SÃO PAULO STATE UNIVERSITY (UNESP)
INSTITUTE OF BIOSCIENCES

GESLAINE RAFAELA LEMOS GONÇALVES

ECOLOGY AND TROPHIC STRUCTURE OF THE SPIDER CRAB *Libinia ferreirae* (DECAPODA: MAJOIDEA) ON THE SOUTH COAST OF SÃO PAULO

PhD Thesis

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PhD Thesis

Ecology and trophic structure of the spider crab *Libinia ferreirae* (Decapoda: Majoidea) on the South Coast of São Paulo

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“Knowledge we learn from masters and books.
Wisdom is learned through life and the humble”
Cora Coralina

“Be the change you wish to see in the world!”
Mahatma Gandhi

I dedicate this work to my family for being the inspiring source of force and determination in my life.

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PROLOGUE

This thesis is the conclusion of my journey since my undergraduate to my PhD, with an interest in studying marine biology, stimulated by the opportunity to have new experiences in this area. Under the supervision of Professor Dr. Antonio Leão Castilho, I fell in love with the possibility of studying Majoidea crabs in my master's and thesis project.

Following the development of my undergraduate research topic "Biodiversity of Penaeoidea shrimp (Crustacea: Decapoda) in the region of São Francisco do Sul, Santa Catarina, Brazil", as part of BSc Biological Sciences, I became curious in studying the Majoidea crab of the genus *Libinia*. This curiosity came about during sampling expeditions on a shrimp trawler, where I was exposed to the different life habits of this species - crabs associated with jellyfish, and crabs of the same species caught outside the jellyfish, who had a different colour than those associated with the jellyfish, also carrying numerous other organisms on the carapace. This observation served as a basis for questions and fascination about symbiosis of marine animals, which led me to store the data obtained on these expeditions, thinking about future research in the area.

After my undergraduate BSc I moved to another city to work in collaboration with UNESP (São Paulo State University) and USP (São Paulo University) where I had a FAPESP scholarship (2013 / 08517-2) for technical training over one and a half years, working in the project "Decapod crustaceans: multidisciplinarity in the characterization of marine biodiversity in the State of São Paulo (taxonomy, spermiotaxonomy, molecular biology and population dynamics - São Paulo", under the supervision of Professor Dr. Fernando Luis Medina Mantelatto. With the team of Prof. Antonio Leão Castilho I was responsible in collecting, organizing, sorting, classifying and analysing the animals collected by trawl fishing for commercially valued shrimp, in the Cananéia and Ubatuba

region (both in the state of São Paulo), and in Macaé (in the state of Rio de Janeiro). I also collected and analysed samples of environmental factors such as sediment, salinity, temperature, among others. During this period, I had opportunities to learn numerous techniques for collecting and analysing abiotic and biotic samples. Also, because I was responsible for managing the communication and distribution of the sample data from the project to the other team members, I created links with countless researchers who in the future became friends and helped me throughout my career. Moved by the interest in studying and discovering more about the symbiotic relationships that the Majoidea crab of the genus *Libinia* has during its life, under the authorization of Prof. Antonio Castilho and other researchers involved in the project, I collected as many samples and as much data as possible about this spider crab and the other animals involved in symbiotic relationships. This area is seldom studied, because the species has no commercial interest and because they are not abundant in the samples, when compared to other crabs. The breadth of samples and data generated during the field activities I participated in served as a basis for the preparation and production of my Master dissertation, PhD thesis and other works developed in partnership with colleagues in the laboratory.

Thus, after my technical training experience, I started a master's degree in MSc Biological Sciences (Zoology) under the guidance of Prof. Antonio Castilho and co-supervision of Professor Dr. Maria Negreiros-Fransozo. Here my research with Genus *Libinia* began – "Populational Ecology to *Libinia ferreirae* (Brachyura; Majoidea) on the southeastern coast of Brazil" (funding by FAPESP 2014/13770-1) – using all the samples and results obtained since the initiation of my scientific career. Due to my curiosity and passion to try to understand the life habits and symbiotic relationships of this crab with different organisms, we worked on and published the following works: **(1)** Gonçalves, G. R. L., Wolf, M. R., Costa, R. C. & Castilho, A. L. (2016). Decapod crustacean

associations with scyphozoan jellyfish (Rhizostomeae: Pelagiidae) in the Southeastern Brazilian coast. Symbiosis 69: 193-198. DOI 10.1007/s13199-016-0395-x; **(2)** Gonçalves, G. R. L., Grabowski, R. C., Bochini, G. L., Costa, R. C. & Castilho, A. L. (2017). Ecology of the spider crab *Libinia ferreirae* (Brachyura: Majoidea): ontogenetic shifts in habitat use. Hydrobiologia 795:313–25. DOI 10.1007/s10750-017-3153-z; **(3)** Gonçalves, G. R. L., Bolla Jr., E. A., Negreiros-Fransozo, M. L. & Castilho A. L. (2017). 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 97(2):289–95. doi:10.1017/S0025315416000370; **(4)** Moraes, I. R. R., Wolf, M. R., Gonçalves, G. R. L. & Castilho, A. L. (2017). Fecundity and reproductive output of the caridean shrimp *Periclimenes paivai* associated with scyphozoan jellyfish. Invertebrate Reproduction & Development 61:1-7. doi:10.1080/07924259.2017.1282890; **(5)** Gonçalves, G. R. L., Negreiros-Fransozo, M. L., Fransozo, A. & Castilho, A. L. (2019). Feeding ecology and niche segregation of the spider crab *Libinia ferreirae* (Decapoda, Brachyura, Majoidea), a symbiont of *Lychnorhiza lucerna* (Cnidaria, Scyphozoa, Rhizostomeae). Hydrobiologia, 847: 1013-1025. doi.org/10.1007/s10750-019-04158-0; **(6)** Gonçalves, G. R. L., Miazakic, L. F., Bolla Jr., E. A., Grabowski, R. C., Costa, R. C. & Castilho, A. L. (2020). Growth and longevity of the spider crab *Libinia ferreirae* (Majoidea, Epialtidae). Nauplius, 28. doi.org/10.1590/2358-2936e2020003; **(7)** Gonçalves, G. R. L., Palomares, L. F. S., Sousa, A. N., Stanski, G., Sancinetti, G., Costa, R. C. & Castilho, A. L. (2020). Geographical ecology to the symbiont crabs *Libinia ferreirae* and *Libinia spinosa*: patterns in the distribution from south-southeastern Brazil. Marine Ecology. DOI: 10.1111/maec.12606.

After my MSc degree, I decided to continue studying in the PhD the relationships that marine organisms have, using new analysis tools. For my PhD under the guidance of

Professor Antonio Castilho and co-supervision of Professor Maria Lucia Negreiros-Fransozo, we decided to improve the studies on the ecology and life cycle of *Libinia* by adopting new methodologies under the project "Ecology and trophic structure of the spider crab *Libinia ferreirae* (Decapoda: Majoidea) on the South coast of São Paulo", funding by FAPESP (2018/01659-0). Our main objective was to explore in detail the ecological interactions and the status of the marine trophic web exploited directly or indirectly by non-selective trawling. Thus, was inserted in this project together with ecological temporal and behavioural data, analysis of stable isotope for carbon and nitrogen.

A great deal of results from stable isotopes analysis of numerous species was obtained during this PhD project. However, because this area was new for both my supervisor Prof. Antonio Leão Castilho and myself, we decided to seek international partnerships with the objective of acquiring more knowledge in the area, mainly to understand and apply the tools of statistical analysis for stable isotopes in trophic relations. Therefore, a BEPE FAPESP project proposal entitled "Application of the isotopic mixing models in the ecology to trophic structure of the spider crab *Libinia ferreirae* (Decapoda: Majoidea)", in collaboration with Professor Dr. Stuart Bearhop of Exeter University, UK, was submitted and approved by FAPESP (2019 / 00105-3). During this process, I presented my preliminary data at the 10th National Conference on Marine Sciences, at the Faculty of Exact and Natural Sciences at the University of Buenos Aires in Argentina, where I had the opportunity to meet Professor Dr. Marleen De Troch of the University of Ghent (Belgium), who suggested adding fatty acid analysis as a complementary tool to my studies. After some time strengthening this idea, a transnational access request was submitted to the Assemble Plus Scholarship under EU

Horizon 2020, with a proposal to elaborate the project “Fatty acids analysis of shrimp (of co-occurring shrimp) in the South-West to Brazil”.

I started my 6 months of BEPE scholarship at the University of Exeter, and during this time my proposal at Assemble Plus was approved. In view of this, FAPESP was asked to extend the scholarship for another 3 months so that I had time to execute my project in Ghent University, which was approved. During this period of 9 months, I developed the statistical analysis necessary for my work with stable isotopes together with Professor Stuart Bearhop and his team, which were of substantial importance for the structuring and elaboration of the work. After this, at the University of Ghent, Belgium, I learned from Professor Marleen De Troch and her laboratory the fatty acid analysis of three species of shrimp. Professor De Troch added excellent knowledge to my career and my work, assisting me in the preparation of important research papers for the area.

Both international collaborations presented me with vast knowledge that brought invaluable personal and professional maturity to my research and career. These experiences have shown me that in trying to overcome your limits, more complete and broader research can be generated, in which apparently distinct topics become closely related to the relationships experienced by marine organisms. Therefore, the research presented in this PhD thesis was divided into 5 chapters in article format, in order to answer the following questions:

- How is the symbiotic relationship of the spider crab *L. ferreirae* with the host jellyfish maintained and how does this symbiosis can influence the life of the crab?
- What is the association behaviour of the crab with its host jellyfish?
- Is it possible to define the type of symbiotic relationship that the crab *L. ferreirae* has with the jellyfish and with different organisms that live on the carapace, through stable isotope analysis?

- How does the *L. ferreirae* crab benefit from this symbiotic relationship with the jellyfish?
- How is the trophic web of organisms collected by trawling aimed at the capture of commercial shrimp in Cananéia, São Paulo, Brazil (including *L. fereirae*) structured? Is there an ecological overlap during the winter and summer of the guilds of the organisms belonging to this trophic web?
- What is the fatty acid profile of three species of shrimp exploited by trawling in the Cananéia region, São Paulo, Brazil?
- As per the fatty acid profile of shrimp *Xiphopenaeus* spp., does it change according to sex, age and the region according to the state of conservation of the environment?

All these questions are proposed with the aim of exploring the intra and interspecific relationships of the organisms caught by trawl fishing, highlighting the importance of energy transfer in the food web between different levels of the trophic structure in which these organisms are involved.

Chapter I

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

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

Abstract

Marine crustaceans can use jellyfish as habitat, where the type and strength of relationship experienced by organisms is until a mystery. We investigated the importance of the symbiotic association between the spider crab *Libinia ferreirae* and the jellyfish *Lychnorhiza lucerna*, to understand this particular relationship in Cananéia and its adjacent oceanic area in Brazil. Where 654 crabs were collected, mostly in the summer. More than 50 % crabs were associated with *L. lucerna*, mainly in early lifecycle stage of decapodid and juvenile. We observed two color's crab pattern, according to the life habit, i.e., (a) white in color, symbiont crabs in association with a medusa; and (b) dark in color, free-living benthic crabs bearing symbiotic or fouling organisms on carapace. Eleven juvenile crabs (approximately, 2 mm in carapace length) were found in a single medusa, while larger *L. ferreirae* were found just one per jellyfish, suggesting competition among crabs for the resource (jellyfish), as seen as observed in the laboratory experiments. We also analyzed the stomach content of free-living adult's and associated juvenile's crabs to test if different groups present niche specialization related to feeding habits. The interaction network of crab-food resource indicates that juvenile crabs associated to jellyfish have a distinct feeding habit compared to the free-living crabs, regardless the ontogenetic stage. The symbiosis with jellyfish is a benefit for the feeding habits of juvenile crabs, as well as the benefits of post-larval survival, shelter, dispersion, and decreased competition with free-living adult's crab. This relationship was represented for kleptoparasitism (negative effect) for cnidarians, because crabs consume part of the food resource caught by medusae.

Keywords: kleptoparasitism, development, decapodid, medusa, niche segregation, behavior

Introduction

Symbiosis "living together of dissimilarly named organisms" (de Bary, 1879) is ubiquitous in nature, since all organisms are involved in intimate and often obligatory associations with other species during a certain phase of development, where there is an organism that lives intimately throughout its life time or a major life-history stage on or

within a host individual, i.e., a symbiont (Werren & Neill, 1997; Thompson, 2005). This relationship can be neutral, negative, or positive, according to the costs and benefits for the host (Leung & Paulin, 2008). Is possible to find numerous terminologies used according to the specificity of the symbiotic relationship as generalists or specialists and facultative *versus* obligatory symbiosis (Baeza & Thiel, 2007; Leung & Paulin, 2008; Martin & Schwab, 2012; 2013). Nevertheless, symbiotic interactions are not fixed and may change throughout the life cycle of organisms as well as by evolutionary manner (Towanda & Thuesen, 2006).

Ocean shows an infinity symbiosis's relationship such as mutualism of algae and corals reef (Rosenberg et al., 2007; Müller-Parker et al., 2015); commensalism of remoras with elasmobranchs (sharks and rays) and whales (Ritter & Brunnschweiler, 2003; Williams Jr. et al., 2003); commensalism of clown fish and sea anemones (Fautin et al., 1992; Chausson et al., 2018); and isopod's parasitism in fishes (Bunkley-Williams & Williams, 1998).

Symbiotic relationships bring interestingly strategies for survival in deep sea, for example when sponges to develop the ability to capture small invertebrates, so becomes carnivorous (Vacelet, 2006, 2007), or when the anomuran crab *Kiwa puravida* Thurber, Jones & Schnabel, 2011 cultivate their "bacteria farm" to produce food resource in the Costa Rica's deep-sea methane seep (Thurber et al., 2011).

At least 500 genera of crustaceans live in several types of symbiotic associations (Ross, 1983). Many marine decapods maintain symbiotic relationship, highlighting the caridean shrimps (Bauer, 2004) as the only one group among crustaceans showing eusocial behaviour (well-known in bees and ants), living in colony with the queen inside the sponges (Duffy, 1996; Duffy & McDonald, 1999). Caridean shrimps - reef fishes relationship has a specialized interaction network studies on bees and flowers with the also do the cleaning symbiosis in which the "client fish" was cleaned by caridean shrimps, a possible benefit when having parasites removed then tissues (Guimaraes et al., 2006). Recent studies have highlighted the symbiotic relationship that scyphomedusae maintain with different crustaceans. Scyphomedusa *Lychnorhiza lucerna* Haeckel, 1880 is a jellyfish endemic species of the Southwestern Atlantic Ocean considered an important floating nursery for several symbiotic species (Morandini et al., 2005; Gonçalves et al., 2016). Some studies emerged on the symbiotic relationship that this jellyfish maintains with caridean shrimps *Periclimenes paivai* Chace, 1969 and *Leander paulensis* Ortmann, 1897 (Martinelli-Filho et al., 2008; Gonçalves et al., 2016; Moraes et al., 2017; Baeza et

al., 2017) and brachyuran crabs *Libinia ferreirae* Brito Capello, 1871 and *Libinia spinosa* Guérin, 1832. (Nogueira Jr. & Haddad 2005; Sal Moyano et al., 2012; Gonçalves et al., 2016:2017a). Nevertheless, the symbiotic model of these relations is still unclear.

The *L. ferreirae* crab maintains a distinctive behaviour during its life, during the initial life stages they live associated with the jellyfish after this, when they move into adulthood go live in the benthos, becoming hosts of innumerable epibionts (Winter & Masunari, 2006; Gonçalves et al., 2017a). Even with evidences showing that the jellyfish has a great effect on the life of the crab, there is a lack of information about the ecological and evolutionary meaning of the symbiotic relationship with the jellyfish. Therefore, the hypothesis of our study is that the symbiotic relationship of *L. ferreirae* crab and *L. lucerna* jellyfish: i) occur during all the year; ii) is significant to the symbiont in obtaining food resources necessary for survive; iii) brings partition of resources between the juvenile and adult, when occupying different habitats; iv) behaviour for association occur when jellyfish touches the bottom and the crab associates; v) compete for the jellyfish host; vi) is kleptoparasitism, when crabs stole the jellyfish food. To test such a hypothesis, the following procedures were accomplished in this study: 1) describe the temporal variation in amplitude of size and abundance of host and symbiont; 2) analyse the niche separation for the crab population, according to the symbiotic behaviour; 3) investigate in the laboratory the association behaviour of juvenile and adult crabs with the host jellyfish; 4) verify if this symbiosis brings a positive effect for the crab when occupying distinct niche of free-living adults, changing the diet of the associated crabs and free life.

Materials and Methods

Collections of crabs and jellyfish with or without symbionts were conducted each monthly from February 2013 to May 2014, in Cananéia and its adjacent oceanic area in the state of São Paulo, Brazil (approximately, 25°05'S - 47°53'W), using a shrimp fishery boat equipped with double-rig nets (mesh size 20 mm and 15 mm in the cod end) (Castilho et al., 2012; Port et al., 2016; Queirolo et al., 2016). We sorted the animals quickly, but carefully, while still on the boat. Each medusa was examined for the presence of crabs in the following body parts: exumbella, oral arms, oral pillars, gastric cavity, subgenital pouches and gonads. We recorded the number of medusae without crabs and returned them to the environment; those with associated crabs were carefully isolated in labelled

plastic bags. The same species of crabs without jellyfish were isolated in plastics bags too, all the samples were conditionate in thermal box with ice.

We made additional collections during March 2016 to obtain animals for the behavior experiments in laboratory, when, *L. lucerna* jellyfish and *L. ferreirae* were transported alive in thermal boxes with sea water, to the laboratory of the NEBECC (Group of studies on Crustacean Biology, Ecology and Cultivation) - Institute of Biosciences, University of the State of São Paulo, UNESP, Campus of Botucatu.

The identification of crabs was based on the pertinent literature as follows: decapodid (Pohle et al., 1999), juvenile and adult (Melo, 1996; Tavares & Santana, 2012). The identification of the medusa *L. lucerna* was confirmed considering the study by Morandini et al. (2005). The animals were carefully sorted according to the life habit, i.e., (a) white in color, symbiont crabs in association with a medusa (Fig 1b, see below); and (b) dark in color, free-living benthic crabs bearing symbiotic or fouling organisms on carapace (Fig 1c, see below).

After removing the crabs, each medusa was transferred to a slightly inclined tray to remove the excess of seawater. The medusae weight (MWe) was measured on a scale with a precision of 0.01 g, and their umbrella diameter (UD = between two opposite ropalia) was measured using a standard metric ruler. Only undamaged specimens were considered for the study (Nogueira Jr. & Haddad, 2005). UD of jellyfish were separated in size classes to 50 mm.

Crabs were weighed (crab weight = CWe) was measured on an analytic scale (precision of 0.001 g). In order to classify the demographic groups of crabs in the host, carapace width (CW) of crabs greater than 15 mm was measured using a digital caliper (precision of 0.01 mm), whereas decapodid and remaining juvenile crabs had their CW measured using a light microscope (Zeiss®, Axioskop 2 plus) and stereomicroscope (Zeiss®, Stemi SV6), which were both equipped with a digital imaging system (Zeiss®, Stemi 2000-C; precision = 0.001 mm).

The crabs were classified according to sex, based on the shape of the abdomen and the number of pleopods like in Gonçalves et al. (2017b). The classification of juvenile and adult were based on Gonçalves et al. (2017b) work about the gonadal and morphology maturity of these specie.

Symbiotic behavior

The medusae specimens utilized in the behavior experiments measured from 44.98 to 141.95 mm of DU, while the juveniles crabs from 15.74 to 33.04 mm of CW. The only one adult crab utilized in the experiments measured 55.08 mm of CW (in supplementary material, Table 1). Crabs were kept isolated in numbered and connected aquaria, which were provided with a sea water recirculation system (Skimmer; Bubble Magus® model NAC7), a digital temperature control (precision of 0.1 °C) with heaters (300W), and filters with ultraviolet lights (15W). The aquaria were filled with sea water (salinity = 35±1 psu and temperature = 23±1°C) from animals collecting place, that was filtered and aerated at least once a week before the experiments. The medusae (5 specimens) were kept in a recirculation system of pseudokreisel type (see Raskoff, 2003), with a capacity of 250 liters, under the same environmental patterns mentioned for crabs. The photoperiod was kept in 12:12h (light: dark). Crabs were fed daily with pieces of penaeidean shrimps, fish and the squid *Loligo* sp., while medusawere fed with recently hatched *Artemia* sp. nauplii each 4h, throughout the experiments. All specimens were acclimated in the conditions described above for at least 24 hours before experimentation.

Preliminary observations on the symbiotic behavior of the spider crab in relation to the medusa were accomplished in a recycling system (pseudokreisel), with four combinations: one adult crab *vs* one medusa, one juvenile crab (CJ) *vs* one medusa, five CJ *vs* one medusa, and five CJ *vs* five medusae. The animals were monitored by means of two video cameras (GoPro Hero 4 Black; Canon Power Shot SX20IS), for 15 to 30 minutes. The behavior observed was grouped by size categories and captured in videos and photographic documentation. During the observation period, we did not offer food to the experimental animals. The videos on the obtained behavior were edited and reduced to a one minute of exhibition. Nevertheless, each behavior recorded is shown in real-time in the figures.

Crab's stomach contents

We analyzed the stomach contents of *L. ferreirae* sampled from February 2013 to May 2014. The crabs were classified in four demographic categories as follow: juvenile free-living (JF), adult free-living (AF), juvenile associated (JA) and adult associated (AA). Stomachs were removed and fixed in 70% alcohol and observed with either a light microscope (Zeiss® Axioskop 2 plus) or stereomicroscope (Zeiss® Stemi SV6), provided with a digital imaging system (Zeiss Stemi 2000-C; precision = 0.001 mm). The

identification of food items was performed according to Mariscal (1974) and Barros et al. (2008) and Gonçalves et al. (2020a).

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

Data analysis

In the host (medusae) - symbiont (crabs)'s relationship data analyses, we did not use 46 associated crabs because they were found walking on the medusa's exumbrella. The correlation in morphometric relationships of medusa with a single crab were tested as follows: a) crab carapace width (CW) with medusae umbrella diameter (UD) and b) crab weight (CWe) with medusae weight (MWe). A Mann-Whitney U test was used to test size differences between crabs associated with medusae and free-living benthic crabs. A single linear regression was applied to determine the relationships between the symbiont's number in each host and its sizes (CW). Based on the wet weight of jellyfish, the additional wet weight of the 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. Statistical analyses were carried out using R software, package 'cars' (R Core Team, 2017).

The network of food interaction come from the analysis of 339 analyzed crabs across four demographic categories AJ, AA, FJ and FA, in which the occurrence of 13 food items were observed for these animals. To determine the modularity level in this weighted bipartite network, we used the QuanBiMo algorithm (Dormann & Strauss, 2014). This method implements assimilated annealing Monte-Carlo approach to find the best division of species into modules. A maximum of 10^{10}MCMC steps with a tolerance level of 10^{-10} were used in 339 interactions, retaining the iterations with the highest likelihood value as the optimal modular configuration. We tested whether the network was significantly more modular than null networks by running the same algorithm in 1000 random matrices. The significance of modularity was tested for 339 iterations by comparing the empirical *versus* the null modularity indices using a z-score test (Dormann

& Strauss, 2014). After testing the modularity of our network, we determined the number of modules and identified the group for each module. Modularity analyses were performed using R software, packages 'bipartite' and 'vegan' (R Core Team, 2017) (Dormann et al., 2009).

Statistical analyses were performed using the equilibrated dataset (n = 339 sequences per sample). Community-scale multivariate analyses, including non-metric multidimensional scaling (NMDS), were performed in R (R Development Core Team, 2017) using statistical package 'Vegan' (Oksanen, 2009). The model used "food items" as a random factor nested within the fixed factor "demographic categories (AA, AJ, FA, FJ)". The NMDS analysis is a nonparametric ordination-based method for reducing the complexity of ecological community data and identifying meaningful relationships amongst communities. The distance-based permutational multivariate analysis of variance (PERMANOVA) was used to test the difference of food items in the structure of diet among habitat, age and demographic categories of groups. After that, the specialization of the interaction network of the groups of crabs (AA, AJ, FJ, FA) *versus* food items was tested through the specialization index (H2') that is based on the Shannon entropy and allows us to infer how generalist or specialized an interaction network is.

Results

Between the years 2013 and 2014, we collected 654 crabs (357 associated and 297 free-living) in distinct lifecycle stages, including decapodid (last larval stage, Fig. 1a), juveniles (Fig. 1b) and adults (Fig. 1c); and 916 medusa of *L. lucerna*, that only 198 jellyfish (UD average ± standard deviation (SD) = 108.12 ± 54.41 mm, ranging 24 until 328 mm) had 357 associated crabs (98 % juveniles with white color, Fig. 1b) caught throughout the year (Fig. 2). The highest abundance of medusae was August (winter, holding only two associated crabs), followed by October (spring) and March (summer, highest number associated crabs) (Fig. 2).

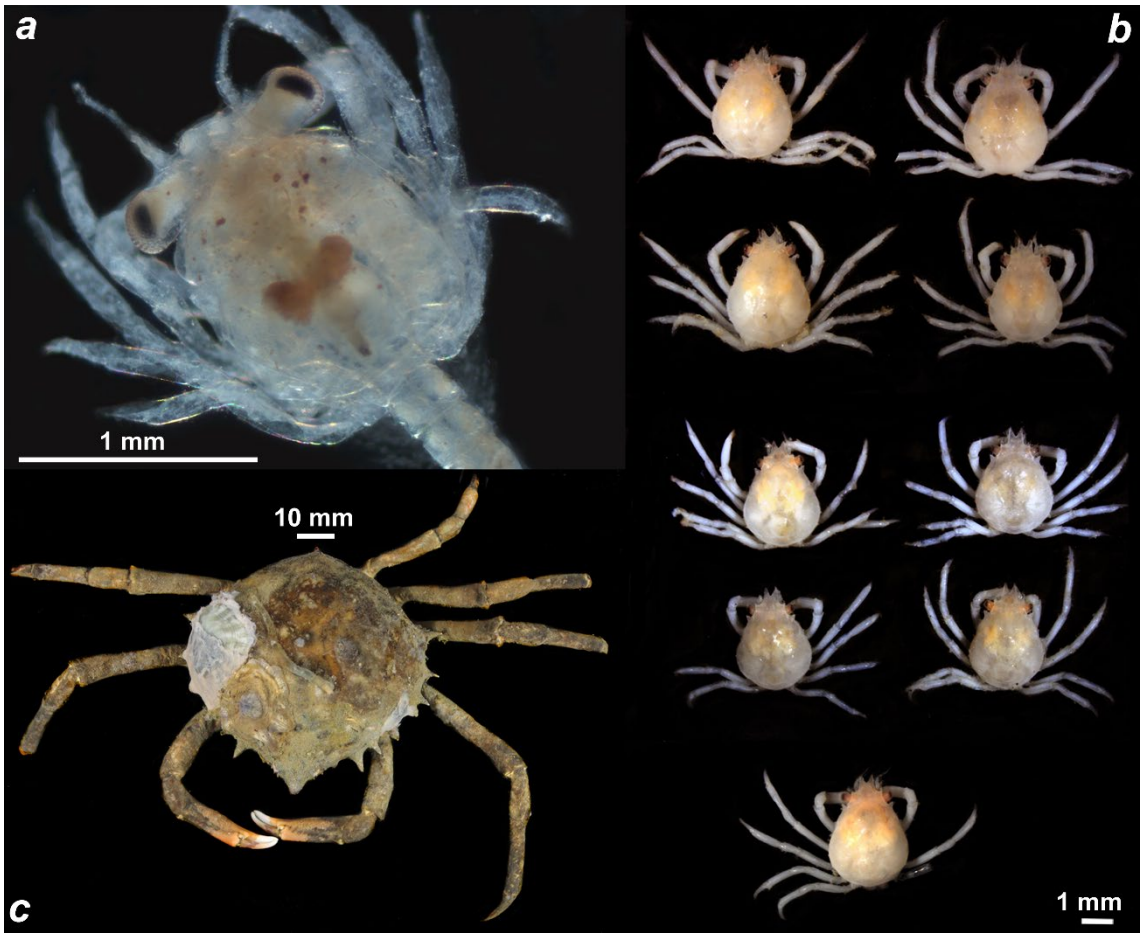


Figure 1. *Libinia ferreirae*. *a*) Decapodid (zooplanktonic stage); *b*) Juvenile individuals (N=9) found associated with a single medusa specimen (note the whitish coloration of the juvenile crab); *c*) A benthic adult crab not associated with the medusa (note the brownish coloration and the presence of epibionts on the carapace).

