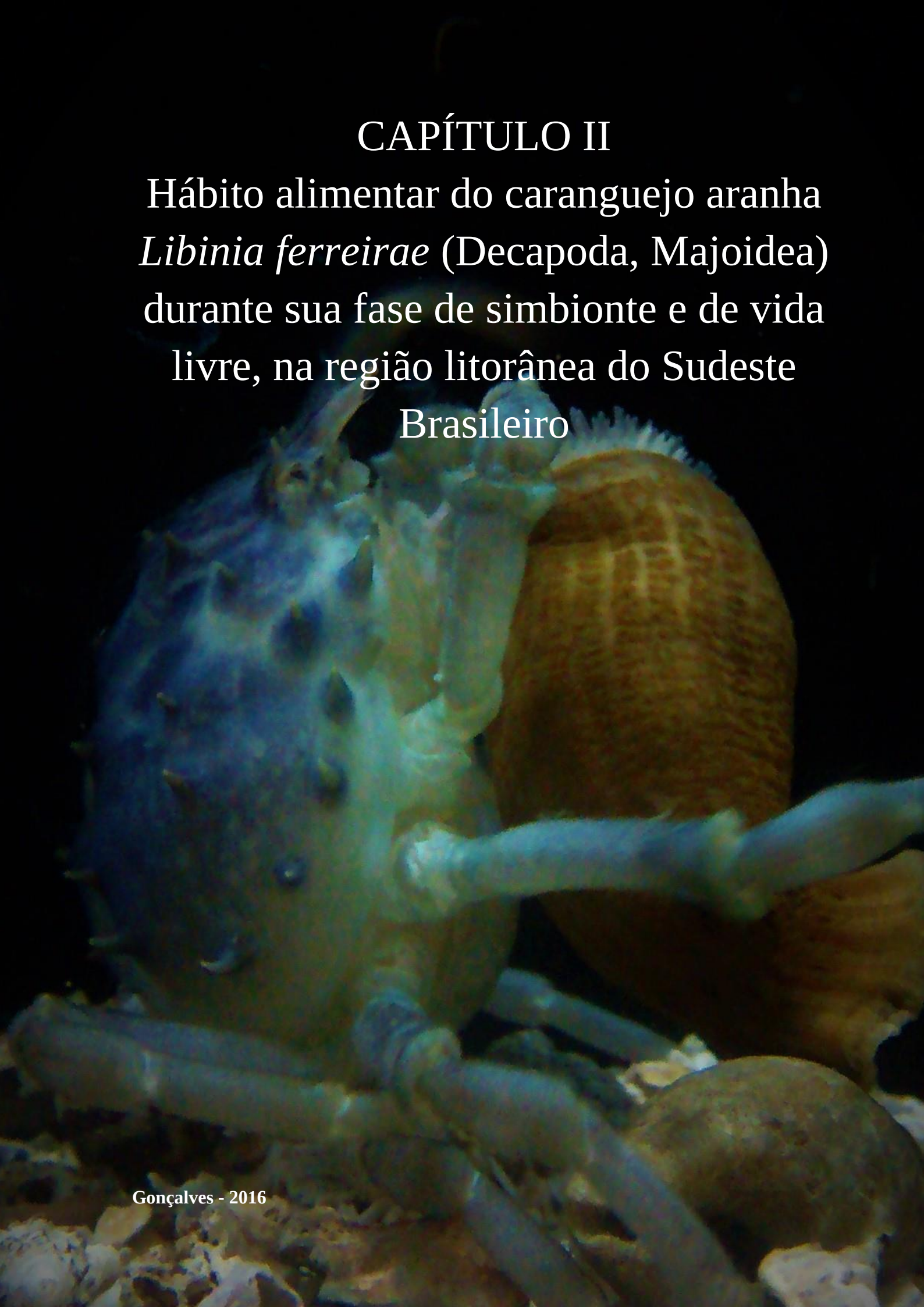
Vega-Perez, L. A., 1993. Estudo do zooplâncton da região de Ubatuba, Estado de São Paulo. Publicação Especial do Instituto Oceanográfico 10: 65–84.

Winter, V. C. & S. Masunari, 2006. Macroepizoismo em Libinia ferreirae (Crustacea, Brachyura, Majidae). Iheringia Série Zoologia 96(2): 135–140.

Zar, J. H., 1999. Biostatistical Analysis. Upper Saddle River: Pratice-Hall.

CAPÍTULO II

Hábito alimentar do caranguejo aranha *Libinia ferreirae* (Decapoda, Majoidea) durante sua fase de simbionte e de vida livre, na região litorânea do Sudeste Brasileiro



Gonçalves - 2016

Resumo: A dieta alimentar do caranguejo aranha *Libinia ferreirae* no litoral de Cananéia/SP – Brasil foi estudada, descrevendo a diversificação alimentar temporalmente, por sexo e quanto ao seu hábito de vida livre bentônico e associado à medusa *Lychnorhiza lucerna*. Os caranguejos foram coletados de jul/2013 a mai/2014, previamente triados no barco, e acondicionados sob resfriamento. Em laboratório foram dissecados 142 estômagos, sendo 99 de indivíduos bentônicos e 43 dos associados à medusa, foi identificado 11 itens alimentares na dieta do caranguejo. Material não identificado, sedimento, crustáceos e cnidários foram os principais itens da dieta, encontrando-se diferença na frequência de ocorrência dos mesmos nos grupos de caranguejos bentônicos e associados às medusas (χ^2 $p < 0,05$). Os caranguejos associados às medusas, diferente dos bentônicos, não apresentaram peixes e poríferas no seu conteúdo estomacal. Em contrapartida, registrou-se nos estômagos desses simbiontes crustáceos Copepoda, além de itens alimentares repletos de nematocistos oriundos da medusa hospedeira. Já os caranguejos bentônicos tinham crustáceos como Cirripedia, Penaeoidea e Brachyura em seu conteúdo estomacal, e os nematocistos encontrados eram das anêmonas provavelmente hospedadas em sua carapaça. Foi demonstrada diferença na frequência de ocorrência entre os hábitos alimentares por sexos dos caranguejos bentônicos (χ^2 $p<0,05$), porém, não foi encontrada diferença significativa temporalmente. Quanto à porcentagem de pontos, os itens alimentares que demonstraram diferença entre os grupos bentônicos e associados foram as algas, foraminíferos, moluscos e sedimento, segundo o Teste de Goodman. Este caranguejo é um predador oportunista e demonstra preferência por crustáceos, tendo distintos hábitos quanto ao sexo e fases de desenvolvimento.

Palavras chave: conteúdo estomacal, método dos pontos, frequência de ocorrência, grupos demográficos, associação

Introdução

A ingestão alimentar para os crustáceos é um fator determinante para o crescimento, pois animais que são privados de alimentação podem comprometer a prole, por influenciar diretamente no investimento reprodutivo. Grande parte da energia originada através da alimentação é usada para os processos de maturação e produção de gametas, além do cuidado parental (Hartnoll 1982).

As necessidades nutricionais dos indivíduos e da população, suas interações com outros organismos, além da importância econômica que podem gerar com novas técnicas para o cultivo de espécies com valor comercial, são alcançados a partir do conhecimento da ingestão alimentar da espécie de interesse (Williams, 1981). Segundo Petti (1990), entender os hábitos alimentares é fundamental para compreensão da participação dos indivíduos na rede trófica. Estre as informações que a alimentação pode trazer sobre a espécie, podemos citar: os padrões de distribuição, migração, muda, reprodução (McLaughlin & Hebard, 1961), mudanças da dieta entre subgrupos da mesma espécie, variação sazonal (Hyslop, 1980), modos ou hábitos usados na ingestão alimentar, característica do ambiente, e disponibilidade de presas no mesmo (Barros *et al*., 2008).

Os hábitos alimentares de muitos crustáceos pode ser um modulador do ambiente, ao controlar populações de outras espécies, como visto por Hotland *et al*. (1980), que a densidade da infauna bentônica aumentava quando peixes e caranguejos eram retirados do ambiente.

No entanto, ter a precisão dos alimentos ingeridos é trabalhoso, pela dificuldade na identificação e a mensuração, especialmente em crustáceos com aparelhos bucais e ossículos do moinho gástrico que geralmente reduzem os alimentos a pequenos fragmentos. Porém, é possível qualificar e quantificar os itens alimentares (Williams 1981).

O caranguejo aranha *Libinia ferreirae* Brito Capello, 1871 tem distribuição geográfica restrita do oceano Atlântico Ocidental, ocorrendo da Venezuela ao Brasil, distribuindo-se

desde o Estado do Pará até Santa Catarina, desde a região costeira até a profundidade máxima de 35 m (Melo, 1996).

As relações ecológicas podem determinar a ocupação e desenvolvimento de *L. ferreirae* em um determinado habitat, pois esta espécie possui uma íntima relação com outros grupos de animais ao longo do seu ciclo de vida.

Segundo Gonçalves et al. (2016), *Libinia ferreirae* durante as fases de megalopa e jovem vivem associadas à medusa *Lychnorhiza lucerna* Haeckel, 1888, onde obtém deslocamento, alimentação e proteção. Durante sua fase adulta vivem em íntima relação com outros organismos, principalmente as anêmonas-do-mar. Embora estes caranguejos demandem um maior tempo e gasto energético por transportar outros indivíduos, conseguem se locomover sem risco de predação por um espaço maior, o que influencia o sucesso evolutivo (Hultgren & Stachowicz, 2011; Winter & Masunari (2006).

O objetivo do presente estudo é identificar qualitativamente e quantitativamente os itens alimentares que compõem a dieta dos caranguejos aranha *L. ferreirae*, na região de Cananéia – SP, levando em consideração variação sazonal da dieta, diferença entre sexos, e fases de vida, com o intuito de verificar se ocorre diferença na ingestão alimentar dos indivíduos associados à água-viva quando jovens e de vida livre, quando adultos.

Material e método

Coleta do material biológico

As coletas foram realizadas de julho/2013 a maio/2014 no complexo Sistema Estuarino-Lagunar de Cananéia-Iguape e área adjacente oceânica (25º04’43”S, 47º50’34”O), estado de São Paulo, través de um barco camaroeiro. Os caranguejos associados à medusa *L. lucerna* e os bentônicos de vida livre foram coletados e individualizados separadamente em sacos plásticos identificados e rapidamente resfriados para parar o processo de digestão Williams (1981).

Os caranguejos capturados foram identificados com base nos estudos realizados por Melo (1996) e Tavares & Santana (2012). Os espécimes foram separados quanto ao sexo, tomando por base o formato do abdômen (padrão alongado para os machos, e oval para as fêmeas) e pelo número de pleópodos (2 pares de pleópodos para os machos e 4 pares para as fêmeas) (Ingle 1977; Sampedro *et al*., 1999).

Os indivíduos foram analisados quanto à aderência do abdômen ao esternito cefalotorácico. Os caranguejos considerados juvenis (inaptos à reprodução) são os que apresentaram abdômen selado através de uma substância cimentante (Bolla & Negreiros-Fransozo, 2015). Por outro lado, os adultos (aptos à reprodução) foram considerados aqueles cujo abdômen não está selado ao esternito cefalotorácico sem substâncias cimentantes (Haefner, 1990b; Guinot & Bouchard, 1998). Os animais foram medidos quanto à largura da carapaça, utilizando-se de um paquímetro digital (precisão: 0,01 mm), incluindo apenas indivíduos maiores que 9.99 mm de largura de carapaça, devido à dificuldade de retirar estômagos de caranguejos menores.

Conteúdo estomacal

Caranguejos usados para esta análise tiveram os estômagos retirados, rebatendo a carapaça e través de uma pinça extraídos com cuidado para que não fosse perfurado, em seguida foram fixados em álcool 70% para se manter o estado de preenchimento do mesmo e de preservação dos itens alimentares (Williams, 1981).

Para a avaliação do preenchimento estomacal foi utilizado um método modificado de Williams (1981) e Mantelatto & Christofoletti (2001), no qual cada estômago foi categorizado em três classes quanto ao volume: Cheio= 70 a 100% preenchido; Incompleto = 20 a 70% preenchido; Vazio= 0 a 20% preenchido. Esta avaliação visual foi possível graças à natureza do tecido estomacal, o qual apresenta um revestimento fino e translúcido. Para tais análises, todos os estômagos obtidos foram utilizados, independentemente da categoria a que

pertenciam. Em seguida seu conteúdo foi observado sob estereomicroscópio com sistema de imagem (Zeiss® Stemi SV6), provido de sistema de captura de imagem (Zeiss Stemi 2000-C) e microscópio (Zeiss® Axioskop 2 plus), sendo que cada conteúdo (presa) encontrado foi identificado até classe ou ordem, utilizando-se literatura específica (González Carman, 2014; Brusca & Brusca, 2007; Rupprt et al., 2005; Mariscal 1974).

O método para a pontuação quantitativa dos itens alimentares foi modificado de Williams (1981) e Mantelatto & Christofoletti (2001). A contribuição relativa de cada categoria alimentar, em relação ao volume total de cada estômago analisado, foi avaliada utilizando uma escala de 1 a 10, sendo: valor 1= contribuição de 0 a 10% do volume estomacal; valor 2= contribuição de 10 a 20% do volume estomacal; valor 3= contribuição de 20 a 30% do volume estomacal, e assim sucessivamente. Em seguida, o valor total de pontos de cada item alimentar foi calculado multiplicando-se o seu valor de contribuição relativa por um valor dependente da categoria de preenchimento estomacal, sendo: Categoria Cheio= valor 1; Categoria Incompleto= valor 0,5; Categoria Vazio= valor 0,1.

Para cada categoria alimentar foram calculadas a porcentagem de pontos e a frequência de ocorrência, ambos descritos por Williams (1981), como se segue:

- Porcentagem de pontos - $p_i = \frac{\sum_{j=1}^{n} a_{ij}}{A} \times 100$, sendo:

  p_i = porcentagem de pontos para o item alimentar *i*;

  n = número total de caranguejos analisados;

  a_{ij} = pontuação do item *i* no estômago do caranguejo *j*;

  A = pontuação total de todos os itens alimentares para todos os caranguejos analisados;

- Frequência de ocorrência - $f_i = \left(\frac{b_i}{n}\right) \times 100$, sendo:

  f_i = frequência de ocorrência do item alimentar *i*;

  b_i = número de indivíduos que continham o item alimentar *i*;

n = número total de caranguejos analisados

A correlação de Spearman foi usada para comparar a porcentagem dos pontos e a frequência de ocorrência dos itens alimentares encontrados.

As porcentagens de pontos de cada item alimentar (quanto por cento de cada item foi encontrado no total das amostras) dos grupos dos caranguejos associados e bentônicos, e a diferença entre a porcentagem de cada item, foram comparadas entre si, utilizando as análises de contraste entre e dentro de proporções multinomiais, proposto por Goodman (1964; 1965) *apud* Curi & Moraes (1981). Esta análise pode ser aplicada para dois casos. Pode-se fixar a classe e verificar o contraste entre as combinações lineares das proporções multinomiais, utilizando-se a estatística G para verificar a significância dos contrastes. Pela comparação do valor calculado de G com o respectivo valor crítico (dependente do número de multinomiais e de classes). Se o valor calculado de G for maior que o seu valor crítico, rejeita-se a hipótese nula, que é a igualdade entre as proporções das multinomiais combinadas par a par.

No segundo caso, pode-se fixar a multinomial e estudar os intervalos de confiança para contrastes entre as proporções das classes, cujos limites inferior e superior são calculados utilizando valores críticos baseados no número de classes. Neste caso, a hipótese nula (igualdade das proporções) é rejeitada caso o intervalo de confiança não inclua o valor zero.

Por fim, o teste de χ^2 foi aplicado para comparar os itens alimentares quanto a frequência de ocorrência, por grupo e entre os grupos (associados e bentônicos) e por sexo (para os caranguejos bentônicos) (Zar, 1999). As análises das estações para cada grupo individualmente, foram testadas pela análise de variância (Kruskal-Wallis test).

Resultados

Foram analisados 142 estômagos de *L. ferreirae*, sendo que destes 99 eram indivíduos bentônicos (66 fêmeas com 38,08 – 71,64 mm de LC, e 33 machos com 21,3 – 71,13 mm de LC) e 43 (25 fêmeas com 10 – 49,91 mm de LC, e 18 machos com 11,7 a 49,59 mm de LC) estavam em associação com a medusa *L. lucerna*, de hábito distinto planctônico. Todos os caranguejos coletados estavam em estágio de intermuda.

Mesmo com itens alimentares dilacerados e em acelerado processo de digestão, foram identificados 11 itens alimentares (tabela 1, figura 1 e 2), sendo que os alimentos que estavam em estado avançado de digestão, não sendo possível as identificações foram classificados como material não identificado (MNI), por conseguinte este item foi o que teve maior frequência de ocorrência em ambos os grupos amostrados.

Tabela 1. Descrição dos itens alimentares encontrados nos estômagos dos caranguejos aranha *Libinia ferreirae* bentônicos de vida livre e associados à medusa *L. lucerna*.

Item Alimentar	**Descrição**
Material não identificado (MNI)	Material muito dilacerado e em avançado estado de digestão
Sedimento (SED)	Grãos de areia
Alga (ALG)	Fragmentos de algas e diatomáceas
Porifera (POR)	Espículas (megascleras e microscleras)
Bryozoa (BRY)	Colônia de autozoóides
Cnidaria (CNI)	Nematocistos, Hydrozoa (hidrocaule e hidroteca)
Mollusca (MOL)	Fragmentos de conchas, Bivalve, partes de Cephalopoda como tentáculo, discos da ventosa e bico córneo
Polychaeta (POL)	Parapódios, e partes de tubos
Crustacea (CRU)	Fragmentos de apêndices, larvas, brânquias, ovos, Copepoda
Peixe (PEI)	Vértebras e escamas
Foraminifera (FOR)	Testas calcárias

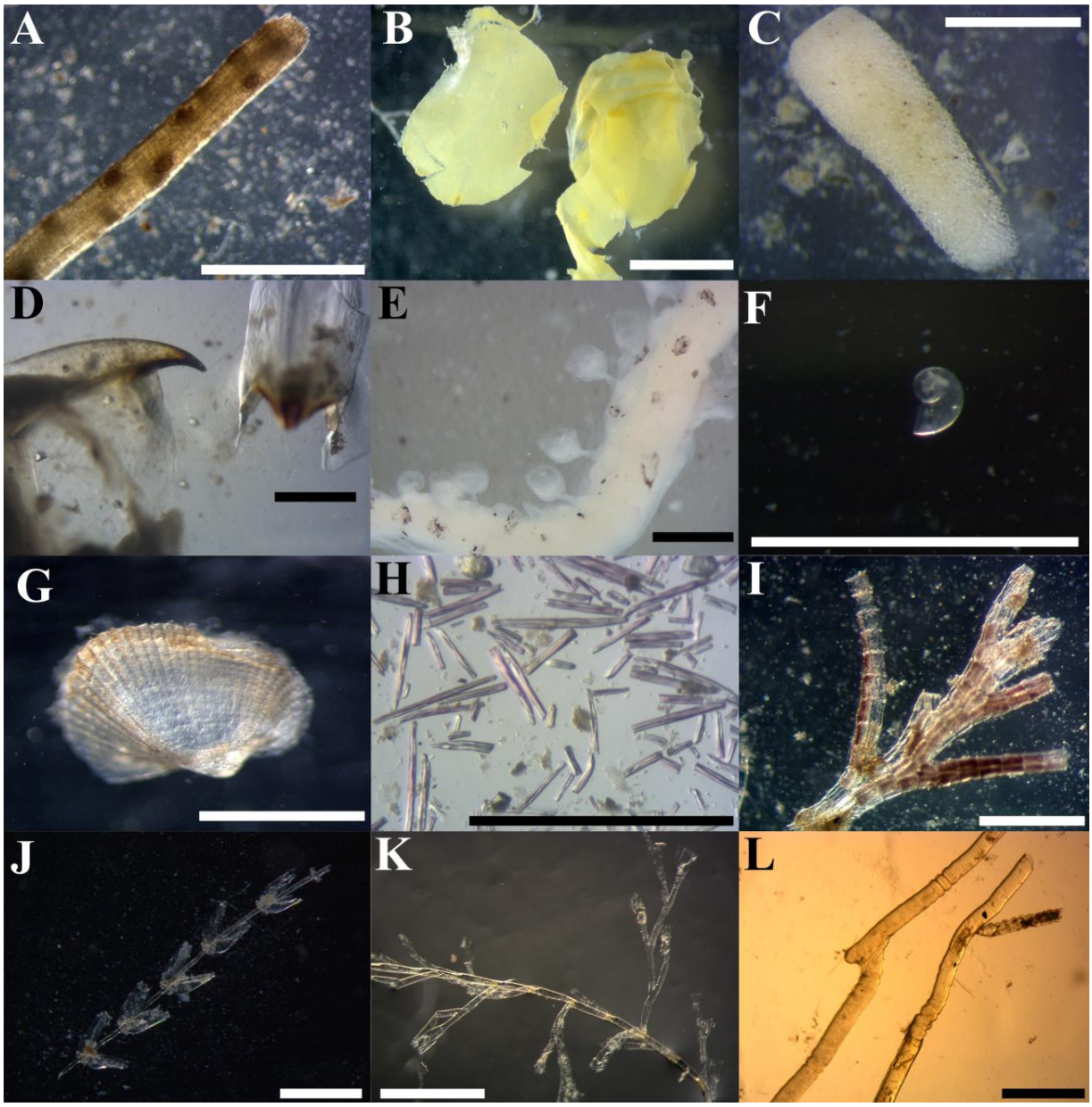


Figura 1. Itens alimentares encontrados no conteúdo estomacal de *Libinia ferreirae* na região de Cananéia SP. A) e B): fragmentos de algas; C) Foraminíferos; D) e E) Mollusca, fragmentos de lulas; F) Mollusca, Gastropoda; G) Mollusca, Bivalve; H) Porifera, espículas; I) Bryozoa; J, K e L) Cnidaria, Hydrozoa. Escala: 1 mm.

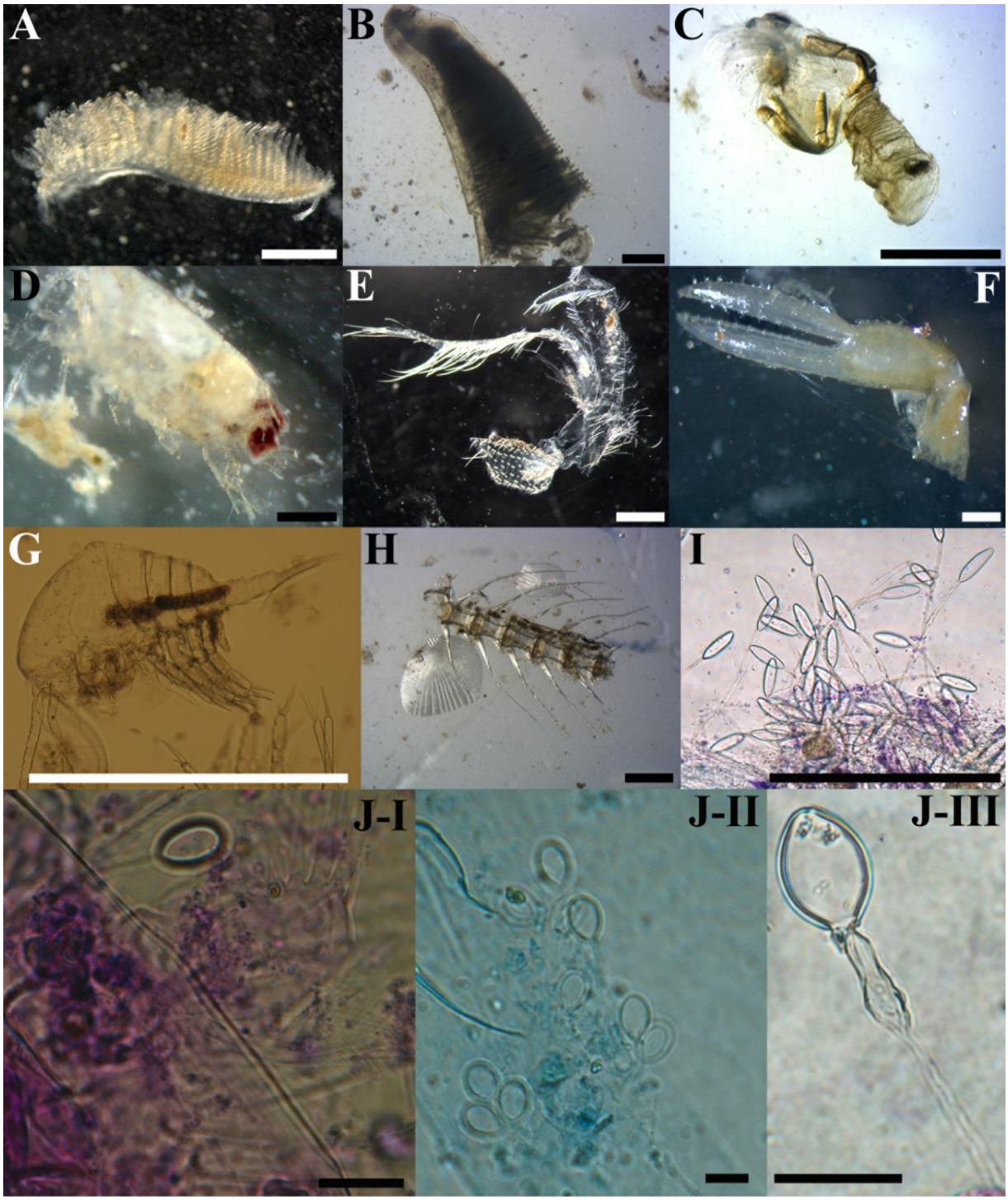


Figura 2. Itens alimentares encontrados no conteúdo estomacal de *Libinia ferreirae* na região de Cananéia SP. A) Crustacea, tricobrânquias; B) Crustacea, filobrânquias; C) Crustacea, Cirripedia; D) Crustacea, Penaeoidea; E) Crustacea, maxilípede; F) Crustacea, Brachyura; G) Crustacea, Copepoda; H) Peixe; I) Cnidaria, Anthozoa, nematocistos; J-I, J-II e J-III) nematocistos de *Lychnorhiza lucerna* aderidos com seus flagelos em presas capturadas pela medusa. Escalas: D = 0.2 mm; G = 0.7 mm; I = 0.3 mm; J = 20 μm; A, B, C, E, F, H = 1 mm.

Correlação positiva foi encontrada entre a porcentagem de pontos e a frequência de ocorrência dos itens alimentares dos caranguejos de vida livre (Spearman: r = 0,89) e associados (Spearman: r = 0,82). Desta maneira, foram mais concentradas nossas análises na frequência de ocorrência.

Foi demonstrado diferença significativa na frequência de ocorrência dos itens alimentares entre os grupos dos caranguejos associados a medusa e aqueles que são bentônicos (χ^2 teste: P<0,05). Sendo que, entre os itens alimentares de cada grupo houve diferença significativa entre: alga, cnidário, foraminífero, peixes, poríferas e sedimento (χ^2 teste: P<0,05).