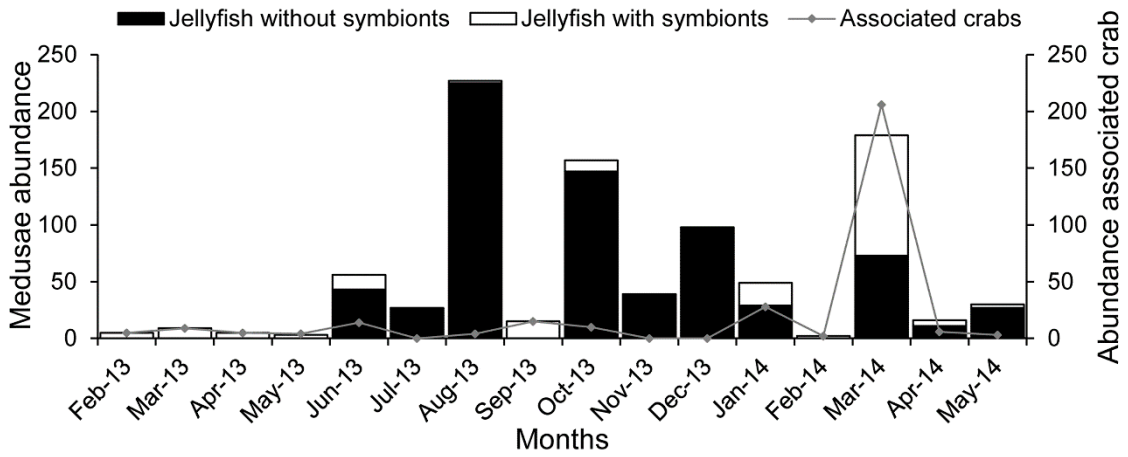


Figure 2. Monthly abundance of *Lychnorhiza lucerna* medusae hosting the symbiotic crab *Libinia ferreirae* and the abundance the medusae with not have crabs.

Associated crabs were smaller than free-living benthic individuals (Mann-Whitney U test, U = 1214.0, n1 = 297, n2 = 357; $P < 0.05$). The associate's crab ranging

0.89 (decapodid) until 49.59 mm with average 7.85 mm (SD ± 8.41 mm) of CW (Fig 3), in which 88.75% showed CW <15 mm. The smallest juvenile crab specimen associated with a medusa had 1.38 mm of CW. Three females (CW 40.17 until 48.25 mm) and four males (CW 35.16 until 49.59 mm) with sizes close to maturity were found associated with medusa. The free-living crabs ranged from 16.35 to 76.37 mm, average in 49.27 (SD ± 8.67 mm) for CW (Fig. 3).

The number of crabs associated with a medusa varied from one to 11 with a average size of 1.59 (SD ± 1.53 mm). Eight medusae hosted from 5 to 11 crabs totaling 60 crabs. Variation of symbiont's sizes were inversely correlated with number of symbionts in each host (Linear Regression, $r^2 = 0.29$; df = 308; F = 128.6; P = 3.9E-25), i.e., crabs occurred as singletons were larger than crabs occurring in counts of 2 or more on medusae. One crab associated with a medusa carried the anemone *Carcinactis dolosa* Riemann-Zurneck, 1975. The medusae with higher numbers of associated crabs had umbrella diameters ranging from 100 to 150 mm.

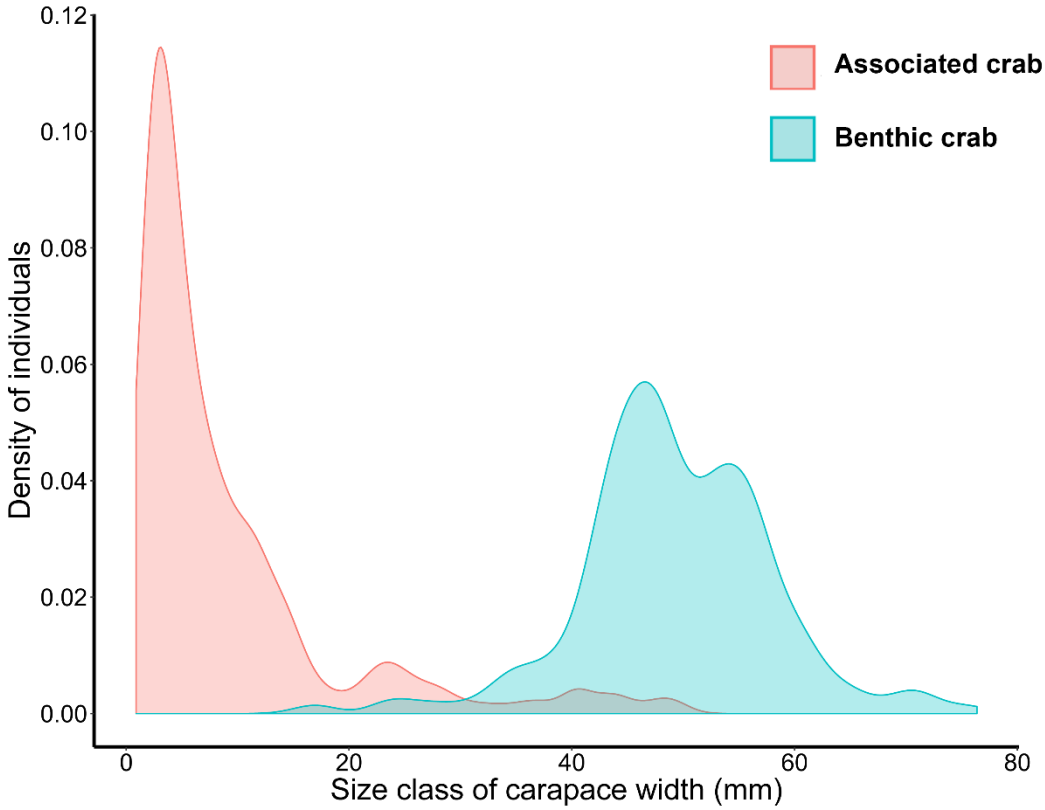


Figure 3. Size frequency distribution based on the carapace width (CW) of crabs (*Libinia ferreirae*) associated with medusae (*Lychnorhiza lucerna*), and free-living.

The monthly UD of medusae and CW of their associated crabs were positively correlated (Linear Regression, $r^2 = 0.76$; df = 11; F = 35.3; P < 0.001). Thus, there was a

tendency in crabs (Fig. 4a) growing along with their hosts over the months (Fig. 4b). Linear regressions revealed relationships between crab's carapace width (CW) and medusa's diameter (UD) (Fig. 5a, Linear Regression, $r^2 = 0.61$; df = 158; F = 249.4; P < 0.001), as well as for crab's weight and medusa's weight (Fig. 5b, $r^2 = 0.66$; df = 158; F = 309.9; P < 0.001) (Fig. 5).

We found that the lower weight (CWe) from crabs carried by a jellyfish host was 0.01% and the greater weight was 14.4%, with a overage of 1.57%. The majority of the sampled medusae (86%) contained crabs, comprising less than 3% of their own body weight. Only 14% (*n* = 27) of the sampled medusae carried crabs comprising more than 3% of their own weight. The weight carried by the host varied seasonally, i.e., larger crabs were obtained during May (CWe= 5.8 %) and October 2013 (CWe= 3.2 %) while smaller symbionts were obtained in March 2014 (CWe= 1.2 %).

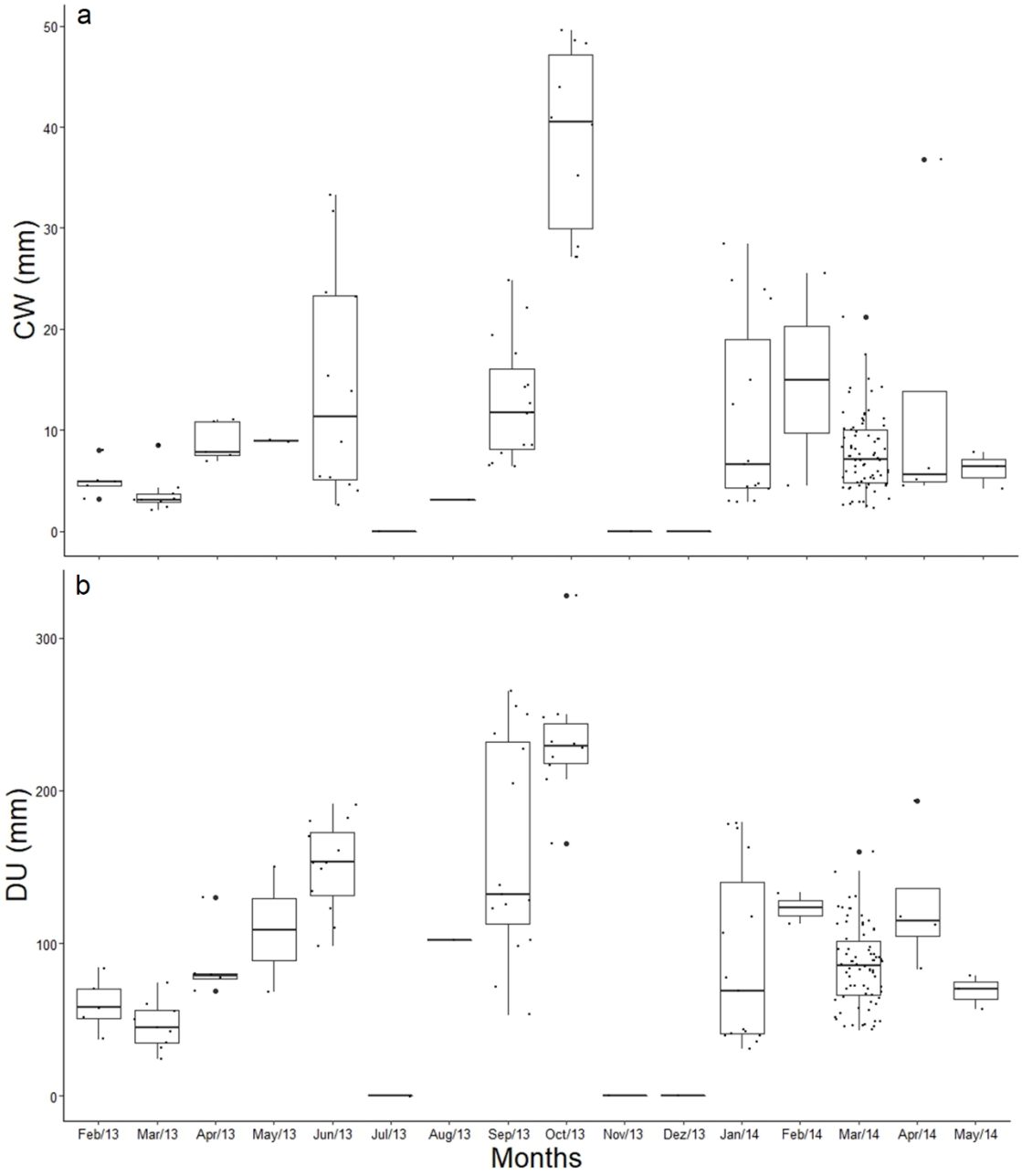


Figure 4. *a*) Monthly mean range of the carapace width (CW) of the *Libinia ferreirae* crabs associated with the medusae *Lychnorhiza lucerna*. *b*) Monthly mean range of the umbrella diameter (UD) of the medusae *L. lucerna*. (Median; percentile 25% and 75%; Min-Max = minimum and maximum values).

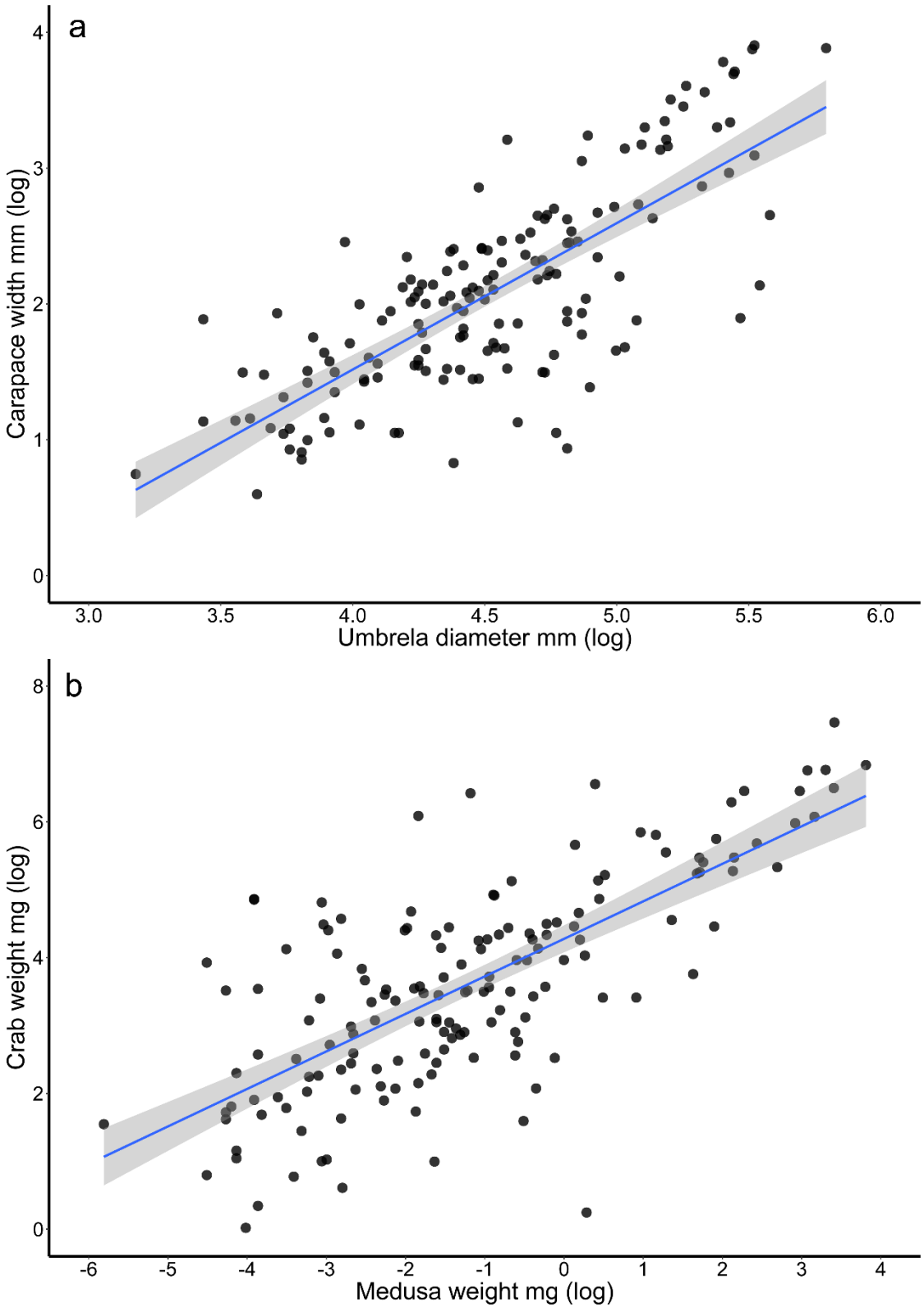


Figure 5. *Libinia ferreirae* and *Lychnorhiza lucerna. a*) Relationship between umbrella diameter (UD) and carapace width (CW) in associated crabs; *b*) Relationship between medusa weight and crab weight.

Symbiotic behavior

The adult crab did not show any interest in relation to the medusa (Video 1). But the juvenile crabs quickly recognized the host. After that, they associated with the medusa in the following ways: i) lifting up their chelipeds towards the medusa and grabbing itself to the host (Figs. 6b and 6d; Video 2); ii) the crabs release their selves from the bottom, opening their pereiopods and, they can "glide", being carried by the current facilitated by the water current (Fig. 6e and 6f; Video 3). Thus, when they reach the medusa, they associate to them (this "glide" was possible just in the smaller crabs, i. e., juveniles >15 mm of CW) (Figs. 6e and 6f; Video 3). When having just one crab as symbiont the medusa moves uniformly (umbrella turned down and tentacles and oral arms toward the bottom) (Video 2). Otherwise, when the medusa had five symbiont crabs it showed difficulty in to move, it produced mucus excessively, and increased the number of pulsations of the body (Figs. 6h, Video 4). The crabs in association with the medusa showed compatible sizes to the host and quickly moved to the oral arms and remained there.

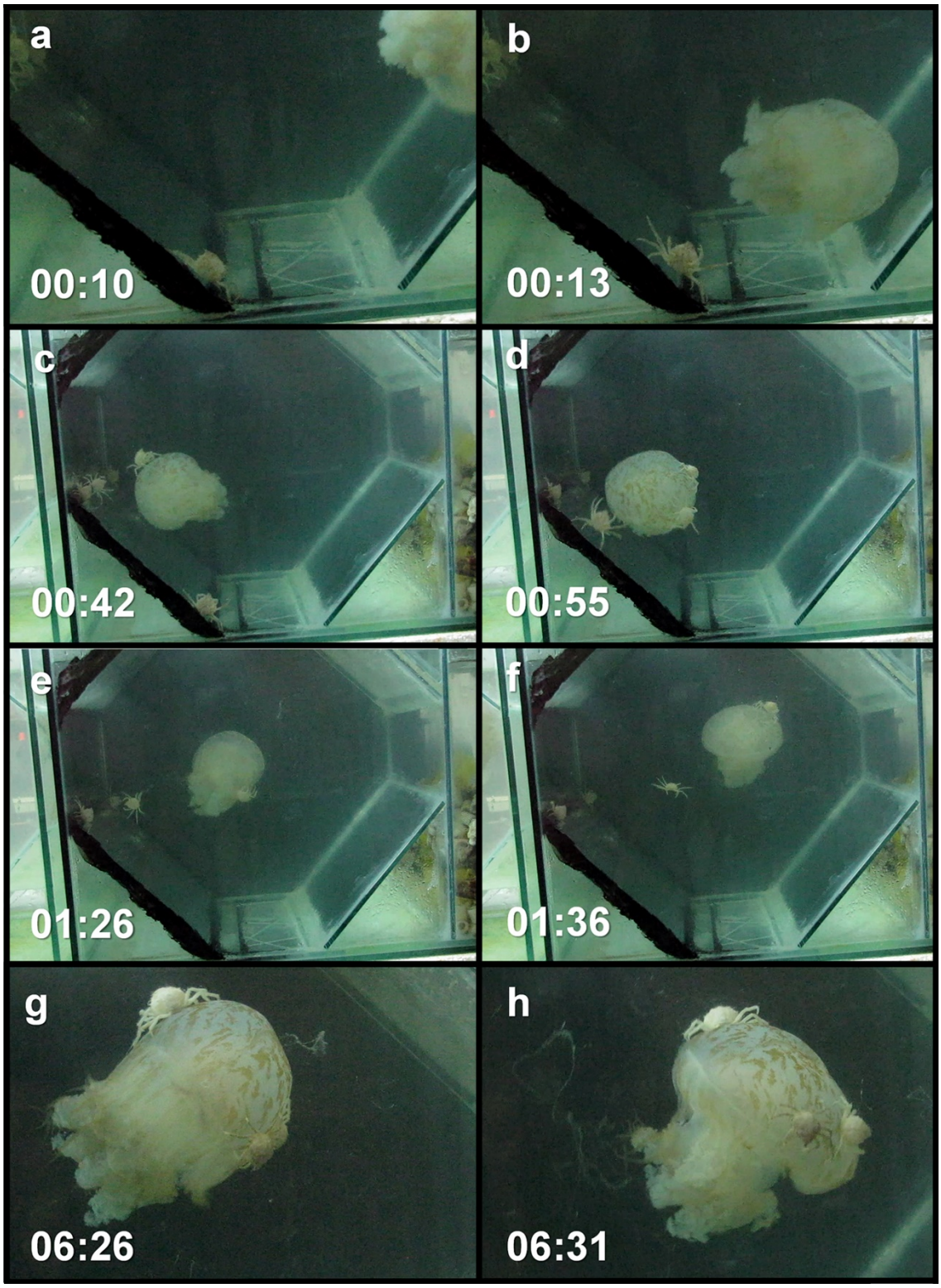


Figure 6. Sequence of *Libinia ferreirae* crab association behavior with *Lychnorhiza lucerna* jellyfish host. This association occurs approximately in the first minute in contact with medusae. Time presented in hh: mm format. (a) introduction of jellyfish into the aquarium with 5 more crabs. (b) crab perceives the presence of the host. (c) first crabs are associated during approximation to jellyfish. (d) two associated crabs note the behavior to the dissociated crab of lifting the chelipeds in the attempt to associate. (e) and (f) observe the planar behavior of the crabs in the attempt to associate. (g) and (h) more than one associated crab. More details in the video 3.

The competition pattern among the juvenile crabs varied according to the host size. There was no dispute among symbionts (N = 5), when just one large sized medusa (DU 142 mm) was tested in an experiment. However, when a small sized medusa (DU 45 mm) was available for crabs, they competed for this resource. In such case, the smaller crabs were quickly turned out from the host by the larger crabs. Still, the expelled smaller crabs tried to associate once again (Videos 3 and 4). The largest CJ (CW 23 mm) strongly competed for the resource, entering in confrontation, and killing the other juvenile (Fig. 7; Video 4). In this case, it can be notice that the fighting crab caused damage to the umbrella of the host (cutting it) during this confrontation. In such a situation, we decided to add more medusa to the experiment facilitating the search of hosts for other crabs. This alteration caused the ceasing of the competition. In this last experiment, after the association of crabs and host, the larger medusa showed increase to 21 to 28 pulsations, while the smaller one increase to 32 to 37 pulsations/20 seconds.

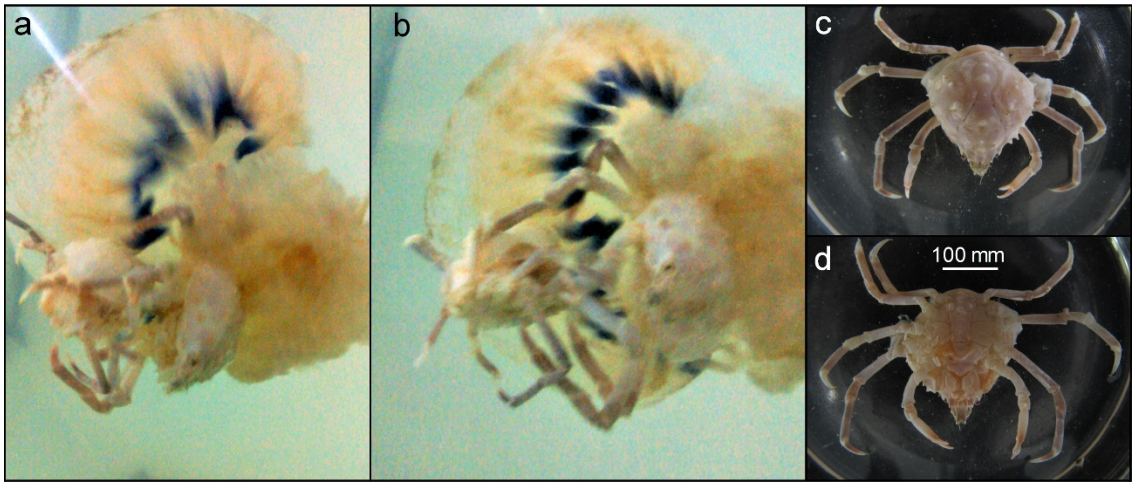


Figure 7. *Libinia ferreirae* crab competition behavior when associated with *Lychnorhiza lucerna* jellyfish host. (a) dorsal view and (b) ventral view of the killed crab after dispute, lateral margin of the cut carapace and get off right pereiopod.

Modularity and NMDS

The modularity of the data matrix (339 individuals, AA, AJ, FA, FJ) found in this study was 0.52 ($P < 0.001$), indicating a difference in the consumption of certain types of food resources in the detriment of others. For better visualization we used NMDS that shows how the groups are distributed in a multidimensional space in relation to the alimentary items that they consume (Fig. 8). It was possible to verify differences in the food preference of crabs, according to their habitat (associated with jellyfish (A) and free-

living (F)) (PERMANOVA, habitat: df = 1, F = 51.7549, P = 9.999e-05). However, age and habitat vs demographic categories (juvenile and adult), were not significant (PERMANOVA: df = 1, F = 0.55, P = 0.72 and df = 1, F = 1.22, P = 0.27, respectively). As for the specialization of network crab interaction food item *vs.* the network showed a specialization value of H2 '= 0.626 (P <0.001).

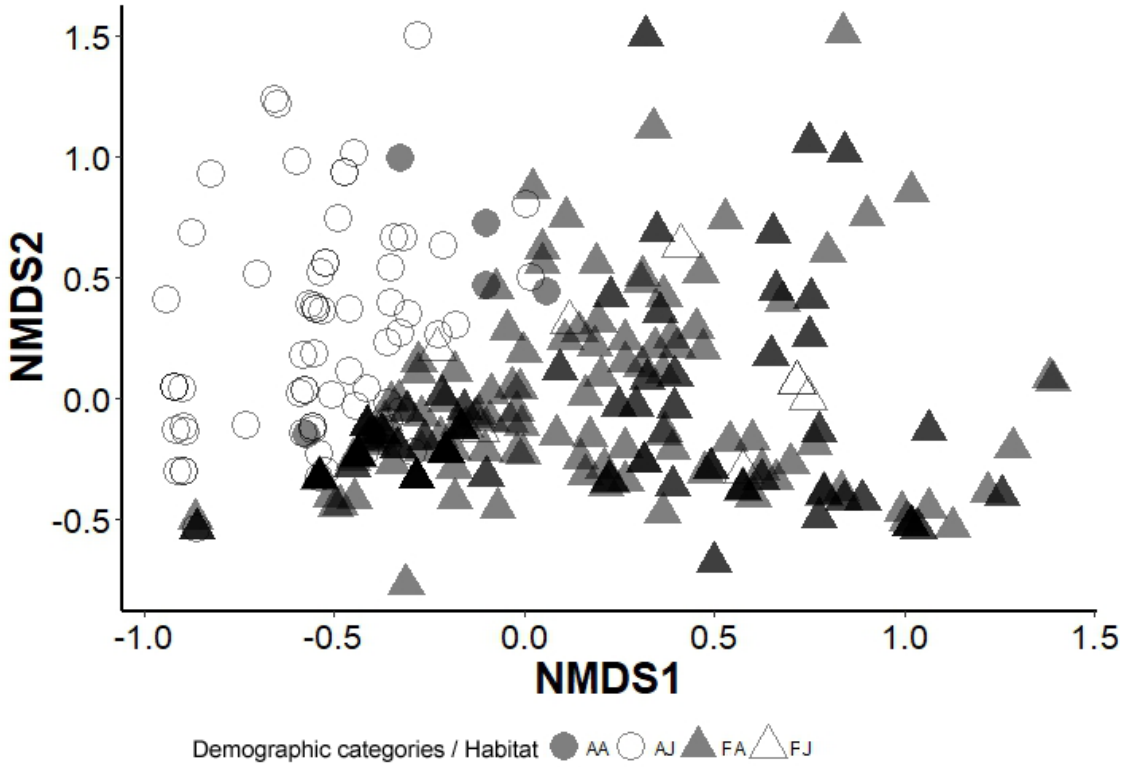


Figure 8. Food habits of the crab *Libinia ferreirae*, during distinct life habits i.e: AA – associated adult; AJ – associated juvenile; FA – free-living adult; FJ – free-living juvenile. This data was analyzed by non-metric multidimensional scaling analysis (NMDS). Circles: crabs associated with jellyfish, triangles: free-living crabs. Gray fill: adult crabs, white fill: juvenile crabs.

Discussion

Our study demonstrated the close relationship that the crab *L. ferreirae* maintains with its host jellyfish, where it was possible to prove the importance of this ecological relationship to the life of these crabs in the larval and juvenile phase. The association peak was found during the hot periods, when marine organisms invest more in reproduction and the growth of the crab followed the growth of the jellyfish. A large abundance of juveniles has been found in a single jellyfish, which has decrease over the course of development, which demonstrates the competition for resource among these juveniles as demonstrated in laboratory tests. An overlapping food niche between crabs associated

with jellyfish and free-living crabs was not found in our study, that is, this symbiotic relationship leads to niche sharing regarding symbiotic behaviour. This study proposes that the symbiotic relationship between the crab and its host jellyfish could be a kleptoparasitism, where the crab steals food from its host.

The abundance of *L. ferreirae* living with *L. lucerna*, in the present study was greater than that obtained by Nogueira Jr. & Haddad (2005), and for *L. spinosa* by Sal Moyano et al. (2012). The high abundance observed in the present study is probably due to the recruitment period of the crabs, which occurred during summer (peaking in March), in agreement with the high abundance of ovigerous crab females in the benthos two months earlier (January) (Gonçalves et al., 2017a). However, symbiotic crab was not found during all the year, refuting our first hypotheses. The crab *L. ferreirae* utilizes the medusa (as a host) and planktonic organisms (as food), when both are in high abundances (spring and/or summer) (Mills, 2001; Nagata et al., 2015). During these seasons, an increase in primary and secondary productivity in the plankton provides food for larvae and juvenile crabs (Thorson, 1950; Gonçalves et al., 2017a). Another advantage of *L. ferreirae* - *L. lucerna*'s association is the geographic dispersal. Ohtsuka et al. (2009) suggested that medusae can transport the ophiuroids *Ophiocnemis marmorata* (Lamarck, 1816) up to 1000 km from their point of colonization. Such observation evidence that migration and gene flow with other populations can be favored as well as colonization of new environments. Gonçalves et al. (2020b), after studying the geographic distributions of *L. ferreirae* and *L. spinosa*., proposed that, in many regions, the population was sustained by crabs carried by *L. lucerna* hosts to maintain crab species connection between metapopulations, but genetic studies need to be accomplished to clear up this hypothesis.

In our study medusa and crab sizes increased simultaneously at the beginning of the sampling period, followed by a sharp decrease in size of both species, and then a gradual increase again during the last's months of study. We believe the sharp decrease was due to host death, followed by a subsequent recruitment and growth of both species during the productive spring and summer months. *Libinia spinosa* recruits on the medusa, *L. lucerna*, during its larval phase and can remain associated throughout the life span of the medusa, which lasts 5 to 6 months, or as long as the cnidarian is able carry it, which depends on the crab's size (Sal Moyano et al., 2012, Schiariti et al., 2008). However, our results show that *L. ferreirae* crabs live in association with *L. lucerna* only until they reach sexual maturity. Crabs leave the medusa when the inner spaces of the medusa are

not sufficiently large for the crabs to remain inside (Corrington, 1927; Gutsell, 1928; Nogueira Jr. & Haddad, 2005). Subsequently, the crabs continue their life cycle in the benthic environment as free-living crabs, like is showed the size differences between the groups in the figure 3.

In the present study the crabs can associate with jellyfish when the host reaches near the juvenile crabs which are situated on the bottom of the aquarium, corroborating with our hypotheses four. However, in our study it was observed that these juvenile crabs can use the other strategy to gliding on the water, when they sense the approximation of the jellyfish host. After that, they push up from the substrate and they are carried out by the water current creating opportunities to reach a jellyfish. The high number of associations, likely, occurs when the *L. ferreirae* crabs were found in the decapodid phase, as they can easily find the *L. lucerna* jellyfish in the zooplankton, since at this stage, they share the same niche as the jellyfish (Carrizo et al., 2016). Anger et al. (1989) studied the larval development of *L. ferreirae* and registered a massive larval mortality in laboratory during the decapodid phase, proposing that during the transition from the decapodid to juvenile phase this species depends on the stimulus generated by the host jellyfish.

This study shows a single medusa hosted up to eleven crabs similar in size, when multiple symbionts were found on a medusa. In the studies by Vaz Ferreira (1972) and Sal Moyano et al. (2012), no more than four crabs were found in the same medusa, whereas in Nogueira Jr. & Haddad (2005), only one crab was found to be associated with the medusa. These observations suggest two possible reasons for these patterns are, the competition for the resource (medusa) or territorial disputes among the crab developmental stages, which could lead to competition and cannibalism (Towanda & Thuesen, 2006). These theories were observed in our laboratory experiments, where we saw the competition by the crabs to remain in the host jellyfish, confirming the hypotheses for crab competition by the jellyfish host.

This study found all the crabs associated with medusae pale or whitish in color, this can be caused by the jellyfish nematocysts, which suggests a cryptic camouflage or adaptive coloration (Bauer, 1981; Stuart-Fox & Moussalli, 2009; Stevens, 2016), with the crab coloration blending into the background of the medusa's pale to whitish tissue.

We did not observe in this study apparent negative effects during the association of one *L. ferreirae* crab in medusa *L. lucerna*, but when more than one crab associates with jellyfish, this make it difficult for medusa to swim; although, this could result in an extra expenditure of energy during locomotion. Towanda & Thuesen (2006) studying the

medusa (*Phacellophora camtschatica* Brandt, 1835), found one specimen carrying 32 decapodids of *Cancer gracilis* (Dana, 1852), with less than 2% of the energetic cost to the host, and with no apparent negative impact to it. However, the fact that the crab could be a kleptoparasite implies that it harms the jellyfish, once crab uses part of the food and consequently energy captured by the host (Ingram et al., 2017).

In this study the habitat in which the crabs were living defined the food preference, since *L. ferreirae* is generalist (Gonçalves et al., 2020a) it feeds on what is available in the environment. Thus, when associated with jellyfish (its environment = host), it uses the prey of jellyfish as a food source, which are mostly copepods, one of the main food resources of jellyfish. This confirm the first hypothesis that jellyfish is significant to crab have food resources. Thus, when they are in the free-living phase, they capture what is available in the environment, with a greater range of resources as seen for *L. ferreirae* by Gonçalves et al. (2020a) and for *L. spinosa* by Barros et al. (2008). Supporting the second hypothesis that the different life habits bring partition of resources between the juvenile and adult crab, because they occupy different habitats. This study, show that not overlap in the use of habitats between crabs that live in the association with the jellyfish and *L. ferreirae* that have a free-living habit on the benthos,

Adult crabs, as seen in the laboratory, were not seen to show an interest in jellyfish and only the juveniles try to associate. The occupation of distinct habitats by juveniles and adults reduces intraspecific resource competition and provides an ecological segregation of the population (Gonçalves et al., 2020a; 2020b). Nevertheless, maybe this is not obligatory, mainly especially the juvenile crabs' stages, as we found medusa-associated crab carrying a small anemone on its carapace, indicating it had lived in the benthos at some point.

Finally, we suggest that the association of *L. ferreirae* and *L. lucerna* is probably kleptoparasitic, as the symbiont steals food captured by the host medusa, as seen in the symbiotic crab's stomach contents by Gonçalves et al. (2020a), as proposed in our last hypotheses. This is not a commensalistic interaction as suggested by Gonçalves et al. (2017a). Sal Moyano et al. (2012) for crab *L. spinosa*. Gonçalves et al. (2020a) observed nematocysts in the crabs' stomach contents, suggesting that during feeding, juvenile crabs may ingest nematocysts accident (Sal Moyano et al., 2012), by stealing prey or scavenging detritus from the oral arms of their hosts. When a crab steals food from its host, this displays a kleptoparasitism behavior, as observed by Ingram et al. (2017) in the ophiuroid *O. marmorata* and the jellyfish *A. aurita.* Thus, the pompom or boxer crab of

the genus *Lybia* H. Milne Edwards, 1834 whose chelipeds are modified to hold an anemone, characterizing a relationship of kleptoparasitism, stealing the food that anemone captures the environment. This is an obligatory relation, as in the anemone's absence, crabs are incapable of feeding themselves alone, and thus they could become extinct without this symbiosis' relationship so specialized that the boxer crabs stimulate the anemone's asexual reproduction (Schnytzer et al., 2013; 2017). Marine slugs which feed on an *Vaucheria litorea* C. Agardh, 1823 algae perform an endosymbiosis through kleptoparasitism, incorporating the chloroplasts of the algae into the cytoplasm of the cells of their own body, keeping the cytoplasm in operation; thus, they are able to make photosynthesis, getting long periods without food (Pierce et al., 2007; Pierce & Curtis, 2012). This showing the wide range of kleptoparasitism relationships that occur in the sea.

In our study, the symbiosis between the crab and jellyfish was absence just in some months of the year, and in some months (summer 2014) was more abundant and was possible see that both animals growing together. This symbiotic relationship reduces or cancels the competition between juvenile and adults of *L. ferreirae* crab, and this probably relationship of kleptoparasitism proved to be beneficial for the crab in countless ways, but not for the jellyfish, however we do not know how harmful the jellyfish this relationship is. However, only a small number of captured jellyfish had symbionts. Our study was the first to describe the interesting association behaviour that juveniles of *L. ferreirae* have when detecting the jellyfish, and the disinterest that the adult phase. Finally, this strategy can be vital for the survival and development of *L. ferreirae*. And can also be an important source of transport which maintains the metapopulations of the species in different regions, as proposed by Gonçalves et al., (2020b).

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Supplementary Material

Table 1. Short compartmental experiments about relationship symbiotic crab *Libinia ferreirae* and Host *Lychnorhiza lucerna*, representing information about the animals and time in the videos. DU - umbrella diameter, N – crab number, CW - carapace width.

Experiment	Time	Medusa			Crab		
		DU (mm)	Wight (g)	Pulsations (20")	N	CW (mm)	Wight (g)
Video 1	30 min	141.95	383.95	31	1	55.08	93.36
Video 2	30 min	141.95	383.95	31	1	19.38	3.47
Replica of Video 2	30 min	141.95	383.95	37	1	33.04	14.45
Video 3	15 min	123.08	268.42	28	5	19.38	3.47
						22.92	5.38
						15.74	1.75
						15.84	2.01
						16.65	1.75
Video 4	15 min	44.98	100.78	37	5	19.38	3.47
						22.92	5.38
						15.74	1.75
						15.84	2.01
						16.65	1.75

Video 1. Adult of *L. ferreirae* and they epibionts together the jellyfish *L. lucerna*. Crab does not show any interest for the jellyfish.

Video 2. Association by the one *L. ferreirae* juvenile with the jellyfish host when lifting up their chelipeds.

Video 3. Association by the 5 *L. ferreirae* juvenile with the bigger jellyfish host when lifting up their chelipeds and opening their pereiopods and, they can “glide”, being carried by the current facilitated by the water current.

Video 4. Association by the 5 *L. ferreirae* juvenile with the small jellyfish host, showing the competition by the host.

Chapter II

Trophic relationships between the crab *Libinia ferreirae* and its symbionts

Trophic relationships between the crab *Libinia ferreirae* and its symbionts

Abstract

Symbioses are ubiquitous in the animal kingdom. However, in most cases the role played by each member of the partnership is relatively fixed and it is rare for the same species to show different symbiotic behaviours during ontogeny. In this study, we investigated the relationships between the spider crab *Libinia ferreirae* its jellyfish host and sea anemones a carapace epibiont. We ask the following questions: How do the food sources used by the crab change between the juvenile and adult phase? How does the symbiotic relationship change when the develops from a juvenile into an adult? Stable isotope analyses (SIMMr package in R) were used to determine the extent to which *L. ferreirae* juveniles were feeding on medusae hosts versus planktonic prey during the symbiont phase and feeding on their epibionts during the free-living phase. $\delta^{13}C$ of *L. ferreirae* differed between juvenile (associated with the jellyfish) and adult stages (free-living), indicating little isotopic niche overlap between these life phases. SIMMr models using $\delta^{13}C$ and $\delta^{15}N$ of the crab and their potential food sources suggested that juvenile crab symbionts did not derive any significant nutrition from medusae and relied predominantly from zooplankton prey. As juvenile crab does not have structures to capture zooplankton for feeding, we suggest that the juvenile crab could be kleptoparasites stealing zooplankton food from their host, but more food sources need be tested before confirm the type of the relationship. The nutrition of free-living crab was predominantly derived from fee-living algae and shrimps and not epibionts. Likewise, the epibionts appeared to rely on similar food resources, suggesting a symbiotic relationship more akin mutualism. Thus, the crabs move from a symbiosis where they can act as parasites to one where they act as hosts. These different relationships that *L. ferreirae* live bring food sources, protection from predators and dispersion for a wider range of environments, increasing the chances of survival. Such flexible approaches to symbioses may be much more common than currently described, particularly in species where the functional roles of individual life stages differ.

Keywords: symbiosis, nitrogen-15, carbon-13, brachyuran crab, Cnidaria, stable isotope analysis

Introduction

Symbiotic relationships organisms are a basis for the survival and coexistence of numerous species. These can be considered as comprising a host species (i.e., the provider or basis of resources) and a symbiont species (i.e., the consumer of such resources) (De Barry, 1879). Two species forming a symbiotic association can have different types of symbiotic interaction, for instance a species with a one obligate symbiotic interaction and species with a facultative symbiotic interaction (Kooi et al., 2014). For obligate symbiosis both the nutrient and the products of the partner are essential to survival. However, facultative symbiotic interaction the products excreted by the partner and the nutrient of the prey are non-essential to survive (Kooi et al., 2014). Obligatory symbiotic relationships have been demonstrated in many systems as an example: mutualistic relations of algae with reef corals (Rosenberg et al., 2007; Müller-Parker et al., 2015); fungus-gardening ants in the Attini tribe (Formicidae), dependent upon the cultivation of fungus for food (Chapela et al., 1994; Mueller et al., 1998); legume associated rhizobial (Kiers & Denison, 2008), cleaners fish that removes parasits for them clients (Bshary & Grutter, 2002) and obligate pollination mutualisms (Goto et al., 2010). At the same time, the facultative symbioses are less well understood, and little is known about the relative roles played by the actors in them. Here we address this by investigating the trophic relationships among the spider crab *Libinia ferreirae* Brito Capello, 1871 with the host jellyfish *Lychnorhiza lucerna* Haeckel, 1880 during the juvenile phase and with the crab epibionts in the adult phase.

The spider crab *L. ferreirae* is one species that maintains different symbiotic relationships during ontogeny. In common with many congeners in the Majoidea superfamily, *L. ferreirae* has a variety of epibionts that adhere to the hooked setae on its exoskeleton, including algae, bryozoans, hydrozoans, and polychaetes (Winter & Masunari 2006). Among these, anemones are the most common, comprising several distinct species and occurring in large numbers in the carapace of most individuals (Hultgren & Stachowicz, 2011; Winter & Masunari, 2006; Nogueira Jr. et al., 2006). The crab is thought to benefit from protection (camouflage and the stinging cells of the anemone) whereas the epibionts gain dispersal opportunities and left over food from the crab (Hultgren & Stachowicz, 2011; Winter & Masunari, 2006; Nogueira Jr. et al., 2006). However, Hultgren & Stachowicz (2011) have also suggested that majoidean crabs may

use their epibionts as food in cases of food shortages. However, it is not common the symbiotic relationship for this crab superfamily with the planktonic jellyfish.

The symbiosis with the jellyfish *L. lucerna* occurs during the larval megalopa and juvenile phase of the *L. ferreirae* crab (Nogueira Jr. & Haddad, 2005; Gonçalves et al., 2017; 2019). Anger (1990) propose that jellyfish is the specific substrate needed for this crab's larval to metamorphose in juvenile. This relationship provides protection (camouflage), feeding, and displacement for these crabs that live with their jellyfish during the early stages of life (Doyle et al., 2014; Gonçalves et al., 2017), around 6 months or more. On the other hand, it is unclear if jellyfish has any benefit from this relationship because crabs were an additional weight for the medusae and competitors by medusae food too. Though, Towanda and Thuesen (2006) proposed that the medusa's associated crabs benefit the host when using the medusa's isopods parasitic as a food source, but no prejudice or benefit has yet been proven.

The host jellyfish *L. lucerna* have large aggregations during the Autumn and Summer in the Southwest of Brazil (Schiariti et al., 2008; Gonçalves et al., 2020a). This jellyfish is a host for others animals as, crabs *Libinia spinosa* Guérin, 1832, caridean shrimps *Periclimenes paivai* Chace, 1969 and *Leander paulensis* Ortmann, 1897 (Martinelli-Filho et al., 2008; Gonçalves et al., 2016) and fish *Chloroscombrus chrysurus* (Linnaeus, 1766) (D'Ambra et al., 2015). Defined in relationships as: parasitism, mutualism, and commensalism with different animals (Ohtsuka et al., 2009, D'Ambra et al., 2015, Gonçalves et al., 2016). The symbiosis of the fish *C. chrysurus* with the jellyfish bring for protection from predators Increasing survival (Purcell et al., 2000). D'Ambra et al. (2015) showed through Stable Isotope mixing model that the average ~90 % assimilated diet for fish come to the scyphozoan hosts contribution.

Recently, Gonçalves et al. (2020b) analysed the food habits of these crabs through stomach contents during their different stages of development (juvenile associated with jellyfish and benthic adults), showing that these crabs do not have niche overlap, not competing for resources. Juvenile crabs associated with jellyfish had a high intake of planktonic crustaceans (copepods) and adults had a diet rich in benthic crustaceans (shrimps, crabs, and hermit crabs), sediment, and others. However, nematocysts were found in the crab's stomach content in both phases of development (Gonçalves et al., 2020b). The authors propose that juveniles ingest nematocysts by using the resources captured by jellyfish as food. Gonçalves et al. (2020b) propose that this crab associated with the jellyfish host have a kleptoparasitism relationship, when steals the food form the

jellyfish. In the other hand, others reseachs studing the relationship between the spider crabs of the genus *Libinia* Leach, 1815 and medusae to be considered this relationship a commensalistic interaction (Corrington, 1927; Vaz Ferreira, 1972; Nogueira Jr. & Haddad, 2005; Sal Moyano et al., 2012; Gonçalves et al., 2016, 2017), predation by crabs on the medusa (parasitism/predation) (Gutsell, 1928; Jachowski, 1963; Shanks & Graham, 1988) and seasonal occupancy (Vaz Ferreira, 1972), have also been reported. According Gonçalves et al. (2020b) the ingestion of the nematocysts by the adults, comes from their symbiotic anemones that often try to steal the crab's food with its tentacles. The relationship between the crab and they anemones epibionts is mutualism is described (Winter & Masunari 2006).

However, even in the face of recent studies with this Majoidea *L. ferreirae* crab, the symbiotic relationships that it maintains during their life are still unclear, the benefit or neutrality for the species involved is questionable (Nogueira Jr & Haddad, 2005; Nogueira Jr. et al., 2006; Winter & Masunari, 2006; Hultgren & Stachowicz, 2011; Gonçalves et al., 2017; 2020b).

Stomach content analysis can clarify the type of relationship the animal has and is a good method to shows what the animal is using as a resource at the time it was sampled (Gonçalves et al., 2020b). But this analysis has limitations for identifying organisms that do not have rigid tissue, such as some Cnidaria, due to laceration when ingested and accelerated soft-tissue digestion. Therefore, to detect the assimilation of the food eaten months before sampling, and to detect food resources that are difficult to see and identify, stable isotope analyses (SIA) of nitrogen and carbon are used (Peterson & Fry 1987; Pitt et al. 2009). SIA is a helpful tool for tracing energy and nutrients from bottom producers to top predators, as well as for estimating trophic levels, resource use, and diet composition, showing as the food web functioning (Bearhop et al., 1999; 2004; Bouillon et al., 2008). As such, it can be a research tool to clarify symbiotic relationships (Fleming et al. 2014; Riascos et al. 2015; D'ambra et al., 2015; Ingram et al., 2017).

The ratio of carbon isotopes in the food web can move around little +1‰ (DeNiro & Epstein 1981; Peterson & Fry 1987; France & Peters 1997), typically used to distinguish carbon fixed by terrestrial C3 plants from that fixed by C4 plants or marine C3 plants (Peterson & Fry 1987). However, consumers are typically enriched by 3–4‰ for ratio of nitrogen, relative to their diets, commonly used to estimate trophic level (DeNiro & Epstein; 1981; Minagawa & Wada, 1984; Peterson & Fry, 1987; Bearhop et al., 2004). Discrimination factor estimates are the difficult determination, the

discrimination may vary because of the consumer's nutritional status, diet quality, lipid extraction, size, age, ontogeny, and tissue and elemental composition (Vanderklift & Ponsard, 2003). The use of fixed discrimination factors is much used because it is difficult to obtain species-specific discrimination factors for different diets.

Given this information, our work proposes to test through SIA of carbon and nitrogen the two hypotheses. (i) During the adult free-living stage, the crabs are both host and predator with respect to their relationship with cnidarian epibionts and (ii) During the juvenile stages the crabs do not derive any significant nourishment directly from their cnidarian hosts. Thus, we predict that i) crab host stable isotope signatures should be higher than their cnidarian epibionts and ii) than juvenile symbiont stable isotope signatures should be similar to those of their host cnidarians.

Material and methods

Sample collections were made on the Cananéia coast in the São Paulo State, Brazil (25º80′43″S, 47º85′34″W), over two seasons (winter/2018 and summer/2019). Representatives of the various taxa associated with the relationships described above (including potential food sources) were captured using a fishing boat outfitted with double-rig shrimp nets. Phytoplankton and zooplankton were collected with phytoplankton nets (diameter = 40 cm; mesh size = 60 µm), and then sorted in nets of 500 to 60 µm (adapted from Ingram *et al.* 2017).

The organisms were identified using the pertinent literature and taxonomic experts. The crabs *L. ferreirae* were classified into associated crabs (white crabs assocated with the jellyfish *L. lucerna*) and free-living crabs (that use different epibionts on their carapace and have a dark colour). Organisms used for isotopic analyses were transported in a box with ice to the laboratory, except for jellyfish that were live transported and in subsequently dissected for analyses.

Stable isotopic analysis -SIA

Where possible muscle tissue was used for stable isotope analyses. For fish symbionts living with associated crabs the muscle between the dorsal and caudal fins was selected. In the case of macroinvertebrates, the guts were discarded, and samples used were as follows: pedal mass for molluscs; abdominal muscle for shrimps; cheliped muscle for crabs; umbrella for jellyfish. All samples were dried at 50°C (in a greenhouse with

circulation and air renewal) until they reached constant weight, for 96 hours for gelatinous zooplankton and 48 hours for other samples (Nagata et al., 2015).

Dried samples were then homogenized via grinding to a fine powder in in a cryogenic mill. Samples of small organisms with a carapace, like *L. ferreirae* cheliped and zooplankton, for just for carbon analyses, were soaked in 1 M HCl for 3 hours. To remove carbonates and exoskeletons, and these samples were re-dried (Ingram et al., 2017). The homogeneous material was analysed in duplicate for $\delta^{13}C$ and $\delta^{15}N$, where it was placed in tin capsules (5.0 x 3.5 mm), except for the material with HCL, which used silver capsules inside tin capsules (Carabel et al., 2006; Mateo et al., 2008), the capsules containing being 50 – 70 μg for $\delta^{13}C$ and 500 – 700 μg for $\delta^{15}N$.

After weighing the capsules were measured using a continuous-flow isotope ratio mass spectrometry system at the Stable Isotopes Center of São Paulo State University in Brazil. The samples were placed on an elemental analyser (Flash 2000 - Thermo Scientific, Germany) coupled to an isotope ratio mass spectrometer (Delta V Advantage - Thermo Scientific, Germany) with an interface (ConFlo IV - Thermo Scientific, Germany) that determines the isotopic ratio (R_{sample} = $^{13}C/^{12}C$ or $^{15}N/^{14}N$) of each sample. The results are given in the relative difference of isotope ratio (δ) of a standard value ($R_{standard}$) expressed in ‰ accordance with:

$$\delta^{13}C \text{ or } \delta^{15}N = \frac{R_{sample}}{R_{standard}} - 1$$

For $\delta^{13}C$ the $R_{standard}$ is V-PDB and $\delta^{15}N$ is atmospheric air. The standard uncertainty (n=10) of the analyses was ±0.2‰ for $\delta^{13}C$ and ±0.15‰ for $\delta^{15}N$. Additionally, in the present study, the lipids were not chemically extracted from our samples. For this reason, the carbon-to-nitrogen (C: N) ratio was used as a proxy of lipid content, indicating the need to apply lipid extraction or correction to $\delta^{13}C$ values, if C: N >3.5 (Post et al., 2007), so, when necessary, the $\delta^{13}C$ values were mathematically corrected.