Para os caranguejos bentônicos, após a maior frequência de ocorrência MNI, foram encontrados, sucessivamente, sedimento, crustáceos, cnidários e algas (figura 3). Os itens menos representativos foram Polychaeta e Bryozoa. Os caranguejos associados tiveram maior frequência de ocorrência, após MNI, de cnidário, crustáceos e sedimento (figura 3), entretanto, além da diferença da frequência de ocorrência, houve ausência de representantes de peixes e Porifera, quando comparados aos bentônicos. Ainda os indivíduos associados tinham Copepoda no conteúdo estomacal (figura 2) e pedaços de larvas, diferindo dos bentônicos que tinham grandes pedaços de Brachyura, Penaeoidea e Cirripedia. Também os nematocistos dos cnidários encontrados diferiram entre os dois grupos, já que os de vida livre possuíam nematocistos oriundos de anêmonas (50% dos machos e 75,5% das fêmeas, tinham anêmonas na carapaça) e os caranguejos associados continham nematocistos advindos de sua hospedeira *L. lucerna*.

Entre os sexos dos caranguejos aranha bentônicos foram encontradas diferenças significativas (χ^2 teste: P<0,05) nos hábitos alimentares, sendo que a frequência de ocorrência foi diferente para os itens: Cnidaria, Foraminifera e Policheta (χ^2 teste: P<0,05). Sendo que, para as fêmeas ocorreu maior frequência de ocorrência de crustáceos nos itens alimentares do

que para os machos, porém essa frequência foi menor em Cnidaria, predominando para os machos. No entanto, a fêmeas foram as únicas com representantes de Bryozoa (figura 4).

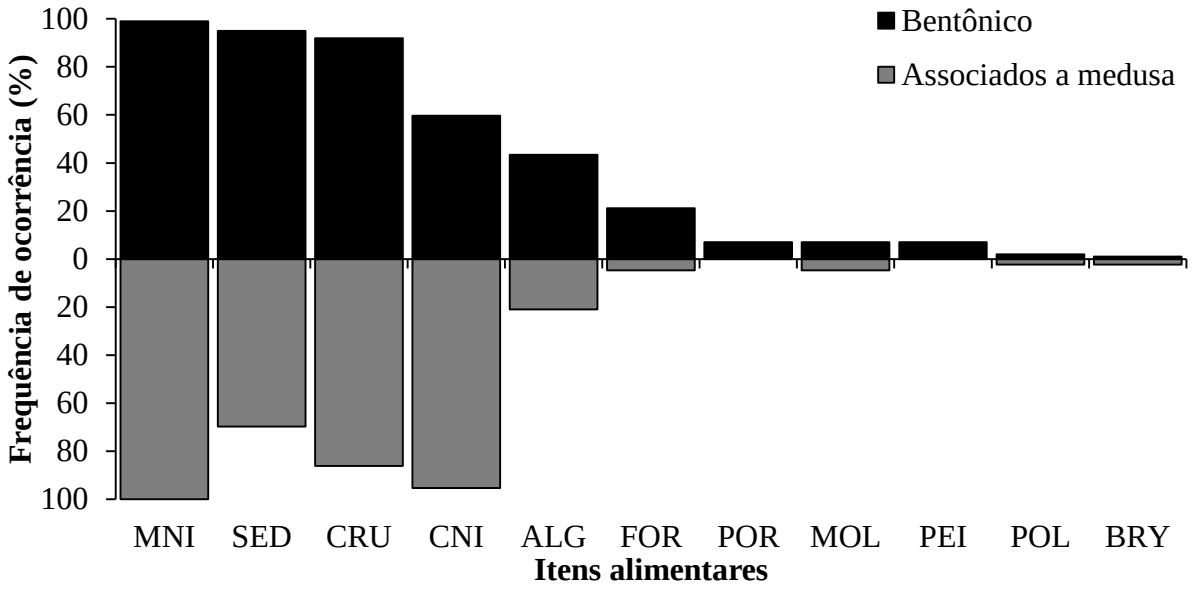


Figura 3. Frequência de ocorrência de cada item alimentar encontrado nos estômagos de *Libinia ferreirae* bentônico de vida livre e associado à medusa *L. lucerna* na região de Cananéia – SP.

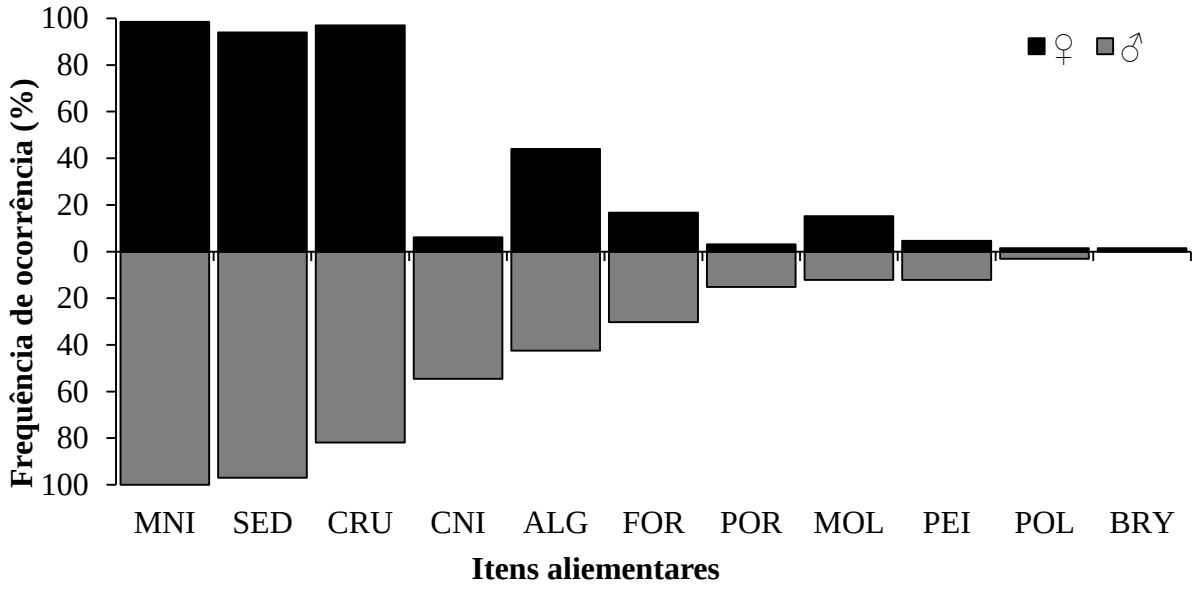


Figura 4. Frequência de ocorrência dos itens alimentares para cada sexo do caranguejo aranha *Libinia ferreirae,* pertencente ao grupo de vida livre, coletados na região de Cananéia – SP. Diferenças significativas entre os hábitos alimentares de cada sexo (χ^2 teste $P<0,05$).

Em relação às estações do ano, para os caranguejos bentônicos a frequência de ocorrência não demonstrou diferença significativa (Kruskal-Wallis test H, P=0,58). Já entre os itens alimentares por estação foi encontrado diferença significativa (Kruskal-Wallis test, H, P<0,05). A primavera foi a estação que se demonstrou mais diversa já que conteve todos os itens alimentares descritos no estudo, porém o outono foi aquele com menor variedade de itens alimentares encontrados, onde a frequência de ocorrência de crustáceos na dieta da *L. ferreirae* foi alta (tabela 2).

Tabela 2. Análise da frequência de ocorrência de itens alimentares por estação anual do caranguejo aranha *Libinia ferreirae* de vida livre (bentônico), durante o período de jul/2013 a mai/2014. MNI – Material não identificado, SED – Sedimento, CRU – Crustáceo, CNI – Cnidária, ALG – Alga, FOR – Foraminífera, POR –Porifera, MOL – Mollusca, PEI – Peixe, POL – Polychaeta, BRY – Bryozoa.

Estação	N	MNI %	SED %	CRU %	CNI %	ALG %	FOR %	POR %	MOL %	PEI %	POL %	BRY %
Inverno	54	100	96	96	72	72	26	6	9	4	0	0
Primavera	24	96	96	92	38	8	8	4	13	8	4	4
Verão	12	100	83	67	67	17	42	17	0	8	0	0
Outono	9	100	100	100	33	0	0	0	0	0	0	0

Os caranguejos associados à medusa também não demonstraram diferença significativa entre à frequência de ocorrência por estação do ano (Kruskal-Wallis test: P=0,52). Já entre os itens alimentares por estação do ano foi encontrado diferença significativa (Kruskal-Wallis test: P=0,00).

Para as *L. ferreirae* associadas à medusa, a frequência de ocorrência de itens alimentares por estação, mostrou-se similar aos bentônicos por também apresentarem a primavera como a estação que conteve maior número de itens alimentares descritos no estudo. Contudo, para este grupo o inverno e o verão tiveram apenas 4 itens alimentares encontrados (tabela 3).

Tabela 3. Análise da frequência de ocorrência de itens alimentares por estação anual do caranguejo aranha *Libinia ferreirae* associados à medusa *L. lucerna*, durante o período de jul/2013 a mai/2014. MNI – Material não identificado, SED – Sedimento, CRU – Crustáceo, CNI – Cnidária, ALG – Alga, FOR – Foraminífera, MOL – Mollusca, POL – Polychaeta, BRY – Bryozoa.

Estação	N	MNI %	SED %	CRU %	CNI %	ALG %	FOR %	MOL %	POL %	BRY %
Inverno	9	100,0	66,7	88,9	100,0	0,0	0,0	0,0	0,0	0,0
Primavera	18	100,0	94,4	83,3	88,9	27,8	11,1	16,7	11,1	5,6
Verão	11	100,0	18,2	90,9	72,7	0,0	0,0	0,0	0,0	0,0
Outono	5	1000	100,0	80,0	60,0	80,0	0,0	0,0	0,0	0,0

Muitos alimentos foram ingeridos em grandes pedaços e assim reconhecíveis, sendo que sua totalidade de volume foi perceptível, desta maneira o método de pontos foi adequado para estimar o quanto cada item alimentar está presente na dieta de *L. ferreirae*.

Foi contabilizado quanto ao preenchimento estomacal para os bentônicos: vazio = 27, incompleto = 38 e cheio = 32. Para os associados à medusa foram contabilizados: vazio = 29, incompleto = 5 e cheio = 8. Na porcentagem de pontos de cada item alimentar (quanto por cento de cada item foi encontrado no total das amostras) os itens que se demonstraram diferentes segundo o teste de Teste de Goodman, foram algas, foraminíferos, moluscos e sedimentos. Entre todo percentual dos itens alimentares encontrados, aqueles que compuseram a contribuição relativa na porcentagem de pontos tanto dos caranguejos bentônicos quanto para os associados foram MNI, CRU e SED sendo a soma destes itens representaram 69,2% e 87,9%, para caranguejos bentônicos e associados a medusa, respectivamente (figura 5).

Os crustáceos encontrados na composição alimentar mesmo tendo uma frequência de ocorrência de 90% nos dois grupos estudados foram representados por um porcentual de 25,7% para os caranguejos bentônicos e 29,5% para os caranguejos associados, de todo o volume de itens alimentares encontrados, entretanto, ocorreu diferença das espécies de crustáceos capturados e ingeridos em cada grupo. Os moluscos e cnidários tiveram sua representatividade de 10,4% e 9,5% para os bentônicos, e 1,3% e 8,4% para os associados.

Mesmo a frequência de ocorrência dos moluscos sendo baixo para os caranguejos bentônicos se teve uma representatividade relativamente alta, o que pode estar relacionado ao fato desta presa quando encontrada estar representando todo o conteúdo alimentar presente no respectivo estômago. Os demais itens como algas, foraminíferos, peixes, poríferas, briozoários e poliquetas, parecem ser de menor importância para a alimentação, sendo representados por 11% e 2,4% para bentônicos e associados, respectivamente (figura 5).

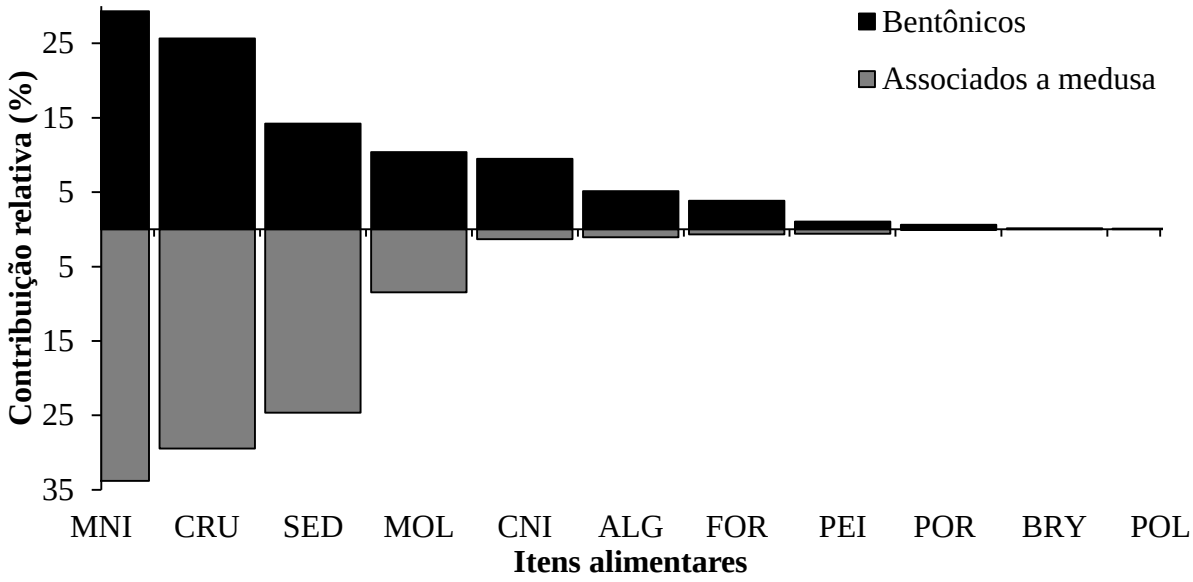


Figura 5: Porcentagem de pontos por item alimentar encontrado no conteúdo dos caranguejos aranha *Libinia ferreirae* analisados na região de Cananéia - SP. A) de vida livre (bentônicos N=99); B) caranguejos associados à medusa *L. lucerna* (N=43).

Discussão

Foi possível observar neste estudo que os caranguejos bentônicos de ambos os sexos foram maiores que os caranguejos encontrados associados à medusa. Esta diferença ocorre devido a maior abundância de indivíduos associados a medusa serem jovens e consequentemente não terem atingido seu máximo potencial de crescimento (Gonçalves et al., 2016b; Nogueira Jr & Haddad, 2005). Outro fator que limita o tamanho dos jovens associados é o espaço restrito dentro de sua hospedeira, pois eles podem permanecer associados até onde seu tamanho é comportado pela medusa, desta maneira caranguejos que estão nesta distinta

fase de desenvolvimento são menores que aqueles de vida bentônica (Sal Moyano et al., 2012).

A diversidade de itens alimentares encontrados para *L. ferreirae* sugere que a espécie é um predador oportunista como encontrado para *Libinia spinosa* H. Milne Edwards, 1834 por Barros *et al*. (2008), no entanto, Petti (1990) trata de *L. spinosa* como tendo hábitos carnívoros. Foi possível observar que existe uma diferença do hábito alimentar dos caranguejos bentônicos e associados à medusa em relação a alguns itens alimentares, demonstrando que ocorre a repartição de nichos na população, já que a dieta alimentar se modifica ao longo do ciclo de vida, sendo que a tendência na fase adulta é uma dieta mais diversa, quanto aquela encontrada para os jovens (Fontes Filho, 2011).

O método de pontos adotado no estudo se demonstrou viável, já que utilizados todos os caranguejos coletados, inclusive aqueles que continham os estômagos pouco preenchidos. Paul (1981) observou em laboratório que o conteúdo orgânico contido no estômago de *Callinectes arcuatus* Ordway, 1863 leva cerca de 6 horas para ser totalmente evacuado, demonstrando a rápida digestão. O preenchimento do estômago dos caranguejos pode ser influenciado pelo período do dia, visto que podem ter hábitos alimentares noturnos, assim como encontrado em *Cancer magister* Dana, 1852 por Stevens *et al*., (1982). Entretanto, Petti (1990) verificou o grau de repleção do estômago de *L. spinosa* em um período de 24 horas, sendo que não encontrou diferenças significativas entre os períodos do dia.

A grande ocorrência de material não identificado em nosso estudo se deu devido as dificuldades de se identificar itens alimentares que continham tecidos moles, por terem sido dilacerados pelas peças bucais e ossículos gástricos dos crustáceos, além de sofrerem acelerado processo de digestão (Barros *et al*., 2008; Petti, 1990; Williams, 1981). No entanto, foi possível determinar uma maior gama de itens alimentares ingeridos por *L. ferreirae*, do que encontrado no trabalho de Barros *et al*. (2008) com *L. spinosa*. Ennis (1973) descreveu

que a porcentagem de ocorrência de espécies de presas no conteúdo estomacal se reflete a partir da abundância relativa das presas no habitat.

Os itens que compuseram a maior parte da dieta do caranguejo aranha *L. ferreirae* identificado neste estudo foi sedimento, crustáceos e cnidários, diferente de Petti (1990) ao estudar hábitos alimentares de *L. spinosa*, que encontrou maior frequência de ocorrência de crustáceos e peixes. A grande frequência de sedimento é justificável pelos caranguejos serem comedores de depósito, além do sedimento ser colonizado por uma ampla variedade de organismos (Branco & Verani,1997). No entanto, alguns autores tratam desta ingestão como acidental ou como item que vai auxiliar na quebra mecânica do alimento encontrado no estômago (Mantelatto & Chistofoletti, 2001; Carqueija & Gôuveia, 1998). Haefner (1990a) e Willimas (1981) tratam do sedimento como um recurso adicional, importante para a produção do novo exoesqueleto no processo de muda, juntamente com pedaços de conchas e estruturas calcárias. Os crustáceos são muito diversos no ambiente, assim sua ingestão consequentemente é alta. A presença de ovos no conteúdo estomacal pode ter ocorrido em resposta da fêmea frente uma situação de stress, que induz a ingestão de seus ovos, ou pela necessidade de uma fonte rica em energia, ou ainda por serem ovos sem sucesso ao desenvolverem uma larva (Barros *et al*., 2008; Petti 1990).

A alta frequência de ocorrência de itens alimentares de cnidários pode ocorrer por este caranguejo predar tais animais. Segundo Hultgren & Stachowicz (2011), os majoideos irão decorar sua carapaça com itens que preferencialmente tragam alguma defesa química e/ou que sirvam como fonte alimentar para estes animais, pois no caso de escassez de recursos disponíveis, estes caranguejos usam sua decoração como alimento. Porém, *L. ferreirae* pode não se alimentar diretamente das medusas e anêmonas, e sim o caranguejo associado à medusa ingeris o alimento que é capturado pelo hospedeiro (*L. lucerna*), o qual contém uma grande quantidade de nematocistos (Sal Moyano *et al*., 2012). Quanto aos caranguejos bentônicos e a ocorrência de cnidários nos itens alimentares pode estar relacionado às

anêmonas epibióticas (Winter & Masunari, 2006) que muitas vezes pode competir com o caranguejo pelo recurso alimentar, tentando capturar parte do alimento do caranguejo aranha. Desta maneira, *L. ferreirae* pode predar pequenos pedaços dos tentáculos das anêmonas ou ingerir nematocistos lançados sobre a presa na tentativa das anêmonas em capturar alimento (observações pessoais). Contudo, futuros estudos devem ser realizados para definir se estas relações envolvem realmente a competição e predação das espécies.

Mesmo sendo menos frequentes os moluscos foram representativos quanto ao seu volume encontrado em alguns estômagos, o que nos mostra que o caranguejo aranha quando encontra presas como lulas as ingerem o máximo possível, porém tal presas parece ser de difícil acesso para estes caranguejos. A baixa presença de animais ágeis como lulas e peixes pode se dar por *L. ferreirae* não ser um predador rápido, já que o alimento é capturado segundo sua proximidade após o contato físico, podendo se alimentar de presas já mortas ou debilitadas (Barros *et al*., 2008). Petti (1990) descreveu que *L. spinosa* se alimenta principalmente de animais sésseis. Os demais itens podem ser acessórios e vão ser ingeridos se estiverem disponíveis no ambiente, como os poríferos e foraminíferos parecem contribuir pouco para a dieta, embora tenham sido encontrados em grande volume em alguns casos, corroborando com Williams (1981).

Diferente de nosso estudo, Petti (1990) teve como itens mais frequentes no conteúdo estomacal de *L. spinosa* ofiuroides, poliquetas, hidrozoários e crustáceos, o que é justificável já que a dieta vai se diversificar conforme o que está disponível no ambiente (Barros *et al*., 2008).

A dieta dos jovens caranguejos associados diferiu dos bentônicos, pelo fato de não conterem peixes nem poríferas em seu conteúdo, sendo que os crustáceos no conteúdo estomacal dos associados a medusa eram Copepoda, que nos demonstra claramente que eles usam como fonte alimentar o que está disponível na coluna d'água, ou seja, os recursos alimentares que são capturados pelos tentáculos da medusa. Stevens *et al*. (1982) encontrou

diferença na alimentação dos menores indivíduos de *Cancer magister* com os maiores indivíduos. Já Haefner (1990a) não encontrou diferença significativa no conteúdo estomacal de jovens e adultos de *Callinectes ornatus* Ordway, 1863.

A dieta se demonstrou diferente entre os sexos dos indivíduos bentônicos, sendo possível observar que apenas as fêmeas ingeriram Bryozoa e que a ingestão de cnidários nas fêmeas foi menor que nos machos, mesmo as fêmeas tendo maior número de anêmonas na carapaça (Nogueira Jr & Haddad, 2006). Ainda foram encontradas diferenças para foraminíferos e poliquetos, no entanto, os demais itens alimentares se demonstraram iguais, então é provável que ambos ocupem o mesmo ambiente (Haefner, 1990a).

Segundo Nogueira Jr & Haddad (2006), as fêmeas possuem uma maior decoração sobre a carapaça, diferentemente dos machos, pois esta relação traz uma maior proteção para as mesmas que se deslocam menos e que precisam proteger seus embriões, o que justificaria e menor ingestão de tal item alimentar. Desta forma, nossos resultados corroboram com os resultados de Petti (1990), que encontrou diferença na alimentação de machos e fêmeas de *L. spinosa*, sendo peixe principal item para machos e crustáceos para as fêmeas, o que a autora justifica pelo fato dos machos serem maiores e possuírem quelípodos mais desenvolvidos, proporcionando a captura de diferentes itens para sua alimentação.

A primavera foi o período de maior diversidade de itens alimentares encontrado para os dois grupos de *L. ferreirae*, bentônicos e associados à medusa, entretanto, não foi comprovada diferença entre as estações. Petti (1990) em estudo de hábitos alimentares de *L. spinosa* em Ubatuba - SP, encontrou a maior diversidade de itens no verão. Nas estações de temperaturas mais elevadas (primavera e verão) se registra maior diversidade de espécies sob condições ótimas para reprodução, aumentando a abundância e diversidade de integrantes da teia trófica marinha, proporcionando maior disponibilidade alimentar para níveis tróficos superiores (Fontes Filho, 2011).

A maior porcentual (volume) encontrado foi o de MNI seguido de CRU e SED, para ambos os grupos de caranguejos estudados, demonstrando que a preferência alimentar está em crustáceos e sedimento, porém os outros itens alimentares também são importantes para estes caranguejos, pois não sabemos se foram ingeridos em menor quantidade por serem de difícil captura (como no caso de alguns moluscos) ou por serem uma das únicas opções disponíveis naquele momento no ambiente.

O caranguejo aranha *L. ferreirae* desempenha um importante papel na cadeia trófica da região de Cananéia – SP, podendo desempenhar um papel fundamental na cadeia trófica controlando demais populações. Petti (1990) em seu estudo de hábitos alimentares expõe que *L. spinosa* juntamente com outras 3 espécies de caranguejos aparentam ser predadores de topo de cadeia, tendo papel fundamental na transferência de energia.

A dieta do caranguejo aranha *L. ferreirae* nos mostrou ser bastante ampla, sendo que os alimentos mais consumidos são sedimento e crustáceos. A diferença encontrada nas dietas entre os grupos (associados à medusa e bentônicos) e entre os sexos dos caranguejos bentônicos, mostra que estes indivíduos conseguem obter o alimento e consequentemente a energia necessária para sua sobrevivência a partir do ambiente sem que haja disputa pelo recurso durante as diferentes fases de desenvolvimento ou grupos populacionais. As relações ecológicas que vive *L. ferreirae* é uma estratégia de extrema importância para a sobrevivência da espécie, minimizando as competições que podem ocorrer pelos recursos durante o seu desenvolvimento.