In our study some prey species were grouped according to ecological function as shown in Table 1 (Phillips et al., 2005), to facilitate the statistical analyses and the data visualization in isotope mixing models.

Statistical analyses

MANOVA was applied to test the differences in the isotopic signatures of $\delta^{13}C$ together $\delta^{15}N$ between the ecological groups and the species that have symbiotic relationship. Following this test, residue analysis was performed to verify the normality

in data distribution. When differences were found in the MANOVA test, we applied the analysis of variance (ANOVA), separately for $\delta^{13}C$ and $\delta^{15}N$, followed by the Tukey test to find if there is a difference in the isotopic signature between the guilds.

To estimate proportional contributions of potential food sources to the diet of *L. ferreirae* crab associated with the jellyfish, free-living crab and the *L. lucerna* medusa and the likely food sources for these animals, we used the Stable Isotope Mixing Model (SIMMr, version) analysis in R package, using a Bayesian statistical framework (Parnell et al. 2008, 2010; Parnell 2016; Parnell & Inger 2016). Package SIMMr allows the use of multiple diet sources with adjustable source-specific trophic fractionation factors.

As the Trophic Discrimination Factors (TDFs) have not been determined for the trophic relationships of interest, we used four different TDFs. These were as follows: generalized TDF published by Post (2002) (mean ± SD: $\Delta^{13}C = 0.4 \pm 1.3‰$; $\Delta^{15}N = 3.4 \pm 1.0‰$); crustacean TDF in Vanderklift & Ponsard (2003) (mean ± SD: $\Delta^{13}C = 1.4 \pm 1.0‰$; $\Delta^{15}N = 3.0 \pm 1‰$) and two by McCutchan et al. (2003) first for generalized TDF and second for consumers raised on other high-protein diets (carnivores) (mean ± SD: $\Delta^{13}C = 0.5 \pm 1.3‰$; $\Delta^{15}N = 2.3 \pm 1.5‰$; $\Delta^{13}C = 1.3 \pm 0.3‰$; $\Delta^{15}N = 3.3, \pm 0.3‰$) (Models A, B, C and D, respectively). These four sets of values helped us assess the sensitivity of the SIMMr model outputs to uncertainty in our TDFs (Riascos et al., 2015, Ingram et al., 2017). All statistical analyses were carried out using R software version 3.4.2 in package SIMMr (for isotopic analyses) and car, arm, lsmeans and multcompView (for MANOVA and Tukey test) (R Core Team, 2017), the level of statistical significance was set at 0.05.

Results

We analysed 16 associated *L. ferreirae* crabs (4.21 - 20.46 mm carapace width) found inside the medusa in the gastric cavities, 3 free-living crabs (53 - 68.53 mm carapace width) and 7 *L. lucerna* jellyfish (53 - 138 mm bell diameter). We combined the isotopic data from the various potential dietary items into six functional groups to assess the trophic interactions that may exist between crabs and jellyfish (Fig 1, Table 1).

Table 1. Isotopic values for all organisms separated in guilds groups collected through trawl fishing at sea of Cananéia in the São Paulo State, Brazil (25º80′43″S, 47º85′34″W), N spe. number of the organisms sampled; N rep. number of samples repetition, mean of isotopic values of $\delta^{13}C$ and $\delta^{15}N$, standard deviation (±), FL: free-living, ASS: associated.

Marine organisms	N spe.	N rep.	δ ^{13}C	δ ^{15}N	C:N
Lychnorhiza lucerna Haeckel, 1880	7	6	-20.46 ±1.07‰	15.11 ±0.63‰	4.79
Chloroscombrus chrysurus (Linnaeus, 1766)	2	4	-19.16 ±0.52‰	12.52 ±1.37‰	2.5
Libinia ferreirae Brito Capello, 1871 - FL	3	6	-17.79 ±0.12‰	12.72 ±0.71‰	3.4
Libinia ferreirae Brito Capello, 1871 - ASS	16	8	-20.96 ±1.02‰	11.34 ±0.36‰	3.5
Zooplankton					
Zooplankton 500-60µ	-	18	-22.64 ±1.50‰	11.91 ±1.78‰	3.66
Peisos petrunkevitchi Burkenroad, 1945	20	4	-20.11 ±0.06‰	11.04 ±0.35‰	3.59
Benthic Algae					
Bryopsis sp. J.V.Lamouroux, 1809	1	2	-19.70 ±0.06‰	9.08 ±0.08‰	-1.67
Ulva sp. Linnaeus, 1753	1	2	-20.24 ±0.31‰	8.25 ±0.44‰	-0.45
Suspensivoros					
Calliactis tricolor (Le Sueur, 1817)	1	2	-19.08 ±0.16‰	12.38 ±0.26‰	1.66
Carcinactis dolosa Riemann-Zürneck, 1975	2	4	-19.59 ±1.51‰	12.25 ±0.66‰	2.09
Porcellana sayana (Leach, 1820)		2	-19.84 ±0.01‰	11.95 ±0.58‰	4.25
Generalist Shrimps					
Rimapenaeus constrictus (Stimpson, 1874)	2	4	-19.32 ±0.51‰	10.25 ±0.29‰	2.64
Sicyonia dorsalis Kingsley, 1878	2	4	-18.79 ±0.23‰	10.99 ±0.62‰	2.79
Xiphopenaeus kroyeri Heller 1862	2	4	-18.88 ±0.56‰	11.40 ±0.74‰	3.06
Mollusc pred/detri					
Olivancillaria urceus (Röding, 1798	2	4	-17.11 ±0.08‰	11.45 ±0.44‰	1.45
Phalium granulatum (Von Born, 1778)	2	4	-18.86 ±1.50‰	12.60 ±2.77‰	1.66
Filters					
Amathia sp.	2	4	-21.61 ±0.82‰	9.36 ±0.65‰	3
Chione cancellata (Linnaeus, 1767)	1	2	-18.71 ±0.03‰	9.81 ±0.39‰	2.17

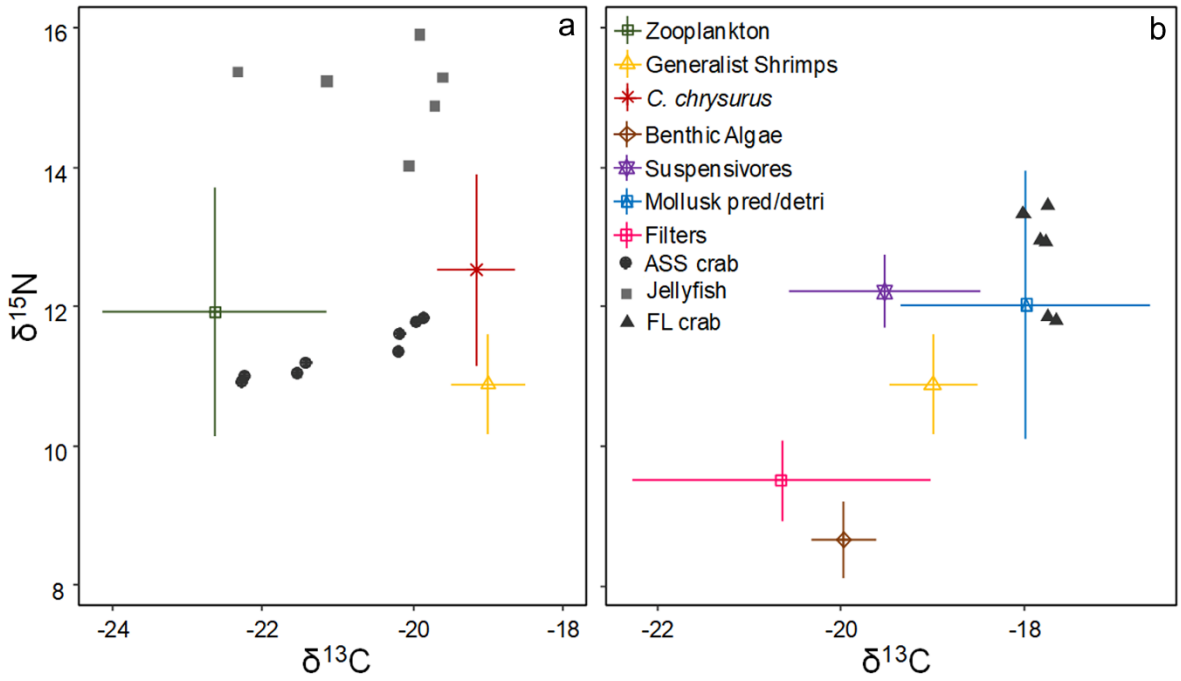


Figure 1. Isotopic biplot of variation in mean (±SD) $\delta^{13}C$ and $\delta^{15}N$ (‰) values calculated for a) *Libinia ferreirae* Brito associated with medusa *Lychnorhiza lucerna* and b) free-living crab with the epibionts together with their potential food sources.

We observed differences in the ecological groups and symbiotic species (MANOVA, F = 15.19, df = 9, p = 2.2e-16) in the isotope values (C;N, together). The residual analyses of our data showed normal distribution for the data. In the univariate analysis, $\delta^{13}C$ and $\delta^{15}N$ differed among the groups and animals that have symbiotic relationship (ANOVA, F = 26.02, df = 9, p = 2e-16 and F = 17.43, df = 9, p = 6.16e-11, respectively), these difference are shown in Fig 2. *Libinia ferreirae* associated and *C. chrysurus* fish symbionts not have statistically difference in $\delta^{15}N$ values between them, but the crab was different to the their *L. lucerna* host (jellyfish *L. lucerna* was ~3.77‰ more enriched in $\delta^{15}N$ than *L. ferreirae)*, however, the fish not have statistically difference in $\delta^{15}N$ values between the host jellyfish (Fig 2). However, *L. ferreirae* decreasing ~0.57‰ (not show statistic difference) in $\delta^{15}N$ than zooplankton and *L. lucerna* was ~3.30‰ more enriched in $\delta^{15}N$ than zooplankton, having statistic difference. *Libinia ferreirae* had ~0.5‰ decreasing in $\delta^{13}C$ than the medusae, not being statistically different, but was statistically different to the zooplankton, ~1.68‰ more enriched in $\delta^{13}C$. While *C. chrysurus* was ~1.30‰ and ~3.46‰ more enriched in $\delta^{13}C$ than the jellyfish and zooplankton, respectively, but was statistically different just for the zooplankton. The jellyfish was different in $\delta^{13}C$ than zooplankton, had ~2.18‰ more enriched (Fig 1 and 2). The free-living *L. ferreirae* and their epibionts grouped here as suspension feeders

shared similar $\delta^{15}N$ values (12.72‰ and 12.21‰, respectively). Even the carbon in *L. ferreirae* free-living was ~1.73‰ more enriched in $\delta^{13}C$ than their epibionts, not being statistically different for $\delta^{15}N$ or $\delta^{13}C$ (Fig 1 and 2). However, between *L. ferreirae* associated to the jellyfish and free-living have difference between the $\delta^{13}C$ but not between the $\delta^{15}N$ (Fig 2).

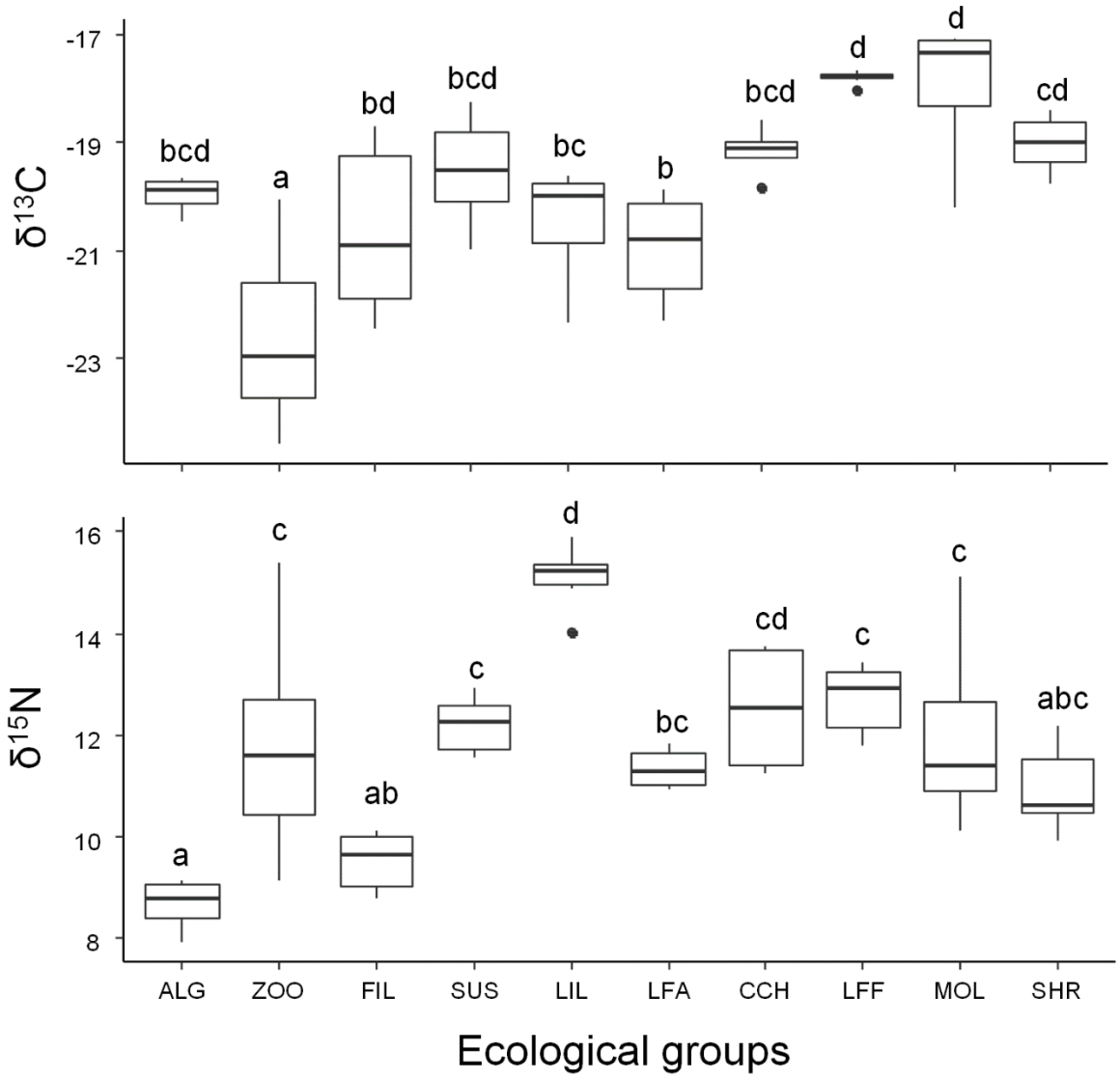


Figure 2. Variation in $\delta^{13}C$ and $\delta^{15}N$ between the species in association and ecological groups in the Cananéia sea, São Paulo Brazil. Median values are represented by central bars between the first quartile and third quartile, the ends of the whiskers representing minimum and maximum. Different letters indicate significant statistical differences (post hoc Tukey test).

All the SIMMr models (Fig 3) showed that the zooplankton had the greater contribution as a food source for the associated crabs (around 10-15%, credible interval (CI) = 95%)) followed by shrimps (around 10%, credible interval (CI) = 95%)). These

models indicated that the dietary contribution of *L. lucerna* was negligible (<5%), showing the crab is not a jellyfish's predator, because they ate the host's zooplankton resource. However,

All models (A, B, C and D, Fig 3) indicated similarity in food source contributions for free-living crabs and jellyfish. The jellyfish models indicated the zooplankton contributed the greatest amounts to the diet (around 17% - 27%, (CI = 95%)). Other diets sources probably made a minor contribution (<10%, CI = 95%), not showing evidence that use the symbiont crab as a food source. Benthic algae have the biggest contribution to the free-living crab diet (around 11 – 28% (CI = 95%)), followed by generalist shrimps and mollusc predator/detritivore (both <10%, CI = 95%). The epibionts of these crabs (suspension feeders group) were not used as food (Fig 3). These epibionts use the part of a food captured by a crab as a resource since it is possible to see the proximity between suspension feeder and free-living crabs in figure 1b.

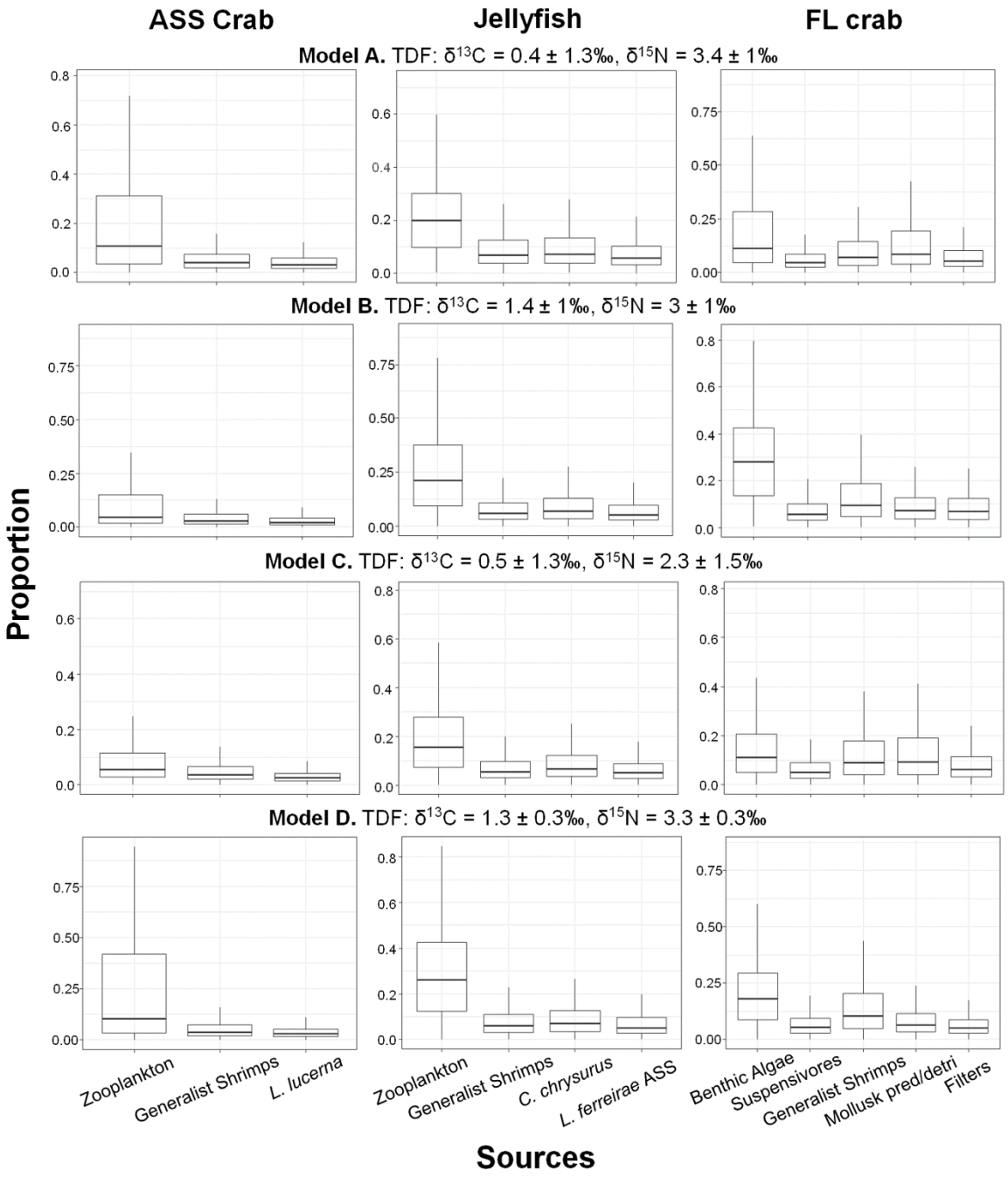


Figure 3. Median (horizontal line within the box), first and second quartile (boundaries of the box), and 95% credibility interval (whiskers of the box) of predicted proportion of food source contribution to the diets of *Libinia ferreirae* associated crab, free-living crab and for jellyfish *Lychnorhiza lucerna*, using different TDF values proposed in the literature: Post (2002); Vanderklift and Ponsard (2003) and two by McCutchan et al. (2003) (Models A, B, C, and D, respectively). ZOO = Zooplankton, BA = Benthic algae, SU = Suspensivore, GS = Generalist shrimps, MO = Mollusk predator/detritivore, FI = Filters.

Discussion

The ontogenic shift in symbioses shown by *L. ferreirae* have been described in recent years, but the forms that these relationships take have been unclear until now. We show that as a juvenile, the crab is unlikely to derive much in the way nourishment from its host and likely feeds on zooplankton and other planktonic organisms. As an adult host the diet comprises mostly benthic algae, small crustacea and molluscs, with the shell epibionts being relatively unimportant in the diet. As showed in our results differences in $\delta^{13}C$, but not for $\delta^{15}N$ between the associated and free-living crab, this show that they use different food resource during the different symbiotic behaviour.

In view of the results found in our study, we suggest that kleptoparasitism could be the crab-jellyfish's relationship, in which the symbiont steals the host's food resource (Iyengar, 2008). Due to the contribution of zooplankton to the associated crab and the jellyfish. This factor reinforces the importance of this symbiotic relationship for crab since in the benthic environment such a diet would not be available. In the other way, the occurrence of different isotopic values for the jellyfish and the symbiotic crab, can't confirm the kleptoparasitism relationship. Still, the contribution from zooplankton to the crab was still low, which shows that some source of food resources for this crab was not sampled in our study or the crab maybe selecting food items captured by the jellyfish. Further studies with a wider range of likely food sources for this symbiotic crab should be explored, to confirm the relationship. Gonçalves et al. (2020b) analysing the stomach content of crabs associated with jellyfish found a large amount of sediment, where they propose that this material can be accidentally consumed when adhering to prey or intensively for: food crushing and to use the organic material associated with the sediment. Still, the contribution of copepod crustaceans was quite representative in this study, with less occurrence of unicellular algae and molluscs in the planktonic phase (Gonçalves et al., 2020b). Thus, the hypothesis that the crab uses jellyfish's food resources was proved, instead, the proposal that this crab uses the jellyfish as a food source was refuted, because the isotopic signal from the associated crab was low than the host jellyfish.

Was possible see in our study that for the jellyfish's main food source was zooplankton, as expected according to Nagata & Morandini (2018). However, this jellyfish does not use symbiont crab as a food source, just like the symbiote fish. Nagata & Morandini (2018) found that dietary proportions in the gut of *L. lucerna*, were generally

similar to the proportions of these food items in the surrounding environment, mostly copepods (81%) in this instance.

The jellyfish demonstrate higher levels of nitrogen in this study, several environmental factors may be contributing to this but need to be tested, as proposed by Ferrier-Pagès & Leal (2019) that organisms with the aid of bacteria, can dissolve inorganic nitrogen sources, such as ammonium, nitrate, and urea, have a higher $\delta^{15}N$, incorporating around to 4–6‰. Thus, the jellyfish could be using ammonia excreted by its crab and symbiotic fish as a nitrogen resource, but studies need to be done to test this hypothesis. Even, particulate detrital or living nitrogen sources and dissolved organic sources taken up by animal hosts display an even higher $\delta^{15}N$ value 6‰ to14‰ (Corbisier et al., 2006; Sigman et al., 2009).

We also show that free-living *L. ferreirae* are generalists, similar proportions of the putative food items but with algae and, to a lesser extent, generalist shrimps being slightly more important. There is little evidence for any significant consumption of the suspension feeding epibionts, refuting the second hypothesis, was not showing representative contribution for epibionts in the free-living crabs diet. Thereby, the free-living crab *L. ferreirae* and its epibionts appear to exist in something more akin to a mutualistic relationship, as previously proposed by Winter & Masunari (2006) and Nogueira Jr. et al. (2006). During the free-living phase *L. ferreirae* and their epibionts have absence of differences in $\delta^{13}C$ and $\delta^{15}N$ between, showing that these symbionts assimilate similar food components than their host crab.

The sensitivity analyses indicated that our models were particularly robust to uncertainties around TDFs as they all produced qualitatively similar results. However, the absence of studies of TDF for a wide range of species is still a problem for studies such as ours, these parameters are necessary to minimize errors in the proposed estimates for the food resource use. Caut et al. (2009) compiling data show that the TDFs differed among the consumer classes for carbon for groups of mammals, birds, fishes, and invertebrates. Nitrogen appears to be a bit more predictable in this respect with mean $\Delta^{15}N$ between 2.5‰ and 3.0‰ (Caut et al., 2009).

The absence of difference between the jellyfish and the *C. chrysurus* symbiont for $\delta^{13}C$ and $\delta^{15}N$ suggest that these symbiont could use the same food resource than the host. Our results differ from the results of D'Ambra et al. (2015), which show that the relationship between the fish in the juvenile phase and the jellyfish is parasitism, because fish feed the jellyfish. However, in our study when analysing adult fish this relationship

may have changed to kleptoparasitism, given the absence of difference between $\delta^{13}C$ and $\delta^{15}N$ in the symbiont and host. Showing that the feeding behaviour of this fish can change throughout its development.

This study shows that the symbiotic relationship to the crab *L. ferreirae* keeps with the host jellyfish bring protection and feeding benefits to crab and fish, yet crabs can benefit from having a wide dispersion (Ohtsuka et al., 2009; Gonçalves et al., 2020a), which would not be possible with usual benthic development. Riascos et al. (2018), treat the jellyfish *Stomolophus meleagris* (Agassiz, 1862), as a floating nursery, because several animals can associate with this host, this provides potentially increases in the survival and recruitment of the symbionts to the adult population. However, these relationships to the host jellyfish may have their contrast, apparently having no benefit for the jellyfish, that has its food stolen and increased energy expenditure in hosted these crabs, this can affect their fitness. During benthonic life, crabs benefit from the protection of epibionts, by going unnoticed in the environment, so host and symbionts can exploit new resources and disperse new generations in different environments and the epibionts can benefit from their host's food scraps, clearly demonstrating the mutual benefits of this relationship. All of these symbiotic behaviour strategies have the objective to decrease competition and increase the survival of these animals.