Referências

Barros SP, Cobo VJ & Fransozo A (2008) Feeding habits of the spider crab *Libnia spinosa* H. Milne Edwards, 1834 (Decapoda, Brachyura) in Ubatuba Bay, São Paulo, Brazil. Braz Arch Biol Technol, 51 (2): 413-417

Bolla Jr. EA & Negreiros-Fransozo ML (2016) Morphology of juvenile phase of *Achelous spinimanus* (Latreille, 1819) (Crustacea, Decapoda, Portunidae) reared in laboratory, J Mar Biol Assoc UK, 03: 615-631

Branco JO & Verani JR (1997) Dinâmica da alimentação natural de *Callinectes danae* Smith (Decapoda) na Lagoa da Conceição Florianópolis, Santa Catarina, Brasil. Rev Bras Zool, 14: 1003-1018

Brusca R & Brusca G (2007) Invertebrados. 2ª Edição, Ed Guanabara Koogan S.A

Carqueija CRG & Gouvea EP (1998) Hábito alimentar de *Callinectes larvatus* Ordway (Crustacea, Decapoda, Portunidae) no manguezal de Jiribatuba, Baía de todos os Santos, Bahia. Rev Bras Zool, 15: 273-278

Curi PR & Moraes RV (1981) Associação, homogeneidade e contrastes entre proporções em tabelas contendo distribuições multinomiais. Ciênc Cult, 33(5): 712-722

Ennis GP (1973) Food, feeding, and condition of lobsters, *Hornarus americanus*, through out the seasonal cycle in Bonavista Bay, Newfoundland. J Fish Res Board Can, 30: 1905-1909

Fonteles-Filho AA (2011) Oceanografia, biologia e dinâmica populacional de recursos pesqueiros. Fortaleza: Ed. Expressão Gráfica

Gonçalves GRL, Wolf MR, Costa RC & Castilho AL (2016) Decapod crustacean associations with scyphozoan jellyfish (Rhizostomeae: Pelagiidae) in the Southeastern Brazilian coast. Symbiosis, 69: 193-198

Gonçalves GRL, Bolla Jr EA, Negreiros-Fransozo ML & Castilho AL (2016b) Morphometric and gonadal maturity of the spider crab Libinia ferreirae Brito Capello, 1871 (Decapoda: Majoidea: Epialtidae) at Southeastern Brazilian coast. J Mar Biol Assoc UK, doi:10.1017/S0025315416000370

González Carman V, Botto F, Gaitán E, Albareda D, Campagna C & Mianzan H (2014) A jellyfish diet for the herbivorous green turtle *Chelonia mydas* in the temperate SW Atlantic. Mar Biol, 161: 339-349

Guinot, D & Bouchard J.M. (1998) Evolution of the abdominal holding systems of brachyuran crabs (Crustacea, Decapoda, Brachyura). Zoosystema, 20, 613–694

Haefner PA Jr. (1990a) Natural diet of *Callinectes ornatus* (Brachyura: Portunidae). Bermuda J Crust Biol, 10 (2): 236-246

Haefner PA Jr. (1990b) Morphometry and size at maturity of *Callinectes ornatus* (Brachyura, Portunidae) in Bermuda. Bull Mar Sci, 46: 274-286

Hartnoll RG (1982) Growth. Pp 111–196, in The Biology of Crustacea: embryology, morphology and genetics. DE Bliss, ed. Vol. 2, Academic Press, New York

Hotland AF, Mountford NK, Hiegel MH, Kaumeyer KR & Mihursky JA (1980) Influence of predation on infaunal abundance in upper Chesapeake Bay, USA. Mar Biol, 57: 221-235

Hultgren K & Stachowicz J (2011) Camouflage in decorator crabs. Pp. 214–238 in the Animal Camouflage: Mechanisms and Function, M. Stevens and S. Merilaita, eds. Cambridge University Press, Cambridge

Hyslop EJ (1980) Stomach contents analysis—a review of methods and their application. J Fish Biol, 17 (4): 411-429

Ingle RW (1977) The larval and post-larval development of the scorpion spider crab, *Inachus dorsettensis* (Pennant) (family: Majidae), reared in the laboratory. Bulletin of the British Museum (Natural History): Zoology, London 30: 329-348

Mantelatto FLM & Christofoletti RA (2001) Natural feeding activity of the crab *Callinectes ornatus* (Portunidae) in Ubatuba Bay (São Paulo, Brazil): influence of season, sex, size and molt stage. Mar Biol, 138(3): 585-594

Mariscal R (1974) Nematocysts. Pp 129–178 in Coelenterate biology: reviews and new perspectives, L Musatine, H Lenhoff, eds. Academic Press, New York

Mclaughlin PA & Herbard JF (1961) Stomach contents of the Bering Sea king crab. Bulletin INPFC, 5: 5-8

Melo GAS (1996) Manual de identificação dos Brachyura (caranguejos e siris) no litoral brasileiro. São Paulo: Ed. Plêiade, FAPESP

Nogueira Jr. M & Haddad MA (2005) *Lychnorhiza lucerna* (Scyphozoa, Rhizostomeae) and *Libinia ferreirae* Brito Capello (Brachyura, Majidae) association in southern Brasil. Rev Bras Zool, 22(4): 908-912

Nogueira Jr. M, Robert MC & Haddad MA (2006) *Calliactis tricolor* (Anthozoa, Acontiaria) epibionte em Brachyura (Crustacea, Decapoda) no litoral sul do Paraná e Norte de Santa Catarina. Acta Biol Paraná, 35 (3–4): 233-248

Paul RKG (1981) Natural diet, feeding and predatory activity of the crabs *Calhnectes arcuattls* and *C. toxotes* (Decapoda, Brachyura, Portunidae). Mur Ecoi Prog Ser, 6: 91-99

Petti MAV (1990) Hábitos alimentares dos crustáceos decápodos braquiúros e seu papel na rede trófica do infralitoral de Ubatuba (litoral norte do estado de São Paulo, Brasil). Dissertação de Mestrado - Oceanografia Biológica, São Paulo, SP, USP, 150p

Ruppert EE, Fox RS & Barnes RD (2005) Zoologia dos Invertebrados. 7ª ed. Roca

Sal Moyano MP, Schiariti A, Gilberto DA, Diaz Briz L, Gavio MA & Miazan HW (2012) The symbiotic relationship between *Lychnorhiza lucerna* (Scyphozoa, Rhizostomeae) and *Libinia spinosa* (Decapoda, Epialtidae) in the Río de la Plata (Argentina-Uruguay). Mar Biol 159: 1933-1941

Sampedro MP, González-Gurriarán E, Freire J & Muinõ R (1999) Morphometry and sexual maturity in the spider crab *Maja squinado* (Decapoda: Majidae) in Galicia, Spain. J Crust Biol, 19: 578–592

Stevens BG, Armstrong DA, & Cusimano R (1982) Feeding habits of the Dungeness crab *Cancer magister* as determined by the Index of Relative Importance. Mar Biol, 72: 135–145

Tavares M & Santana W (2012) On the morphological differentiation between *Libinia spinosa* and *L. ferreirae* (Crustacea: Brachyura: Majoidea: Epialtidae). Zoologia (Curitiba) 29 (6): 577-588

Williams MJ (1981) Methods for analysis of natural diet in portunid crabs (Crustacea: Decapoda: Portunidae). J Exp Mar Biol Ecol, 52(1): 103-113

Winter VC & Masunari S (2006) Macroepizoismo em *Libinia ferreirae* (Crustacea, Brachyura, Majidae). Iheringia Sér Zool, 96(2): 135-140

Zar JH (1999) Biostatistical Analysis. Upper Saddle River, NJ: Pratice-Hall

Considerações finais

De acordo com nosso estudo, o caranguejo *Libinia ferreirae* apresenta reprodução contínua, sendo que sua desova foi efetiva. A população durante a fase jovem demonstrou ter uma razão sexual igual, porém, quando adultos se encontrou uma tendência a favor das fêmeas.

Os fatores abióticos que demonstraram influenciar com maior força as fêmeas ovígeras foi a salinidade e o Phi.

Foi possível observar neste estudo uma clara repartição de nicho entre os jovens e adultos, não ocorrendo sobreposição das categorias demográficas, devido ao distinto hábito pelágico simbionte e de vida livre bentônico.

A relação simbiótica deste caranguejo com a medusa traz inúmeras vantagens para seu crescimento e desenvolvimento, porém não se sabe ao certo se este comportamento pode prejudicar a medusa. A associação da *L. ferreirae* ocorre inicialmente na fase de megalopa e durante a fase juvenil, sendo que os caranguejos demonstraram ter um crescimento contínuo juntamente com suas hospedeiras. Experimentos comportamentais devem ser realizados afim de demonstrar se a medusa é um substrato específico necessário para a sobrevivência do caranguejo, e ainda se realmente o caranguejo não prejudica sua hospedeira.

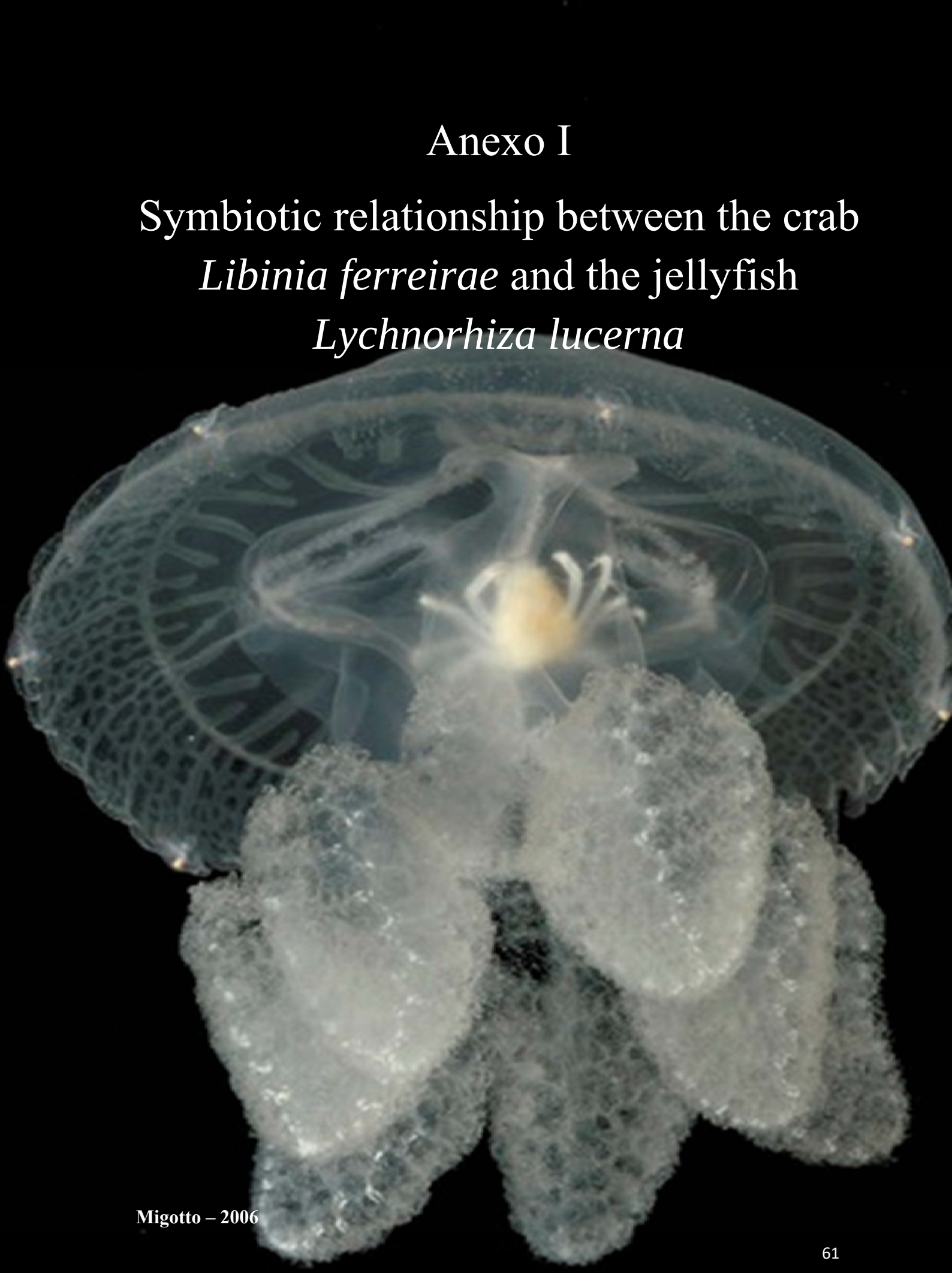
Libinia ferreirae teve dois grupos morfológicos separados para os machos, sendo machos adolescentes que possuem maturidade gonadal mais não morfométrica e os machos maduros que possuem maturidade gonadal e morfométrica concomitantemente. Os dois tipos de machos aparentemente são ativos na população e podem copular caso haja a oportunidade, contudo, testes de comportamento de cópula e histológicos devem ser realizados para confirmar se a cópula por machos adolescentes ocorre e se pode gerar prole viável. Já as fêmeas demostraram possuir a maturidade gonadal e morfométrica simultaneamente.

Este estudo demonstrou a clara diferenciação de tamanho entre os sexos e de indivíduos do mesmo sexo, mesmo que estes grupos possuam a muda terminal que o impede de crescer continuamente até sua morte, tal característica pode ser influenciado por inúmeros fatores.

O hábito alimentar da *L. ferreirae* foi composto por 11 itens distintos com uma alta frequência de crustáceos. A dieta dos indivíduos associados foi distinta dos caranguejos adultos bentônicos. Os animais associados tiveram ausência na dieta de porífera e peixes, sendo que os crustáceos que estavam no conteúdo estomacal eram representantes apenas de Copepoda e todo o material do estômago destes animais estava repleto de nematocistos oriundos da sua medusa hospedeira. Ambas as fases de desenvolvimento de *L. ferreirae* tiveram distintos hábitos alimentares, demonstrando ter diferença significativa da dieta durante as estações do ano. A dieta também foi diferenciada quanto ao sexo para os caranguejos de vida livre.

Anexo I

Symbiotic relationship between the crab *Libinia ferreirae* and the jellyfish *Lychnorhiza lucerna*



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Abstract:	The association between the crab Libinia ferreirae and the jellyfish Lychnorhiza lucerna is poorly documented in the literature. We therefore investigated the following aspects of this relationship: demographic groups, morphometric relationship, larval development and the diet composition of L. ferreirae associated with L. lucerna. Collections of both species - crabs and medusa - were performed each month from February 2013 to May 2014 in Cananéia municipality (approximately 25°05'S; 47°53'W) of São Paulo, Brazil.; of the 654 individuals obtained, 357 were white, indicating an association with L. lucerna. The size distribution of these crabs revealed that 88.75% had a carapace width (CW) of less than 15 mm. The majority of L. lucerna¬-associated juvenile individuals were collected during the summer (March 2014, N = 206), 96% of which exhibited a CW <15 mm. There were significant relationships (Linear regression, P <0.05) between L. ferreirae CW and weight vs. jellyfish umbrella diameter and weight. Competition by resource (jellyfish) was reflected when there was only one L. ferreirae individual in a single jellyfish, but when the crabs were <10 mm they occurred in more than one individuals. The crab stomach had a high quantity of nematocysts. The crab larvae obtained in the laboratory were larger than those of wild crabs. This symbiotic relationship reflects the evolutionary success of this crab species, the juveniles of which obtain protection and food in a peculiar environment. It also promotes the fitness of early juvenile stages by avoiding intraspecific competition with adult crabs for food resource.
Suggested Reviewers:	Maria Páz Sal Moyano Universidad Nacional de Mar del Plata paz.salmoyano@gmail.com Because she has many papers considering the general biology of the genus Libinia.
	Tommy Leung

	University of Otago, New Zealand tleung6@une.edu.au Because he has many papers about ecological relationships .
	Ingo S. Wehrtmann Universidad Nacional de Costa Rica ingowehrtmann@gmx.de Because he studies the Aquatic biodiversity with emphasis on decapod crustaceans at marine environments.
Opposed Reviewers:	

Running hed: Relationship of crab with medusa

Symbiotic relationship between the crab *Libinia ferreirae* and the jellyfish *Lychnorhiza lucerna*

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Key words: commensalism, development, megalopa, niche segregation, nematocysts

Abstract. The association between the crab *Libinia ferreirae* and the jellyfish *Lychnorhiza lucerna* is poorly documented in the literature. We therefore investigated the following aspects of this relationship: demographic groups, morphometric relationship, larval development and the diet composition of *L. ferreirae* associated with *L. lucerna*. Collections of both species - crabs and medusa - were performed each month from February 2013 to May 2014 in Cananéia municipality (approximately 25°05'S; 47°53'W) of São Paulo, Brazil.; of the 654 individuals obtained, 357 were white, indicating an association with *L. lucerna*. The size distribution of these crabs revealed that 88.75% had a carapace width (CW) of less than 15 mm. The majority of *L.*

lucerna-associated juvenile individuals were collected during the summer (March 2014, $N = 206$), 96% of which exhibited a CW <15 mm. There were significant relationships (Linear regression, $P < 0.05$) between *L. ferreirae* CW and weight *vs.* jellyfish umbrella diameter and weight. Competition by resource (jellyfish) was reflected when there was only one *L. ferreirae* individual in a single jellyfish, but when the crabs were <10 mm they occurred in more than one individuals. The crab stomach had a high quantity of nematocysts. The crab larvae obtained in the laboratory were larger than those of wild crabs. This symbiotic relationship reflects the evolutionary success of this crab species, the juveniles of which obtain protection and food in a peculiar environment. It also promotes the fitness of early juvenile stages by avoiding intraspecific competition with adult crabs for food resource.

Introduction

Crustaceans exhibit several types of association with other invertebrates that contribute to their evolutionary success. It is estimated that at least 500 genera have evolved through such symbiotic interactions (Ross, 1983). The medusae, on the other hand, act as hosts for a wide diversity of organisms such as fishes, cestodes, echinoderms, and, most commonly among crustaceans, amphipods, crabs and carideans (Arai, 1997; Ohtsuka *et al.*, 2009).

In cases of interspecific symbiotic relationships (i.e., the species live together; De Bary, 1879 *apud* Martin and Schab, 2013), there is a host organism (i.e., the provider or basis of resources) and a symbiont organism (i.e., the consumer of such resources). According to Baeza and Tiel (2007), in the marine environment, symbiotic relationships usually involve small organisms that live inside the host or in biotic

refuges such as sea anemones, ascids, corals, sponges, sea urchins, oysters, and some crustaceans. These relationships are varied and can be classified based on the costs and benefits for their hosts and the visitors (Baeza *et al.,* 2015). Such symbiotic interactions are referred to as mutualistic when the benefits are reciprocal; comensalistic when the symbiont (visitor) benefits with no loss to the host; and parasitic when the symbiont uses the host as a resource, thereby resulting in harm to but not the death of the host (Leung and Paulin, 2008). Additionally, other classifications have been used based on the number of involved species participating in the relationship (generalists or specialists) and in the degree of interdependence between the associated organisms (e.g., facultative versus obligatory symbiosis) (Baeza and Tiel, 2007).

The benefits of symbiosis interactions are diverse for individuals capable of invading the body cavity of another living organism (i.e., an endosymbiont), as they are able to extract food, are protected against specialized predators, and are isolated from the external environment (Nogueira Jr. and Haddad, 2005; Towanda and Thuesen, 2006; Martin and Schab, 2013).

With respect to animal evolution, the symbiotic life style is the result of the appearance of complex and diverse phenotypes that were able to colonize new environments, selecting features that allowed the exploitation of new resources and the expansion of the ecological niche in the process (Moran, 2007).

Symbiotic relationships can differ according to habitat and environmental adversities. Thus, the organisms that obtain an advantage from the association can occasionally become harmful to their host, or vice versa (Thiel and Baeza, 2001; Leung and Paulin, 2008; Lee *et al.,* 2009). The division of interspecific relationships into immutable groups is often fallible; these grouping frequently exhibit high plasticity, as

evidenced in the literature by cases of mutualism and commensalism that evolved naturally to parasitism (Ewald, 1987; Pellmyr *et al.*, 1996).

The relationship between brachyuran crabs and the Scyphomedusa *Lychnorhiza lucerna* Haeckel, 1880 has been recorded in Brazilian coastal areas (Nogueira Jr. and Haddad, 2005). The majority of investigators (Corrington, 1927; Vaz Ferreira, 1972; Nogueira Jr. and Haddad, 2005; Sal Moyano *et al.*, 2012) have considered the relationship between spider crabs of the genus *Libinia* Leach, 1815 and medusa to be a commensalistic interaction. However, predation by crabs on the medusa (parasitism/predation) (Gustsell, 1928; Jachowski, 1963; Shanks and Graham, 1988) as well as seasonal occupancy (Vaz Ferreira, 1972) have been reported.

The spider crab *Libinia ferreirae* Brito Capello, 1871 has a geographic distribution restricted to the Atlantic Ocean ranging from Venezuela to Brazil (from Pará to the Santa Catarina states) and from the littoral region to 35 meters deep (Melo, 1996). Moreira (1961) described the first observed association of *L. ferreirae* with the medusa *Phyllorhiza punctata* von Lendelfeld, 1884. The spider crab *L. ferreirae* can associate with *L. lucerna* during the megalopa or juvenile stages (Nogueira Jr. and Haddad 2005; Gonçalves *et al.*, 2016a). Species of spider crab can display a wide range of symbiotic relationships, acting as a host when it decorates its own carapace with algae, sponges, anemones and other ectosymbiont organisms (Winter and Masunari, 2006; Hultgren and Stachowicz, 2011). In this manner, *L. ferreirae* represents an important model for the study of interspecific interactions due to its lifelong cultivation of symbiotic relationships.

Scyphomedusa *L. lucerna* is endemic of the Southeastern Atlantic (Morandini *et al.*, 2005), which makes it a unique ecological resource and a key species of the marine trophic chain, predating on fishes and other organisms and as a floating nursery for

several symbiotic species. Notably, this species of medusa has a high economic value in the pharmaceutical and medical industries, and is a human food item in some countries (Omori and Nakano 2001; Purcell *et al.,* 2007; Schiariti *et al.,* 2008).

The peculiar coevolution of *L. ferreirae* and *L. lucerna* denotes the importance of detailed studies of this association. Both species are commonly caught by shrimp fisheries in non-selective devices (i.e., double-rigged nets and otter trawls) in the southeastern and southern regions of Brazil. This phenomenon results in the trophic destruction of marine resources (Branco and Verani, 2006).

This study analyzed several seasonal aspects of the life history of *L. ferreirae* and its abundance, as well as the association between *L. ferreirae* and the medusa *L. lucerna* in the Cananéia region of southeastern Brazil. In addition, we analyzed other features of the crab and the medusa, including crab larval development, the stomach content of *L. ferreirae*, and the morphometric relationship between *L. ferreirae* and *L. lucerna*.

Materials and Methods

Biological material sampling

Collections of both species - crabs and medusa - were performed each month from February 2013 to May 2014 in seven locations within the Cananéia municipality (approximately 25°05'S; 47°53'W) of São Paulo, Brazil. Samplings were carried out using a shrimp fishery boat equipped with a named double-rig net.

The identification of medusa (*Lychnorhiza lucerna*) was based on the work of Morandini *et al.* (2005). Each medusa was examined for the presence of crabs in the following body cavities: exumbrella, oral arms, oral pillars, gastric cavity, subgenital

pouch and gonads. Medusa with no associated crabs were quantified and returned to the environment, while those with associated crabs were kept in carefully labelled plastic bags. The obtained crabs (i.e., those not associated with medusa) were also kept and were stored in plastic bags, labelled and isolated, in two groups: a) white in color, denoting recent release from the medusa after removal from the net; and b) dark in color and bearing symbiont organisms on the carapace (i.e., the benthic crabs that live freely). After, all animals (medusa and crabs) were anesthetized by cold. They were then transported in thermic boxes on ice to the laboratory.

After searching for crabs, each medusa was transferred to a slightly inclined tray to remove excess seawater. Then, the medusae were weighed (MWe) in a regular scale (precision = 0.01 g), and their umbrella diameter (UD; between two opposite ropalia) was measured using a regular ruler. The medusae were then grouped into 50 mm-amplitude size classes. Only animals in good condition with no damage were considered for the study (Nogueira Jr. and Haddad, 2006).

The identification of crabs was based on the pertinent literature describing megalopa (Pohle *et al.*, 1999), juvenile crabs (Tavares and Santana, 2012), and adult crabs (Melo, 1996). After, they were weighed (crab weight = CWe) in an analytic scale (precision = 0.001 g), and their carapace width (CW) was measured using a digital caliper (precision of 0.01 mm). The crabs were then grouped into 1.5- and 3 mm-amplitude size classes. The crabs were classified according to sex based on the shape of the abdomen and the number of pleopods (Ingle, 1977; Sampedro *et al.*, 1999). The classification of individuals about the maturation followed the descriptions of Gonçalves *et al.* (2016b).

Morphometric relationships

The morphometric relationships analyzed were performed (only for the medusae associated with only one crab) as follows: a) medusae umbrella diameter (UD) *vs*. crab carapace width (CW), and b) the medusae weight (MWe) *vs*. crab weight (CWe). The relationships between the quantitative variables were examined using single linear regressions according to a parametric analysis (Zar, 1999).

Because the crab size distribution did not fit a normal distribution, the sizes of the associated medusae and non-associated crabs were evaluated via the Mann-Whitney test.

In all statistical analyses, we adopted a significance level of 5% (Zar, 1999).

The abundance of crabs and medusa is represented according to size classes during the collection months using box plots (Statistica, version 10.0).

The additional weight of associated crabs carried by each medusa was calculated and represented by the ratio of the sum of the crabs' weight to that of their respective medusa.

Crab larval development

Four adult females *Libinia ferreirae* (free-living in a benthic region) carrying embrionated eggs attached to the pleopods (i.e., ovigerous) were captured in November 2013 and transferred to the wet laboratory at NEBECC (Study Group for the Biology, Ecology and Culture of Crustaceans) at the Biosciences Institute of UNESP in Botucatu, São Paulo, Brazil. These females were maintained in aquaria filled with seawater (salinity: 35±1 ppm; temperature: 25 ± 1ºC) and fed pieces of shrimp, squid and fish until hatching. The rearing techniques followed the procedures adopted by Bakker *et al.* (1990), Negreiros-Fransozo *et al.* (1989, 2008) and Flores *et al.* (2002).

After hatching, we transferred the most active zoeae using a Pasteur pipette to glass receptacles (250 ml) at a density of 20 zoeae each. We maintained the rearing recipients at 35±1 ppm salinity and 22±1ºC and changed the water daily to new filtered seawater. The zoeae were fed newly hatched *Artemia* sp. We fixed several megalopa at this stage, while the rest were maintained alive to obtain juvenile individuals.

All cultivated larvae were measured for carapace width (CW) and all size measurements were assessed as follows: minimum, maximum, mean and standard deviation. After, the sizes of the megalops raised in the laboratory were compared to the juveniles (up to 2 mm of CW) obtained in a natural environment in association with the medusa *L. lucerna*.

Stomach contents of crabs associated with medusae

Samples from the stomachs of *L. ferreirae* (N = 20) were counted and measured (for more details, see Williams, 1981) under a light microscope (Zeiss® Axioskop 2 plus).

For smaller individuals (< 5 mm), larvae measurements and some stomach contents we used a stereomicroscope (Zeiss® Stemi SV6) equipped with a digital imaging system (Zeiss Stemi 2000-C; precision = 0.001 mm).

Results

In this study, we obtained 654 specimens of *Libinia ferreirae*. The crabs associated with medusa presented with whitish coloration comprised 357 individuals (311 were found inside medusa and 46 were separate from medusa). The non-associated crabs (i.e., benthic) comprised 297 individuals and exhibited a dark brownish

coloration; most of these crabs exhibited a carapace covered with invertebrate symbionts (Fig. 1).

Distinct stages of crab development were identified among the samples, including megalopa, juveniles and adult specimens, of which 88.75% were smaller than 15 mm CW.

Of all 916 medusa (*Lychnorhiza lucerna*) obtained, only 22% (n = 198) were associated with crabs (*L. ferreirae*). The number of associated crabs varied from one to 11 per host. The observed pattern of associated crabs revealed that their size depends on their number in the medusa: when they were numerous, their size was smaller (CW varying from 1 to 3 mm) (Fig. 2). Crabs larger than 6 mm CW were not found in groups larger than four in a given medusa.

Other interesting findings include: 1) the capture of medusae hosting more than four crabs each (up to 11 crabs in a same medusa); 2) one crab in the intermolt stage carrying a *Carcinactis dolosa* Riemann-Zurneck, 1975, anemone on its carapace; and 3) 3 crabs exhibiting an exoskeleton in the newly post-molt stage.

Seasonal variation

Throughout the year, we caught crabs associated with medusa across the entire study area. The highest number of crabs found in association with medusa was observed during summer (March 2014; n= 206); 96% of these crabs were smaller than 15 mm CW, indicating the occurrence of crabs developing in association. The recruitment period of *L. ferreirae* is probably during the summer, as we found a high number of ovigerous females two months before summer (Gonçalves, unpubl data). In contrast, we detected the highest abundance of medusa during winter 2013, followed by spring 2013 and summer 2014 (Fig. 3).