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Chapter III

Trophic web of organisms caught through commercial shrimp fishing in southeastern of Brazil

Trophic web of organisms caught through commercial shrimp fishing in southeastern of Brazil

Abstract

Emerging anthropic issues related to global climate change, pollution and the exploitation of environmental resources through fishing require an urgent advance in the knowledge and understanding of the marine trophic web, where numerous organisms are involved in relationships along the structure of the food network. Understanding the trophic nodes and energy pathways are essential for understanding complex and highly biodiverse ecosystems. Isotopic analysis was applied to all organisms collected through commercial shrimp fishing (shrimp trawl fisheries), during winter and summer seasons, in the Cananéia region (southeastern Brazil), in order to: i) understand how the stable isotopes reflect the diet of these animals, ii) what is the trophic structure and isotope signature of these organisms, iii) estimate the niche overlap between the animals groups. An exceptionally high number of nodes in the food chain (78), divided into 11 guilds was analysed. Trophic nitrogen enrichment along the chain was ~ 7 δ and ~ 10 δ during the winter and summer, respectively. Three trophic levels (TL) were found in our study. Zooplankton and invertebrates were the main nodes responsible for transferring energy from the primary producers to other TLs, with fish being the main dominant consumers, where some species of fish and jellyfish were the top predators. Based on $\delta^{13}C$ signature, phytoplankton is one of the main responsible for sustaining the food chain in the region. Some guilds in the trophic webs showed seasons were statistically different for $\delta^{13}C$ and $\delta^{15}N$ between the seasons. An overlapping of equal guilds between winter and summer were found, showing that food resources have little change for vagile benthic invertebrates and demersal benthic fish. The other guilds show less overlap, indicating changes in food resources during the seasons. However, within each season, analysing the sob reposition between all guilds some overlap was found. This shows that species may be competing for resources, especially in winter. Crustaceans such as shrimps can be one of the key species in the transmission of energy along the food chain. Our study exhibit important results in the relationship of these organisms in the Cananía region, and due to climate change, pollution and the potential over-exploitation of fishing resources, changes to this food chain may have disruptive effects for the future. Thus, it is important to monitor the species within this trophic web continuously.

Keywords: stable isotopes, SIBER, food web structure, diet composition, trophic level

Introduction

In Brazil, coastal fisheries are of great benefit to the economy and society. The total annual landings of Brazilian coastal fisheries in 2010 were around 208,422 tonnes (INE, 2011). According to Ávila-da-Silva et al. (2018), caught coastal fish totalled 12,380 tonnes for the state of São Paulo in 2017. In Cananéia specifically this was around 1913 tonnes in the same year. The capture of shrimp by trawling is important to the local economy of Cananéia (Mendonça & Katsuragawa, 2001). However, shrimp's trawling has been demonstrated as a destructive method, changing the sea bottom environment and changing the behaviour of populations (Mendonça & Katsuragawa, 2001, Keller, 2005, Boss et al., 2016) and there are many cases of fish population destabilization related to the fisheries history (Myers et al., 1997).

According to the Food and Agriculture Organization of the United Nations (FAO, 2015), the bycatch is around 3 to 15 times larger than the total biomass of the tropical target species, i.e., crush and kill a high biomass of discarded animals because have little or no commercial value (Kaiser et al., 2000). The impact of this fishing pressure on the environment depends on its intensity and the robustness of the ecosystem. So, trawling can change the trophic structure of the community, where predator populations exhibit a lower mean trophic level (TL), as a result of the decline in the TL of their preys (Jannings et al., 2001; Pinner et al., 2002). Consequently, trawling can change the relative abundance of prey for benthic and demersal top predators (Kaiser et al., 2002) and the relative composition of prey assemblages that can affect the health and productivity of predator populations (Pruell et al., 2003), leading to a decline in the biomass, density, diversity, and size of individuals, and reductions in food-web connectivity (Johnson et al., 2015; Zeug et al., 2017).

The answers for recovering destructured communities lie in the understanding of the function - and consequently the structure - of species that compose these marine communities, as well as the impacts of fishing on the dynamics of interspecific interactions (Smith et al., 2011). Identifying and understanding the key species that may be threatened by fishing pressure is extremely important for the recovery of populations as well as for the subsistence of families that depend on fishing and the regional economy (Smith et al., 2011). Ecological studies in this area can help to restore these species, creating a balanced environment with guidelines and norms for fisheries that will not lead

to overexploitation and local extinction of countless species.

The estuary-marine complex of Cananéia, Iguape and Ilha Comprida (São Paulo state, Brazil) has highly important ecological environments with faunal and floral biodiversity's hotspots (Diegues, 1987) that provides nursery, refuge, and natural development areas for many species (Chevillot et al. 2019). The mangroves play a significant role in estuarine and coastal food webs and are among the most important habitats in tropical and subtropical estuaries in terms of primary production, exporting this primary production to adjacent habitats as leaves, detritus and dissolved organic matter (Alongi, 1998). These factors can cause an increase in primary production of phytoplankton and benthic microalgae (Flemming 2002). However, this ecosystem suffer from varying impactful factors such as waste, overfishing, habitat destruction, and bioinvasion which can decrease the populations of animals, such as macrobenthos and small crustaceans, which are essential to the cycling of energy and consequently the transfer energy from of organic matter within the trophic web (Faria and Jardim 2012; Chevillot et al. 2019).

Cananéia is impacted by the pressure of shrimp fisheries (Mendonça and Katsuragawa, 2001), where seabob shrimp *Xiphopenaeus* spp. is the main fishery resource in the region - 70% of the trawlers boats were adapted to fish it (Mendonça and Katsuragawa, 2001). Shrimp trawling kills countless other animals via accessory fishing (bycatch), of which most are involved in complex trophic webs.

Knowledge of the food-web structure in marine ecosystems is fundamental to giving insight into how energy is transferred from low trophic positions to upper trophic-level consumers (Schückel et al., 2015). Food web is a representation of the trophic interactions in an ecosystem, where "nodes" indicate species, and are connected by "pairwise interactions" or "links" (Dunne, 2009). Food web analysis has been a powerful tool to achieve a fisheries ecosystem approach as well as to understand the ecosystem under various historic and contemporary scenarios (Pauly et al., 1998).

The food sources and trophic niche overlaps can be characterized by stomach content data coupled with other descriptive indexes such as those for diversity and evenness (Bearhop et al., 2004). However, this shows only the food resources used around the time that the animals are sampled. Stable isotope analysis (SIA) is a method for tracing the energy flux through the trophic web, providing insightful information on the use of food resources at different time periods, depending on the type of tissue from the analysed animal (Newsome et al., 2007). SIA tools using carbon and nitrogen analysis have often

been used in ecological studies, as they present a straightforward mechanism for giving an overview of trophic levels, trophic iterations and consequently possible top predators and key species of a community. SIA integrates trophic studies' information from large time scales and proves accurate in identifying general sources of diet (Fry, 2006). It is worth noting that this analytical tool does not bring great damage to the environment, because the number of samples required is low, and in many circumstances, it is not necessary to sacrifice the organisms (Bearhop et al., 2004).

The carbon and nitrogen reflect the assimilation of the food and consequently the position of the trophic level and food habit of the animals (Bearhop et al., 2004). Stable isotope analysis benefits studies of resource competition in community structures and helps to characterize population-level connectivity, crucial for the successful conservation of migratory and/or rare species (Newsome et al. 2007). Nitrogen isotopes ($\delta^{15}N$) are a powerful signature for estimating an organisms' trophic position, exhibit dietary inputs, stepwise enrichment in the trophic transfers and trophic relationships. $\delta^{15}N$ values in the tissues of consumers tend to be between 2.5‰ and 5‰ greater than those of their diets (Bearhop et al. 2002) and $\delta^{15}N$ is an enrichment of around 3‰ (Post, 2002), but can have variation. Ratios of carbon isotopes ($\delta^{13}C$) can be used to determine ultimate sources of dietary carbon, varying among primary producers with different photosynthetic pathways (e.g., C3 vs. C4 plants) (Layman et al., 2007). $\delta^{13}C$ shows a tendency to increase in trophic levels too, but to a lesser degree than in the case of $\delta^{15}N$ (Fry, 2006).

The isotopic niche position of consumers in the isotopic space illustrates some aspects of habitat and resources used (Newsome et al. 2007). The isotopic values of the community members can be analysed to quantify aspects of their trophic ecology, such as species niche breadth and niche overlaps (Newsome et al. 2007, Jackson et al. 2011, Newsome et al. 2012). Analysing different species together and the responses that these species have in relation to changes in the disposition of food resources (prey) can bring clarifications at the community level (Bearhop et al., 2004). Thus, it is possible to observe the variation in niche overlap, the similarities in the use of resources and the trophic diversity in a food web (Layman et al., 2007a). Studies with a wide range of species allow for the verifying of different responses between generalist and specialist species, which is indicated by the breadth of the diet's niche. These types of studies provide an understanding of interactions and competitions for resources that change with the availability of prey (Chesson and Kuang, 2008).

We utilize $\delta^{15}N$ and $\delta^{13}C$ to investigate the food-web structure in the coastal region around Cananéia, an ecosystem exploited by trawling. We hypothesize that: 1) trophic structure of the organisms studied will change during winter and summer, changing the isotopic composition; 2) competition for resources are evidenced within season's studied, being higher in winter because the food resources tend to be lower; and 3) crustaceans are important links for transferring energy along the trophic web. Thus, our objectives are: i) to understand how the stable isotopes reflect the diet of these animals; ii) what is the trophic structure of these organisms between the winter and summer seasons; iii) how the animals' isotope signature varies between the seasons; and vi) to estimate the overlap of the niche between the animals groups during the winter and summer seasons.

Material and methods

Study area: Coastal area of Cananéia, in São Paulo state, Brazil (25°80'43"S, 47°85'34"W)

The estuary-marine complex of Cananéia, Iguape and Ilha Comprida (São Paulo state, Brazil) represents a remarkably diverse environment with high floral and faunal diversity recognized as an Atlantic Forest Biosphere Reserve by UNESCO (United Nations Educational, Scientific and Cultural Organization) since 2005. The mangrove ecosystem is the third-largest productive marine ecosystem in the South Atlantic. It is well-conserved and brings large amounts of nutrients and organic matter to the marine system, helping to maintain biodiversity (Diegues, 1987; Mendonça et al., 2010). However, these coastal regions suffer from urbanization and the growth of the human population, who dispose pollutants into this environment (Mendonça et al., 2010).

Sampling

The collections were carried in two seasons (winter/2018 and summer/2019), in a depth between 11 to 15 meters. During the sampling period, the surface temperature in the sea of Cananéia ranged from 20 to 26 °C and the salinity from 33 to 35, from winter and summer, respectively. The organisms involved in the trophic web were captured using a fishing boat outfitted with double-rig shrimp nets. Zooplankton were collected with phytoplankton nets (diameter = 40 cm; mesh size = 60 µm), and then sorted in nets of 500 to 60 µm (adapted from Ingram *et al.* 2017). The organisms were identified using pertinent literature and with the support of researchers that work with specific taxa.

Isotopic analysis

Muscle tissue material used for the isotopic analyses was removed from the organisms, similar to the muscle between the dorsal and caudal fins of a fish. For macroinvertebrates, the guts were discarded and samples from different tissues were used, including pedal mass for molluscs; abdominal muscle for shrimps; cheliped muscle for crabs; umbrella for jellyfish, among others. All samples were dried at 50° C (in a greenhouse with air circulation and renewal) until they reached constant weight, allowing 96 hours for gelatinous zooplankton and 48 hours for other samples (Nagata et al., 2015).

The dried samples were ground for six minutes in a cryogenic mill to complete homogenization. For carbon analysis, samples of organisms with a carapace, (e.g. crab chelipeds and zooplankton) were soaked in 1 M HCL for 3 hours to remove carbonates and exoskeletons, and these samples were re-dried (Ingram et al., 2017). The homogeneous material was analysed in pairs, in tin capsules (5 mm, Sercon), with the exception of the material with HCL, which used silver capsules inside tin capsules (Carabel et al., 2006; Mateo et al., 2008). The capsules contained 50 - 70µg of $\delta^{13}C$ and 500 – 700µg of $\delta^{15}N$.

After weighing, the capsules were measured using a continuous flow isotope ratio mass spectrometry system at the Stable Isotopes Centre of São Paulo State University in Brazil. The samples were placed on an elemental analyser (Flash 2000 - Thermo Scientific, Germany) with an interface (ConFlo IV - Thermo Scientific, Germany) that determines the isotopic ratio (R_{sample} = $^{13}C/^{12}C$ or $^{15}N/^{14}N$) of each sample. The results are given in the relative difference of isotope ratio (δ) of a standard value ($R_{standard}$) expressed in ‰ accordance with:

$$\delta^{13}C \text{ or } \delta^{15}N = \frac{R_{sample}}{R_{standard}} - 1$$

For $\delta^{13}C$ the $R_{standard}$ is V-PDB and $\delta^{15}N$ is atmospheric air. The standard uncertainty (n=10) of the analysis was ±0.2‰ for $\delta^{13}C$ and ±0.15‰ for $\delta^{15}N$.

To provide a broad overview of the trophic structure of organisms collected though shrimp trawling, the trophic level was estimated in relation to the lowest nitrogen values found for the primary producer sample (*Ulva* sp.), and sequentially the primary and secondary consumers. The trophic levels were calculated based on the study by Post (2002):

$$TL = \lambda + (\delta^{15}N_{consumer} - \delta^{15}N_{Ulva\ sp.})/\Delta$$

Where λ is the trophic position of the primary producer (*Ulva* sp.); $\delta^{15}N_{consumer}$ is the value of each consumer, which is measured directly; $\delta^{15}N_{Ulva\ sp.}$ is the value for this sample; Δ is the enrichment in $\delta^{15}N$ per trophic level. The constant enrichment factor is (Δ) of 3.4‰, according to Minagawa & Wada (1984).

We classified the species into 11 trophic guilds: primary producers (PPR), zooplankton (ZOO), gelatinous zooplankton (GZO), sessile benthic invertebrates (SesBI), sedentary benthic invertebrates (SedBI), vagile benthic invertebrates (VBI), benthic fish (BFI), demersal benthic fish (DBI), pelagic benthic fish (PBF), pelagic neritic fish (PNF), cephalopod (CEP) according to taxonomy and habitat (following Sherwood & Rose 2005).

Statistical analyses

MANOVA was applied to test the differences in the isotopic signatures of $\delta^{13}C$ together with $\delta^{15}N$ between the guilds and the seasons (winter and summer). The PPR was excluded from the analysis because it does not occur in both seasons. Following this test, residue analysis was performed to verify the normality in data distribution. When differences were found in the MANOVA test, we applied the analysis of variance (ANOVA), separately for $\delta^{13}C$ and $\delta^{15}N$, followed by the Tukey test to find if there is a difference in the isotopic signature between the guilds and seasons. The T-test was used to test the differences in the isotopic signature (C and N) for the species that occurred in both seasons. All statistical analyses were performed in R packages (car, arm, lsmeans and multcompView) and R studio (version 1.0.153, R Development Core Team). The level of statistical significance was set at 0.05.

To produce the biplot with the signatures of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) for the guild groups in the winter and summer the R package Stable Isotope Mixing Models, SIMMr, (Parnell and Inger, 2016) was used.

We used the Stable Isotope Bayesian Ellipses (SIBER) of $\delta^{15}N$ and $\delta^{13}C$ to allow us to make statistical comparisons of dispersion within and among communities and groups (Layman et al. 2007; Jackson et al. 2011). The trophic niches of the sampled organisms to the communities and guild groups were inferred from the 'isotopic niche space' occupied by each of the guild groups on a $\delta^{13}C/\delta^{15}N$ biplot and calculated as the Standard Ellipse Area (SEA). The SEA were probability tested to see if they were significantly different as well as comparing area overlap between the guild's groups. For

the analyses of the SEA, the groups that have ≥10 values of isotopes for both seasons were used, thus the guilds of PPR, SesBI, PBF and CEP were excluded from analyses.

The seasons (winter and summer), were used as a winter community and a summer community, where the total area (TA) and corrected standard ellipse area calculated (SEAc) were determined. These communities were also used for calculating dietary niche overlap, Bayesian Standard Ellipse Area (SEAb), and the analysis of niche breadth, for each guild group of each season community. To evaluate possible niche overlap between the same guilds between the seasons (winter and summer), and between the different guilds in the same season, we estimated the niche overlap of overlapping SEAc between these guilds group. The function maxLikOverlap returned three values each: the computationally calculated area of the first ellipse, second ellipse, and the overlap between them. The overlap as a proportion of the sum of the non-overlapping areas of the ellipses seems suitable in a general sense, since this will range from 0 when the ellipses are completely distinct, to 1 when the ellipses are completely coincidental, we use the overlap between the corresponding 95% prediction ellipses. These analyses were performed using SIBER, package R (Jackson et al., 2011; R Development Core Team 2011). The results were compared between guild groups and seasons based on analysis of the credibility intervals and showed in table and graphs. Similarities or dissimilarities between de guild groups in the same seasons or between winter and summer were showed through the degree of overlap between the Bayesian distributions (Abrantes et al., 2014).

Results

We analysed 78 different species and additionally 1 group of zooplankton, collected in two seasons (see Fig. 1 and Table S1 in the Supplement). These organisms were separated into 11 guilds, the more diverse guilds being VBI and DBF. The signatures of carbon and nitrogen (mean ± standard deviation) of the species of each trophic guild are shown in Fig. 1. It was possible to calculate three trophic levels (TL) for the species in both seasons (winter and summer) (Fig 1). The primary producers in our study are two algae species (*Ulva* sp. and *Bryopsis* sp.). Between the TL1 and TL2 were found species from SBI, SedBI, VBI (more abundant guild), BFI, DBF and PBF. Between TL 2-3 had animals of groups ZOO, ZGO, SedBI, VBI, BFI, DBF (more representative group), PBF, PNF and CEP. Finally, the TL >3 had GZO and DBF (Fig 1, Table supplementary 1). Mainly zooplankton and invertebrates are shown to be responsible for the transfer of

energy from these primary producers to other levels (Fig. 2). The animals in the higher TL were the demersal benthic fish *Genidens barbus* (Lacépède, 1803) and gelatinous zooplankton *Tamoya haplonema* (F. Müller, 1859) (Fig. 1).

The lowest values of carbon were for the zooplankton and the crab *Callinectes sapidus* Rathbun, 1896 and the highest values were found in the gastropod *Olivancillaria urceus* (Röding, 1798) and hermit crab *Isocheles sawayai* Forest & de Saint Laurent, 1968.

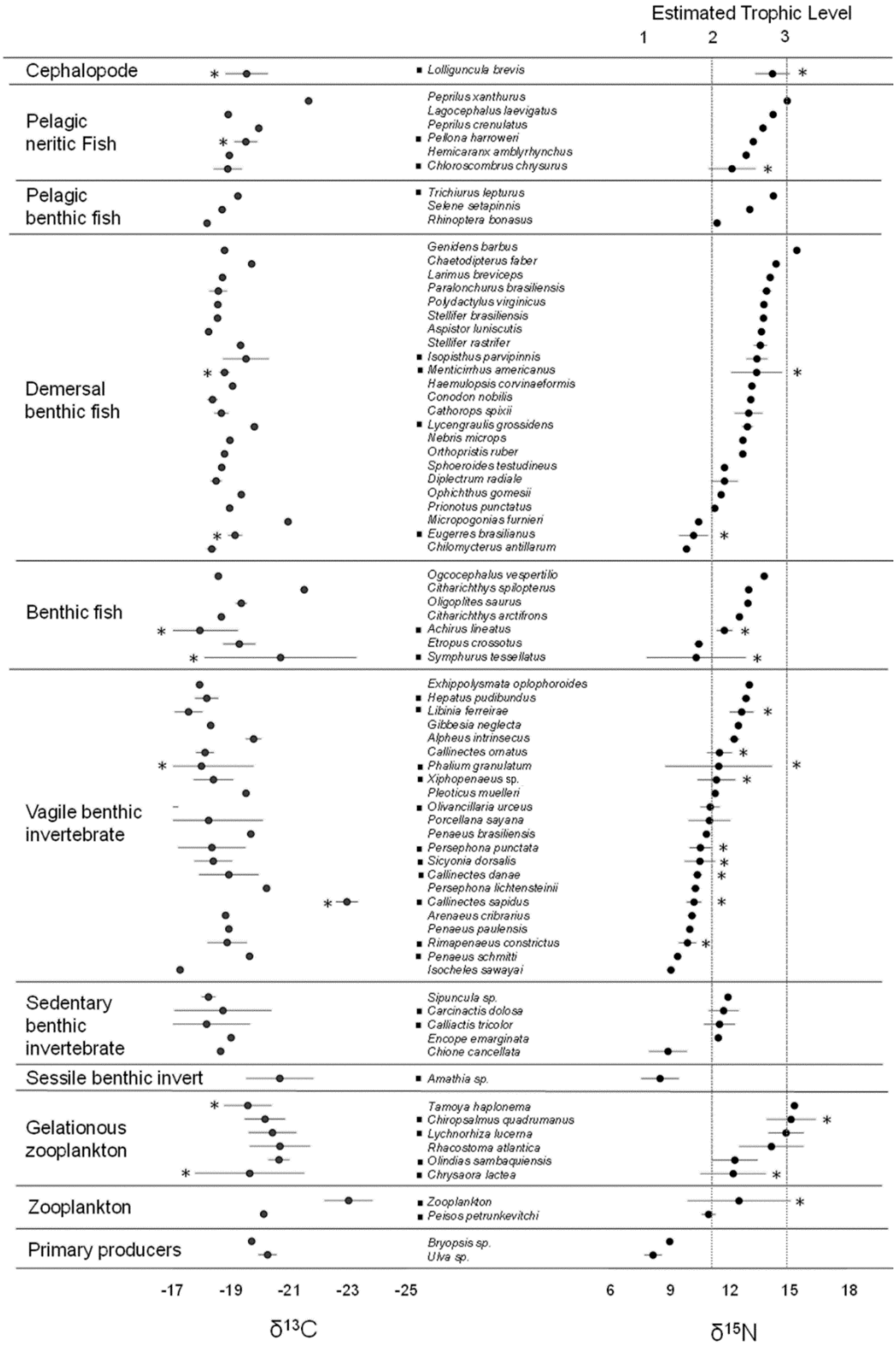


Figure 1. Isotope signatures of $\delta^{13}C$ and $\delta^{15}N$ (mean ± SD) for the organisms collected through trawl fishing of the Cananéia sea, São Paulo Brazil, separated in functional groups (guilds). Data presented for each trophic guild. TL = trophic-level estimates. Squares represent the species sampled in both stations and * = $p < 0.01$ (Student's t-test) represent differences between $\delta^{13}C$ and $\delta^{15}N$ of this species.

Trophic group

The guilds separated by seasons (Fig. 2) provide a general picture of the food web structure, where we found 49 and 60 species during winter and summer, respectively. Many species occurred only in one season (Fig. 1), e.g., the macroalgae (primary producer) was found only summer.

Indeed, when considering all the individuals together, the $\delta^{15}N$ values range from ~7‰ to more than ~17‰, showing a continuous enrichment of this isotope over the trophic web entire. ZOO, ZGE, SedBI, DBF and PNF had a $\delta^{15}N$ increase from winter to summer and ZGE, DBF and PBF had a $\delta^{13}C$ increase. In winter and summer, the $\delta^{13}C$ mean of ZGE was separated by ~2.23 δ and ~2.39 δ units, respectively, for the ZOO, and ~1.14δ (winter) and ~0.77δ (summer) of $\delta^{15}N$. The main primary producers were separated by ~1.71δ units of $\delta^{13}C$ for the VBI in the summer. The carbon and nitrogen values vary around ~4δ in winter and ~6δ in summer, from the organisms in the base of the trophic web to the top predators. All the guild consumers probably were aligned with phytoplankton carbon (with amplitude from -23 to -16‰) (Fig. 2). The guild groups show the more variation in the displacement of the groups regarding the elevation of nitrogen (carbon maintained its pattern), since in the summer the diversity of organisms, consequently food resources is higher and there is a greater supply of food resources.

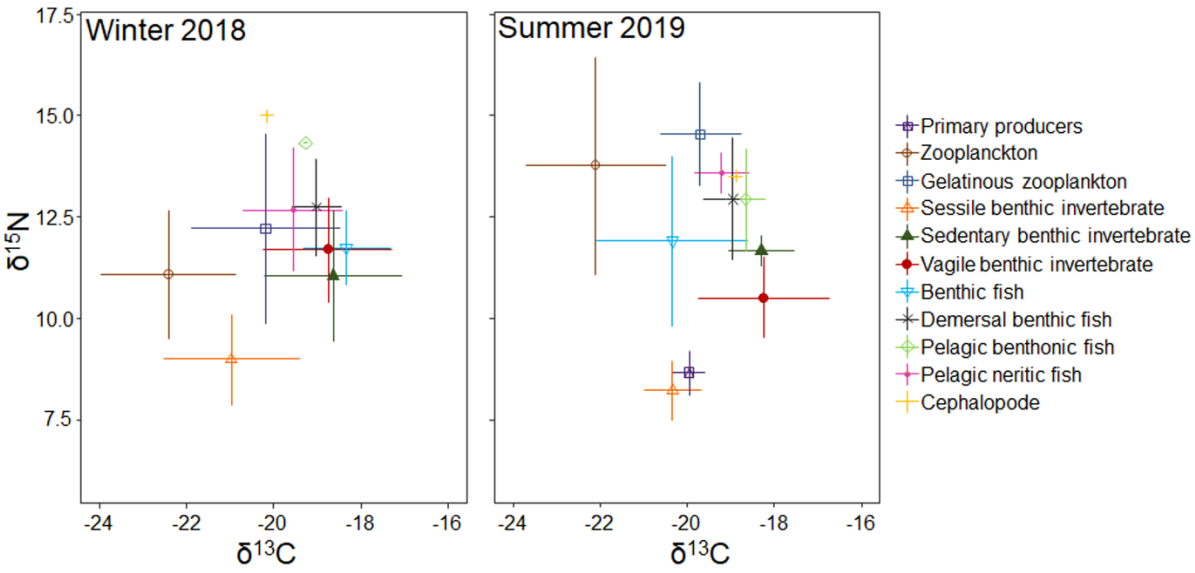


Figure 2. $\delta^{13}C$ and $\delta^{15}N$ (mean ± SD) estimates of trophic guild groups of consumers from the Cananéia sea Brazil, during the winter 2018 and summer 2019. The groups were defined in guilds by a similarity in the habitat (see Fig. 1)

We observed seasonal differences in the guilds (MANOVA, F = 5.66, df = 18, p = 2.96e-3) in the isotope values (C:N together). The residual analysis of our data showed normal distribution for the data. In the univariate analysis, $\delta^{13}C$ and $\delta^{15}N$ differed among the guilds (ANOVA, F = 2.58, df = 9, p = 0.003 and F = 5.35, df = 9, p = 0.013, respectively), the difference is shown in Figure 3. Seventeen species occurred only in the winter, and 28 were found only in summer (Table S1). For the 32 species that occurred in both seasons, 10 had differences in the $\delta^{13}C$ signatures, and 18 in the $\delta^{15}N$ signatures between the seasons (Fig. 1).