Morphometric relationships

The medusa with a higher number of associated crabs exhibited umbrella diameters ranging from 100 to 150 mm (Fig. 2). Crabs obtained in association with medusa (i.e., those with whitish coloration) were smaller in CW size compared to those of benthic crabs (i.e., those with a dark brownish coloration) (Mann-Whitney U test, $U = 1214.0$, $n1 = 297$, $n2 = 357$; $P < 0.05$). In addition, free-living crabs were considered adult in size (Fig. 4).

Medusa and their associated crabs size were positively correlated durim the months (Spearman, $rs = 0.82$; $n = 16$; $P < 0.001$). Thus, there was a monthly tendency of growth for animals in association, as seen in Fig. 5.

The tested Linear regressions revealed a significant relationship between medusa diameter (DU) and the size of the *L. ferreirae* ($r2 = 0.61$; $F = 249.4$; $P < 0.001$), as well as in the relationship between medusa weight and crab weight ($r2 = 0.66$; $F = 309.9$; $P < 0.001$) (Fig. 6).

By considering the weight of each crab associated with a medusa, we determined that the minimum weight carried by a host was 0.01% and the maximum was 14.42% (percentile 25 = 0.34% and percentile 75 = 1.76%), with a median of 0.90% and a mean of 1.57%. The majority of the medusae (86%) contained crabs comprising less than 3% of their own body weight. Only 14% ($n = 27$) of the medusae carried crabs comprising more than 3% of their own weight.

Larval development of L. ferreirae

The zoeae reared in the laboratory took between 15 to 17 days to reach the megalopa stage ($n = 17$), which lasted four days. The size of the megalopae obtained in

the laboratory was greater than the observed for associated ones (n = 4), being similar to the observed size for the smaller associated juveniles (n = 20) (Fig. 7).

Associated crab stomach content

The analysis of stomach content for each sex of crab revealed fragments of organic origin such as algae, mollusks, polychaetes, fishes, crustaceans, and a high quantity of nematocysts (identified as *L. lucerna* according to Mariscal 1974). The majority of nematocysts found were in fragments of body tissue, whereas others were loose (Fig. 8).

Discussion

The literature has documented the association between brachyuran crabs and pelagic cnidarians worldwide in which approximately 1/3 of all majoid crabs participate, primarily those belonging to Epialtidae. However, the relationship between these symbiotic organisms are poorly characterized (Towanda and Thuesen, 2006). The majority of these associations involve Majoidea and are treated as facultative or commensal (Phillips *et al.,* 1969; Nogueira Jr. and Haddad, 2005; Sal Moyano *et al.,* 2012; Gonçalves *et al.,* 2016a). However, some authors (Jachowski, 1963; Coleman, 1977) have mentioned that crabs feed on their hosts, and thus consider the association to be predation or parasitism.

We observed that the association of *Libinia ferreirae* with *Lychnorhiza lucerna* is a remarkable protection strategy for survival during the juvenile phase, because a wide range of marine animals preys on these crabs (Vaz Ferreira, 1972; Nogueira Jr. and Hadadd, 2005; Sal Moyano *et al.,* 2012). The end of the molt phase in crustaceans

is a critical period in the life of these organisms. The association during the post-molt stage of these crabs with the medusa reinforces the hypothesis that the host constitutes a shelter for such individuals (Corrington, 1927; Sal Moyano *et al.*, 2012).

In this manner, the medusa acts as a floating nursery, which explains the difficulty in finding and capturing *L. ferreirae* juveniles in the benthic environment. Other decapods are frequently caught by the same fishery devices used to catch crabs, as pointed by Castilho *et al.* (2008), who captured small specimens of *Sicyonia dorsalis* Kingsley, 1878 (>5 mm of length carapace). Nevertheless, there is no overlap between the niche of juvenile (54%) and adult *L. ferreirae* (Anger *et al.*, 1989). Thus, there is no competition for resources. Distinctly from Phillips *et al.* (1969), who reported the same size classes for *Libinia dubia* Milne Edwards, 1834, either in association or not with the medusa *Stomolophus meleagris* Agassiz, 1862. This suggests a distinct evolutionary mechanism for these relationships that is dependent on the species under consideration.

In the present study, the close relationship existing between medusa and crab species indicates an adaptation of the *L. ferreirae* to the nematocysts produced by *L. lucerna*, mainly because we observed the occurrence of such nematocysts in the crabs' stomach contents, which indicates probable predation or competition for food resources, as mentioned by Sal Moyano *et al.* (2012). To date, no mechanisms against nematocysts have been identified in *L. ferreirae*. However, Shanks and Graham (1988), studying *L. dubia*, suggested that this crab species is immune or tolerant to nematocysts.

In the absence of information in the literature and on the stomach contents that would support the predatory habit of the crab on the host (medusa), we suggest the association of *L. ferreirae* and *L. lucerna* is instead a commensalism. Probably, *L. ferreirae* is an endosymbiont that uses the medusa as a shelter against predators and a source of food items (zooplankton) and transport (saving energy for growth, for

instance), with no damage to the *L. lucerna,* as already noted by Towanda and Thuesen (2006) and Sal Moyano *et al.* (2012). Nevertheless, this type of association needs further experimental studies to examine the existence of damage or deficit to the medusa when hosting crabs.

One of the most remarkable advantages of the association between pelagic and benthic organisms is the facilitation of the dispersion process for the latter, favoring the genic change among their populations (Hultgren and Stachowicz, 2011). In this study, we observed an advantageous association for the crabs once we found that 50% of the sampled crabs (*L. ferreirae*) inhabited medusa (*L. lucerna*).

We pointed that these associations are dynamic. A series of variables could influence several relevant changes, such as the type of symbiotic relationship, the crabs' growth, and the ingestion of possible parasites of the medusa, in addition to the defense provided by the medusa to the crabs that lead to a mutualistic relationship (Towanda and Thuesen, 2006). Additionally, it could be changed to a parasitism, in the case of competition for food resources between the crab and the medusa. Notwithstanding, detailed experimental studies should be conducted to gain a better understanding of the association of medusae and crabs.

The abundance of the crab, *L. ferreirae*, within the medusa, *L. lucerna*, in the present study was more representative (53.5%) than that obtained for the same species by Nogueira Jr. and Haddad (2005) [time of catch per unit effort (cpue) = 30 months]; and by Sal Moyano *et al.* (2012) (cpue = 8 months) for *Libinia spinosa* H. Milne Edwards, 1834 and *L. lucerna*. The high abundance observed in the present study is probably due to the high quantity of juvenile specimens associated with the medusa in the period of recruitment, which occurred during summer (peaking in March), when the environmental factors are favorable to the crabs' development.

Our results showed more than one specimen of associated crab in medusae (from one to eleven), and when this occurred, the crabs had similar sizes. In the studies by Vaz Ferreira (1972) and Sal Moyano *et al.* (2012), no more than four crabs were found in the same specimen of medusa, while in Nogueira Jr. and Haddad (2005) only one crab was found to be associated with the medusa. Although, as larger crabs were found to inhabit medusae, there was a tendency toward fewer crabs per medusa, with a single crab per medusa being more common. This finding indicates a competition for the resource (medusa) or a territorial dispute among the crab developmental stages, which could lead to competition and cannibalism (Towanda and Thuesen, 2006).

The association of the medusa *L. lucerna* with the crab *L. ferreirae* cannot be considered as species-specific, as other crustaceans (crabs and shrimps) were found to inhabit medusae (Nogueira Jr. and Haddad, 2005; Gonçalves *et al.*, 2016a), but preferentially, those crustaceans belonged to Rhizostomeae (Moreira, 1961; Schiariti *et al.*, 2012). Thus, *L. ferreirae* might share its pelagic habitat (medusa) with other symbionts or would be aggressive against other crab specimens or distinct species. Several symbionts are solitary but aggressive, defending their territory against any intruder, but is possible to find two associated specimens (a couple) or a structured community (i.e., one male taking care of several females) that cooperate in the defense of their territory (Thiel and Baeza, 2001).

The crab *L. ferreirae* could utilize the medusa (as host) and planktonic organisms (as food), mainly when they are in high abundance (spring and/or summer), as mentioned by Millis (2001). During such warm and hot seasons, an increase occurs in the primary productivity (phytoplankton) that serves as food to zooplankton that consequently, is a resource base for many early stages of several animals, favoring the growth of their larvae and juveniles (Thorson, 1950). Thus, the increase in the water

temperature would be a factor that explains the low number of obtained crabs associated with the medusa during winter, despite a high abundance of medusa in this period.

Considering the hypothesis of the obligatory symbiotic relationship between *L. ferreirae* and *L. lucerna*, during the megalopa, i.e., if this stage needs the host protection to molt to juvenile and to develop throughout the juvenile phases, any variation in the medusa abundance will affect the recruitment/development of the symbiont crab. According to Thiel and Baeza (2001), the host's movement, distribution and abundance influence the frequency with which the symbiont is found and, thus, drastic alterations could damage the symbiotic relationship, affecting the most dependent species in such an association.

According to Sal Moyano *et al.* (2012), the crabs *L. spinosa* enter the medusa *L. lucerna* during their larval phase and can remain associated throughout the life span of the medusa, which lasts 5 to 6 months (Schiariti 2008), or while the cnidarian could carry it, depending on the crab's size. However, the present authors do not agree that the crabs should live associated with *L. lucerna* until they reach sexual maturity (approximately 12 months). Crabs leave the medusa when the inner space is not sufficiently large to remain inside it, or when the medusa expels them (Corrington, 1927; Gutsell, 1928; Nogueira Jr. and Haddad, 2005). Thus, the crabs continue their life in the benthic environment.

The present study shows that the medusa and crab sizes increase along with the sampling period and, posteriorly, they decrease. We suggest the medusae are ending their life span, based on the results of the box plot monthly distribution. In this sense, these results reinforce the idea that *L. ferreirae* enter the medusa in a higher frequency during the end of the larval phase because the spawn, hatching and subsequent larval

development coincide with the increase in the primary productivity of the sea and indirectly with the development of the medusa (hosts).

The association of crabs with medusa occurs when the later go down near the ocean floor or when they are drag by marine currents touching the benthic environment (Corrington, 1927; Colombo *et al.*, 2003; Towanda and Thuesen, 2006). Additionally, the megalopa stage could move down and up (Queiroga, 1998) and in this way reach the medusa. As stated by Begon *et al.* (2007), the occupation of distinct niches by juveniles and adults diminishes the resource competition, providing an ecological segregation of the population. Nevertheless, this is not obligatory after the early crabs' stages, as we found one crab *L. ferreirae* associated carrying a small anemone on its carapace (this indicates the crab dissociation and subsequently a new association) (see Sal Moyano *et al.*, 2012).

The difference among the associated *L. ferreirae* crabs compared to free-living crabs was remarkable; the latter were much larger. In addition, the associated crabs exhibited a whitish coloration, which could indicate that the crabs are attempting to reproduce the host color to be invisible to predators (Hultgren and Stachowicz, 2011).

The medusa *L. lucerna* is not apparently damaged by carrying *L. ferreirae* crabs. In several cases, the medusa was found to carry more than 3% of its body weight. However, this may result in the medusa expending a greater amount of energy for locomotion; further experimental studies should be performed to evaluate this energetic loss. Towanda and Thuesen (2006) have reported a medusa (*Phacellophora camtschatica* Brandt, 1835) carrying a total of 32 megalopae of the crab *Cancer gracilis* (Dana, 1852), which consumed less than 2% of the energetic content of the host with no negative impact.

The similar sizes among the megalopae raised in laboratory and the first juvenile obtained associated with the medusa are probably related to the great nutritional value of the laboratory food (*Artemia* newly hatched nauplii) (Suprayudi *et al.,* 2002; Figueiredo *et al.,* 2009). The increase in the temperature is another key factor that shortens the larval development. However, increasing temperature, although it shortens the larval stage, generating low cost, it leads to a decrease in the final size of the larvae in the long term (Barría et al. 2005; Negreiros-Fransozo et al. 2008). This way, the associated crabs seem to show a reduction in duration of development, causing smaller size, but are advanced with regard to their stage of development.

The *L. ferreirae* larvae reared in the laboratory did not successfully molt from megalopa to juveniles; they died in the pre-molt stage. The megalopa stage is a critical period of brachyuran life due to the occurrence of morphological alterations of the mouth appendages and the consequent importance for feeding habits. In addition, some crab species require a host stimulus to trigger their developmental progression, and in this case, the high mortality rate in laboratory could be attributed to the absence of medusa. Anger *et al.* (1989) mentioned the megalopae mortality likely caused by the absence of the Scyphozoa medusa, which provides specific substratum favorable to the metamorphosis and development of juveniles. Nevertheless, these assumptions must be experimentally tested.

This study provides evidence that *L. lucerna* is vital importance to the settlement of *L. ferreirae,* particularly at the end of its larval phases (megalopa), and is a decisive factor in whether individuals reach juvenile phases. The entrance of *L. ferreirae* megalopa to the medusa *L. lucerna* is an important strategy for maintaining the crab population; it prevents competition for food and/or shelter with other brachyuran species and protects against predation when compared with the benthic habitat.

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Literature Cited

Anger, K., Harms, J., Montú, M., and Bakker, C. 1989. Growth and respiration during the larval development of a tropical spider crab, *Libinia ferreirae* (Decapoda: Majidae). *Mar. Ecol. Prog. Ser.* **54:** 43–50.

Arai, M. N. 1997. A functional biology of Scyphozoa. Chapman and Hall, London.

Baeza, J. A., Hemphill, C. A., and Ritson-Williams, R. 2015. The sexual and matin–g system of the shrimp *Odontonia katoi* (Palaemonidae, Pontoniinae), a symbiotic guest of the ascidian *Polycarpa aurata* in the coral triangle. *PloS one.* **10:** 1–18.

Baeza, J. A., and Thiel, M. 2007. The mating system of symbiotic crustaceans. A conceptual model based on optimality and ecological constraints. In: Duffy JE, Thiel M (eds) Reproductive and Social Behavior: Crustaceans as Model Systems. Oxford: Oxford University Press.

Bakker, C., Montu, M., Anger, K., and Harms, J. 1990. Larval development of a tropical spider crab, *Libinia ferreirae* Brito Capello, 1871 (Decapoda: Majidae), reared in the laboratory. *Meeresforschung.* **33:** 90–103.

Barría, E. M., Jara, C. G., and Paschke, K. A., 2005. La temperatura como factor de variabilidad en el desarrollo y morfología de larvas zoeas de *Acanthocyclus hassleri* Rathbun (Decapoda, Brachyura, Atelecyclidae) cultivadas en laboratorio. *Invest. Mar.* **33:** 25–4.

Begon, M., Harper, J. L., and Towsend, C. R. 2007. Ecology: from individuals to ecosystems. 4th ed., London, Blackwell Publishing.

Branco, J. O., and Verani, J. R. 2006. Pesca do camarão sete-barbas e sua fauna acompanhante, na Armação do Itapocoroy, Penha, SC, In: Branco JO and Marenzi AWC (eds) Bases ecológicas para um desenvolvimento sustentável: estudos de caso em Penha, SC. Itajaí, Editora Univalli. 291: 153−170.

Castilho, A. L., Furlan, M., Costa, R. C., Fransozo V (2008) Reproductive biology of the rock shrimp *Sicyonia dorsalis* (Decapoda: Penaeoidea) from the southeastern coast of Brazil. *Invertebr. Reprod. Dev.* **52:** 59−68.

Coleman, N. 1977. A field guide to Australian marine life. Rigby, Adelaide.

Colombo, G. A., Mianzan, H., and Madirolas, A. 2003 Acoustic characterization of gelatinous-plankton aggregations: four case studies from the Argentine continental shelf. *ICES J. Mar. Sci.* **60:** 650–657.

Corrington, J. D. 1927. Commensal association of a spider crab and a medusa. Biol Bull 53:346–350

Ewald P (1987) Transmission modes and evolution of parasitism-mutualism continuum. *Ann. NY. Acad. Sci.* **503:** 295–306.

Figueiredo, J., van Woesik, R., Lin, J., and Narciso, L. 2009. *Artemia franciscana* enrichment model—how to keep them small, rich, and alive? *Aquaculture.* **294**: 212–220.

Flores, A. A. V., Marques, F. P. L., and Negreiros-Fransozo, M. L. 2002. Postlarval stages and growth patterns of the spider crab *Pyromaia tuberculata* (Brachyura, Majidae) from laboratory-reared material. *J. Crustacean Biol.* **22:** 314–327.

Gonçalves, G. R. L., Wolf, M. R., Costa, R. C., and Castilho, A. L. 2016a. Decapod crustacean associations with scyphozoan jellyfish (Rhizostomeae: Pelagiidae) in the Southeastern Brazilian coast. *Symbiosis.* doi 10.1007/s13199-016-0395-x.

Gonçalves. G. R. L., Bolla Jr. E. A., Negreiros-Fransozo, M. L., and Castilho, A. L. 2016b. Morphometric and gonadal maturity of the spider crab *Libinia ferreirae* Brito Capello, 1871 (Decapoda: Majoidea: Epialtidae) at Southeastern Brazilian coast. *J. Mar. Biol. Assoc.* UK. 1–7, doi:10.1017/S0025315416000370.

Gutsell, J. S. 1928. The spider crab, *Libinia dubia*, and the jellyfish *Stomolophus meleagris*, found associated at Beaufort, North Carolina. *Ecology.* **9:** 358–359.

Hultgren, K., and Stachowicz, J. 2011. Camouflage in decorator crabs. Pp. 214–238 in the Animal Camouflage: Mechanisms and Function, M. Stevens and S. Merilaita, eds. Cambridge University Press, Cambridge.

Ingle. R. W. 1977. The larval and post-larval development of the scorpion spider crab, *Inachus dorsettensis* (Pennant) (family: Majidae), reared in the laboratory. *Bull. Br. Mus. Nat. Hist.* **30:** 329–348.

Jachowski, R. 1963. Observations on the moon jelly, *Aurelia aurita*, and the spider crab, *Libinia dubia*. *Chesapeake Sci.* **4:** 195.

Lee, J., Kim, T., and Choe, J. 2009. Commensalism or mutualism: conditional outcomes in a branchiobdellid–crayfish symbiosis. *Oecologia.* **159:** 217–224.

Leung, T. L. F., and Poulin, R. 2008. Parasitism, commensalism, and mutualism: exploring the many shades of symbioses. *Vie et Milieu.* **58:** 107–115.

Mariscal, R. 1974. Nematocysts. Pp. 129–178 in Coelenterate biology: reviews and new perspectives, L. Musatine and H. Lenhoff, eds. Academic Press, New York.

Martin, B. D., and Schwab, E. 2013. Current usage of symbiosis and associated terminology. *Int. J. Biol.* **5:** 32–45.

Martinelli Filho, J. E., Stampar, S. N., Morandini, A. C., and Mossolin, E. C. 2008 Cleaner shrimp (Caridea: Palaemonidae) associated with scyphozoan jellyfish. *Vie et Milieu.* **58:** 133–140.

Melo, G. A. S. 1996. Manual de identificação dos Brachyura (caranguejos e siris) no litoral brasileiro. São Paulo, Ed. Plêiade, FAPESP.

Millis, C. E. 2001. Jellyfish blooms: are populations increasing globally in response to changing ocean conditions? *Hydrobiologia.* **451:** 55–68.

Moran, N. A. 2007. Symbiosis as an adaptive process and source of phenotypic complexity. *Proc. Natl. Acad. Sci.* **104:** 8627–8633.

Morandini, A. C., Ascher, D., Stampar, S. N., and Ferreira, J. F. V. 2005. Cubozoa e Scyphozoa (Cnidaria: Medusozoa) de águas costeiras do Brasil. *Iheringia Sér. Zool.* **95**: 281–294.

Moreira, M. G. B. S. 1961. Sobre *Mastigias scintillae* sp. nova (Scyphomedusae, Rhizostomeae) das costas do Brasil. Bolm. *Inst. Oceanogr. S. Paulo.* **11:** 5–29.

Negreiros-Fransozo, M. L., Fransozo, A., and Hebling, N. J. 1989. Larval development of *Persephona mediterranea* (Herbst, 1794) (Brachyura, Leucosiidae) under laboratory conditions. *Crustaceana*. **57:** 177–193.

Negreiros-Fransozo, M. L., Fransozo, A., and Hirose, G. L. 2008. The megalopa and the early juvenile development of *Hepatus pudibundus* (Herbst, 1785) raised from neuston samples. *Rev. Bras. Zool.* **25:** 608–616.

Nogueira Jr. M., and Haddad, M. A. 2005. *Lychnorhiza lucerna* (Scyphozoa, Rhizostomeae) and *Libinia ferreirae* Brito Capello (Brachyura, Majidae) association in southern Brazil. *Rev. Bras. Zool.* **22:** 908–912.

Nogueira Jr. M., and Haddad, M. A. 2006. Macromedusae (Cnidaria) from the Paraná Coast, Southern Brazil. *J. Coast. Res.* **39:** 1161–1164.

Ohtsuka, S., Koike, K., Lindsay, D., Nishikawa, J., Miyake, H., Kawahara, M., Mulyadi., Mujiono, N., Hiromi, J., and Komatsu, H. **2009.** Symbionts of marine medusae and ctenophores. *Plankton. Benthos. Res.* **4:** 1–13.

Omori, M., and Nakano, E. 2001. Jellyfish fisheries in Southeast Asia. *Hydrobiologia.* **451**: 19–26.

Pellmyr, O., Thompson, J. N., Brown, J. M., and Harrison, R. G. 1996. Evolution of pollination and mutualism in the yucca moth lineage. *Am. Nat.* **148:** 827–847.

Phillips, P. J., Burke, W. D., and Keener, E. J. 1969. Observations on the trophic significance of jellyfishes in Mississippi Sound with quantitative data on the associative behavior of small fishes with medusae. *Trans. Am. Fish. Soc.* **4:** 703–712.

Pohle, G., Mantelatto, F. L., Negreiros-Fransozo, M. L., and Fransozo, A. 1999. Larval Decapoda (Brachyura). Pp. 1281–1351 in South Atlantic Zooplankton, D. Boltovskoy, eds. Leiden, Backhuys Publisher.

Purcell, J. E., Uye, S. I., and Lo, W. T. 2007. Anthropogenic causes of jellyfish blooms and their direct consequences for humans: a review. *Mar. Ecol. Progr. Ser.* **350:** 153–174.

Queiroga, H. 1998. Vertical migration and selective tidal stream transport in the megalopa of the crab *Carcinus maenas*. *Hydrobiologia*. **375/376:** 137–149.

Ross, D. M. 1983. Symbiotic Relations. Pp. 163–2012 in biology of Crustacea Behavior and Ecology, F. Vernberg, W. B. Vernberg, eds. Academic Press, New York.

Sal Moyano, M. P., Schiariti, A., Gilberto. D. A., Diaz Briz, L., Gavio, M. A., and Miazan, H. W. 2012. The symbiotic relationship between *Lychnorhiza lucerna* (Scyphozoa, Rhizostomeae) and *Libinia spinosa* (Decapoda, Epialtidae) in the Río de la Plata (Argentina-Uruguay). *Mar. Biol.* **159:** 1933–1941.

Sampedro. M. P., González-Gurriarán, E., Freire, J., and Muiño, R. 1999. Morphometry and sexual maturity in the spider crab *Maja squinado* (Decapoda: Majidae) in Galicia, Spain. *J. Crustacean Biol.* **19:** 578–592.

Schiariti, A., Kawahara, M., Uye, S., and Mianzan, H. W. 2008. Life cycle of the jellyfish *Lychnorhiza lucerna* (Scyphozoa: Rhizostomeae). *Mar. Biol.* **156:** 1–12.

Schiariti, A., Sal Moyano, M. P., Giberto, D. A., Mianzan, H. W. 2012. Primer registro de la asociación entre *Lychnorhiza lucerna* (Scyphozoa, Rhizostomeae) y *Cyrtograpsus affinis* (Decapoda, Varunidae). *Lat. Am. J. Aquat. Res.* **40:** 1090–1093.

Shanks, A. L., and Graham, W. M. 1988. Chemical defense in a scyphomedusa. *Mar. Ecol. Prog. Ser.* **45**: 81–86.

Suprayudi, M. A., Takeuchi, T., Hamasaki, K., and Hirokawa, J. 2002. Effect of *Artemia* feeding schedule and density on the survival and development of larval mud crab *Scylla serrata*. *Fish. Sci.* **68:** 1295–1303.

Tavares, M., and Santana, W. 2012. On the morphological differentiation between *Libinia spinosa* and *L. ferreirae* (Crustacea: Brachyura: Majoidea: Epialtidae). *Zoologia*. **29:** 577–588.

Thiel, M., and Baeza, J. A. 2001. Factors affecting the social behavior of crustaceans living symbiotically with other marine invertebrates: a modelling approach. Symbiosis. **30:** 63–190.

Thorson, G. 1950. Reproductive and larval ecology of marine bottom invertebrates. Zoological Museum, University of Copenhagen.

Towanda, T., and Thuesen, E. V., 2006. Ectosymbiotic behavior of *Cancer gracilis* and its trophic relationships with its host *Phacellophora camtschatica* and the parasitoid *Hyperia medusarum*. *Mar. Ecol .Progr. Ser.* **315:** 221–236.

Tunberg, B. G., and Reed, S. A. 2004. Mass occurrence of the jellyfish *Stomolophus meleagris* and an associated spider crab *Libinia dubia*, eastern Florida. FL. *Scientist.* **67:** 93–104.

Vaz Ferreira, C. 1972. Relaciones de conveniencia entre *Libinia spinosa* Milne Edwards (Decapoda, Brachyura) y Discomedusae. *Bol. Soc. Zool. Uruguay.* **2:** 64–66.

Williams, M. J. 1981. Methods for analysis of natural diet in portunid crabs (Crustacea: Decapoda: Portunidae). *J. Exp. Mar. Biol. Ecol.* **52:** 103–113.

Winter, V. C., and Masunari, S. 2006. Macroepizoismo em *Libinia ferreirae* (Crustacea, Brachyura, Majidae). *Iheringia Ser. Zool.* **96:** 135–140.

Zamponi, M. O. 2002. The association between the medusa *Lychnorhiza lucerna* (Scyphomedusae, Rhizostomeae) and the decapod *Libinia spinosa* (Brachyura, Majidae) - the first record for neritic waters of Argentina. *Russ. J. Mar. Biol.* **28:** 267–269.

Zar, J. H. 1999. Biostatistical analysis. 4th edition. Upper Saddle River, NJ: Prentice-Hall.

Figure 1. *Libinia ferreirae*. The pictures show the specimens utilized in the present study. *a*) Megalopa stage (obtained in laboratory); *b*) Juvenile individuals (N=9) found associated with a single medusa specimen (note the whitish coloration of the juvenile crab specimens); *c*) An adult crab specimen from a benthonic habit not associated with the medusa (note the brownish coloration and the presence of ectosymbionts on the carapace). The organisms were sampled at Cananéia, São Paulo, Brazil, between February 2013 and May 2014.

Figure 2. *a*) *Libinia ferreirae*. Size frequency distribution, based on carapace width (CW), of the crabs obtained only of medusae with 5 through 11 hosted crabs (N=60). *b*) *Lychnorhiza lucerna*. Size frequency distribution of the umbrella diameter (UD) according to size class and the respective abundance of the crab *L. ferreirae* inhabiting such medusae. The organisms were sampled at Cananéia, São Paulo, Brazil, between February 2013 and May 2014. The X axis' values represent the midpoint of size classes.

Figure 3. *Libinia ferreirae* and *Lychnorhiza lucerna*. The monthly abundance of *L. lucerna* medusae hosting crabs (*L. ferreirae*) and medusae with no crabs. The data were collected at Cananéia, São Paulo, Brazil, between February 2013 and May 2014.

Figure 4. *Libinia ferreirae*. A size frequency distribution based on the carapace width (CW) of crabs (*L. ferreirae*) associated with medusae (*L. lucerna*). The animals were sampled at Cananéia, São Paulo, Brazil, between February 2013 and May 2014The X

axis' values represent the midpoint of size classes. The arrow indicates the approximate size at gonad and morphometric maturity according to Gonçalves *et al.* (2016).

Figure 5. *a*) *Libinia ferreirae*. The monthly mean range of the carapace width (CW) of the *L. ferreirae* crabs associated with the medusae *L. lucerna*. *b*) *Lychnorhiza lucerna*. The monthly mean range of the umbrella diameter (UD) of the medusae *L. lucerna*. (Median; percentile 25% and 75%; Min-Max = minimum and maximum values). The organisms were sampled at Cananéia, São Paulo, Brazil, between February 2013 and May 2014.

Figure 6. *Libinia ferreirae* and *Lychnorhiza lucerna*. *a*) The relationship between umbrella diameter (UD) and carapace width (CW) in associated crabs; *b*) The relationship between medusa weight and crab weight. The organisms were sampled at Cananéia, São Paulo, Brazil, between February 2013 and May 2014.

Figure 7. *Libinia ferreirae*. Mean values of the carapace width (CW) of the megalopae. First: megalopae obtained in the laboratory (M lab) and isolated from medusa; second: megalopae of nature (M nat) associated with medusa in the wild; and third: juvenile of nature (J nat) specimens (smaller than 2 mm CW and associated with medusa *L. lucerna*). SE= standard error; min-max= minimum and maximum values. The organisms were sampled at Cananéia, São Paulo, Brazil, between February 2013 and May 2014.

Figure 8. Stomach contents of the *Libinia ferreirae* crab specimens associated with the medusae *L. lucerna. a* and *b* are nematocysts fixed to the exoskeleton of Copepod

crustaceans, and *c* is a nematocyst of the medusa *L. lucerna*. Scales = 20 μm. The organisms were sampled at Cananéia, São Paulo, Brazil, between February 2013 and May 2014.

Figure 1

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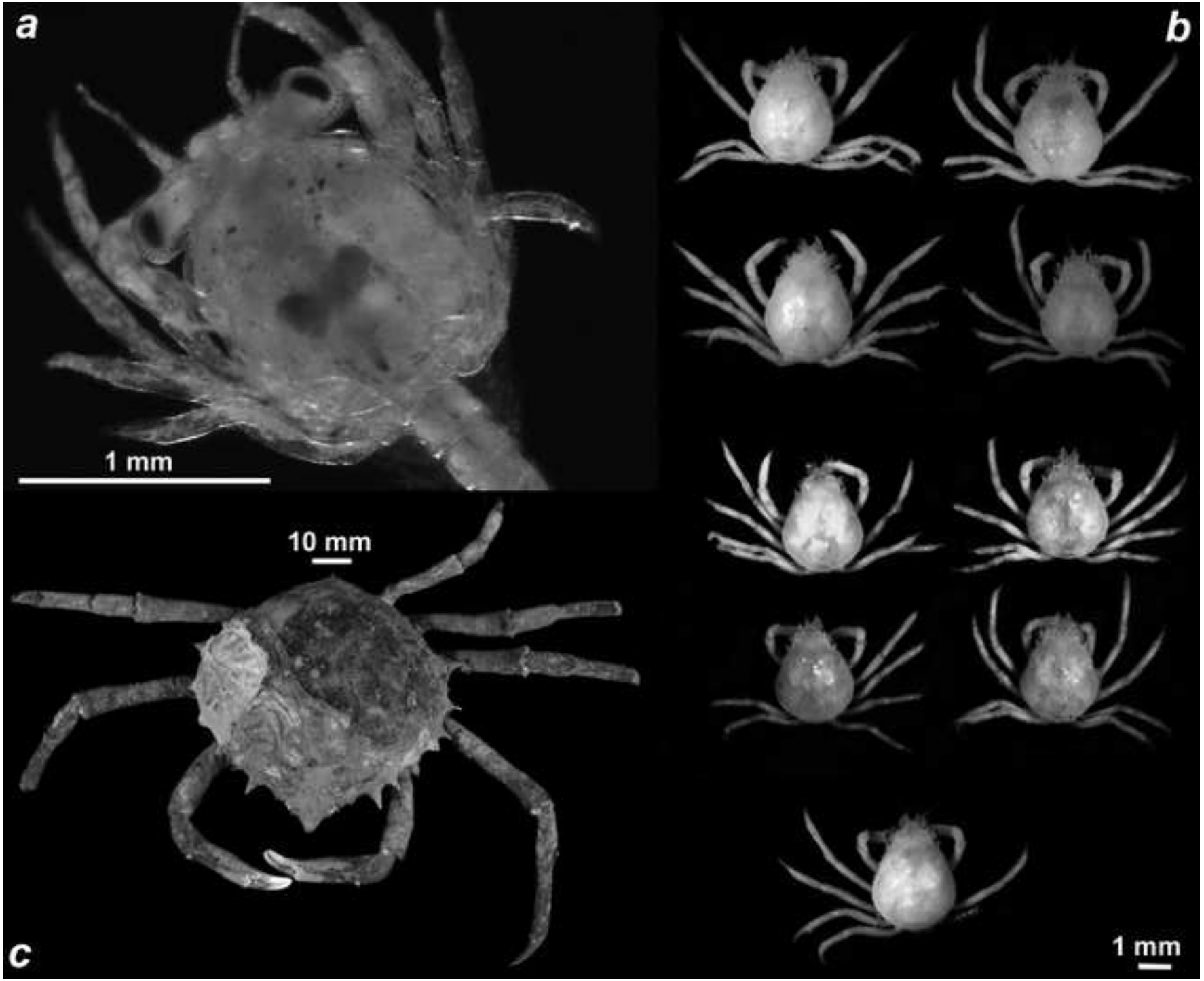

Figure 2

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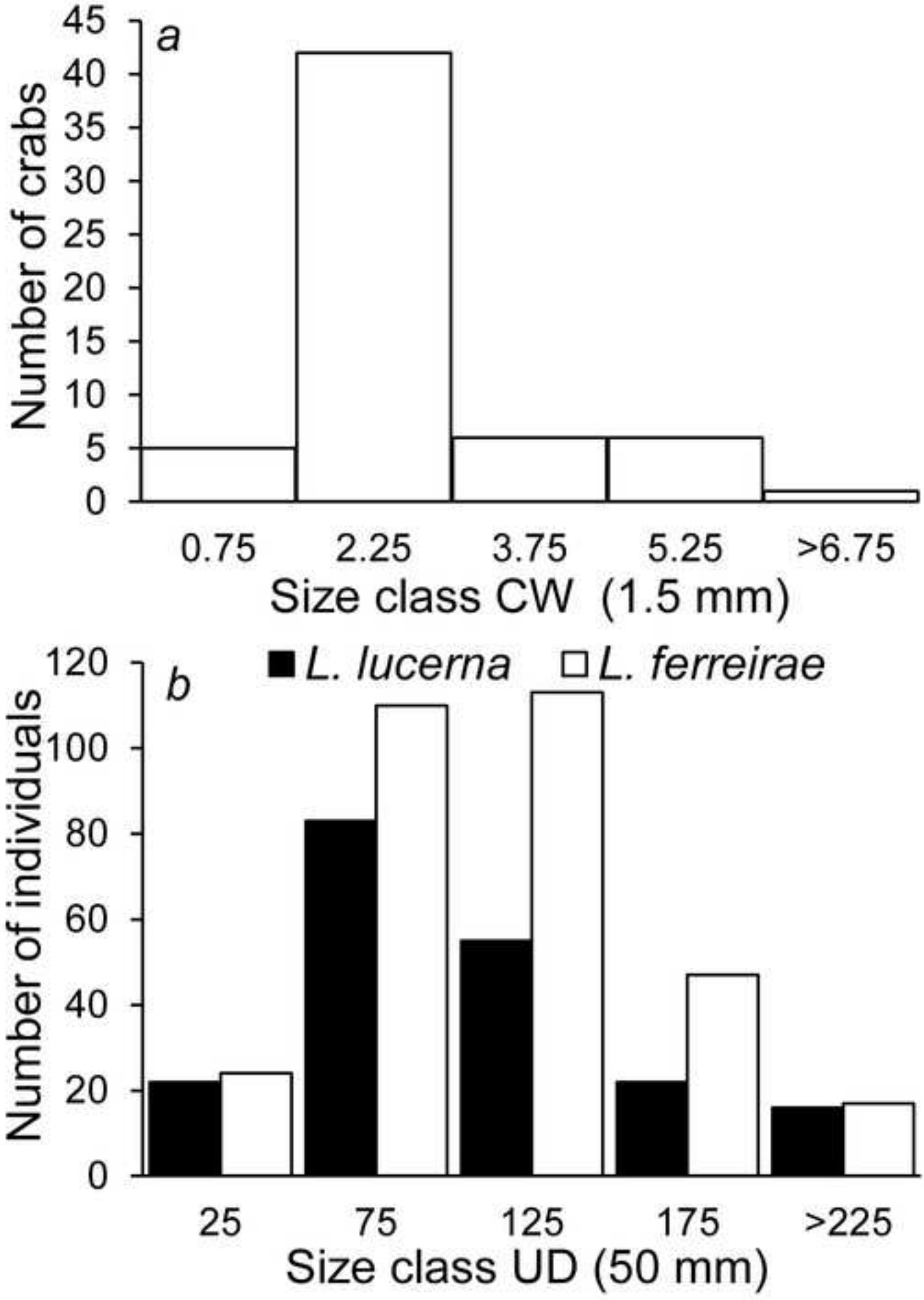

Figure 3

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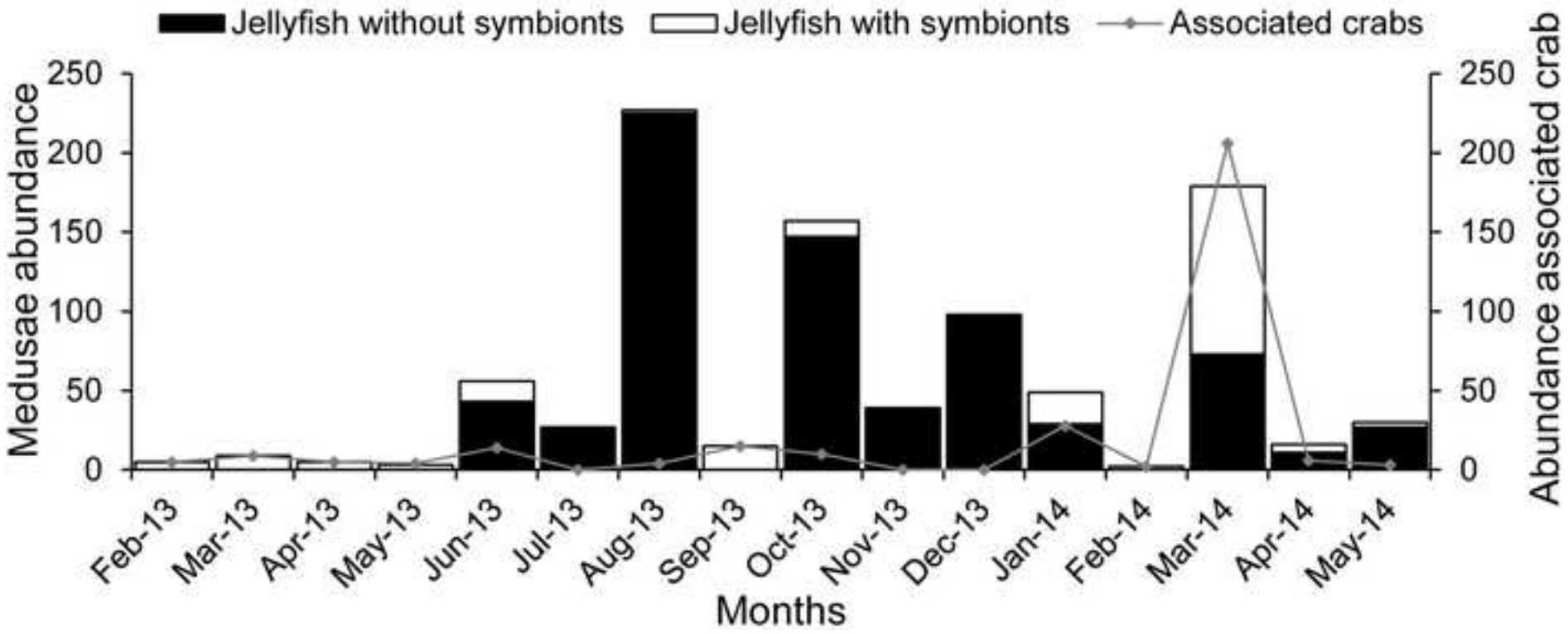

Figure 4

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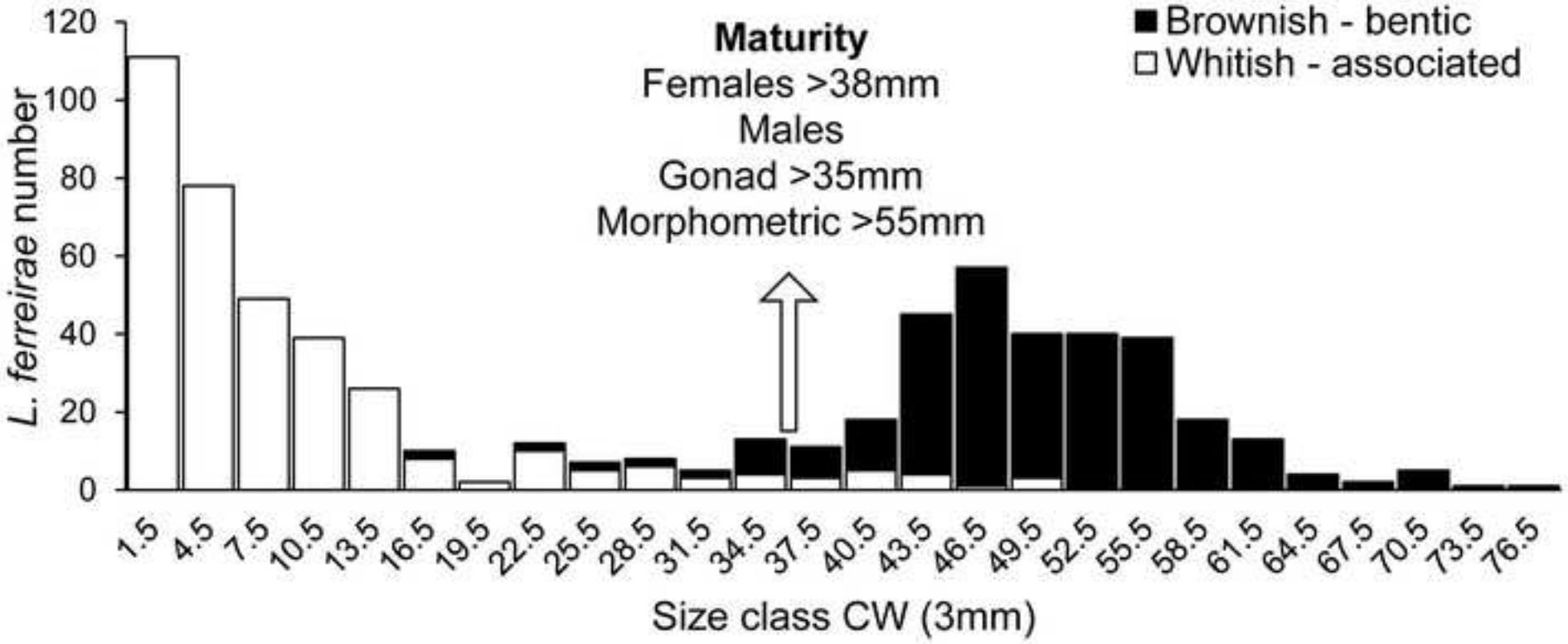

Figure 5

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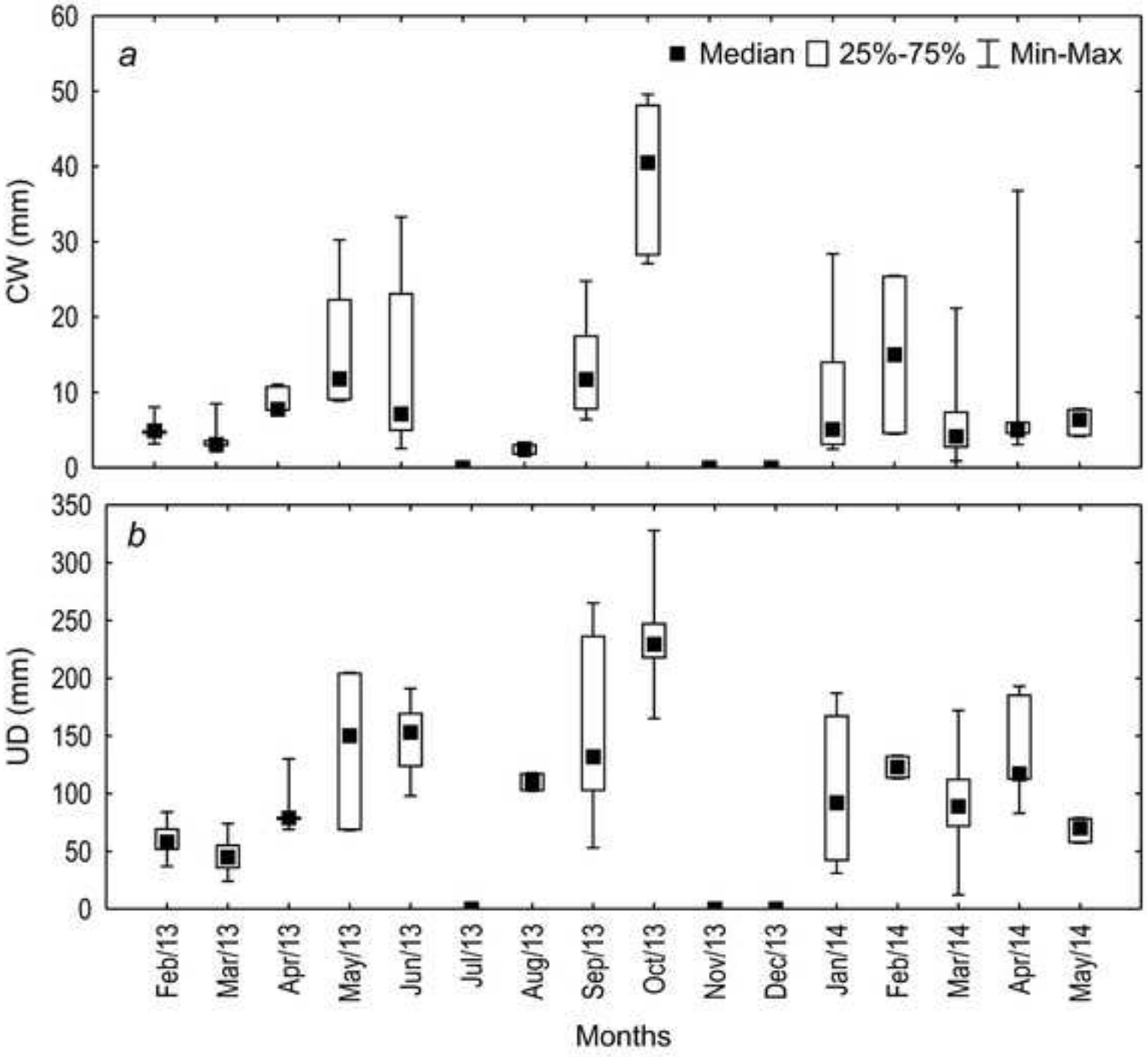

Figure 6

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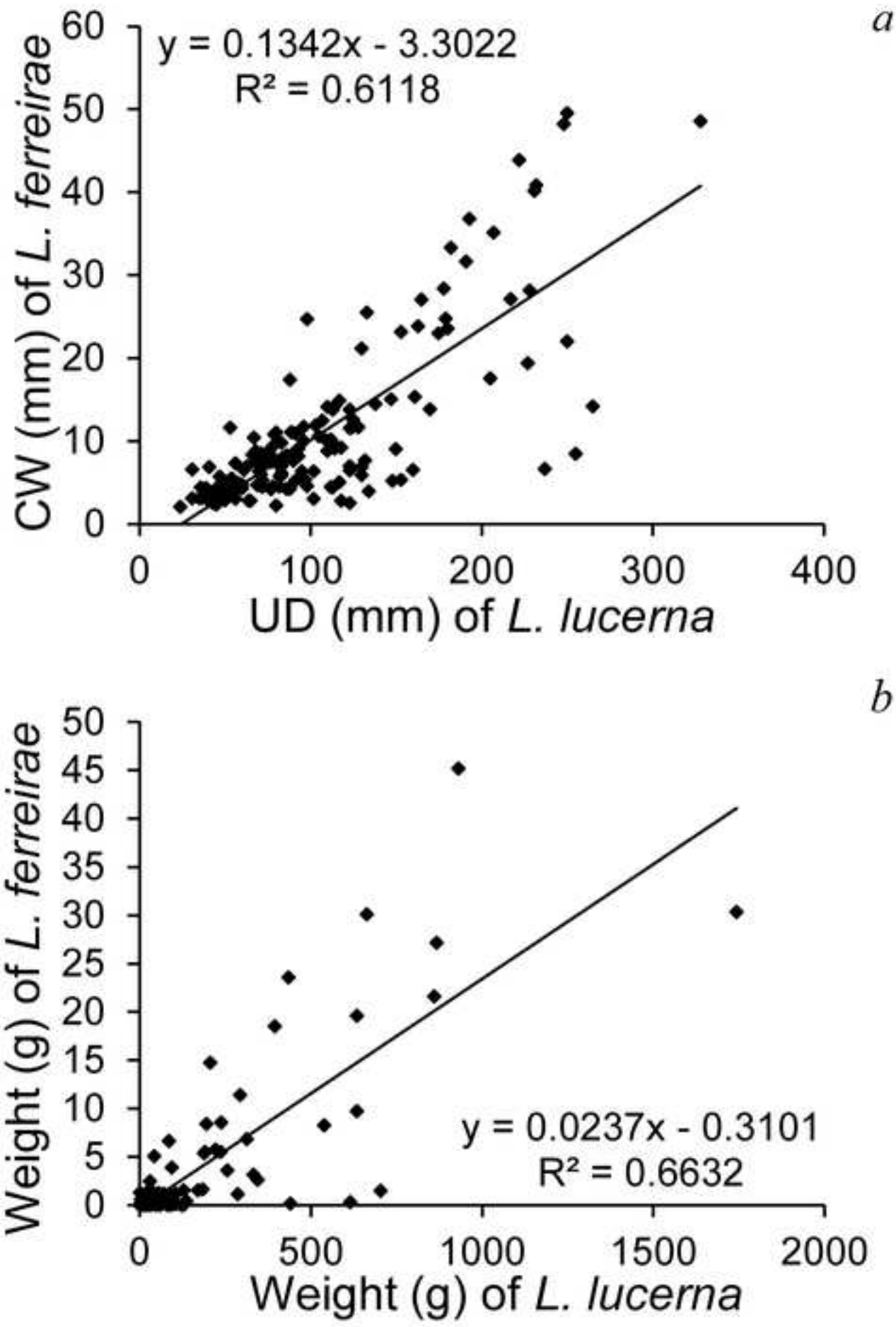

Figure 7

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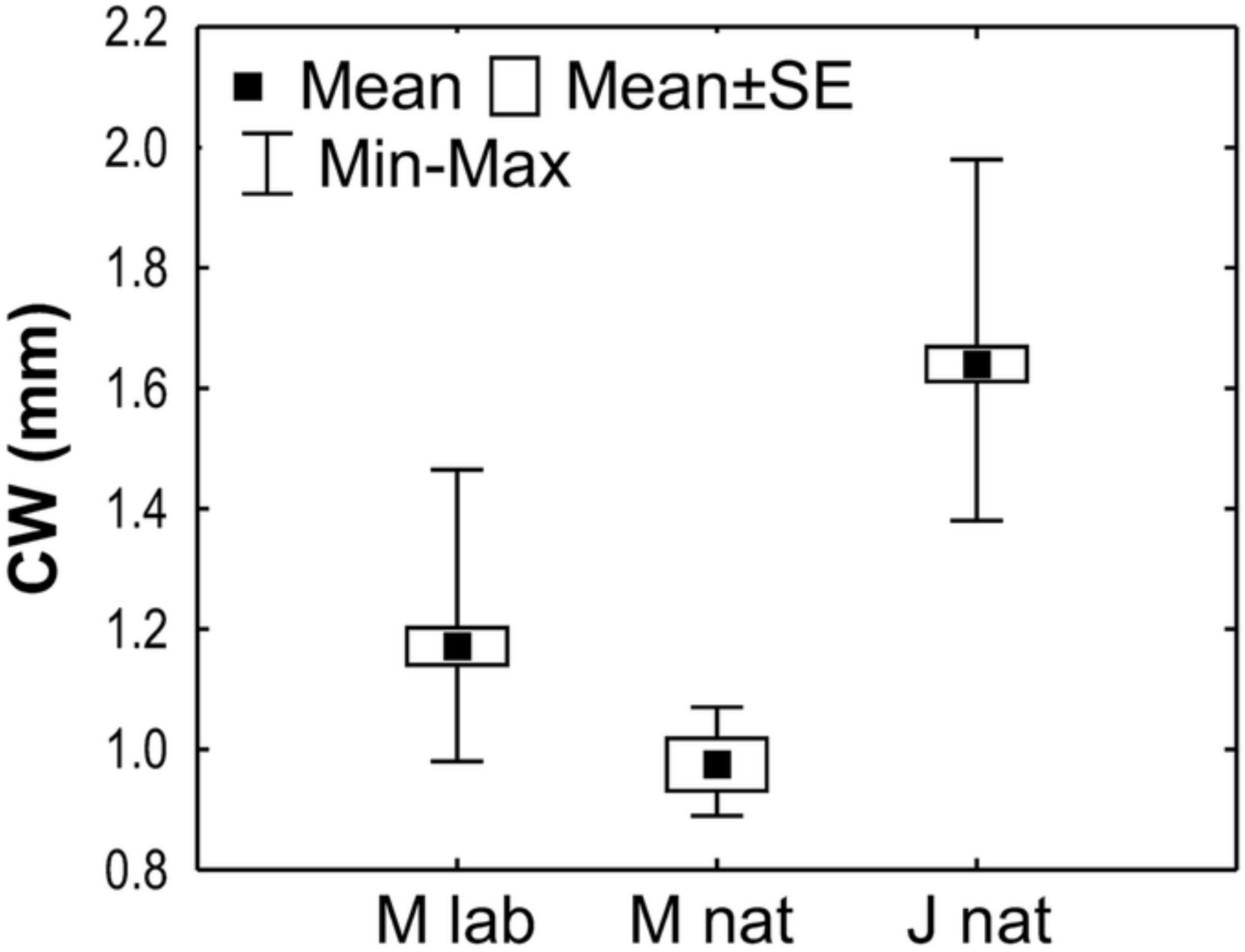