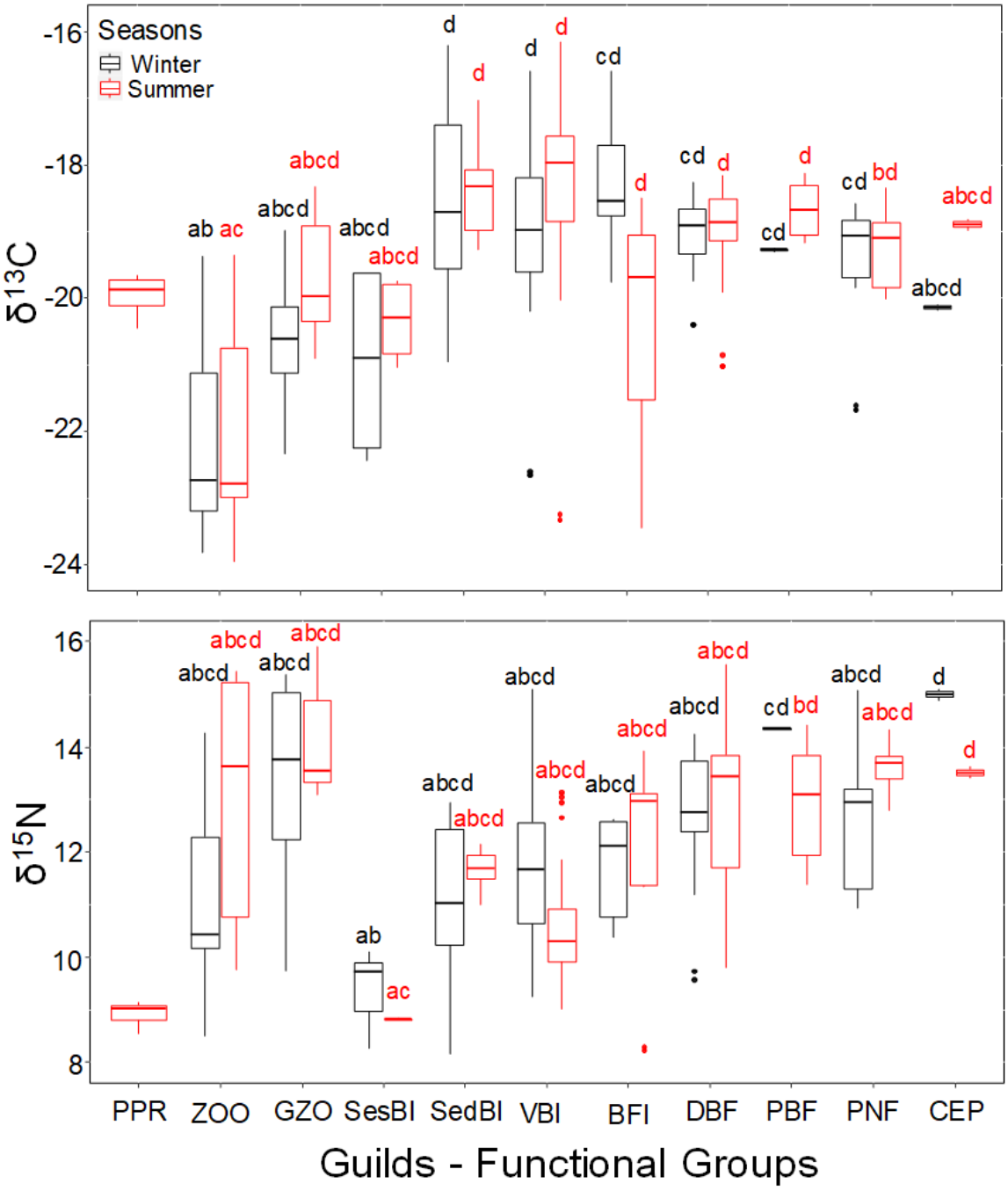


Figure 3. Variation in $\delta^{13}C$ and $\delta^{15}N$ between the guilds in winter/2018 (black) and summer/2019 (red) in the Cananéia sea, São Paulo Brazil. Median values are represented by central bars between the first quartile and third quartile, the ends of the whiskers representing minimum and maximum. Different letters indicate significant statistical differences (post hoc Tukey test).

The size of the TA and SEA of the guilds differed between the seasons. ZOO, ZGE, SedBI, VBI and PNF had higher TA and consequently higher SEA in winter than summer, but BFI and DBF had these higher parameters in the summer (Table 1).

The size of the SEAb and SEAc of the congener guilds differed between seasons,

and the winter had higher TA and SEA than the summer (Table 1). Bayesian Standard Ellipse Area generated large area for the same guild's groups during the seasons (Table 1 and Fig. 4). The largest and smallest niche overlap occurred in DBF and SedBI, respectively. In Fig. 4 we can see the SEAc of DBF, ZOO and VBF, had the greatest overlap. DBF had a large overlap between winter and summer, showing the isotopic niche did not change much during the seasons. However, ZOO, GZO, SedBI, BFI and PNF had a slight overlap between the niches, but ZOO had increase in nitrogen in summer, which shows the niche differentiation of these groups during the seasons. Some groups have a small niche during the summer, probably due to the abundance of a certain food.

Winter was the season with the highest overlap of the isotopic niche between the guilds, the largest being SedBI with VBI, followed by VBI with BFI. The summer had a smaller overlap than winter, and during this season ZGE with DBF and BFI with DBF showed the largest niche overlap. The isotopic niche overlap between distinct guilds showed a likely niche overlap and competition for resources.

Table 1. Trophic-niche width as the sample size-corrected. Total Area (TA), Standard Ellipse Area (SEA), Corrected Standard Ellipses (SEAc), Bayesian Standard Ellipse Area (SEAb) and credible intervals (95%), of the guild groups in two seasons Winter/2018 and Summer/2019. Showing their potential competitors, and the no overlapping are of the two ellipses based on 95% probability ellipse area of isotopic niche. Zooplankton (ZOO), gelatinous zooplankton (GZO), sedentary benthic invertebrates (SedBI), vagile benthic invertebrates (VBI), benthic fish (BFI), demersal benthic fish (DBI), pelagic neritic fish (PNF).

Winter 2018

						Overlap on SEAc (δ units^2)						
Guilds	N	TA	SEAc	SEAb	CI 95%	ZOO	ZGE	SedBI	VBI	BFI	DBF	PNF
ZOO	22	16.49	8.2	7.46	4.94							
ZGE	14	11.52	5.6	5	2.84	32.68						
SedBI	14	9.86	7.52	6.68	3.84	24.82	29.44					
VBI	46	23.56	6	5.78	4.29	24.06	27.89	62.84				
BFI	12	3.74	2.86	2.56	1.27	11.86	11.07	34.02	47.8			
DBF	28	5.91	2.15	2.01	1.38	10.18	20.49	24.96	31.13	36.6		
PNF	10	4.75	3.79	3.6	4.81	16.13	31.44	39.48	39.01	32.4	40.71	

Summer 2019

						Overlap on SEAc (δ units^2)						
Guilds		TA	SEAc	SEAb	CI 95%	ZOO	ZGE	SedBI	VBI	BFI	DBF	PNF
ZOO	14	13.42	8.86	8.66	4.81							
ZGE	14	5.02	3.23	2.99	1.7	10.91						
SedBI	10	0.98	0.79	0.67	0.35	2.14	11.48					
VBI	44	16.29	4.78	4.58	3.4	19.18	7.78	16.69				
BFI	10	13.36	8.65	8.03	3.84	25.25	23.61	6.18	20.62			
DBF	36	10.38	3.21	30.8	2.17	12.17	41.78	15.89	23.56	35.36		
PNF	10	1.33	1.04	0.83	0.45	3.89	32.23	3.65	2.79	12.05	29.01	

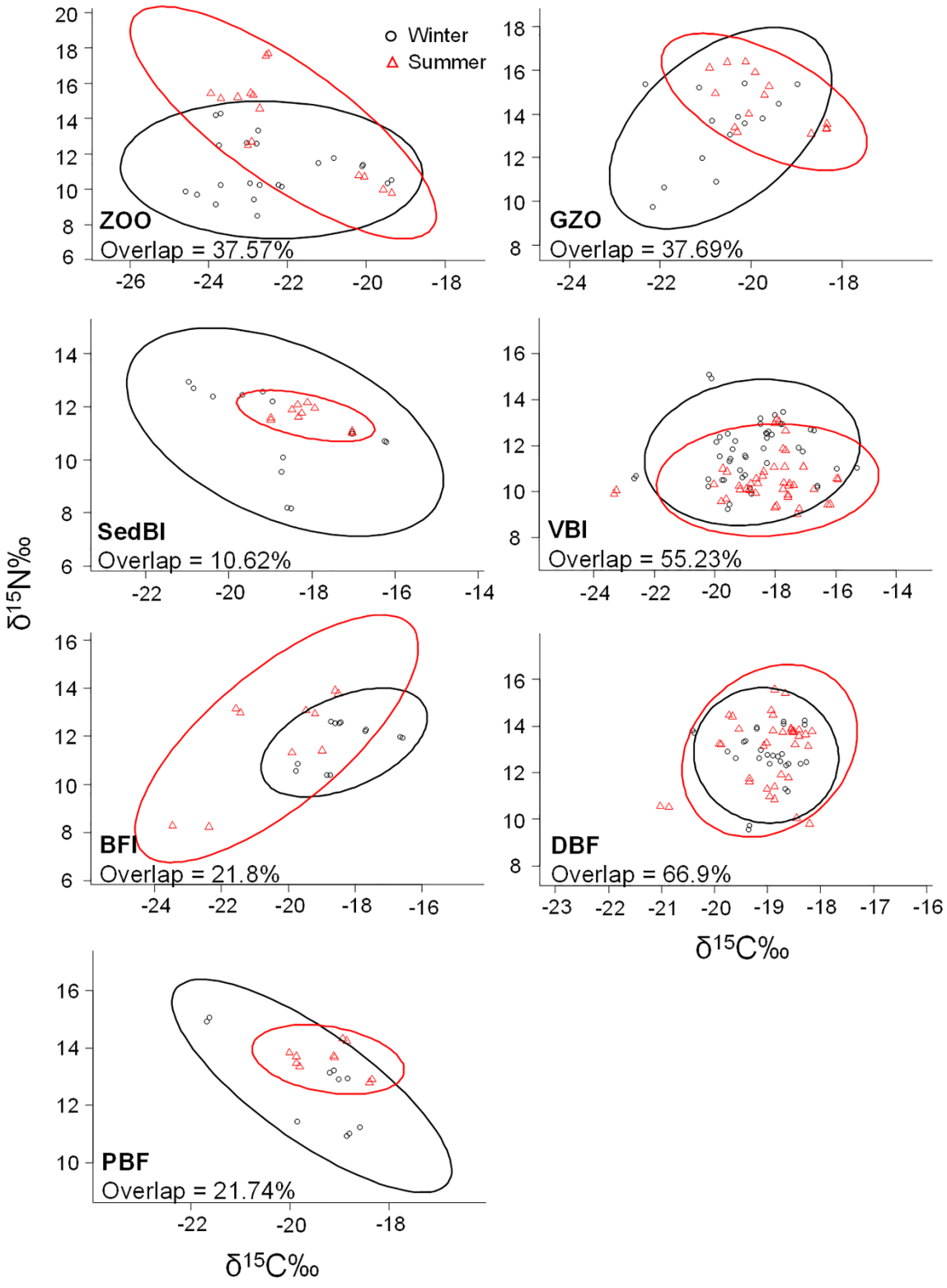


Figure 4. Isotopic niches of the functional guild groups between winter/2018 (black) and summer/2019 (red), calculated with standard ellipse area for small sample size (SEAc), show their isotopic niche non-overlapping for the same groups, from the Cananéia sea, São Paulo Brazil.

Discussion

We demonstrated a complex trophic network in Cananéia's sea, where it is possible to see how different species interact during different seasons, showing significant change in nitrogen and carbon composition within the trophic guilds. This demonstrates the variation in available resources as well as the difference in species composition between seasons, which confirms our first hypothesis. The species composition can change the structure of the trophic web in this region between the seasons, probably because a smaller range of food resources during the cold season, what generate less variation of nitrogen in the winter than in summer. The lowest food supply consequently created greater overlap between the guilds in winter.

The energy transfer in our study was represented by three trophic levels, as shown by Vinagre et al. (2018) in intertidal rocky shores in Ceará, Brazil. This shows that a great diversity of organisms found in the same environment will not necessarily mean a greater number of trophic levels; however, the region sampled here is a subset of a broader food network, which includes rays, sharks and cetaceans that were not sampled in this study. Nagata et al. (2015) found 4 trophic levels, but the top predator was represented by the Delphinidae mammal. In this study the second trophic level had the highest abundance of species, the top predators in the highest trophic levels were represented by the fish *Trichiurus lepturus* Linnaeus, 1758, *G. barbus* and a Cubozoa medusa *T. haplonema*.

Among the primary producers and the other groups, we had invertebrates as important nodes in the chain connecting energy to the other trophic levels, as already proposed in the work of Nagata et al. (2015). The isotopic signatures of primary producers were similar to others found in subtropical regions (Bouillon et al. 2002). The designation of the -19.40 ‰ value (by Barcellos et al. 2009) for the phytoplankton $\delta^{13}C$ is indirectly supported by the $\delta^{13}C$ of the shrimp *Peisos petrunkevitchi* Burkenroad, 1945 (-20.10 ‰), however our results (-23 to -16 ‰) were close to the expected range for phytoplankton in tropical regions (from -22 to -18 ‰) (Peterson & Fry 1987). As was found for Nagata et al. (2015), what observed the trophic web of his study aligned from phytoplankton carbon. Although isotope values were not recorded for organic matter, it is likely that they also provide an important source of food for animals that can eat directly from this source, as demonstrated by studies in shrimp and crabs (Willems et al. 2016; Chevillot et al. 2019). In the study of fatty acids for different shrimps in the same region (Gonçalves et al., in press), it is proposed that the suspended organic matter from the mangrove provides the

highest resuspension of phytoplanktonic cells, as well as nutrients deposited at the bottom, incrementally increasing the algal biomass, the base of the food chain.

The difference between the ZOO during the season was one of factors responsible for the changes between the structure of the trophic webs between the seasons. During the summer, primary productivity is higher, which provides a greater supply of food resources. Fast responses to the changing marine environment are found in the zooplankton, consisting of passively drifting small organisms and fish larvae (Taylor et al., 2002). At the base of the marine pelagic food web, they form the plant-animal interface and any change in the structure of this community is expected to propagate to higher trophic levels (Hays et al., 2005; Taylor et al., 2002). Changes in the phytoplankton are expected to cascade to the zooplankton and to higher trophic levels (Brett et al., 2009). An increase in phytoplankton and zooplankton biomass could have strong positive influence on the rest of the food web (Bueno-Pardo et al. 2018). As shown in this study, ZOO has higher values of $\delta^{15}N$ during the summer that reflect the enrichment for the most of other guild groups in this season. The ZOO enrichment in our study was reflected in the trophic elevation mainly of the jellyfish. In this study it was possible to see the higher amplitude for nitrogen in the GZO guild, the GZO represent the trophic level 2 and 3. *Chiropsalmus quadrumanus* (F. Muller, 1859) and *T. haplonema* were the top predators in this study. The elevated values of nitrogen in summer can be justified by the elevation in the nitrogen for zooplankton, due to greater primary production and consequently the reproductive period of countless species, such as the fish that have their spawning during this period.

As jellyfish feed on fish eggs and larvae, they may enact top-down control - this is a possible explanation for the inverse relationship between these populations (Möller, 1980). The different predation impact of jellyfish species make that jellyfish occupy a variety of trophic levels, as shown in this study, feeding on micro- and mesozooplankton, epibenthic invertebrates, other jellyfish, and fish (Purcell 1997), and potentially using the same resources as many other consumers (such as crustaceans and fishes). Our results show an overlap of more than 40% between the ZOG and DBF during the summer, demonstrating the use of the same resource in this season. The trophic overlap between planktivorous jellyfish and fish populations was demonstrated by Nagata eta al. (2015) and Shoji et al. (2009) with stable-isotope analysis. In ecosystems dominated by fish, jellyfish populations are kept in check through predation and competition (Richardson et al. 2009).

However, organisms such as SBI, SedBI, VBI and BF are probably not strongly influenced by this nitrogen enrichment found in the ZOO, as they probably use other food sources, such as: microalgae, pelagic particulate, organic matter or direct food sources found from benthic organic matter, such as debris, bacteria, dinoflagellates and others, as suggested of shrimp in this same region by Gonçalves et al. (in press). However, it is possible to see an increase in the trophic level of VBI to DBF, PNF, PBF and CEP, demonstrating that these groups of fish and cephalopods use some food resources from the group of VBI, which are mainly crustaceans. Gonçalves (unpublished) analysed the stomach of fish, including: *Oligoplites saurus* (Bloch & Schneider, 1801), *Conodon nobilis* (Linnaeus, 1758), *Paralonchurus brasiliensis* (Steindachner, 1875), *Menticirrhus americanus* (Linnaeus, 1758), *Isopisthus parvipinnis* (Cuvier, 1830), *Polydactylus virginicus* (Linnaeus, 1758) (all from the DBF guild), *T. lepturus* (PBF) and *Chloroscombrus chrysurus* (Linnaeus, 1766) (PNF). In the stomach of these fish, crustaceans as food resources were found, e.g., Sergestoidea (*Acetes americanus*), Penaeoidea (*Sicyonia dorsalis* and *Xiphopenaeus* spp.); Brachyura and Stomatopoda were found as a food resource only in the stomach of fish *P. brasiliensis* and *M. americanus*. In this way, it is possible to demonstrate how certain groups of species such as shrimp are extremely important as energy transfer nodes in this complex trophic web studied here.

The differences found between the seasons can be influenced by the seasonality of the species. We cannot rule out that some fish species moved into our study locations shortly before the sample collection, but the isotopic signatures for carbon did not indicate this. Fish can move among habitats and this could reflect different isotopic baseline for the carbon, with the characteristics of the previously occupied habitat (Silberberger et al., 2018). However, our data did not show a general great breadth or different patterns for ^{13}C, thus, migration can be excluded as a possible explanation.

Differences between the guilds are shown in our results, where the food sources change over the seasons. The amplitude of the guilds between the seasons was higher in the summer (~10 δ), than in the winter (~7 δ). Larger niche for some groups can show the versatility in the feeding habit of these species (Nagata et al., 2015). Groups of predators exhibited a narrowing of the niche breadth in the diet, indicating reduced prey diversity (Layman et al., 2007a).

The niche overlap between equal guild groups from different seasons (i.e. ZOO in winter and summer) shows us that some of the groups tend to use the same resources during the seasons, however most groups have niche differentiation during the seasons

(Fig. 4). According to Bearhop et al. (2004), niche parameters can respond very rapidly to changes in intraspecific and interspecific competition as well as prey abundance. During the same season, it was possible to observe that different groups of guilds have niche overlap (Table 1), with the greatest overlaps being found in winter. This was as expected, since in colder temperatures the diversity of resources tends to decrease, so some groups have similarity in the use of resources. This answered our second hypothesis that the competition for resources was evidenced within the seasons studied, with more competition in winter.

Some species that occurred in both seasons showed differences in their isotopic signature, in our study, they may also be influenced by changes in the supply of resources in the environment or they may have differed due to the age and / or size of the organisms. The narrowing of food niches occurs according to changes in the disposition of key species of prey in the environment (Gulka et al., 2017). Theoretically, the food partitioning processes in an environment with limited resources occur in functionally related species (Losos, 2000). However, when resources are abundant, niche overlap (i.e., dietary overlap) between species may not trigger interspecific competition but instead the sharing of prey (Lucena et al., 2000). As Gonzales et al (2019) showed, there are more pronounced SEAc overlaps between snook fish species during the rainy season, which may be attributable to a broader availability of prey during this period. Other studies have found that population niche width expands when resources become scarce or intraspecific competition otherwise increases (Svanbäck & Persson 2004).

Key species in environments are nodes of connection and transmit energy to a wide range of organisms, as well as those that control certain populations. Determining key species in environments with great diversity of organisms is difficult and requires long periods of study. However, here we propose that one of the key bottom-up species is the Sergestoidea *P. petrunkevitchi* component of zooplankton. It is an energy transfer link, and this small shrimp is a food source for countless fish and crustaceans, as was proposed by our third hypothesis. We cannot forget the diversity and abundance of the other most abundant species of shrimp in the region: *Xiphopenaeus* spp., *Penaeus schmitti* Burkenroad, 1936; *Penaeus brasiliensis* Latreille, 1817; *Penaeus paulensis* (Pérez Farfante, 1967). The shrimp found in this trophic web are also important in the transfer of energy from the benthos through the use of food resources found in organic matter (Gonçalves et al., (in press); Willems et al., 2016). Segundo Willems et al. (2016) noted that the benthic microalgae from intertidal mudflats and offshore sedimentary organic

matter were important primary food sources for shrimp *Xiphopenaeus kroyeri* (Heller, 1862), off the coast of Suriname. *Xiphopenaeus kroyeri* is the main epibenthic organism found at high densities in nearshore waters in Suriname, the species plays a crucial role in transferring energy from low trophic level prey and primary food sources up to higher levels in the food chain (Willems et al., 2016). Abarca-Arenas et al. (2007) showed that the shrimp is a species with an important position in the food web, being a food for many different predators. This means that penaeids could be responsible for the structural and functional cohesion of the food webs. Shrimps, besides being an important human resource, are an important link in the trophic network of the area, for this reason the depletion of shrimp biomass could negatively impact the overall community structure and function of the area (Abarca-Arenas et al. 2007).

In a study of the food chain in southern Brazil, Bornatowski eta al. (2016) highlight the importance of some key species such as crabs and squids that are closely connected with several species in the food web as opportunistic predators. They are linked through the energy transfer for higher predators. Squids, for instance, occupy a large range of trophic levels in marine food webs with large trophic width. Removing species that transmit energy to a wide range of other consumers can cause negative effects on marine ecosystems. The top predatory species that have top-down control are likely jellyfish and some fish (Nagata et al., 2015). However, this is difficult to determine in a region that suffers from fishing, the natural balance between species is prevented from occurring to analyse this factor.

We used the globally accepted value of $\Delta^{15}N$: 3.4‰ in sea studies (Minagawa & Wada 1984, Post, 2002). However, reviews demonstrated that ^{15}N in organisms can vary (from 2 to 4.5‰), and could be related to the consumer's nutritional status, diet quality, size, age, dietary ontogeny, and the biochemical form of nitrogen excretion (Minagawa & Wada 1984, Bearhop et al., 1999; 2004). For more accurate estimates of Trophic Enrichment Factors (TEF), C and N need to be experimentally studied in different taxonomic groups. Still, without the common application of 3.4‰, a scaled approach would hinder a direct comparison with most of the existing literature (Vander Zanden and Fetzer, 2007).

The species studied here suffer from great environmental pressure through non-selective fishing, which can lead to a drastic reduction and extinction of species due to overexploitation by fishing, causing a cascading effect on the other species in the trophic web (Boss et al. 2016). Mendonça and Katsuragawa (2001) and Mendonça et al. (2010)

showed a decline of animals fished over the past few years in the Cananéia region, indicating high levels of fishery effort, without taking into account the sustainable use and to allow them for reproduction for the species. In a study of trawled and untrawled environments (Funes, 2019), higher TLs and broader isotopic niches in the trawled environment were found, a factor that may be caused by the continuous discarding of fish. Long-term trawling can cause losses that are often irreversible in the environment.

With the importance of shrimps in the trophic web in mind, people responsible for fishery policies should consider the important role that shrimp has in the ecosystem. This work serves as a basis for the future monitoring of populations of species that occur in this environment, as well as the interactions along the food chain. Conservation of intertidal areas and their associated mangrove systems benefit coastal shrimp production and fisheries in tropical ecosystems.

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Table 1. Isotopic values for all organisms separated in guilds collected through trawl fishing along the 2 seasons (winter 2018 and summer 2019), at Cananéia Lagoon Estuarine System. n: number of samples analyzed, mean of isotopic values of ^{15}N and ^{13}C, standard deviation (SD) and, - no measurement.

Guilds	Seasons											
	Winter						Summer					
Primary producers	n	$\delta^{13}C$	SD ^{13}C	δ ^{15}N	SD ^{15}N	TL	n	$\delta^{13}C$	SD ^{13}C	δ ^{15}N	SD ^{15}N	TL
Bryopsis sp. J.V.Lamouroux, 1809	-	-	-	-	-	-	2	-19.70	0.06	9.08	0.08	1.25
Ulva sp. Linnaeus, 1753	-	-	-	-	-	-	2	-20.24	0.31	8.25	0.44	1.00
Zooplankton												
Zooplankton	16	-23.00	0.99	11.14	1.75	1.85	10	-23.04	0.47	15.16	1.70	3.03
Peisos petrunkevitchi Burkenroad, 1945	4	-19.76	0.39	10.88	0.54	1.77	4	-19.79	0.40	10.31	0.51	1.61
Gelatinous zooplankton												
Chiropsalmus quadrumanus (F. Muller, 1859)	2	-19.58	0.25	14.15	0.47	2.74	2	-20.72	0.27	16.24	0.19	3.35
Chrysaora lactea Eschscholtz, 1829 C	2	-22.04	0.18	10.18	0.63	1.57	4	-18.43	0.17	13.33	0.18	2.49
Lychnorhiza lucerna Haeckel, 1880 C	4	-20.97	1.01	14.51	0.91	2.84	6	-20.03	0.42	15.23	0.84	3.05
Olindias sambaquiensis Müller, 1861	2	-20.93	0.22	11.44	0.77	1.94	2	-20.34	0.04	13.28	0.14	2.48
Rhacostoma atlantica Agassiz, 1851	2	-20.67	0.29	13.38	0.45	2.51	-	-	-	-	-	-
Tamoya haplonema F. Müller, 1859	2	-19.57	0.81	15.36	0.02	3.09	-	-	-	-	-	-
Sessile benthic invertebrate												
Amathia sp.	4	-20.97	1.55	8.98	1.10	1.21	4	-20.35	0.66	8.21	0.71	0.99
Sedentary benthic invertebrate												
Calliactis tricolor (Le Sueur, 1817)	4	-17.65	1.64	11.53	0.99	1.97	2	-19.15	0.19	11.72	0.31	2.02
Carcinactis dolosa Riemann-Zürneck, 1975	4	-19.32	1.83	12.09	0.84	2.13	4	-17.66	0.72	11.36	0.38	1.91
Encope emarginata (Leske, 1778)	-	-	-	-	-	-	2	-18.99	0.00	11.53	0.07	1.97
Chione cancellata (Linnaeus, 1767)	4	-18.63	0.10	9.00	0.97	1.22	-	-	-	-	-	-
Sipuncula sp	-	-	-	-	-	-	2	-18.22	0.24	12.02	0.12	2.11
Vagil benthic invertebrate												
Olivancillaria urceus (Röding, 1798)	4	-16.39	0.93	11.42	0.48	1.93	4	-16.50	0.65	10.82	0.31	1.75

Phalium granulatum (Von Born, 1778)	2	-20.15	0.06	15.00	0.14	2.98	4	-16.89	0.79	9.83	0.45	1.46
Alpheus intrinsecus Spence Bate, 1888	2	-19.76	0.27	12.33	0.25	2.20	-	-	-	-	-	-
Arenaeus cribrarius (Lamarck, 1818)	-	-	-	-	-	-	2	-18.81	0.01	10.21	0.21	1.58
Callinectes danae Smith, 1869.	2	-19.04	0.06	10.67	0.06	1.71	2	-18.79	1.75	10.29	0.03	1.60
Callinectes ornatus Ordway, 1863	2	-18.33	0.07	12.11	0.31	2.14	2	-17.87	0.25	11.07	0.01	1.83
Callinectes sapidus (Rathbun, 1896)	2	-22.63	0.03	10.62	0.07	1.70	2	-23.28	0.06	9.99	0.11	1.51
Gibbesia neglecta (Gibbes, 1850)	2	-18.29	0.03	12.54	0.03	2.26	-	-	-	-	-	-
Exhippolysmata oplophoroides (Holthuis, 1948)	-	-	-	-	-	-	2	-17.92	0.06	13.09	0.07	2.42
Hepatus pudibundus (Herbst, 1785)	2	-18.48	0.00	13.06	0.17	2.41	2	-17.84	0.26	12.80	0.21	2.34
Isocheles sawayai Forest & de Saint Laurent, 1968	-	-	-	-	-	-	2	-17.25	0.05	9.15	0.18	1.26
Libinia ferreirae Brito Capello, 1871	6	-17.48	0.56	13.00	0.33	2.40	2	-17.70	0.06	11.83	0.04	2.05
Penaeus schmitti Burkenroad, 1936	2	-19.55	0.04	9.34	0.14	1.32	2	-19.71	0.12	9.63	0.08	1.41
Penaeus brasiliensis Latreille, 1817	-	-	-	-	-	-	2	-19.67	0.11	10.94	0.11	1.79
Penaeus paulensis (Pérez Farfante, 1967)	-	-	-	-	-	-	2	-18.92	0.06	10.09	0.03	1.54
Persephona lichtensteinii Leach, 1817	2	-20.21	0.01	10.38	0.21	1.63	-	-	-	-	-	-
Persephona punctata (Linnaeus, 1758)	2	-18.72	0.62	11.08	0.22	1.83	2	-17.95	1.74	10.17	0.10	1.56
Pleoticus muelleri (Bate, 1888)	2	-19.50	0.03	11.37	0.07	1.92	-	-	-	-	-	-
Porcellana sayana (Leach, 1820)	4	-18.22	1.86	11.08	1.06	1.83	-	-	-	-	-	-
Rimapenaeus constrictus (Stimpson, 1874)	4	-19.27	0.53	10.27	0.28	1.59	4	-18.44	0.58	9.68	0.39	1.42
Sicyonia dorsalis Kingsley, 1878.	2	-18.99	0.02	11.52	0.07	1.96	4	-18.08	0.60	10.14	0.38	1.56
Xiphopenaeus sp. Smith, 1869	4	-18.77	0.70	12.29	0.34	2.19	4	-18.01	0.46	10.58	0.25	1.68
Benthic fish												
Achirus lineatus (Linnaeus, 1758)	4	-17.16	0.62	12.10	0.18	2.13	2	-19.45	0.63	11.37	0.06	1.92
Citharichthys arctifrons Goode, 1880	2	-18.66	0.08	12.59	0.03	2.28						
Citharichthys spilopterus (Günther 1862)	-	-	-	-	-	-	2	-21.50	0.09	13.06	0.12	2.41
Etropus crossotus Jordan & Gilbert, 1882	4	-19.27	0.55	10.55	0.22	1.68	-	-	-	-	-	-
Ogcocephalus vespertilio (Linnaeus,1758)	-	-	-	-	-	-	2	-18.55	0.07	13.84	0.09	2.64
Oligoplites saurus (Bloch & Schneider, 1801)	-	-	-	-	-	-	2	-19.34	0.20	13.02	0.09	2.40
Symphurus tessellatus (Quoy & Gaimard, 1824)	2	-18.47	0.04	12.58	0.02	2.27	2	-22.91	0.77	8.26	0.03	1.00

Demensal benthic fish												
Cathorops spixii (Agassiz, 1829)	2	-18.86	0.13	12.44	0.07	2.23	-	-	-	-	-	-
Aspistor luniscutis (Valenciennes, 1840)	-	-	-	-	-	-	2	-18.22	0.08	13.70	0.08	2.60
Cathorops spixii (Agassiz, 1829)	-	-	-	-	-	-	2	-18.47	0.09	13.66	0.13	2.59
Chaetodipterus faber (Broussonet, 1782)	-	-	-	-	-	-	2	-19.70	0.05	14.44	0.04	2.82
Chilomycterus antillarum Jordan & Rutter, 1897	-	-	-	-	-	-	2	-18.33	0.17	9.93	0.17	1.49
Conodon nobilis (Linnaeus, 1758)	-	-	-	-	-	-	2	-18.36	0.18	13.17	0.05	2.45
Diplectrum radiale (Quoy & Gaimard, 1824)	4	-18.48	0.19	11.84	0.68	2.06	-	-	-	-	-	-
Eugerres brasilianus (Cuvier, 1830)	2	-19.35	0.01	9.66	0.12	1.41	2	-18.92	0.06	10.91	0.08	1.78
Genidens barbus (Lacepède, 1803)	-	-	-	-	-	-	2	-18.77	0.13	15.48	0.12	3.13
Haemulopsis corvinaeformis (Steindachner, 1868)	-	-	-	-	-	-	2	-19.05	0.04	13.22	0.09	2.46
Isopisthus parvipinnis (Cuvier, 1830)	2	-19.77	0.73	13.28	0.58	2.48	2	-18.97	0.81	13.86	0.02	2.65
Larimus breviceps Cuvier, 1830	2	-18.70	0.01	14.14	0.08	2.73	-	-	-	-	-	-
Lycengraulis grossidens (Spix & Agassiz, 1829)	2	-19.68	0.11	12.78	0.19	2.33	2	-19.90	0.02	13.22	0.03	2.46
Menticirrhus americanus (Linnaeus, 1758)	2	-18.63	0.04	12.35	0.07	2.21	2	-18.91	0.03	14.57	0.16	2.86
Micropogonias furnieri (Desmarest, 1823)	-	-	-	-	-	-	2	-20.94	0.11	10.54	0.01	1.67
Nebris microps (Cuvier. 1830)	2	-18.95	0.07	12.77	0.02	2.33	-	-	-	-	-	-
Ophichthus gomesii (Castelnau, 1855)	-	-	-	-	-	-	2	-19.34	0.00	11.67	0.10	2.01
Orthopristis ruber (Cuvier, 1830)	2	-18.77	0.05	12.76	0.08	2.33	-	-	-	-	-	-
Paralonchurus brasiliensis (Steindachner, 1875)	2	-18.30	0.01	14.16	0.13	2.74	2	-18.81	0.13	13.76	0.06	2.62
Polydactylus virginicus (Linnaeus, 1758)	-	-	-	-	-	-	2	-18.54	0.02	13.83	0.10	2.64
Prionotus punctatus (Bloch, 1793)	-	-	-	-	-	-	2	-18.94	0.10	11.35	0.06	1.91
Sphoeroides testudineus (Linnaeus, 1758)	-	-	-	-	-	-	2	-18.67	0.10	11.85	0.10	2.06
Stellifer brasiliensis (Schultz, 1945)	-	-	-	-	-	-	2	-18.53	0.04	13.80	0.06	2.63
Stellifer rastrifer (Jordan, 1889)	4	-19.32	0.13	13.65	0.34	2.59	-	-	-	-	-	-
Pelagic benthic fishs												
Rhinoptera bonasus (Mitchill, 1815)	-	-	-	-	-	-	2	-18.17	0.04	11.47	0.14	1.95
Selene setapinnis (Mitchill, 1815)	-	-	-	-	-	-	2	-18.69	0.02	13.11	0.01	2.43
Trichiurus lepturus Linnaeus, 1758	2	-19.28	0.04	14.35	0.02	2.80	2	-19.17	0.01	14.25	0.26	2.76

Pelagic niritic fishs												
Chloroscombrus chrysurus (Linnaeus, 1766)	4	-19.03	0.56	11.16	0.23	1.86	4	-18.73	0.43	13.28	0.49	2.48
Hemicaranx amblyrhynchus (Cuvier, 1833)	2	-18.93	0.13	12.94	0.02	2.38	-	-	-	-	-	-
Lagocephalus laevigatus (Linnaeus, 1766)	-	-	-	-	-	-	2	-18.90	0.06	14.29	0.05	2.78
Pellona harroweri (Fowler, 1917)	2	-19.16	0.06	13.18	0.06	2.45	2	-19.84	0.05	13.41	0.08	2.52
Peprilus crenulatus Cuvier, 1829	-	-	-	-	-	-	2	-19.94	0.10	13.78	0.10	2.63
Peprilus xanthurus (Quoy & Gaimard, 1825)	2	-21.65	0.04	15.00	0.10	2.99	-	-	-	-	-	-
Cephalopod												
Lolliguncula brevis (Blainville, 1823)	2	-20.15	0.06	15.00	0.14	2.99	2	-18.90	0.11	13.51	0.16	2.55

Chapter IV

Species-specific fatty acid profiles of three consumption shrimp from Southeastern Brazil reveal their role in a complex local food web

Species-specific fatty acid profiles of three consumption shrimp from Southeastern Brazil reveal their role in a complex local food web

Abstract

Estuaries and mangroves are transition areas where numerous species are maintained and raised. In view of the intensive fisheries and environmental changes, this study aimed to describe the fatty acid (FA) composition of shrimp species that form part of fished resources within one of the largest mangrove forests in Brazil. Through this knowledge the nutritional quality of the shrimp, as well as its importance and role in the transfer of energy in the trophic web will be evidenced. The fatty acid profile of three shrimps with different life cycles were analysed: *Xiphopenaeus* spp., *Penaeus schmitti* and *Penaeus paulensis*, in the region of Cananéia, São Paulo, Brazil. Overall, 24 different FAs were found for all species, with the FAs 16:00, 18:00, 18:1ω9, 20:4ω6, 20:5ω3 and 22:6ω3 being the most abundant. There was a species-specific difference in the abundance of the FAs. FAs 16:0, 18:0, 20:5ω3 and 22:6ω3 were responsible for significant differences between these species. The restricted occurrence of *Xiphopenaeus* spp. in the marine part of the study region explained the difference in absolute FA concentration with the other shrimp species. The study highlights also that these shrimps are important sources of FAs that are essential to human health such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

Keywords: benthic consumers, *Xiphopenaeus* spp., *P. schmitti*, *P. paulensis,* mangrove

Introduction

Coastal regions and estuaries are productive ecosystems that receive input of organic matter and nutrients from various sources that finally flow to the ocean. These factors cause an increase in the growth of phytoplankton and the primary production from benthic microalgae (Flemming, 2002). Associated with many ecological functions, these regions and estuaries provide nursery, refuge, and growing areas for many species (Chevillot et al., 2019). Estuaries can be considered essential to the establishment of many organisms, since this ecosystem is a natural habitat for crustaceans, fish, birds and mammals; a migration route for fish; a spawning region, and home to highly diverse fauna (Miranda et al., 2002). However, these systems suffer from varying impactful factors such as pesticides, toxic elements, waste, overfishing, habitat destruction, acidification of the

water and bioinvasion. These factors can decrease the populations of animals, such as macrobenthos and small crustaceans, which are essential to the cycling of energy and consequently transfer of organic matter within the trophic web (Faria & Jardim, 2012; Chevillot et al., 2019).

The Cananéia region in São Paulo state, Brazil has been recognized worldwide as the third largest productive marine ecosystem in the South Atlantic, due to its large mangrove and well-conserved environmental resources (Mendonça et al., 2010). The highly diversified environments with their faunal and floral biodiversity are of great ecological importance (Diegues, 1987). Despite of the recognition of this diversity and the importance of its conservation, this rich region is impacted by the pressure of shrimp fishery (Mendonça & Katsuragawa, 2001).

The shrimps commonly caught by commercially valuable fisheries in and off the coast of the Cananéia region are *Xiphopenaeus kroyeri* (Heller, 1862), *Penaeus schmitti* Burkenroad, 1936, *Penaeus paulensis* (Pérez Farfante, 1967) and *Penaeus brasiliensis* Latreille, 1817 (Mendonça & Katsuragawa, 2001; Bochini et al., 2019; Barioto et al., 2017). Exploitation by artisanal fisheries of these shrimp species is high, because they are an easily accessible resource for coastal fisheries, with emphasis on *X. kroyeri*, being the main target species for artisanal fisheries in southern Brazil (Dias-Neto, 2011; Silva et al., 2013; Miazaki et al., 2018). According to Ávila-da-Silva et al. (2018), caught fish totalled 12380 tonnes for the coast of São Paulo state in 2017 and in Cananéia specifically this was around 1913 tons in the same year. Costa et al. (2016) and Boss et al. (2016) discussed the decrease of *P. paulensis* and *P. brasieliensis* catches along the Brazilian coast, especially in the southeastern and southern regions and indicated that the fishing pressure is dangerously high. These studies pointed out that species of this genus are not included in the Red List of Threatened Species Animals of the International Union for Conservation of Nature but they should be, due to great fishing pressure throughout their development.

The shrimp species *P. paulensis* and *P. schmitti* are usually caught as bycatch of fishing on *X. kroyeri* (Boss et al., 2016). They are caught during the juvenile phase as they leave the estuary, thus they do not have a chance to breed (Costa et al., 2007). This impact on the juvenile recruitment brings serious consequences for the survival, reproduction and maintenance of populations and the energy transfer throughout the trophic web. Consequently, there is a declining trend in the shrimp populations in all regions of Brazil due to fishing (Boos et al., 2016).

Studies suggest that *X. kroyeri* in this region of Brazil is spawning and reproducting in inshore areas, corresponding to a type 3's life cycle (Costa et al., 2011; Castilho et al., 2015) - in other words this species is restricted to truly marine environments, with individuals migrating from inshore to offshore areas during spawning. This is different to the type 2's life cycle of *P. schmitti* and *P. paulensis*, with the species depending on estuarine, coastal, and marine habitats to complete its ontogenetic development (Dall et al., 1990; Costa et al., 2016).

Recent studies with *X. kroyeri* revealed that they are not only one species, but a species complex (Kerkhove et al., 2019a, b; Carvalho-Batista et al. 2019). In Cananéia region Carvalho-Batista et al. (2019) recently registered the occurrence of two species of this complex, *X. kroyeri* and *Xiphopenaeus dincao* nov. sp., but the only morphologic character to separate these species is the petasma and *appendix masculina*, for this reason in our study we will use the terminology for the genus *Xiphopenaeus* spp. Smith, 1869.

Besides the commercial value, shrimps play a key role in the coastal food web (Abarca-Arenas et al., 2007, Nagata et al., 2015). They are an important primary consumer and as opportunistic omnivores these shrimps consume detritus, benthic microalgae from intertidal mudflats and offshore sedimentary and organic matter (Dall et al., 1990; Abreu et al., 2007; Willems et al., 2016). They can also be a predator of small benthic animals such as hyperbenthic crustaceans (Dall et al., 1990; Soares et al., 2005; Abreu et al., 2007; Willems et al., 2016). As such, food resource for fish and crustaceans, these shrimps are responsible for the transfer of energy to the higher trophic levels (Nagata et al., 2015).

Shrimp have considerable nutritional importance in the trophic web and for human consumption, since they are a source of proteins, minerals and polyunsaturated fatty acids (PUFA – fatty acid (FA)) with two or more double bonds in the aliphatic chain (Bragagnolo & Rodriguez-Amaya, 2001). Lipids play a role as a secondary energy source and ensure cell-membrane integrity, mainly consisting of cellular and sub-cellular membranes (Teo et al., 2015). The FAs that compose the lipids can be separated into three different categories: 1) Essential FAs (EFA) including ω3 and ω6 that cannot be synthesized by the organism and need to be obtained from their diet; 2) conditionally indispensable EFAs that are widely available in the diet or can be endogenously synthesized from short chain precursors; and 3) non-EFAs that are constantly available in the diet or readily synthesized from short-chain precursors (Iverson, 2009; Teo et al., 2015).

FAs are key descriptors of food quality in aquatic environments and represent a potentially important link between primary producers and consumers (Bell et al., 2007: Willems et al., 2016). Polyunsaturated fatty acids (PUFA) in aquatic webs originate in the primary producers, so essentially in the plants (Tocher, 2009). Highly unsaturated fatty acids (HUFA - a subset of PUFA) may be key nutritional constituents of zooplankton diets that can determine energy efficiency across the plant–animal interface, secondary production and the strength of trophic coupling in aquatic pelagic food webs (Brett & Müller-Navarra, 1997; De Troch et al., 2012). Herbivores and omnivores can convert the short-chain PUFA (SC-PUFA, PUFA with <20 carbons) α-linolenic acid (18:3ω3) into long-chain PUFA (LC-PUFA, PUFA with ≥20 carbons), such as eicosapentaenoic (EPA, 20:5ω3) and docosahexaenoic (DHA, 22:6ω3) acids (Brett & Müller-Navarra, 1997; De Troch et al., 2012). In particular, the EFA, arachidonic acid (ARA, 20:4ω6), EPA and DHA, are considered to be important drivers of ecosystem health and stability (Parrish, 2013). Fatty acids are important trophic markers in marine ecosystems providing several applications for resolving ecosystem dynamic processes (Dalsgaard et al., 2003), understanding the importance of top-down and bottom-up controls on populations of key trophic players (Brett & Goldman, 1997; De Troch et al., 2012).

A lack of information does not allow to clearly understand the trophic ecology, and hence the ecological role of commercial shrimps in food webs. Some penaeid shrimp have different life cycles, undergoing migrations during the reproductive and development period, changing habitats and food sources as they grow from post larvae to adults (Dall et al., 1990). Understanding these interactions are key to provide an ecological approach for sustainable fisheries. Predicting the potential effect of environmental changes and recruitment success are factors related with food availability for the species. This knowledge about the trophic ecology can bring new approaches to the yield of shrimp fisheries (Pinnegar et al., 2000).

From an ecological point of view, it is interesting to know the FA of different species of shrimp that co-exist in the same region to understand their niche segregation, and to estimate the origin and transfer of PUFA in the local marine food web. Studies like this are scarce, thus, our study will be the first to disclose information on the composition and fatty acids of three species of commercial value in the Cananéia region. Due to the different life cycles of shrimp *Xiphopenaeus* spp., *P. schmitti* and *P. paulensis*, our hypothesis is that there is also variation in the FA profile among these species that were sampled in Cananéia littoral.

Material and methods

Study area

The estuary-marine complex of Cananéia, Iguape and Ilha Comprida (São Paulo state, Brazil) represents a remarkably diverse environment with high floral and faunal diversity recognized as an Atlantic Forest Biosphere Reserve by UNESCO (United Nations Educational, Scientific and Cultural Organization) since 2005. The mangrove ecosystem is the third-largest productive marine ecosystem in the South Atlantic as it is well-conserved and brings large amounts of nutrients and organic matter to the marine system that help to maintain biodiversity (Diegues, 1987; Mendonça et al., 2010). However, these coastal regions suffer from urbanization and growth of the human population, who dispose pollutants into this environment (Mendonça et al., 2010).

Sample collection

Samples were collected in Cananéia (São Paulo, 25º 04' S 47º 50' W), using trawling fishing gear (mesh size: 20 mm knot-to-knot in the body and 15 mm in the cod end) in September 2019. *Xiphopenaeus* spp. was identified according Carvalho-Batista et al. (2019), others shrimp species were identified at species level based on Costa et al. (2003). The carapace length (CL) was measured using a digital calliper (precision of 0.01 mm).

Fatty acids analysis

Tissue samples of each species constituted of the abdominal muscle (without the exoskeleton and bowel). This muscle tissue was frozen at -80 °C and later freeze-dried for 48 hours in the Stable Isotopes Center of São Paulo State University (Brazil). In the Marine Biology research group at Ghent University (Belgium), each shrimp sample was ground individually to a fine powder and 10 mg of every sample was weighed for the analyses. Fatty acid methyl esters were prepared from the freeze-dried samples using a direct transesterification procedure with 2.5% (v:v) sulfuric acid in methanol as described by De Troch et al. (2012). An internal standard (1 mg/ml of methylnonadecanoate 19:0, Sigma-Aldrich, Bornem, Belgium) was added prior to the transesterification procedure. Fatty acid methyl esters were extracted twice with hexane.

FA composition analysis of the shrimp fatty acids was carried out using a gas chromatograph (HP 7890B, Agilent Technologies, Diegem, Belgium) (GC) connect to a flame ionization detector (FID) and an Agilent 5977A Mass Selective Detector (Agilent Technologies, Diegem, Belgium) (MS). The GC was further equipped with a PTV injector (CIS-4, Gerstel, Mülheim an der Ruhr, Germany). A 60m×0.25mm×0.20µm film thickness HP88 fused-silica capillary column (Agilent Technologies, Diegem, Belgium) was used for the gas chromatographic analysis, at a constant Helium flow rate (2 mL/min). The injected sample is split equally between the MS and FID detectors at the end of the GC column using an Agilent capillary flow technology splitter. The oven temperature was programmed as follows: at the time of sample injection the column temperature was 50°C for 2 min, then gradually increased by 10°C/min to 150°C, followed by a second increase of 2°C/min to 230°C. The injection volume was 2 µL. The injector temperature was held at 30°C for 0.1 min and then ramped at 10°C/s to 250°C and held for 10 min. The transfer line for the column was maintained at 250°C. The quadrupole and ion source temperatures were 150 and 230°C, respectively. Mass spectra were recorded at 70 eV ionization voltage over the mass range of 50 - 550 m/z units.

Chromatogram analysis was done with the Agilent MassHunter Qualitative and Quantitative Analysis software (Agilent Technologies, Diegem, Belgium). The signal obtained with the FID detector was used to generate quantitative data of all compounds. Peaks were identified based on their retention times, compared with external standards as a reference (Supelco 37 Component FAME Mix, Sigma-Aldrich, Overijse, Belgium) and by the mass spectra obtained with the Mass Selective Detector. Quantification of fatty acid methyl esters (FAME) was based on the area of the internal standard (19:0) and on the conversion of peak areas to the weight of the fatty acid by a theoretical response factor for each fatty acid (Ackman & Sipos, 1964; Wolff et al., 1995).

Shorthand FA notations of the form A:BωX were used, where A represents the number of carbon atoms, B gives the number of double bonds, and X gives the position of the double bond closest to the terminal methyl group (Guckert et al., 1985; De Troch et al., 2012).

Statistical analyses

Prior to the data analysis, some FA were summed: 15:0 (sum of 15:0 + iso15:0 + antiso15:0), 16:0 (sum of iso16:0 + 16:0), 17:0 (sum of 17:0 + iso17:0 + antiso17:0) and 18:0 (sum of 18:0 + iso18:0). The results were expressed as untransformed, absolute FA

data. Data were analysed for normality and homogeneity through the Shapiro test (Sokal & Rohlf, 1987).

Analysis of variance (ANOVA) was applied, followed by the Tukey post-hoc test to find if there is a difference in total fatty acids (TF); Saturated FA (SFA); Monosaturated FA (MFA), Polyunsaturated FA (PUFA), ω3 FA, ω6 FA, PUFA/SFA, ω3/ω6 between the shrimp species in the same region. The analyses were performed using the "car", "arm", "lsmeans" and "multcompView" packages in the R software (R Development Core Team 2013).

Multivariate analysis of the FA data was conducted by non-metric multidimensional scaling (nMDS) based on Bray-Curtis similarity. Next, ANOSIM (analysis of similarities) was used to assess the similarities of FA composition between the three species of shrimps in the same region. Percentages of similarity (SIMPER) were calculated for untransformed, absolute FA data to determine the main FAs contributing to any differences. These analyses were performed using "ggplot2" and "vegan" packages in the R software (R Development Core Team 2013).

Results

In total, 23 shrimps of three different species were analysed - *Xiphopenaeus* spp. (n = 9, carapace length (LC) from 21.8 - 26.1 mm), *P. schmitti* (n = 9, LC from 27.1 – 44.3 mm), *P. paulensis* (n=5, LC from 22.3 – 28 mm). In total, 24 different FAs were detected and quantified for these shrimps (see Table 1).

Table 1. Average (± standard deviation - SD) of absolute fatty acids concentrations in *Xiphopenaeus* spp. (Xs), *Penaeus schmitti* (Ps), *Penaeus paulensis* (Pp) from the littoral of the Cananéia, São Paulo - Brazil. DW = Dry Material. ARA: arachidonic acid, DHA: docosahexaenoic acid, EPA: eicosapentaenoic acid. Significant differences are given by asterisk: *p < 0.05; **p < 0.01 and ***p < 0.001 using the ANOVA followed by Tukey test - different letters indicate statistical significant differences, df = 23.

Cananéia, São Paulo – Brazil							
Fatty acids	*Xiphopenaeus* spp.	*P. schmitti*	*P. paulensis*	ANOVA	Tukey test		
mg/g DW	(Xs)	(Ps)	(Pp)		Xs	Ps	Pp
14:0	0.28 ± 0.06	0.27 ± 0.04	0.21 ± 0.03	*	a	b	ab
15:0	0.29 ± 0.04	0.33 ± 0.03	0.57 ± 0.11	***	a	b	a
16:0	4.4 ± 0.42	3.25 ± 0.36	3.23 ± 0.41	***	a	b	b
16:1ω7	1.11 ± 0.11	1.31 ± 0.22	0.8 ± 0.22	***	a	b	a
17:0	0.68 ± 0.04	0.79 ± 0.13	1.07 ± 0.16	***	a	b	c
17:1ω8	0.21 ± 0.03	0.28 ± 0.06	0.63 ± 0.18	***	a	b	a
18:0	2.52 ± 0.11	2.89 ± 0.18	3.37 ± 0.34	***	a	b	c
18:1ω9	2.01 ± 0.27	1.94 ± 0.18	1.19 ± 0.07	***	a	b	a
18:1ω7	0.84 ± 0.15	0.59 ± 0.16	0.69 ± 0.08	**	a	ab	b
18:2ω6	0.23 ± 0.04	0.2 ± 0.03	0.19 ± 0.08				
18:3ω3	0.12 ± 0.02	0.07 ± 0.03	0.11 ± 0.04				
18:4ω3	0.05 ± 0.01	0.05 ± 0.02	0.05 ± 0.01				
19:1ω10	0.06 ± 0.01	0.06 ± 0.02	0.23 ± 0.07	***	a	b	a
20:0	0.12 ± 0.02	0.13 ± 0.01	0.12 ± 0.02				
20:1ω11	0.07 ± 0.01	0.13 ± 0.03	0.25 ± 0.05	***	a	b	c
20:1ω9	0.13 ± 0.02	0.11 ± 0.02	0.06 ± 0.01	***	a	b	c
20:1ω7	0.09 ± 0.02	0.11 ± 0.03	0.15 ± 0.06	**	a	a	b
20:2ω6	0.17 ± 0.05	0.16 ± 0.02	0.14 ± 0.02				
20:4ω6 ARA	1.26 ± 0.17	1.52 ± 0.32	1.62 ± 0.21				
20:5ω3 EPA	2.74 ± 0.23	3.16 ± 0.44	3.67 ± 0.26	***	a	b	b
22:0	0.22 ± 0.04	0.18 ± 0.01	0.08 ± 0.01	***	a	b	c
22:5ω6	0.22 ± 0.03	0.24 ± 0.06	0.18 ± 0.03	*	ab	a	b
22:5ω3	0.19 ± 0.02	0.47 ± 0.09	0.48 ± 0.07	***	a	b	b
22:6ω3 DHA	4.48 ± 0.5	3.09 ± 0.35	2.83 ± 0.51	***	a	b	b

The most abundant FAs in the three shrimp species were 16:00, 16:1ω7, 17:00, 18:00, 18:1ω9, 20:4ω6, 20:5ω3 and 22:6ω3. The major PUFA present in the shrimps were ARA, EPA and DHA (Table 1 Fig. 1). With the average of concentration for *Xiphopenaeus* spp., *P. schmitti* and *P. paulensis* of 1.26, 1.52 and 1.62 mg/g DW for ARA; 2.74, 3.16 and 3.67 mg/g DW for EPA; and 4.48, 3.09 and 2.83 mg/g DW for DHA. However, *Xiphopenaeus* spp. showed more abundance of 16:00 and DHA, while *P. schmitti* was rich in 18:00 and DHA and *P. paulensis* had high concentrations of 18:00

and EPA (Fig 1a). When FA were summed per category (see Fig. 1b), there were no species-specific differences (ANOVA, $p > 0.05$).