Figure 8

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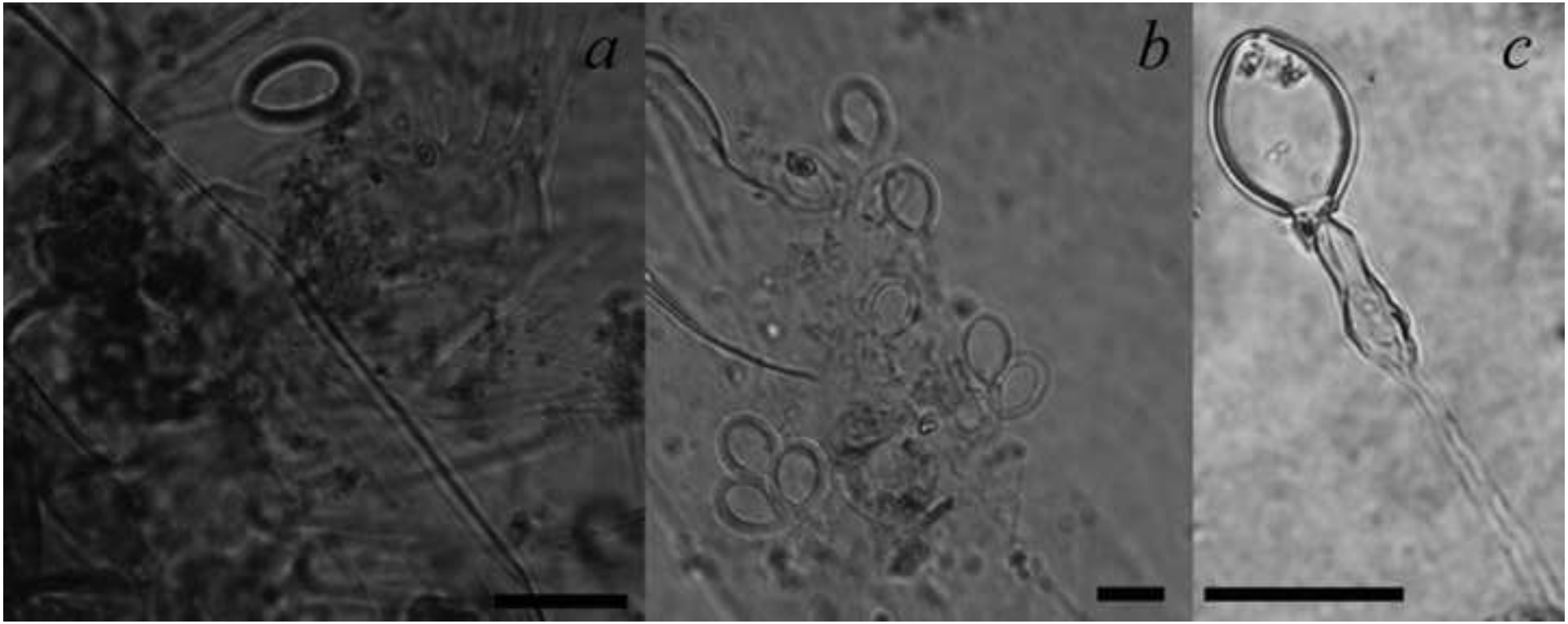
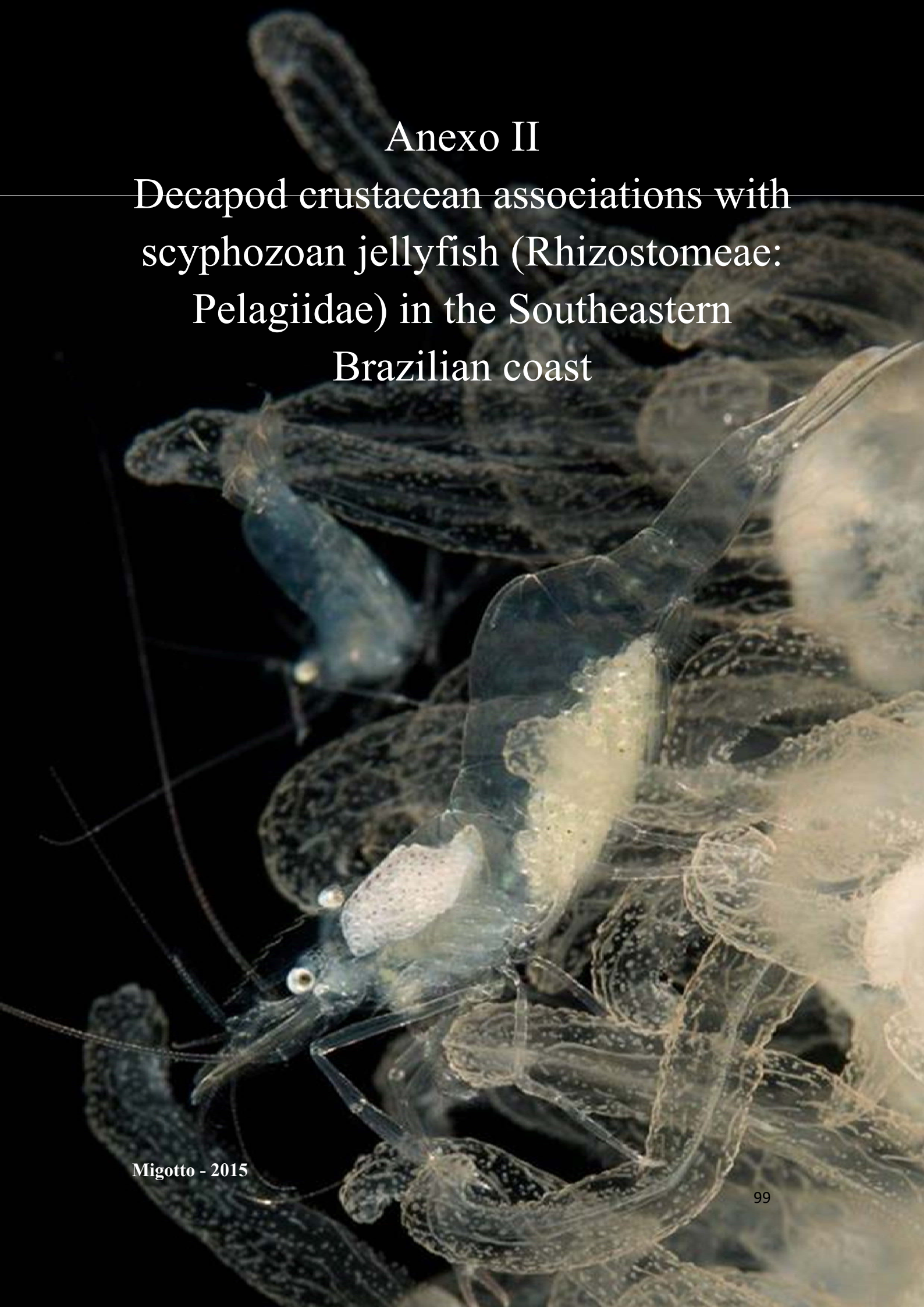


Anexo II

Decapod crustacean associations with scyphozoan jellyfish (Rhizostomeae: Pelagiidae) in the Southeastern Brazilian coast



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Symbiosis
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SHORT COMMUNICATION

Decapod crustacean associations with scyphozoan jellyfish (Rhizostomeae: Pelagiidae) in the Southeastern Brazilian coast

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Abstract In southeastern Brazil, decapod crustaceans were found living in association with the scyphozoan jellyfish. In total, 2002 specimens of the Scyphozoa *Lychnorhiza lucerna* were collected of which 511 were associated decapods that were identified as three species of the crab *Libinia ferreirae*, *Libinia spinosa*, and one Grapsoidea sp. and two species of caridean shrimps *Periclimenes paivai* and *Leander paulensis*. This is the first record of an association between the caridean shrimp *L. paulensis* and a scyphozoan and the first report of symbioses involving the crabs *L. spinosa* and Grapsoidea sp. on the Brazilian coast.

Keywords Brachyura · Crab · Caridea · Medusae · Symbiosis · Shrimp

1 Introduction

Associations of a benthic species with a pelagic organisms like the medusae of scyphozoan jellyfish can bring benefits such as greater mobility, shelter and food availability (Nogueira and Haddad 2005; Martinelli Filho et al. 2008; Sal Moyano et al. 2012; and Schiariti et al. 2012). Other potential advantages of symbiosis may include the exploitation of new resources, the occupation of new ecological niches, refuges, and protection from predation (Thiel and Baeza 2001; Martinelli Filho et al. 2008; Sal Moyano et al. 2012).

Previous studies have documented associations of decapod crustaceans such as caridean shrimps (Palaemonoidea) (Marliave and Mills 1993; Moore et al. 1993; Martinelli Filho et al. 2008) and brachyuran crabs (Majoidea and Grapsoidea) (Nogueira and Haddad 2005; Sal Moyano et al. 2012; Schiariti et al. 2012) with scyphozoan in the medusoid-phase. On the southern and southeastern coasts of Brazil, jellyfish, are accidentally captured as bycatch during trawl-fishing for target species such as the seabob shrimp, *Xiphopenaeus kroyeri* (Heller, 1862) (Graça-Lopes et al. 2002; Schroeder et al. 2014). The capture of jellyfish, especially Rhizostomeae, potentially has an impact on any symbiotic species that are associated with them (Mianzan and Cornelius 1999). The community structure may thus be altered as a result of fishing influencing ability of symbionts to complete stages in their life cycle. The aim of the present study was to analyze and characterize the decapod species which live in association with scyphozoan medusae in southeastern Brazil.

2 Materials and methods

The medusoid-phase of scyphozoans and any associated crustaceans were sampled by trawling (30-min duration) using a shrimp boat outfitted with double-rig nets. The collections were done monthly in São Paulo state, Ubatuba (23°35′00″S - 45°12′30″W, July 2013 to August 2014) and Cananéia (25°04′43″S - 47°50′34″W, February 2013 to May 2014), and Rio de Janeiro state, Macaé (22°22′33″S - 41°46′30″W, September 2013 to June 2014), at depths between 5 and 15 m. A total of 216 trawls were executed (i.e., 48 in Macaé, 56 in

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Ubatuba and 112 in Cananéia). In order to standardize the results, the abundance of hosts and symbionts was estimated as the number of individuals caught per standard trawl set (catch per unit effort).

The bottom water was sampled using a Van Dorn bottle and the salinity was measured with an Atago S/1000 optic refractometer. The temperature (°C) was measured using a thermometer attached to the Van Dorn bottle. The medusa collected were examined for the presence of any symbionts and identified with the help of Morandini et al. (2005) and Nogueira and Haddad (2006). Medusa without any symbionts were counted and returned to the sea. Those that had symbionts were individually packed in plastic bags and transported to the laboratory.

In the laboratory, whole jellyfish in good condition were examined macroscopically to check the anatomical location of symbiotic crustaceans. Medusae were measured between two rhopalia (an accuracy of 0.01 mm) to assess the umbrella diameter (UD) (Nogueira and Haddad 2006) and were then grouped into 10 mm size classes. Umbrella diameters of medusae collected in the Cananéia region were compared with those from Macaé using the Kolmogorov-Smirnov two-sample test (Sokal and Rohlf 1995).

The decapod crustaceans were fixed in 70 % alcohol and identified using Williams (1984); Melo (1996) and Tavares and Santana (2012) for Brachyura, Machado et al. (2010) and Chace (1969) for caridean shrimps and Pohle et al. (1999) for larvae. As a result of the difficulty in determining *Libinia* species because of their great similarities during the juvenile phases, we utilized a specific character on the dorsal surface of the thoracic sternum. In *Libinia spinosa* H. Milne Edwards, 1834, the thoracic episternites of IV-VII is armed with strong, posterolateral-projecting, broad teeth, a feature that is absent in the teeth of *Libinia ferreirae* Brito Carpello, 1871 (Tavares and Santana 2012).

The Brachyura were separated by sex, based on the abdomen shape for adults (elongated pattern for males and oval for females) and on the number of pleopods on individuals (two pairs for males and four pairs for females) (Almeida et al. 2013; Melo 1996). Both sexes were assessed for maturity by abdomen adherence to the thoracic sternite. Individuals were considered immature if the abdomen was sealed and cementing substances were present in the abdomen contour. Individuals with an unlocked abdomen were considered adults (for more details, see Gonçalves et al. 2016).

The sex of the Caridea were determined based on the presence or absence of appendices on the base of the endopod of the second pleopod, present in males but absent in females (Bauer 2004). The state of female sexual maturity was determined by the presence or absence of embryos attached to the pleopods.

The carapace width in Brachyura (CW) and carapace length (CL) in the Caridea were measured with a digital caliper to an accuracy of 0.01 mm. Specimens smaller than 5 mm were measured with a stereo microscope (Zeiss® Stemi SV6, fitted with a Zeiss® Stemi 2000-C image capture system) with accuracy within 0.0001 mm.

The proportion of jellyfish that had symbionts was estimated using an index: P = number of individuals with symbionts/total individuals*100, and the density of symbionts per host was calculated as IMS = total number of symbionts/total number of hosts (Bush et al. 1997).

3 Results

3.1 Abundance and richness of associated species

A total of 2002 *Lychnorhiza lucerna* Haeckel, 1880 specimens were collected which had 511 associated decapod crustaceans from the three collecting areas. The crustaceans belonged to the Majoidea, Grapsoidea, and Palaemonoidea superfamilies. They comprised two species of Brachyura crabs *Libinia ferreirae* (N = 492) and *L. spinosa* (N = 1), one species of Grapsoidea crab (N = 1) and two species of caridean shrimps *Periclimenes paivai* Chace 1969 (N = 13) and *Leander paulensis* Ortmann, 1897 (N = 5) (Table 1, Fig. 1). It was not possible to identify the Grapsoidea because it was a small juvenile. More than one species of crustacean was sometimes present on the same medusae in the Cananéia region. *Lychnorhiza lucerna* was host to an average number of 1.55 and 1.06 symbionts in the Cananéia and Macaé areas, respectively and the frequency of finding medusa with symbionts was 22 % and 19 % respectively.

With respect to crab maturity, juveniles of *L. ferreirae* were found associated with medusae from Cananéia (CW = 0.89 mm-36.8 mm) and Macaé (CW = 2.08 mm-4.97 mm) and adults in medusae from Cananéia (CW = 40.17 mm-49.59 mm). The specimens of *L. spinosa* and the Grapsoidea sp. were also juveniles (Table 1). With respect to shrimps, the majority of *P. paivai* were adult females carrying embryos (N = 11). Only one male was found. In contrast only males of *L. paulensis* were collected from Cananéia. In some cases, both caridean shrimp species were found sharing the same host in the Cananéia region. In two cases, two females of *P. paivai* carrying embryos (= ovigerous females) were observed sharing the same *L. lucerna* medusae. However no medusae was found to be host to a male-female pair.

A total of 1076 and 922 *L. lucerna* were collected in Macaé and Cananéia, respectively. Four *L. lucerna* from the Ubatuba region had decapod associates; but only one with an umbrella diameter (UD = 78 mm) was associated with *L. spinosa* (CW = 15.8 mm), 45 % of the associations in Cananéia region were in large medusae with UD 80–130 mm. In Macaé, the 42 % of the associations were found in medusae between 60 and 100 mm in size (Fig. 2). The sizes of *L. lucerna* in the two

Table 1 Number of crustacean decapod species associated by jellyfish species for each region sampled in Brazilian southeastern coast

Region	Host	Decapod Sym	Sex of Sym	Size range of carapace Sym		N° Sym
				<CW/CL mm	>CW/CL mm	
Cananéia-São Paulo (25°04′43″S; 47°50′34″W)	*L. lucerna*	*L. ferreirae*	F	4.23	48.25	101
			M	4.24	49.59	76
			-	0.89	4.3	134
	L. lucerna	*L. paulensis*	M	3.74	4.75	5
	L. lucerna	*P. paivai*	F ovg	2.08	-	12
			M	3.02	5.18	1
	L. lucerna	Grapsidea	-	1.3	-	1
Macaé-Rio de Janeiro (22°22′33″S; 41°46′30″W)	*L. lucerna*	*L. ferreirae*	F	4.59	32.03	87
			M	4.62	20.00	70
			-	2.08	4.97	23
Ubatuba-São Paulo (23°35′00″S; 45°12′30″W)	*L. lucerna*	*L. spinosa*	F	15.8	-	1
Total of animals associated						511

Sym = symbionts; M = males; F = females; F ovg = ovigerous females (−) unsexed animals; CW = carapace width; CL = carapace length; N° = abundance of decapods by species/sex

regions was significantly different (Kolmogorov-Smirnov two-sample test; $d_{max} = 0.27$, $P < 0.01$).

In Macaé, 16 % ($N = 169$) of medusae carried a total of 180 juveniles crabs (CW = 2.08 mm-32.03 mm) in association, of which 73 % were smaller than 10 mm CW. The highest incidence of medusae associated with crabs (134) was found in September 2012 (winter, temperature 21–22 °C; Figs. 3 and 4*a*). During this period, the mean salinity was 38. At this time there was the greatest abundance of *L. ferreirae* (85 % of the entire sample of associations). In January 2014 (summer, temperature 18.5–20 °C and a mean salinity of 36.7), the medusae were also abundant ($N = 329$), but no associations were found (Figs. 3 and 4*a*).

In the Cananéia region, 22 % of the captured medusae ($N = 205$, UD = 24–328 mm) had 330 associated decapods. The most abundant crustacean species was *L. ferreirae* ($N = 311$), composed mainly of juveniles (CW = 1.38 mm-36.8 mm), with 76 % of individuals being less than 10 mm CW, and there were 4 megalopa larvae. Only 2 % of these crabs were adults (CW = from 40.17 to 49.59 mm). This region had medusae with up to 11 associated *L. ferreirae* as well as more than one species in the same host, for example, 1 *L. ferreirae* + 1 *L. paulensis*, 3 *L. ferreirae* + 1 *P. paivai* (females carrying embryos), and 6 *L. ferreirae* + 1 Grapsidae. The average CW of crabs that shared each medusae was 4.21 mm, which is smaller than the average CW of 7.8 mm for crabs captured alone in their host. The highest incidence of crabs associated with *L. lucerna* occurred during March 2014 (summer, temperature 25–26 °C and salinity 34): 179 medusae were associated with 206 crabs. Some had more than one

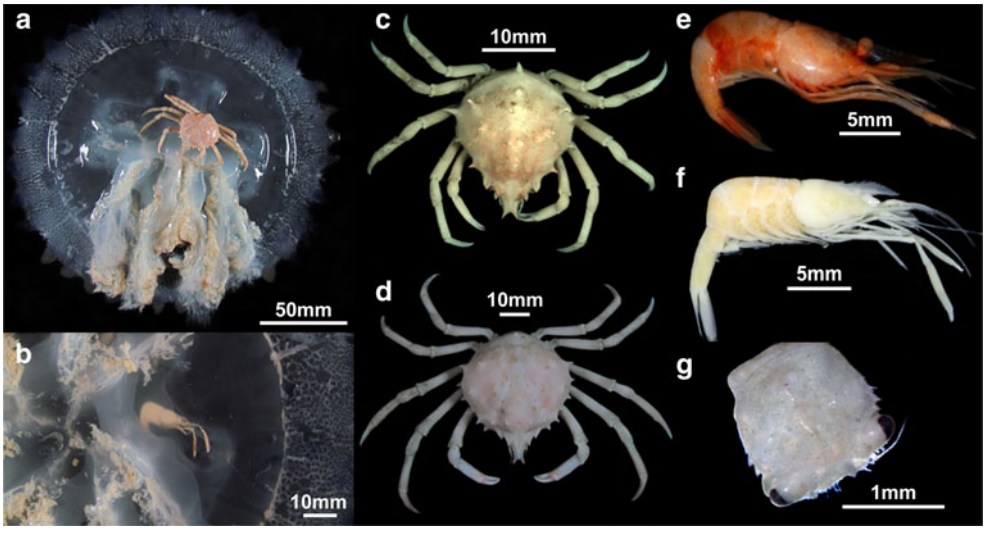

Fig 1 **a**. *Lychnorhiza lucerna* Haeckel, 1880 and *Libinia ferreirae* Brito Capello, 1871 dorsal view; **b**. *L. lucerna* dorsal view with *Periclimenes paivai* Chance, 1969 (female carrying developing embryos attached beneath the abdomen) within the subgenital space of the medusa; **c**. and **d**. *L. ferreirae* and *Libinia spinosa* H. Milne Edwards, 1834 dorsal pictures, respectively; **e**. *Leander paulensis* Ortmann, 1897 lateral view; **f**. *P. paivai* dorsal view; **g**. dorsal view of Grapsoidea MacLeay, 1838

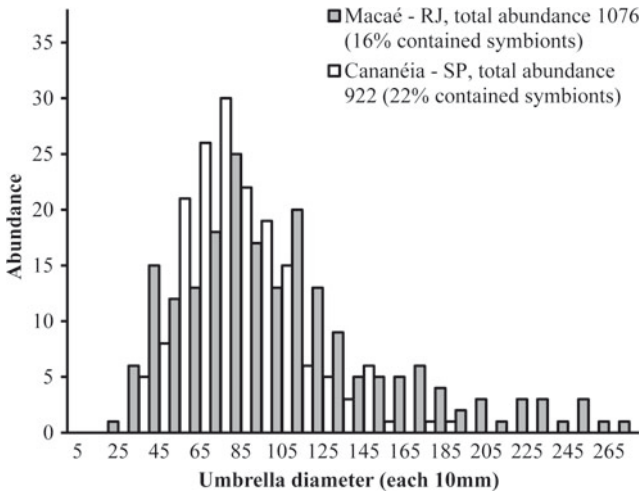

Fig 2 Size–frequency distributions for *Lychnorhiza lucerna* jellyfish with decapods associated from Cananéia, São Paulo, and Macaé, Rio de Janeiro, Brazilian southeastern coast. Intervals of 10-mm size class were used, ranging from 0 to 10 (class 5) to >270 (class 275)

symbiont in each host (Figs. 3 and 4*b*). The highest capture of 226 *L. lucerna* medusae was in August 2013 (winter, temperature 17.5–19 °C and salinity 29.2) (Figs. 3 and 4*b*) but only two medusae were host to four juvenile crabs (CW of 1.51 to 3.21 mm).

4 Discussion

Morandini (2003) studied juvenile *L. lucerna* medusae in the Cananéia region, and observed the jellyfish bloom during the spring season. The initial ephyra phases develops into the medusa phase during the following season and the life span is 5–6 months (Schiariti 2008; Sal Moyano et al. 2012). It is after the ephyra phase, that the *L. lucerna* medusae becomes associated with decapod larvae (Sal Moyano et al. 2012).

The association of shrimps in the Palaemonidae family with Cnidarians has been reported previously, mainly with anemones (Omori et al. 1994; Fautin et al. 1995; Azofeifa-Solano et al. 2014) but occasionally with the scyphozoan medusae (Moreira 1961; Martinelli Filho et al. 2008). In the present study of associations with *L. lucerna*, there was a predominance of adult *P. paivai* females carrying embryos. This has been observed by others (Martinelli Filho et al. 2008 and Ohtsuka et al. 2010).

In the present study, it is possible that female *P. paivai* incubating embryos adopt a sedentary behavior after reproduction and use their scyphozoan host as a means of protection for their offspring (Martinelli Filho et al. 2008). The females may disassociate from the hosts when larval are to be releases as the offspring could be a food source for the medusae (Bauer 2004).

Although the abundance of the shrimp, *L. paulensis*, was low in this study, it is the first record of an association with *L. lucerna*. Furthermore, little is known about this species, except that it is found in shallow waters up to 16 m deep on sandy bottoms and algae. The occurrence of only males in this association with *L. lucerna* in Cananéia could indicate a difference in behavior between the two shrimp species (Ramos-Porto 1986; Zimmermann et al. 2015).

Libinia ferreirae was the most abundant brachyuran crab species associated with *L. lucerna*, and they were predominantly juveniles which are not found freely on the ocean floor. Larval and juvenile individuals may be associated with jellyfish because the latter provide a protected environment during this most vulnerable period of their lives (Nogueira and Haddad 2005). Probably, these crabs colonize the medusae

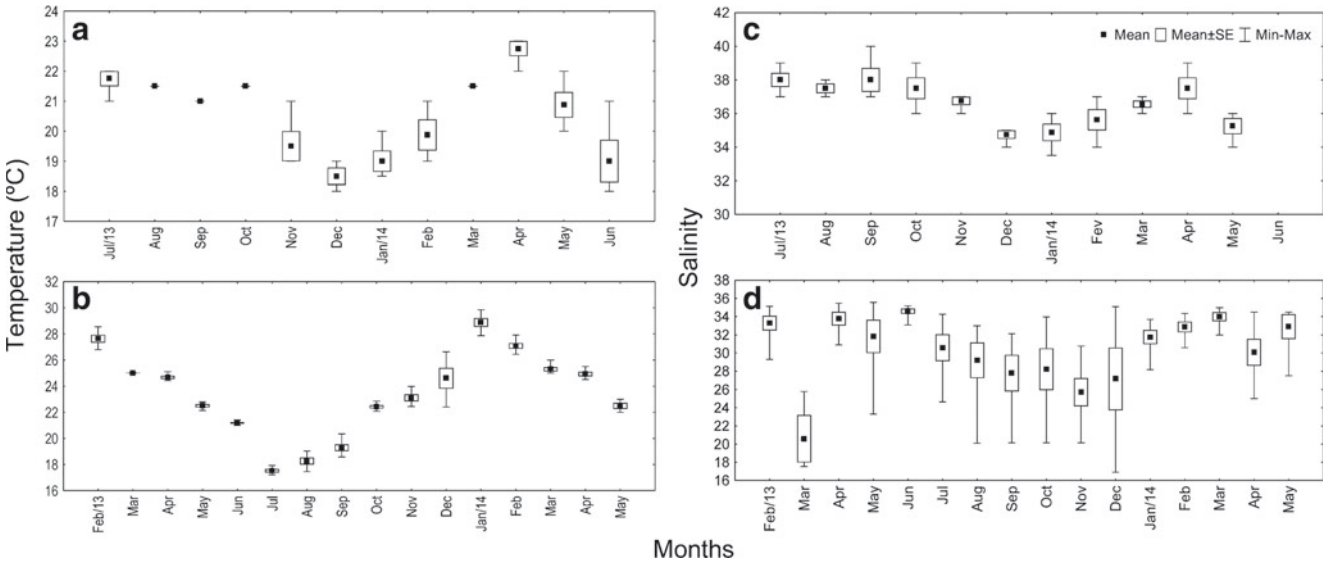

Fig 3 Monthly average temperature **a**, **b** and salinity **c**, **d**) variations. **a** and **c**. Macaé, Rio de Janeiro state; **b** and **d**. Cananéia, São Paulo state, Brazilian southeastern coast. SE: Standard error; Min-Max: the minimum and maximum ranges

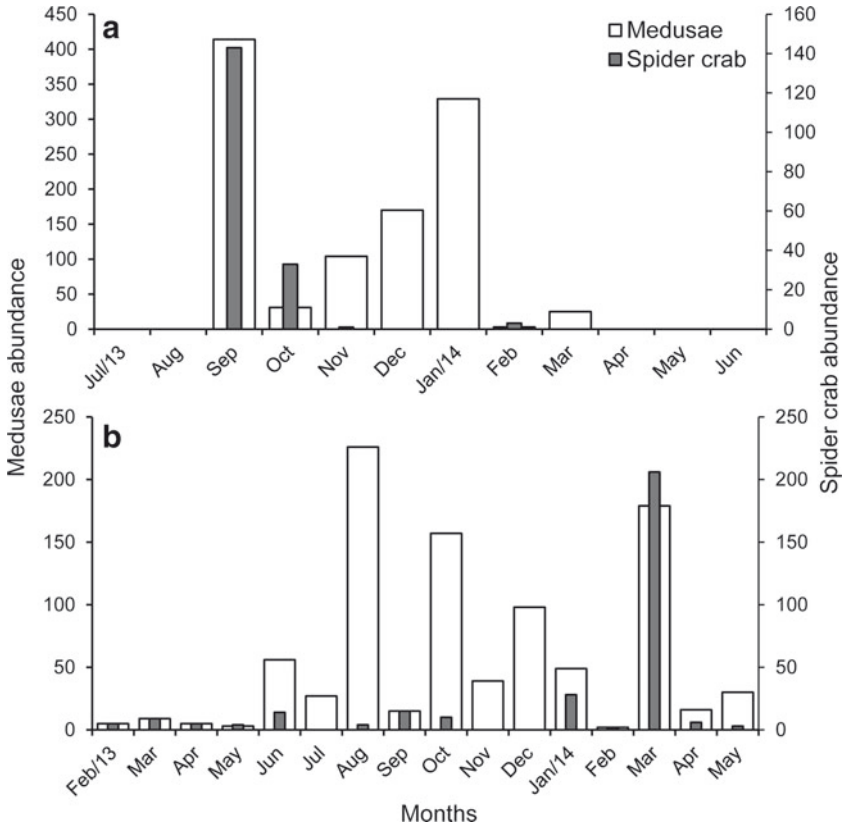

Fig 4 Abundance of *Lychnorhiza lucerna* and *Libinia ferreirae* collected in Macaé **a**, Rio de Janeiro state, and Cananéia **b**, São Paulo state, Brazilian southeastern coast

during their larval phases, they do so from the plankton (Gutsell 1928; Nogueira and Haddad 2005).

Jellyfish are highly mobile animals and this can be a disadvantage as they may be moved by sea currents to areas where the environmental conditions are unsuitable for any symbionts (Berggren 1994). In the present study, a greater abundance of *L. ferreirae* associated with medusae occurred during periods with high water temperatures. The lower abundance of *L. ferreirae* in Macaé during summer can be explained by cooler water derived from the cold ocean currents from the South Atlantic (South Atlantic Central Water) and intensified by upwelling (Campos et al. 2000; Acha et al. 2004). Lower temperatures are likely not favorable for these crustaceans as suggested by other studies (Hartnoll 2001; Nogueira and Haddad 2005; Sal Moyano et al. 2012). It is interesting that the wide salinity variation found between the three sites in the present study appeared not to influence the ability of crabs to associate with medusae.

Schiariti et al. (2012) also found individuals of the Grapsoidea superfamily together with *Libinia* sp. while another Grapsoidea, *Planes major* (MacLeay, 1838), has been found in association with other hosts as turtles (Pfaller et al. 2014). It has been suggested that they use the host to complete the life cycle when associated in the megalopa phase.

Commensalism seems to be the most likely relationship between the decapod crustaceans and *L. lucerna*. This relationship benefits one of the members without harming the other (Parmentier and Michel 2013). Commensalism was proposed for *L. ferreirae* (Nogueira and Haddad 2005). However, the deep sea shrimp *Notostomus robustus* Smith, 1884 associated with the medusoid-phase *of Atolla wyvillei* Haeckel, 1880 (collected at a depth of 790 m) feeds on the jellyfish umbrella so can be parasitic (Moore et al. 1993). Jachowski (1963) also considered the relationship between the crab *Libinia dubia* H. Milne Edwards, 1834 and the jellyfish *Aurelia aurita* (Linnaeus, 1758) to be parasitism, while Shanks and Graham (1988) suggested that crabs prey on jellyfish gonads.

Our results corroborate that *L. lucerna* provide protection during the medusoid-phase for their pelagic crustacean associates during the most vulnerable phases (larvae, juveniles, females carrying embryos and post-molt individuals). Further research is needed to understand these associations and their role in marine ecosystems.

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References

Acha EM, Mianzan HW, Guerrero RA, Favero M, Bava J (2004) Marine fronts at the continental shelves of austral South America physical and ecological processes. J Mar Syst 44:83–105

Almeida AC, Hiyodo CM, Cobo VJ, Bertini G, Fransozo V, Teixeira GM (2013) Relative growth, sexual maturity, and breeding season of three species of the genus persephona (decapoda: brachyura: leucosiidae): a comparative study. J Mar Biol Assoc UK 93:1581–1591

Azofeifa-Solano J, Elizondo-Coto M, Wehrtmann I (2014) Reproductive biology of the sea anemone shrimp *Periclimenes rathbunae* (caridea, palaemonidae, pontoniinae), from the Caribbean coast of Costa Rica. ZooKeys 457:211–225

Bauer RT (2004) Remarkable shrimps: adaptations and natural history of the carideans. University of Oklahoma, Oklahoma

Berggren M (1994) *Periclimenes nomadophila* and *Tulеariocaris sarec*, two new species of pontoniine shrimps (decapoda: pontoniinae), from Inhaca Island, Moçambique. J Crustac Biol 14:782–802

Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis et al. revisited. J Parasitol 83: 575–583

Campos EJD, Velhote D, Silveira ICA (2000) Shelf break upwelling driven by Brazil current cyclonic meanders. Geophys Res Lett 27: 751–754

Chace FA (1969) A new genus and five new species of shrimps (decapoda: palaemonidae, pontoniinae) from the Western Atlantic. Crustaceana 16:251–271

Fautin DG, Guo CC, Hwang JS (1995) Costs and benefits of the symbiosis between the anemone shrimp *Periclimenes brevicarpalis* and its host *Entacmaea quadricolor*. Mar Ecol Prog Ser 129:77–84

Gonçalves GRL, Bolla Jr EA, Negreiros-Fransozo ML, Castilho AL (2016) Morphometric and gonadal maturity of the spider crab *Libinia ferreirae* brito capello, 1871 (decapoda: majoidea: epialtidae) at Southeastern Brazilian coast. J Mar Biol Assoc UK 1–7. doi:10.1017/S0025315416000370

Graça-Lopes R, Tomás ARG, Tutui SLS, Severino-Rodrigues E, Puzzi A (2002) Fauna acompanhante da pesca camaroneira no litoral do Estado de São Paulo, Brasil. Bol Inst Pesca 28:173–188

Gutsell JS (1928) The spider crab, *Libinia dubia*, and the jellyfish *Stomolophus meleagris*, found associated at Beaufort, North Carolina. Ecology 9:358–359

Hartnoll RG (2001) Growth in crustacea – twenty years on. Hydrobiologia 449:111–122

Jachowski R (1963) Observations on the moon jelly, *Aurelia aurita*, and the spider crab, *Libinia dubia*. Chesapeake Sci 4:195

Machado IF, Ferreira RS, Vieira RRR, D'incao F (2010) *Leander paulensis* Ortmann, 1897 e *Periclimenes longicaudatus* (stimpson, 1860) primeiros registros de caridea (crustacea, decapoda, palaemonidae) para o litoral de Santa Catarina, Brasil. Atlântica 32:119–120

Marliave JB, Mills CE (1993) Piggyback riding by pandalid shrimp larvae on hydromedusae. Can J Zool 71:257–263

Martinelli Filho JE, Stampar SN, Morandini AC, Mossolin EC (2008) Cleaner shrimp (caridea: palaemonidae) associated with scyphozoan jellyfish. Vie Milieu 58:133–140

Melo GAS (1996) Manual de identificação dos brachyura (caranguejos e siris) no litoral brasileiro. Ed. Plêiade, FAPESP, São Paulo

Mianzan HW, Cornelius PFS (1999) Cubomedusae and scyphomedusae. In: Boltovskoy D (ed) South Atlantic zooplankton, vol I. Backhuys Publishers, Leiden, pp. 513–559

Moore PG, Rainbow PS, Larson RJ (1993) The mesopelagic shrimp *Notostomus robustus* smith (decapoda: plophoridae) observed *in situ* feeding on the medusa *Atolla wyvillei* Haeckel in the Northwest Atlantic, with notes on gut contents and mouthpart morphology. J Crustac Biol 13:690–696

Morandini AC (2003) Estrutura populacional de *Chrysaora lactea* e *Lychnorhiza lucerna* (cnidaria, scyphozoa) em amostras de plâncton, com a redescrição das espécies. Tesis. Instituto de Biociências da Universidade de São Paulo, São Paulo

Morandini AC, Ascher D, Stampar SN, Ferreira JFV (2005) Cubozoa e scyphozoa (cnidaria: medusozoa) de águas costeiras do brasil. Iheringia, Sér Zool 95:281–294

Moreira MGBS (1961) Sobre *Mastigias scintillae* S*p. nova* (scyphomedusae, rhizostomeae) das costas do brasil. Bolm Inst Oceanogr S Paulo 11:5–29

Nogueira M Jr, Haddad MA (2005) *Lychnorhiza lucerna* (scyphozoa, rhizostomeae) and *Libinia ferreirae* brito capello (brachyura, majidae) association in Southern Brasil. Rev Bras Zool 22:908–912

Nogueira M Jr, Haddad MA (2006) Macromedusae (cnidaria) from the Paraná Coast, Southern Brazil. J Coast Res 39:1161–1164

Ohtsuka S, Kondo Y, Sakai Y, et al. (2010) In situ observations of symbionts on medusa occurring in Japan, Thailand, Indonesia and Malaysia. Bull Hiroshima Univ Mum 2:9–18

Omori K, Yanagisawa Y, Hori N (1994) Life-history of the caridean shrimp *Periclimenes ornatus* Bruce associated with a sea-anemone in Southwest Japan. J Crustac Biol 14:132–145

Parmentier E, Michel L (2013) Boundary lines in symbiosis forms. Symbiosis 60:1–5

Pfaller JB, Alfaro-Shigueto J, Balazs GH, et al. (2014) Hitchhikers reveal cryptic host behavior: new insights from the association between *Planes major* and sea turtles in the Pacific Ocean. Mar Biol 161: 2167–2178

Pohle G, Mantelatto FL, Negreiros-Fransozo ML, Fransozo A (1999) Larval decapoda (brachyura). In: Boltovskoy D (ed) South Atlantic zooplankton. Backhuys Publisher, Leiden, pp. 1282–1351

Ramos-Porto M (1986) Revisão das espécies do género *Leander* E. Desmarest que ocorrem no litoral brasileiro. Trab Oceanogr 19:7–26

Sal Moyano MP, Schiariti A, Gilberto DA, et al. (2012) The symbiotic relationship between *Lychnorhiza lucerna* (scyphozoa, rhizostomeae) and *Libinia spinosa* (decapoda, epialtidae) in the Río de La Plata (Argentina-Uruguay). Mar Biol 159:1933–1941

Schiariti A (2008) Historia de vida y dinámica de poblaciones de *Lychnorhiza lucerna* (scyphozoa) ¿ Un recurso pesquero alternativo? Universidad de Buenos Aires, Thesis

Schiariti A, Sal Moyano MP, Giberto DA, Mianzan HW (2012) Primer registro de la asociación entre *Lychnorhiza lucerna* (scyphozoa, rhizostomeae) y *Cyrtograpsus affinis* (decapoda, varunidae). Lat Am J Aquat Res 40:1090–1093

Schroeder R, Branco JO, Freitas F Jr, Resgalla C Jr (2014) Preliminary assessment of the jellyfish bycatch captured off southern and Southeastern Brazil. Lat Am J Aquat Res 42:289–300

Shanks AL, Graham WM (1988) Chemical defense in a scyphomedusa. Mar Ecol Prog Ser 45:81–86

Sokal RR, Rohlf FJ (1995) Biometry. W. H, Freeman and Company, New York

Tavares M, Santana W (2012) On the morphological differentiation between *Libinia spinosa* and *L. ferreirae* (crustacea: brachyura: majoidea: epialtidae). Zoologia (Curitiba) 29:577–588

Thiel M, Baeza JA (2001) Factors affecting the social behavior of crustaceans living symbiotically with other marine invertebrates: a modelling approach. Symbiosis 30:163–190

Williams AB (1984) Shrimps, lobsters, and crabs of the Atlantic coast of the Eastern United States. Washington, Smithsonian Institution Press, DC (USA), Maine to Florida. Ed

Zimmermann U, Carvalho FL, Mantelatto FL (2015) The reproductive performance of the red-algae shrimp *Leander paulensis* (ortmann, 1897) (decapoda, palaemonidae) and the effect of post-spawning female weight gain on weight-dependent parameters. Braz J Oceanogr 63:207–216

Anexo III

Morphometric and gonad maturity of the spider crab *Libinia ferreirae* Brito Capello, 1871 (Decapoda: Majoidea: Epialtidae) on the south-eastern Brazilian coast

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Morphometric and gonad maturity of the spider crab *Libinia ferreirae* Brito Capello, 1871 (Decapoda: Majoidea: Epialtidae) on the south-eastern Brazilian coast

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Sexual maturity of the spider crab Libinia ferreirae *was assessed for each sex, based on body dimensions and observations of gonad condition. A total of 346 crabs were analysed, of which 68% were females. Immature and adult individuals were recognized based on their allometric growth and gonad development. Abdomen width (AW)* vs *carapace width (CW) and propodus length (PL)* vs *CW were the relationships that best separated allometric groups of females and males, respectively. For females, gonad and allometric morphological maturity were, respectively, 38.77 and 39.43 mm of CW, which is close to the carapace size of the smallest ovigerous female (38.08 mm). For males, gonad maturity was 34.86 mm of CW and three allometric phases were observed: immature (IM♂), adolescent (AD♂) and adult morphometrically mature (MM♂). The IM♂ phase showed lower values of CW and PL than the AD♂ phase, without spermatophores inside the vas deferens; the AD♂ phase exhibited higher CW values than IM♂, but lower CW and PL values than the MM♂ phase, and the presence of spermatophores in the vas deferens; the MM♂ phase had higher values of CW and PL than the AD♂ phase and spermatophores in the vas deferens. Therefore, females showed synchronic morphometric, gonadal and functional maturity, while in males, gonadal maturity was attained before morphometric maturity, which probably could be a reproductive strategy for this species.*

Keywords: Allometry, gonad maturity, morphometric maturity, morphotypes, spider crab

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INTRODUCTION

The onset of sexual maturity for brachyurans is one of the main criteria used to describe population dynamics and structure (Pinheiro & Fransozo, 1998). Knowledge concerning the legal minimum size for fishing, for both commercially exploited and accidentally captured (by-catch) species, is important for preserving marine biodiversity, allowing animals to reproduce before capture (Conan *et al.*, 2001).

Reproductive maturity in crustaceans may be assessed based on three lines of evidence: morphometric, as changes in relative growth of secondary sexual characters (such as the cheliped and abdomen); by the presence of mature oocytes or spermatozoids/spermatophores; and by the capacity to copulate and carry embryos (Hartnoll, 1969, 1974; López-Greco & Rodríguez, 1999; Viau *et al.*, 2006). For brachyuran crabs, morphological changes in male chelipeds and in the female abdomen commonly characterize the transition from morphologically immature to the morphologically mature stage, which may be represented by pubertal moult (Hartnoll, 1974).

However, Majoidea crabs present a terminal moult (or pubertal moult, according to some authors – see Teissier, 1935; Hartnoll, 1978) after the prepubertal moult, featuring morphological maturity and the end of their growth (Hartnoll, 1974, 1982; Sampedro *et al.*, 1999; Sal Moyano *et al.*, 2011). As a consequence, this group reveals some reproductive peculiarities that have frequently been the subject of investigation, mainly concerning (1) the number of allometric stages in males, which varies from two (Comeau & Conan, 1992; Corgos & Freire, 2006) to three stages (Hartnoll, 1963, 1974; Carmona-Suárez, 2003), (2) different mating behaviour patterns for male morphotypes, as observed for *Libinia emarginata* Leach, 1815 and *Libinia spinosa* Milne-Edwards, 1834 by, respectively, Laufer & Ahl (1995) and Sal Moyano & Gavio (2012), or (3) the synchronism between gonad and morphometric maturities, which may occur concomitantly or one preceding the other (Corgos & Freire, 2006).

The species *Libinia ferreirae* Brito Capello, 1871 shows a wide distribution in the western Atlantic, occurring from Venezuela to Brazil (from Pará to Santa Catarina State), from the coastal region to depths down to 35 m, preferably in muddy bottoms (Melo, 1996). As a typical brachyuran, it plays an important role in marine food webs, occupying

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several different levels (Rocha *et al.*, 1998). Additionally, this species may present symbioses with algae, sponges and cnidarians, among other animals, using them as camouflage, which may have contributed to its evolutionary success. This characteristic allows these crabs to move over a wider area with a lower predatory rate, besides increasing the distribution of several sedentary or sessile species with which they are associated (Nogueira Junior & Haddad, 2005; Hultgren & Stachowicz, 2011).

Knowledge of the size at onset of sexual maturity in *L. ferreirae* would enable us to better understand and preserve the marine community, since this species has suffered constant indirect exploitation by the shrimp fishing fleet in the south-eastern Brazilian littoral (Graça-Lopes *et al.*, 2002). Thus, this study investigated the sexual maturity of the spider crab *L. ferreirae* in south-eastern Brazil, based on morphometric and gonadal analyses.

MATERIALS AND METHODS

Biological sampling

Crabs were captured monthly from February 2013 to January 2014, in the complex lagoon-estuarine system of Cananéia-Iguape, and its adjacent oceanic area, in São Paulo state, using a shrimp boat fitted with double-rig nets. In order to capture juvenile and adult individuals, sample stations (i.e. trawls) were previously determined at isobaths from 5 to 15 m deep (25°04′43″S 47°50′34″W).

Captured crabs were frozen and kept in insulated boxes filled with ice until morphometric measurements and gonadal analyses were performed. The specimens were identified according to Melo (1996) and Tavares & Santana (2012), and they were separated by sex, based on the abdomen shape for adults (elongated pattern for males and oval for females) and on the number of pleopods for juvenile individuals (two pairs for males and four pairs for females) (Melo, 1996; Almeida *et al.*, 2013).

Abdomen closure and gonadal maturation

Individuals of both sexes were assessed by abdomen adherence to the thoracic sternite. Individuals considered here as immature presented a sealed abdomen, with cementing substances present in the contour of the abdomen (which do not allow its extension) (Bolla & Negreiros-Fransozo, 2015). Individuals showing an unlocked abdomen were considered here as adults, wherein the abdomen could be easily flexed even with the presence of a press-button mechanism, but without cementing substances. Thus, the adult individuals were considered able to reproduce (Haefner, 1990; Guinot & Bouchard, 1998), as they can display movements which expose their gonopods to females.

After this categorization, a gonad macroscopic analysis was conducted, for both sexes, and development stages were characterized according to shape, colour and size, for ovaries, testes and vas deferens, following Choy (1988), Abelló (1989) and Sal Moyano *et al.* (2011). Thus, the gonad development stages registered, according to the literature above, were: four stages for females, i.e. (1) immature, with sealed abdomen; (2) rudimentary, with thin and whitish-coloured ovaries and unlocked abdomen; (3) developing, with thin and light orange-coloured ovaries and unlocked abdomen; and (4) developed, with thicker and dark orange-coloured ovaries and unlocked abdomen. Likewise, three gonad development stages were characterized for males: (1) immature, with sealed abdomen and unrecognizable vas deferens; (2) rudimentary, with a translucent and thin vas deferens and unlocked abdomen; and (3) developed, with a thicker and white-coloured vas deferens and unlocked abdomen (for more details see Choy, 1988 and Abelló, 1989). We adopted here the following abbreviations in the analysis and graphs: the individuals in immature gonad stages as immature females (GI♀) or males (GI♂); and individuals in rudimentary, developing or developed gonad stages as mature females (GM♀) or males (GM♂).

The presence of ovigerous females (i.e. bearing embryos retained on the pleopods) was recorded, identifying their functional maturity, as well as providing evidence of the reproductive period (Sal Moyano *et al.*, 2011).

Relative growth and morphometric maturity

The measurements used for the morphometric analyses were based on a study carried out by Sal Moyano *et al.* (2011). For females, carapace width (CW) and abdomen width (AW) (closest to the fifth abdominal segment) were measured. For males, the length (PL), width (PW) and height (PH) of the propod, in addition to the carapace width (CW), were measured (Figure 1). Measurements were made using digital callipers (accuracy 0.01 mm) and, for individuals with a CW less than 5 mm, a microscope/stereoscope (Zeiss® Stemi SV6) was utilized, equipped with an image capture system (Zeiss Stemi 2000-C) (accuracy 0.0001 mm).

Relative growth analyses followed the allometric model proposed by Huxley (1950), in which equations were calculated for both males and females separately. Data were ln-transformed to fit a linear model and allometry (positive, negative or isometry) was verified by Student's *t*-test, at a 5% level of significance ($\alpha = 0.05$) (Sampedro *et al.*, 1999).

In order to separate the morphometric phases, data from separated sexes were submitted to a 'K-means clustering' analysis (Sokal & Rohlf, 1979), commonly used in similar studies (see Sampedro *et al.*, 1999; Corgos & Freire, 2006; Hirose *et al.*, 2010). Such analysis is based on the establishment of predetermined groups, attributing each empirical point to any group by means of an iterative process that minimizes

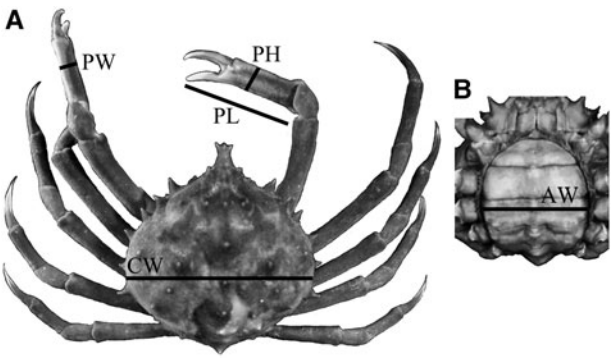

Fig. 1. *Libinia ferreirae* Brito Capello, 1871. Morphological structures measured for morphometric growth analyses. (A) carapace width (CW), length (PL), height (PH) and width (PW) of the propodus; (B) abdomen width (AW).

the variance inside groups and maximizes it among groups. Then, a discriminant analysis is applied in order to discriminate the characteristics of each group, allowing the reallocation of each point to the group that best represents it, classifying each group into distinct categories: immature (MI♂), adolescents (AD♂) and morphometrically mature adults (MM♂) for males; and immature (MI♀) and morphometrically mature adults (MM♀) for females. Subsequently, to verify the accuracy of each group, the slopes (b) and intercepts (a) of the equations of all groups were tested using covariance analysis (ANCOVA), at a 5% level of significance ($\alpha = 0.05$) (Zar, 1999).

It is important to note that, for males, the estimated value of morphometric maturity was based on the relationship, among all tested (PL, PW and PH *vs* CW), that presented the highest determination coefficient (r^2), for all size groups, since this coefficient indicates the best fit of the equations to the empirical data (Zar, 1999).

When an overlap between size groups was detected in all analyses (both gonadal and morphometric), individuals were grouped into size classes (interval: 3 mm of CW for gonadal analyses; 2 mm of CW for morphometric analyses) and the size at the onset of sexual maturity was determined by means of CW_{50} by fitting the following logistic function: $\%Adults = 1/1 + e^{r(CW\text{-}CW_{50})}$, where CW_{50} corresponds to the carapace size in which 50% of the individuals are considered mature and r corresponds to the curve slope (modified from Aguillar *et al.*, 1995; Vazzoler, 1996). For all maturity values, 95% confidence intervals were obtained using the bootstrap interaction method (Macro-supplement for Microsoft Excel®).

Nevertheless, when there was no overlap between size groups, morphometric maturity was determined based on the mean value between the largest individual from a size group and the smallest individual from the next size group.

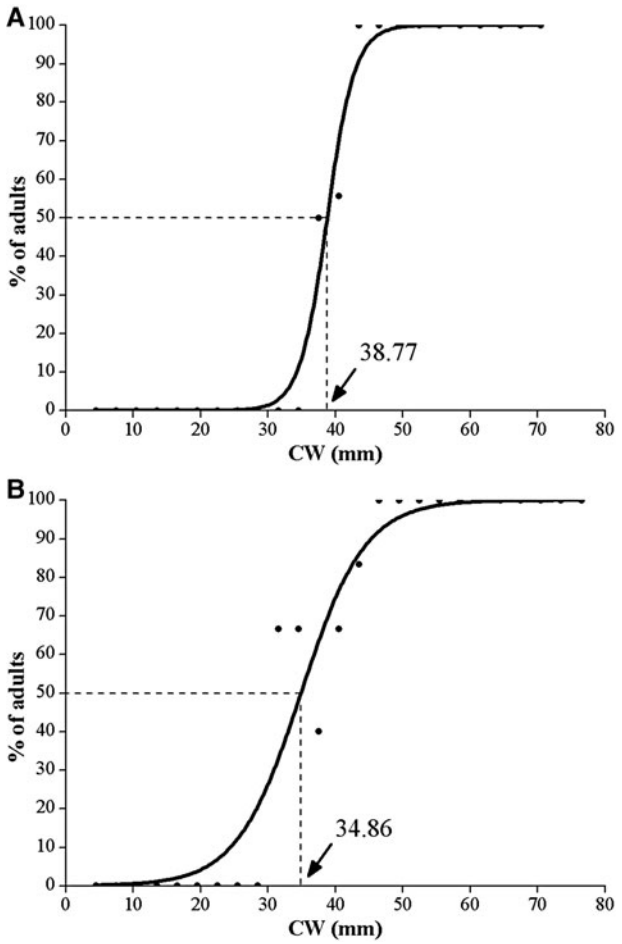

Fig. 2. *Libinia ferreirae* Brito Capello, 1871. Gonadal maturity (CW_{50}) for females (A) and males (B).

RESULTS

A total of 346 crabs were collected. The proportion of females (68%) was significantly ($\chi^2 < 0.001$) higher than that of males (32%). For females (non-ovigerous, N = 97; ovigerous, N = 137), the CW ranged from 3.09 to 71.64 mm; the smallest ovigerous females had a CW of 38.08 mm. A size comparison between sexes showed a greater size variation of CW (6.29 to 77.84 mm) in males (N = 112) than in females. However, males showed a lower mean size (CW = 37.81 mm, against 42.19 mm of females) (Mann–Whitney rank sum test, U statistic = 15294; $P = 0.012$).

Gonadal maturity

Based on the logistic curves, gonadal maturity (CW_{50}) for females was a CW of 38.77 mm, with a 95% confidence interval between 37.49 and 40.12 mm (bootstrap interactions: 10 000) (Figure 2A), which is close to the size of the smallest functionally mature female (smallest ovigerous female: CW of 38.08 mm). The largest GI♀ had a CW of 41.3 mm.

For males, gonadal maturity (CW_{50}) was at 34.86 mm, with a 95% confidence interval between 30.91 and 38.02 mm (bootstrap interactions = 10 000) (Figure 2B). The smallest males with rudimentary and developed gonad were, respectively, a CW of 31.83 and a CW of 35.12 mm; the largest GM♂ had a CW of 43.11 mm.

Relative growth and morphometric maturity

For females, the relative growth analysis indicated that the MI♀ group showed positive allometric growth and there was a terminal moult marked by an elevated change in AW and a decreased degree of allometry (ANCOVA, $P < 0.05$) (Table 1). There was an overlap in CW between 38.08 mm (smallest MM♀) and 45.69 mm (largest MI♀). The size increment from MI♀ to MM♀ was 0.9 mm for CW and 8.73 mm for AW (this calculation was performed with the mean values of the overlapping data). The morphometric maturity estimated was CW_{50} 39.43 mm of CW, with a 95% confidence interval between 38.09 and 40.64 mm (bootstrap interactions: 10 000) (Figure 3).

For males, the relative growth of the right propodus length (RPL), in relation to the CW, presented the highest determination coefficient (r^2), among all relationships tested, and revealed three allometric groups: (1) MI♂ that showed isometry, small CW and PL; (2) AD♂ that showed high positive allometry in the chelipeds; and (3) MM♂, which also showed positive allometry and the largest CW and PL (Figure 4). A similar pattern was observed when analysing the left PL and the PH (both left and right), but only partially

Table 1. *Libinia ferreirae* Brito Capello, 1871. Relative growth analysis between males (♂) and females (♀), for all the relationships.

Relationship	Demographic	N	Linear eq.: lnY = *a* + *b*lnX		r^2	*t*	Allometry	ANCOVA				
	category		*a*	*b*		(*b* = 1)	level	Sex: category	Factor	*F*	*p*	sig.
CW *vs* AW	MI♀	55	−1.639	1.198	0.985	9.776	+	♀: MI *vs* MM	*b*	49.215	0.0000	*
	MM♀	171	−0.125	0.895	0.867	3.889	−					
CW *vs* RPL	MI♂	43	−0.645	1.006	0.991	0.445	0	♂: MI *vs* AD	*b*	27.642	0.0000	*
	AD♂	30	−2.193	1.467	0.862	4.210	+	♂: MI *vs* MM	*b*	14.522	0.0003	*
	MM♂	20	−1.756	1.424	0.885	3.508	+	♂: AD *vs* MM	*b*	0.051	0.8219	
									a	40.514	0.0000	*
CW *vs* RPW	MI♂	44	−1.950	0.817	0.947	6.156	−	♂: MI *vs* AD	*b*	13.790	0.0004	*
	AD♂	34	−3.406	1.278	0.751	2.138	+	♂: MI *vs* MM	*b*	9.708	0.0028	*
	MM♂	21	−3.417	1.393	0.840	2.822	+	♂: AD *vs* MM	*b*	0.245	0.6230	
									a	86.673	0.0000	*
CW *vs* RPH	MI♂	43	−2.058	0.990	0.957	0.310	0	♂: MI *vs* AD	*b*	3.306	0.0731	
									a	15.887	0.0002	*
	AD♂	34	−2.738	1.217	0.756	1.771	+	♂: MI *vs* MM	*b*	7.954	0.0065	*
	MM♂	22	−3.413	1.481	0.873	3.809	+	♂: AD *vs* MM	*b*	1.693	0.1990	
									a	71.653	0.0000	*
CW *vs* LPL	MI♂	39	−0.643	1.005	0.991	0.320	0	♂: MI *vs* AD	*b*	13.205	0.0005	*
	AD♂	35	−1.697	1.324	0.816	2.959	+	♂: MI *vs* MM	*b*	37.930	0.0000	*
	MM♂	24	−2.174	1.527	0.933	6.028	+	♂: AD *vs* MM	*b*	1.415	0.2393	
									a	69.056	0.0000	*
CW *vs* LPW	MI♂	40	−1.984	0.827	0.933	4.801	−	♂: MI *vs* AD	*b*	18.570	0.0001	*
	AD♂	34	−3.838	1.392	0.797	3.154	+	♂: MI *vs* MM	*b*	24.389	0.0000	*
	MM♂	24	−4.567	1.663	0.898	5.540	+	♂: AD *vs* MM	*b*	1.962	0.1670	
									a	62.326	0.0000	*
CW *vs* LPH	MI♂	40	−2.050	0.990	0.976	0.401	0	♂: MI *vs* AD	*b*	13.056	0.0006	*
	AD♂	34	−3.198	1.342	0.854	3.492	+	♂: MI *vs* MM	*b*	8.529	0.0049	*
	MM♂	24	−3.147	1.418	0.779	2.592	+	♂: AD *vs* MM	*b*	0.169	0.6824	
									a	67.734	0.0000	*
									a	5.589	0.0213	*

CW, carapace width; AW, abdomen width; RPL, RPW, RPH, length, width and height of the right propodus; LPL, LPW, LPH: length, width and height of the left propodus, respectively; MI, immature; AD, adolescent; MM, morphometric mature; N, number of individuals; *a*, linear coefficient; *b*, angular coefficient; r^2, determination coefficient; *t*, Student's *t*-test; 0, −, +, isometry, negative and positive allometries, respectively; ANCOVA, covariance analysis; sig., significant for $\alpha = 0.05$.

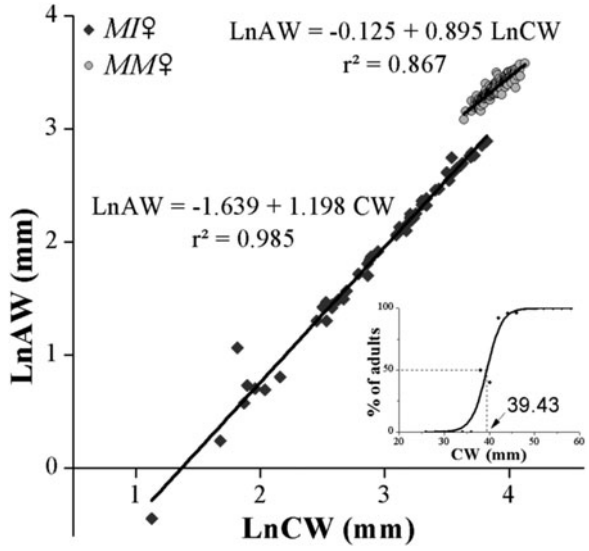

Fig. 3. *Libinia ferreirae* Brito Capello, 1871. Relationship between carapace width (CW) and abdomen width (AW), considering immature (MI♀) and mature (MM♀) females. r^2 = determination coefficient; Inset graph: CW_{50} results on the overlap between MI♀ and MM♀.

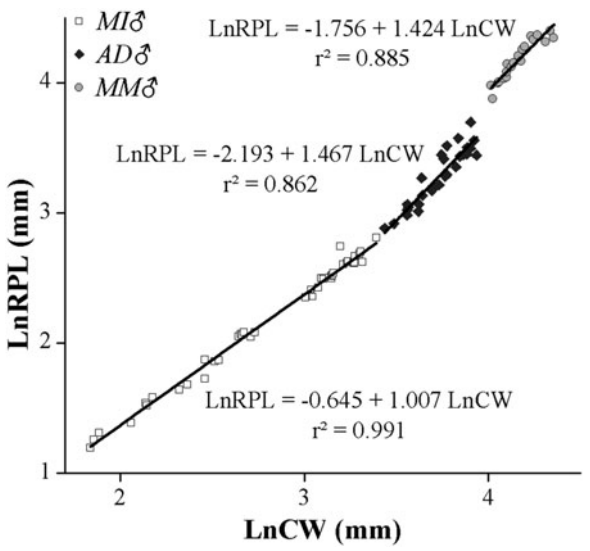

Fig. 4. *Libinia ferreirae* Brito Capello, 1871. Relationship between carapace width (CW) and right propodus length (RPL) for immature (MI♂), adolescent (AD♂) and morphometrically mature (MM♂) males. r^2 = determination coefficient.

found when analysing the PW (both left and right) because the MI♂ group showed negative allometric growth (ANCOVA, $P < 0.05$) (Table 1).

Considering that there was no overlap among the size groups of the males, the morphometric maturity obtained for AD♂ and for MM♂ was, respectively, a CW of 30.41 and 53.25 mm. The covariance analysis (ANCOVA) significantly validated the separation of all groups for both males and females (Table 1).

DISCUSSION

The higher proportion of females, observed in the present results, diverges from previous studies performed for *L. spinosa* and *L. emarginata*, in which the abundance of males was higher (Sal Moyano *et al.*, 2011) or similar to that of females (DeGoursey & Auster, 1992). Therefore, it is likely that either there is a differential occupation of habitats between sexes and the distribution of males was not included in these samples or, indeed, the population has a higher abundance of females. However, reproductive capability may not be, necessarily, injured by the lower abundance of males, since Majoidea females, due to the presence of a seminal receptacle, do not require periodic copulae and can show several spawns with only one spermatic mass from a single copula (González-Gurriarán *et al.*, 1998). Additionally, males can mate with several females in same season (Hartnoll, 1969; Diesel, 1991; Sainte-Marie & Lovrich, 1994).

The sexual dimorphism observed between the largest individuals of each sex of *L. ferreirae* is expected, as females grow to a lesser extent than males in the terminal moult, probably since they allocate a higher amount of energy to egg production (Hartnoll, 1982). Additionally, smaller females could facilitate the mating and post-copulatory guarding behaviour by MM♂, which could offer better protection to females during or after copula (Hartnoll, 1969; Conan & Comeau, 1986).

It was possible to observe that *L. ferreirae* presents different sizes at maturity for both sexes, in addition to the occurrence of the AD♂ group, as already reported for other Majoidea species (see Hartnoll, 1963, 1974; Sampedro *et al.*, 1999; Carmona-Suárez, 2003; Sal Moyano *et al.*, 2011). Sexual maturity of Majoidea crabs is still much discussed, mainly in relation to the sequence in which gonad and morphometric maturity occurs.

Gonad maturity

Studies carried out on the species *Maja squinado* (Herbest, 1788), *Hyas coarctatus* Leach, 1815, *Inachus dorsettensis* (Pennant, 1777) and *L. spinosa* indicated that gonad and morphometric maturity could be coincident with similar body sizes for females (Bryant & Hartnoll, 1995; Sampedro *et al.*, 1999; Sal Moyano *et al.*, 2011), as observed in our investigation. However, this does not seem to be a rule for Majoidea crabs; gonad maturity can occur after morphometric maturity (see Jones & Hartnoll, 1997) or before, as attested by Alunno-Bruscia & Sainte-Marie (1998) studying *Chionoecetes opilio* (O. Fabricius, 1788), in which it was found that females acquired gonad maturity first and subsequently reached morphological maturity with the terminal moult.

For males, gonad maturity was found to occur prior to morphometric maturity. It is proposed that this pattern could favour the reproductive success of AD♂ males, like an opportunistic copula, especially when there is a high abundance of reproductive females in the environment or when MM♂ are rare in the environment, as suggested by Sampedro *et al.* (1999) and Sal Moyano *et al.* (2012). Thus, AD♂ could remain in prepubertal intermoult for a longer time before they perform their terminal moult (Laufer & Ahl, 1995; Sampedro *et al.*, 1999; Sal Moyano & Gavio, 2012). Elner & Beninger (1995) have suggested that *C. opilio* AD♂ bearing spermatophores inside the vas deferens could opportunely copulate or, according to Laufer & Ahl (1995), increase their mating opportunities by 'sneak' mating. Indeed, this behaviour was reported for *L. spinosa* by Sal Moyano & Gavio (2012), as AD♂ successfully copulated when MM♂ were absent or in agonistic behaviour with other MM♂ males. However, further studies should be performed, under laboratory conditions, to verify if these copulas could produce a viable brood.

Relative growth and morphometric maturity

For *L. ferreirae* females, the relative abdominal growth suggests a clear separation between immature individuals and those that are able to reproduce, a pattern commonly observed for Majoidea, wherein such a morphometric split occurs after the terminal moult, concomitant with sudden alterations in the allometric coefficients. Positive allometry ($b = 1.19$) during the immature phase indicates that the abdomen is being prepared to become an embryo-incubating chamber, a process that will be completed after the terminal moult and consequent beginning of the mature phase (Hartnoll, 1974, 1982; Sampedro *et al.*, 1999).

Regarding *L. ferreirae* males, relative growth relationships revealed the existence of two significantly different groups of allometric growth (the MI♂ and AD♂ groups) and one size allometry group (MM♂) where the increase is the result of males of different sizes undergoing their terminal moult. Morphologically mature males presented a higher allometry level, associated with cheliped size, than MI♂ and AD♂. This fact is related to the terminal moult, which provides high sexual dimorphism in males, as was also found in *M. squinado* and *L. spinosa* by Sampedro *et al.* (1999) and Sal Moyano *et al.* (2011), respectively. Many authors have proposed that the male morphotypes are a result of the ontogenetic process they go through to reach maturity and ensure reproductive success (Hartnoll, 1963; Homola *et al.*, 1991; Laufer & Ahl, 1995). Nevertheless, the terminal moult is not necessarily the maturation moult, because spermatophores can be found inside spermatic ducts even before terminal moult, as assumed by Elner & Beninger (1995), Laufer & Ahl (1995), Rotllant *et al.* (2000) and Sal Moyano *et al.* (2010). It is also important to note that AD♂ are grouped based only on morphology because of their small cheliped and are close to the pubertal moult. However, they can include physiologically mature or immature individuals.

A larger cheliped is of great importance in reproduction, because it is useful for both combat and female protection during mating and may be a decisive factor in the selection of the male by females (Sal Moyano & Gavio, 2012). Therefore, although the occurrence of mating by AD♂ crabs could be an important reproductive strategy in

situations where larger animals (with larger secondary sexual characters) are rare or absent (López-Greco & Rodríguez, 1999), only MM♂ present post-copulatory guarding behaviour, such as embracing and female guarding when other males approach, while AD♂ rapidly depart from the female after mating (Laufer & Ahl, 1995; Sal Moyano & Gavio, 2012). In addition, AD♂ seem to imitate female behaviour when they are close to MM♂, thereby avoiding confrontation and so increasing their mating opportunities in opportunistic cohorts (Laufer & Ahl, 1995; Sal Moyano & Gavio, 2012).

Therefore, further laboratory experiments aiming to observe mating among females and the different male morphotypes are necessary to better understand functional maturity and its relationship with the reproductive process. Furthermore, studies concerning population structure, distribution, growth and longevity should be conducted in order to understand which factors drive the population biology of *L. ferreirae*.

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REFERENCES

Abelló P. (1989) Reproduction and moulting in *Liocarcinus depurator* (Linnaeus, 1758) (Brachyura: Portunidae) in the northwestern Mediterranean. *Scientia Marina* 53, 127–134.

Aguillar A.T., Malpica Z.C. and Urbina B.V. (1995) *Dinámica de Poblaciones de Peces*. Lima: Libertad.

Almeida A.C., Hiyodo C.M., Cobo V.J., Bertini G., Fransozo V. and Teixeira G.M. (2013) Relative growth, sexual maturity, and breeding season of three species of the genus *Persephona* (Decapoda: Brachyura: Leucosiidae): a comparative study. *Journal of the Marine Biological Association of the United Kingdom* 93, 1581–1591.

Alunno-Bruscia M. and Sainte-Marie B. (1998) Abdomen allometry, ovary development, and growth of female snow crab, *Chionoecetes opilio* (Brachyura, Majidae), in the northwestern Gulf of St. Lawrence. *Canadian Journal of Fisheries and Aquatic Sciences* 55, 459–477.

Bolla E.A. Jr and Negreiros-Fransozo M.L. (2015) Morphology of juvenile phase of *Achelous spinimanus* (Latreille, 1819) (Crustacea, Decapoda, Portunidae) reared in laboratory. *Journal of the Marine Biological Association of the United Kingdom*. doi: 10.1017/S0025315415000508.

Bryant A.D. and Hartnoll R.G. (1995) Reproductive investment in two spider crabs with different breeding strategies. *Journal of Experimental Marine Biology and Ecology* 188, 261–275.

Carmona-Suárez C.A. (2003) Reproductive biology and relative growth in the spider crab *Maja crispata* (Crustacea, Brachyura, Majidae). *Scientia Marina* 67, 75–80.

Choy S.C. (1988) Reproductive biology of *Liocarcinus puber* and *L. holsatus* (Decapoda, Brachyura, Portu-nidae) from the Grower Peninsula, South Wales. *Marine Ecology* 9, 227–291.

Comeau M. and Conan G.Y. (1992) Morphometry and gonad maturity of male snow crab, *Chionoecetes opilio*. *Canadian Journal of Fisheries and Aquatic Sciences* 49, 2460–2468.

Conan G.Y. and Comeau M. (1986) Functional maturity and terminal molt of male snow crab, *Chionoecetes opilio*. *Canadian Journal of Fisheries and Aquatic Sciences* 43, 1710–1719.

Conan G.Y., Comeau M. and Moriyasu M. (2001) Are morphometrical approaches appropriate to establish size at maturity for male American lobster *Homarus americanus*? *Journal of Crustacean Biology* 21, 937–947.

Corgos A. and Freire J. (2006) Morphometric and gonad maturity in the spider crab *Maja brachydactyla*: a comparison of methods for estimating size at maturity in species with determinate growth. *Journal of Marine Science* 63, 851–859.

DeGoursey R.E. and Auster P.J. (1992) A mating aggregation of the spider crab (*Libinia emarginata*). *Journal of Northwest Atlantic Fishery Science* 13, 77–82.

Diesel R. (1991) Sperm competition and the evolution of mating behavior in Brachyura, with special reference to spider crabs (Decapoda: Majidae). In Bauer R.G. and Martin J.W. (eds) *Crustacean sexual biology*. New York, NY: Columbia University Press, pp. 145–163.

Elner R.W. and Beninger P.G. (1995) Multiple reproductive strategies in snow crab, *Chionoecetes opilio*: physiological pathways and behavioral plasticity. *Journal of Experimental Marine Biology and Ecology* 193, 93–112.

González-Gurriarán E., Fernández L., Freire J. and Muiño R. (1998) Mating and role of seminal receptacles in the reproductive biology of the spider crab *Maja squinado* (Decapoda, Majidae). *Journal of Experimental Marine Biology and Ecology* 220, 269–285.

Graça-Lopes R., Tomás A.R.G., Tutui S.L.S., Severino-Rodrigues E.E. and Puzzi A. (2002) Fauna acompanhante da pesca camaroeira no litoral do Estado de São Paulo, Brasil. *Boletim do Instituto de Pesca, São Paulo* 28, 173–188.

Guinot D. and Bouchard J.M. (1998) Evolution of the abdominal holding systems of brachyuran crabs (Crustacea, Decapoda, Brachyura). *Zoosystema* 20, 613–694.

Haefner J.A. Jr (1990) Morphometry and size at maturity of *Callinectes ornatus* (Brachyura, Portunidae) in Bermuda. *Bulletin of Marine Science* 46, 274–286.

Hartnoll R.G. (1963) The biology of Manx spider crabs. *Proceedings of the Zoological Society of London* 47, 79–300.

Hartnoll R.G. (1969) Mating in the brachyura. *Crustaceana* 16, 161–181.

Hartnoll R.G. (1974) Variation in growth pattern between some secondary sexual characters in crabs (Decapoda, Brachyura). *Crustaceana* 27, 131–136.

Hartnoll R.G. (1978) The determination of relative growth in Crustacea. *Crustaceana* 34, 281–293.

Hartnoll R.G. (1982) Growth. In Bliss D.E. (ed) *The biology of Crustacea: embryology, morphology and genetics*. Volume 2. New York, NY: Academic Press, pp. 111–196.

Hirose G.L., Bolla E.A. Jr. and Negreiros-Fransozo M.L. (2010) Post-larval morphology, growth, and development of *Uca cumulanta* Crane, 1943 (Crustacea, Decapoda, Ocypodidae) under laboratory conditions. *Invertebrate Reproduction and Development* 54, 95–109.

Homola E., Sagi A. and Laufer H. (1991) Relationship of claw form and exoskeleton condition to reproductive system size and methyl farnesoate in the male spider crab, *Libinia emarginata*. *Invertebrate Reproduction and Development* 20, 219–225.

Hultgren K. and Stachowicz J. (2011) Camouflage in decorator crabs. In Stevens M. and Merilaita S. (eds) *Animal camouflage: mechanisms and function*. Cambridge: Cambridge University Press, pp. 214–238.

Huxley J.S. (1950) Relative growth and form transformation. *Proceedings of the Royal Society of London* 137, 465–469.

Jones D.R. and Hartnoll R.G. (1997) Mate selection and mating behaviour in spider crabs. *Estuarine, Coastal and Shelf Science* 44, 185–193.

Laufer H. and Ahl J.S.B. (1995) Mating behaviour and methyl farnesoate levels in male morphotypes of the spider crab *Libinia emarginata* (Leach). *Journal of Experimental Marine Biology and Ecology* 193, 15–20.

López-Greco L.S. and Rodríguez E.M. (1999) Size at the onset of sexual maturity in *Chasmagnathus granulata* Dana, 1851 (Grapsidae, Sesarminae): a critical overall view about the usual criteria for its determination. In Scharan F.R. and Valupel Klein J.C. Von. (eds) *Crustaceans and the biodiversity crisis*. Leiden: Brill, pp. 675–689.

Melo G.A.S. (1996) *Manual de identificação dos Brachyura (caranguejos e siris) do Litoral Brasileiro*, 1st edition. São Paulo, SP: Plêiade/FAPESP.

Nogueira Junior M. and Haddad M.A. (2005) *Lychnorhiza lucerna* (Scyphozoa, Rhizostomeae) and *Libinia ferreirae* Brito Capello (Brachyura, Majidae) association in southern Brasil. *Revista Brasileira de Zoologia* 22, 908–912.

Pinheiro M.A.A. and Fransozo A. (1998) Sexual maturity of the speckled swimming crab *Arenaeus cribrarius* (Lamarck, 1818) (Decapoda, Brachyura, Portunidae), in the Ubatuba litoral, São Paulo State, Brazil. *Crustaceana* 71, 434–452.

Rocha G.R.A., Gasalla M.A., Rossi-Wongtschowski C.L.D.B., Soares L.S.H., Pires-Vanin A.M.S., Muto E.Y., Cergole M.C., Aidar E., Mesquita H.S.L., Mesquita S.M.L., Gianesella-Galvão S.M.F., Vega-Perez L.A. and Jarre-Teichmann A. (1998) Quantitative model of trophic interactions in the Ubatuba shelf system (Southeastern Brazil). *Fishbyte* 21, 25–32.

Rotllant G., Takac P., Liu L., Scott G.L. and Laufer H. (2000) Role of ecdysteroids and methyl farnesoate in morphogenesis and terminal moult in polymorphic males of the spider crab *Libinia emarginata*. *Aquaculture* 190, 103–118.

Sainte-Marie B. and Lovrich G.A. (1994) Delivery and storage of sperm at first mating of female *Chionoecetes opilio* (Brachyura, Majidae) in relation to size and morphometric maturity of male parent. *Journal of Crustacean Biology* 14, 508–521.

Sal Moyano M.P. and Gavio M.A. (2012) Comparison of mating behavior and copulation in male morphotypes of the spider crab *Libinia spinosa* (Brachyura: Majoidea: Epialtidae). *Journal of Crustacean Biology* 32, 31–38.

Sal Moyano M.P., Gavio M.A. and Cuartas E.I. (2010) Morphology and function of the reproductive tract of the spider crab *Libinia spinosa* (Crustacea, Brachyura, Majoidea): pattern of sperm storage. *Helgoland Marine Research* 64, 213–221.

Sal Moyano M.P., Gavio M.A. and Maggi M.D. (2011) Morphometric and gonad maturity of the spider crab *Libinia spinosa* (Crustacea: Brachyura: Majoidea: Epialtidae) in Argentina. *Journal of the Marine Biological Association of the United Kingdom* 91, 837–844.

Sal Moyano M.P., Schiariti A., Gilberto D.A., Diaz Briz L., Gavio M.A. and Miazan H.W. (2012) The symbiotic relationship between *Lychnorhiza lucerna* (Scyphozoa, Rhizostomeae) and *Libinia spinosa* (Decapoda, Epialtidae) in the Río de la Plata (Argentina-Uruguay). *Marine Biology* 159, 1933–1941.

Sampedro M.P., González-Gurriarán E., Freire J. and Muiño R. (1999) Morphometry and sexual maturity in the spider crab *Maja squinado* (Decapoda: Majidae) in Galicia, Spain. *Journal of Crustacean Biology* 19, 578–592.

Sokal R.R. and Rohlf F.J. (1979) *Biometría, principios y métodos estadísticos en la investigación biológica*. Madrid: Blume Ediciones.

Tavares M. and Santana W. (2012) On the morphological differentiation between *Libinia spinosa* and *L. ferreirae* (Crustacea: Brachyura: Majoidea: Epialtidae). *Zoologia* 29, 577–588.

Teissier G. (1935) Croissance des variants sexuels chez *Maia squinado* L. *Travaux de la Station Biologique de Roscoff* 13, 93–130.

Vazzoler A.E.A.M. (1996) *Biologia da Reprodução de Peixes Teleósteos: Teorias e Prática*. Maringá, PR: Eduem.

Viau V.E., López Greco L.S., Bond-Buckup G. and Rodríguez E.M. (2006) Size at the onset of sexual maturity in the anomuran crab, *Aegla uruguayana* (Aeglidae). *Acta Zoologica* 87, 253–264.

and

Zar J.H. (1999) *Biostatistical analysis*, 4th edition. Upper Saddle River, NJ: Prentice-Hall.

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