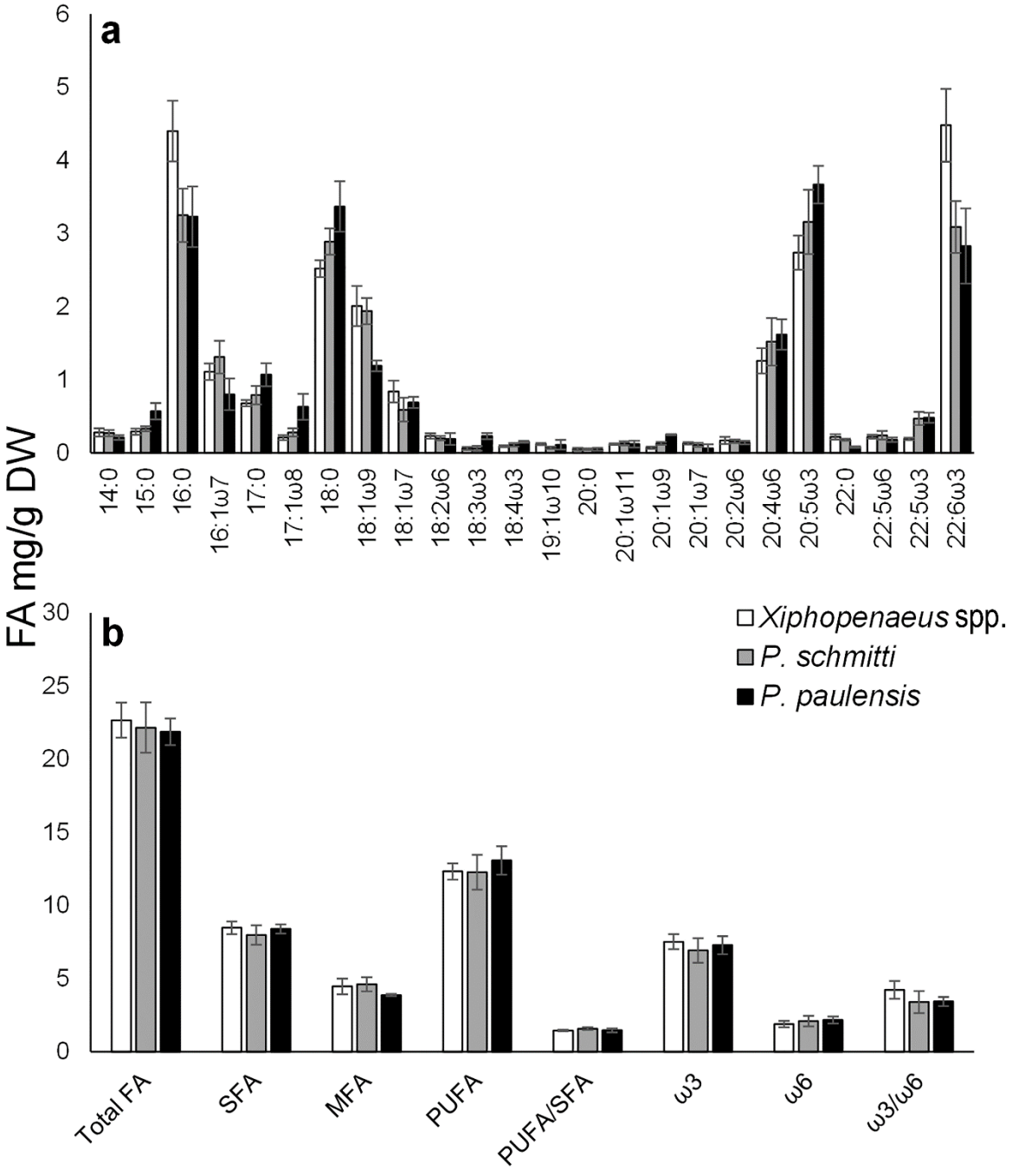


Figure 1. Absolute fatty acids concentrations (average ± SD) in the shrimp species *Xiphopenaeus* spp., *Penaeus schmitti* and *Penaeus paulensis*, a) individual FA concentrations (in mg/g DW) and b) FAs grouped according to the FA classes Saturated FA (SFA); Monosaturated FA (MFA), Polyunsaturated FA (PUFA), ω3 FA, ω6 FA, PUFA/SFA, ω3/ω6.

The non-metric multidimensional scaling (nMDS) applied to the FA profiles of the three shrimps species (Fig. 2) showed a significant separation between the three shrimp species (ANOSIM, R: 0.91, p = $1e^{-04}$). The largest dissimilarity was between *Xiphopenaeus* spp. and *P. paulensis* (18% dissimilarity, Table 2). The FAs that contribute most to the species-specific differences are 16:0, 22:6ω3, 20:5ω3 and 18:0 (Table 2). SIMPER analyses indicated that the FAs that were responsible for about 70% (cumulative contributions) in the difference between species were 16: 0, DHA, EPA and 18: 0 FAs (Table 2).

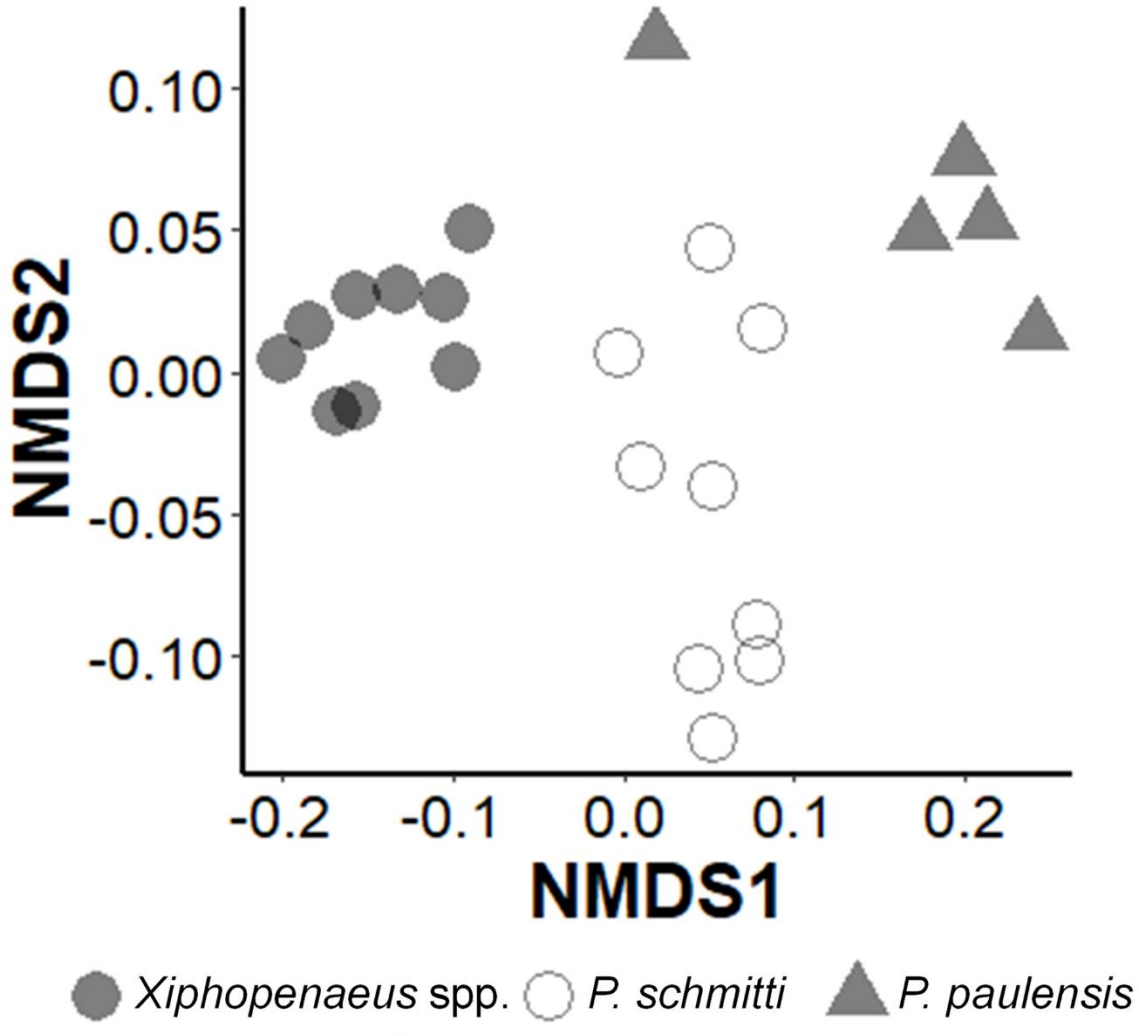


Figure 2. Non-metric multidimensional scaling (nMDS) plot based on Bray-Curtis similarity, for the comparison of untransformed absolute FA concentrations of the shrimps *Xiphopenaeus* spp., *Penaeus schmitti* and *Penaeus paulensis*.

Table 2. Similarity Percentage analysis (SIMPER), showing the average dissimilarity (Av. Diss.) between the species compared and cumulative contributions for the FAs that differentiated between the shrimps *Xiphopenaeus* spp., *Penaeus schmitti* and *Penaeus paulensis*.

Shrimp species	Av. Diss.	Fatty acids	Cumulative Contribution
Xiphopenaeus spp. vs. *P. schmitti*	13%	DHA; 16:00; EPA; 18:00; ARA; 22:5ω3	73%
Xiphopenaeus spp. vs. *P. paulensis*	18%	DHA; 16:00; EPA; 18:1ω9; 18:00; 17:00; 16:1ω7	71%
L. schmitti vs. *P. paulensis*	12%	18:1ω9; EPA; 16:1ω7; DHA; 18:00; 16:00; 17:00; ARA	72%

Discussion

It was clear in our study that shrimp species differ in terms of absolute abundances of certain fatty acids. These species-specific FA profiles can be explained by the difference in their life habits as well as their occurrence in different habitats. The study region is very diverse and directly influences these shrimps due to the differential supply of nutrients from the large mangrove forest.

Based in the behaviour of *X. kroyeri* (seabob shrimp) that have different life strategy restrict to the offshore (type 3), which one probably is the same for the genus *Xiphopenaeus spp.* in this region, is expected that this shrimp has a larger dissimilarity in FA profile with the other species due to the type of life of the other shrimps *P. schmitti* (white shrimp) and *P. paulensis* (pink shrimp), dependent on estuaries (type 2). The larvae and juveniles of the latter two species depend on the mangrove to complete their life cycle. The differences in the absolute FA concentrations differed even more between *Xiphopenaeus* spp. and *P. paulensis*.

Although the three shrimp species under study had the same FAs in their profile, their absolute concentration differed considerably. This can indicate that the shrimps may use the same food resource but depending on their life phase and availability of these resources in the environment, they may rely on different food preferences. Based on their occurrence in the study region, *P. schmitti* and *P. paulensis* are mainly relying on the food sources in the mangrove area while *Xiphopenaeus* spp. are feeding on organic matter in offshore that comes from the mangrove to the sea, generating high primary productivity. The long-chain FAs (LCFAs) with number of carbon atoms ≥22, as reported in the FA

profiles of the shrimp, indicated food resources that originate from mangroves such as the vascular plant matter in sediments and suspended organic matter (Kelly & Scheibling, 2012).

However, our results show that even the sea contributing to the food resources of these shrimp, marine resources are essential sources given the high levels of EPA and DHA found in the species studied. The marine system is characterised by high levels of the ω3HUFA, EPA and DHA (Dalsgaard et al., 2003). The freshwater system is characterised by higher levels of 18:2ω6 and 18:3ω3 and some EPA, but low DHA concentrations. Food with HUFA are essential for marine and freshwater fish, molluscs and crustaceans as they impact survival, growth, feed conservation, fecundity and osmotic stress balance (Bragagnolo & Rodriguez-Amaya, 2001). Some FAs are biomarkers and make it possible to detect possible food resources used by the shrimps. For example, DHA is an indicator of algae while the ratio 16:00/16:1ω7 is often high in flagellates and EPA in diatoms (Kaneda 1991, Ederington et al., 1995; Stübing & Hagen, 2003; Caramujo et al., 2008). The DHA/EPA ratio can be used as a marker for the trophic position and for the proportion of diatoms to flagellates in the diet (El-Sabaawi et al., 2009). The FA 18:1ω7 can result from chain elongation of the diatom marker 16:1ω7 (Nelson et al., 2001; Stübing & Hagen, 2003).

In the profile of *Xiphopenaeus* spp. the most abundant FAs were palmitic acid (16:00) and DHA. This corresponds to the findings of Bragagnolo & Rodriguez-Amaya (2001) for the same species. The same study however reported another FA profile with a high level of EPA in *Penaeus brasiliensis*. In our study, *P. paulensis* and *P. schmitti* use the estuary and mangroves for growth and development, so the difference in abundance of EPA when compared to *Xiphopenaeus* spp. Gatune et al. (2014) reported an increase in ARA, EPA and DHA in *P. monodon* that could be linked to the progressive decomposition of litter from mangrove leaves and the further development of the periphytic biofilm. This biofilm combined with the ability of *P. monodon* to bioconvert FA allows the shrimp to nutritionally upgrade their food source and as such become themselves more nutritious and thus of increasing value to their consumers, including humans. This shows the importance of the food sources from the mangrove to the shrimp. Khériji et al. (2003) found in their laboratory experiments that DHA and EPA increased in low temperature and salinity, which are both environmental characteristics of a typical estuary. In addition to that, the time spends by the shrimp in the mangrove forest or more towards the marine part of the estuary is important for its diet. *Xiphopenaeus* spp. does

not undergo a development phase inside the mangroves, as it spends most of its time in open sea (Kerkhove, 2020). This is different for *P. paulensis* as this species spends considerable time in mangrove and will thus use higher quantity of detritus during its juvenile phase but far less in its adult phase (Soares et al., 2005). The same feeding pattern probably occurs for *P. schmitti*, because both species have life cycle type 2 as they use the estuaries for their larval and juvenile development (Dall et al., 1990).

In our study PUFA were the most abundant FAs, with higher concentrations of ω3 than ω6, and with these results we prove that the shrimps in this region are great sources of essential fatty acids for transfer in the trophic web and for human consumption. PUFA were predominant in all studies on shrimp (Bragagnolo & Rodriguez-Amaya, 2001; Rosa & Nunes, 2003; Sriket et al., 2007; Li et al., 2011; Pires et al., 2016), likely due to the good quality of FA synthesized by the primary producers (e.g. phytoplankton) in these sites and then these FAs will be transferred to other consumers when the shrimp are used as food by predators (Brett and Müller-Navarra, 1997). Our results on higher levels of FAs in shrimps corroborates with the results found by Bragagnolo & Rodriguez-Amaya (2001) for the marine shrimps *P. brasiliensis*, *P. schmitti* and *X. kroyeri*. The only difference was found in the abundance of 18:1ω7 and 17:00, where we found 18:1ω7 in a lower concentration and 17:00 in a higher concentration. The higher level of 17:00 points at a higher use of bacteria as a food resource, probably as it is associated to organic matter (Parrish, 2013). Li et al. (2011) analysed 6 shrimp species and reported ARA was the dominant ω6 PUFA, while EPA and DHA were the major ω3 PUFA in all shrimps analysed. Pires et al. (2016) found in *L. schmitti* that the more abundant FAs were palmitic acid, stearic acid (18:0), DHA, EPA and oleic acid (18:1ω9). This outcome was similar to the results of Rosa & Nunes (2003) for palmitic, oleic, EPA, and DHA with exception to the stearic FA for marine shrimps *Aristeus antennatus* (Risso, 1816) and *Parapenaeus longirostris* (Lucas, 1846). Sriket et al. (2007) reported that palmitic acid and stearic acid were the most abundant SFA in *P. monodon* and *Penaeus vannamei* Boone, 1931. Although SFA can appear in higher concentrations in some species, there may be differences within the same species. These intraspecific differences can be linked to the life stage or the different use of habitats (Pires et al., 2016). In summary, this shows that the shrimp species have similar food sources, this can probably be linked to their benthic behaviour but there are also differences in FA concentrations that can be explained by the interspecific differences in occupation of habitats.

Our study area is strongly influenced by a large supply of freshwater from rivers and this modulates the FA composition of the invertebrates in these waters as the supply of their food resources is different. Marine shrimps had much higher total ω3 PUFA but lower ω6 PUFA levels than most of the freshwater fish and shrimp (Li et al., 2011). This is due to the composition of marine phytoplankton, with higher ω3PUFA, EPA, and DHA, but lower ω6PUFA levels (Li et al., 2011). Environmental conditions and diet have been reported as the factors that influence the FA composition in crustacean muscles most (Li et al., 2011).

Some FAs are essential for the growth and development of the shrimps, as shown in a study by Huang et al. (2008) of *P. monodon*, which demonstrates the correlations between the content of ARA in oocytes and fecundity. The balance between ω3 and ω6 fatty acid families, total PUFA, total SFA, EPA and DHA may play vital roles in maturation and reproductive performance of *P. monodon* broodstock (Huang et al., 2008). The shrimps in our study show a high concentration of these FA, which signals that this region provides good nutrition, that consequently produces a higher quality broodstock. An unbalanced or incomplete diet causes poor reproductive performance or may even stop animals from reproducing (Huang et al., 2008).

In Cananéia, there is an increase in food sources due to the nature of mangroves systems such as sediment trapping, erosion reduction and enhanced coastal accretion, which in turn promote the formation of intertidal mudflats which are important for the entire ecosystem (Alongi, 2008). However, these intertidal areas are being lost by land reclamation, and mangrove forests are being destroyed at a high rate (Blanco et al., 2012). It is worth highlighting that the intertidal mudflats also affect the productivity of offshore fisheries production, due to the impact they have on the energy transfer in the trophic web. Thus, the continued conservation and maintenance of these mudflats and their associated mangrove systems are essential (Blanco et al., 2012).

A probable food sources for the shrimp in our study is related to the organic matter coming from the rivers and mangroves in this region that provides a source of food during the development and growth of different organisms. Penaeid juveniles prefer finer sediments with higher organic-matter content (Dall et al., 1990; Costa et al., 2011). This type of sediment has a higher quantity of different food resources (e.g. diatoms, bacteria, algae, organic matter) for benthic crustaceans (Branco & Moritz Jr., 2001). The biofilm microorganisms, found on the organic matter in the sediment are consumed by shrimp and provide essential nutrients (sterols, essential fatty acids, amino acids and vitamins),

that make them an important complementary food source (Thompson et al., 2002). Garcia et al. (2018) found higher chlorophyll concentration in the estuary of Cananéia than in the sea (adjacent coastal area). The authors correlate this factor with the low temperature variability that occurred in this region, which increases primary productivity. Cananéia estuarine areas have low depth, which can be favourable for the transfer of sunlight and thus the functioning of chlorophyll in the biofilm on the shallow sediment. This creates a higher resuspension of phytoplanktonic cells, as well as nutrients deposited at the bottom, incrementally increasing the algal biomass. In addition, the estuary naturally has a large amount of nutrients that comes from the forest around the rivers in Cananéia and the surrounding islands (Aquino et al., 2012; Garcia et al., 2018). Thus, we emphasize the importance of this ecosystem for the preservation and sustainability of the species that are dependent on the high-quality food resources that Cananéia provides.

From our data, we can conclude that any change in the quality of the environment can change the FA composition of primary consumers. Particularly, the change in the quality and quantity of organic matter available in the study region can cause changes in the composition of the FA as well as knock-on changes in the population, decreasing the quality of reproduction and offspring, a factor that can change the shrimp populations in the region and consequently the FA quality for shrimp consumers. Copepods and shrimps are linked to higher trophic levels, doing the transference of energy absorbed from primary producers, transmitting them when they are used as food source for the predator - second consumers (Tocher, 2010), when the quality of FAs decreases there is a possible effect on the health of secondary consumers and/or their nutritional value for human consumers. Shrimp species are important in the trophic web as they contribute to the diet of demersal coastal fish, but their general importance for higher trophic levels remains unassessed (Camargo & Isaac, 2004). Therefore, it is extremely important that the system contains primary producers with a high-quality FA, that is then transferred to other consumers, building a balanced trophic web. As showed by De Troch et al. (2012), when copepod feeds on diatoms, they incorporate 53-fold more dietary DHA in their tissues than when feeding on bacteria. This has important implications for higher trophic levels feeding on copepods (e.g. other crustaceans and juvenile fish) (De Troch et al., 2012), that will transfer the energy to other secondary consumers.

Our results show the importance of these shrimps in the transfer of essential fatty acids to the other trophic levels of the trophic web, as well as an important source of essential FA in human diets (Bragagnolo & Rodriguez-Amaya, 2001; Nagata el al., 2015).

FA profiles of the three species of commercial interest illustrate that the shrimps in the study region are a good source of FA and especially ω3 PUFA. Also, shrimps are a good source of proteins combined with a lower caloric value (Bragagnolo & Rodriguez-Amaya, 2001; Pires et al., 2016). PUFA are beneficial to health and play vital functions in animals (Brett & Müller-Navarra, 1997). For humans, ω3 FA are essential for normal growth and development and play a role in the prevention and treatment of the coronary artery disease, cancer, hypertension, arthritis and other inflammatory and autoimmune disorders. EPA (20:5ω3) and DHA (22:6ω3) play an important role in the prostaglandin metabolism, normal development of the brain, eyes, nerves, immunology, reduced inflammation, thrombosis and atherosclerosis (Kerr et al., 2015; Narayan et al., 2006; Simopoulos, 1999; Yu et al., 2015). This demonstrates that the shrimps are a marine food that is of importance to human diet and health (Pires et al., 2016).

However, the species of shrimp studied here, as well as others, suffer from great environmental pressure that can lead to a drastic reduction and extinction of species due to overexploitation by fishing, which would cause a cascading effect on the other species in the trophic web (Boss et al., 2016). Mendonça & Katsuragawa (2001) and Mendonça et al. (2010) showed a decline of animals fished over the past few years in the Cananéia region, indicating high levels of effort to fish this resource, without taking into account the need for sustainable use and to allow them for reproduction. The authors suggest more adequate action to preserve these species, as extensive research on the populations of penaeid shrimps from different areas, principally from the species complex *Xiphopenaeus* spp. which still doesn't have much specific information about the different species, to determine where the stocks occur and reproductive cycle (Kerkhove et al., 2019a, b; Carvalho-Batista et al. 2019). Thus, zones and periods of total fishery prohibition can be proposed and implemented so that this important economic resource can be protected, maintained and allowed to flourish in different areas. Still, some recent studies propose the aquaculture growth of the pink shrimp *P. paulensis* and *P. brasiliensis*, as a strategy to decrease the fishing of the natural population and degradation of the environment (Soares et al., 2005).

Thus, that the obtained knowledge on the species-specific FA composition of these shrimps that are rich sources of essential fatty acids and form an important link in the food chain is useless if the conservation of these species does not occur through the preservation of their environment. This also highlights the importance of mangroves, where a reduction of fishing pressure in shrimp species may help to improve all stages of

life, especially the early stages.

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Chapter V

Changes in fatty acid profiles of *Xiphopenaeus* spp. indicates change in pollution levels in the Southeastern Brazilian coast

Changes in fatty acid profiles of *Xiphopenaeus* spp. indicates change in pollution levels in the Southeastern Brazilian coast

Abstract

Aquatic environments, in general, have suffered from anthropic actions. The consequences of this are evident in the changes that occur in organisms, affecting intra and interspecific interactions, throughout the trophic network. In the marine environment, Penaeoidea shrimp perform energy transfer between primary producers and secondary consumers. Considering that the shrimps are rich in sources of protein and essential fatty acids, this study aimed to compare the fatty acid composition of seabob shrimp *Xiphopenaeus* spp. in two coastal sites of Southeastern Brazilian littoral. The samples location are influenced by distinct anthropic actions, i.e., Ubatuba (UBA) is influenced by tourism and Cananéia (CA) is a large preservation area, however both are exploited by fishing. Seabob shrimp in all sampled locations presented 24 different fatty acids, with a large abundance of 16: 0, 18: 0, 18: 1ω9, ARA, EPA and DHA. We found a lower abundance of these FAs in UBA when compared to CA, a site with greater preservation status. Chl and HDO abiotic factors related positively to FAs in CA and inversely in UBA. These factors are bioindicators of the quality of the environment, since in healthy environments the production of phytoplankton increases the production of oxygen and, consequently, generates greater availability of food and survival of these shrimp. In view of these results, we point out seabob shrimp as a key taxon within the trophic web, as well as a good food source for other animals like fish when in healthy environments, as it has high sources of FAs, especially ω3PUFA. However, we emphasize the importance of these penaeidean shrimps remaining in these environments - without pollution and free from fishing - in which they can fulfil their role as a node between trophic levels, transmitting quality energy to their predators.

Keywords: polyunsaturated fatty acids, chlorophyll, oxygen, mangrove, seabob shrimp

Introduction

The impact of human activities on biological communities is an important topic in ecological research (Halpern et al., 2008; Cardinale et al., 2012). Anthropogenic changes cause environmental stress, negatively affecting the function of the ecosystem by causing a loss of species diversity and changes in the community structure, albeit

without necessarily causing species' extinction. These changes decrease the contribution that species can make to the overall functioning of the ecosystem, mainly in energy transfer (Wittebolle et al., 2009; Mensens et al., 2015; De Laender et al., 2016).

Fatty acids (FA) are an efficient tool to explain the energy transfer in the plant-animal inter-face, to clarify how the consumers assimilate and modify the FA in the dietary (Müller-Navarra et al., 2004; De Troch et al., 2012). Polyunsaturated fatty acids (PUFA) with two or more double bonds in the aliphatic chain represent around one third of all FA in the organism and are responsible for developing several physiological functions in the organism, but these PUFA cannot be produced by the animal, and must come from a food resource or be bio-converted from some precursor of FA. In the marine ecosystem, most PUFA originates from primary producers in special photoautotrophs - phytoplankton (Iverson, 2009). Highly unsaturated fatty acids (HUFA - a subset of PUFA) may be a key nutritional constituent of zooplankton diets, as the diatoms, which can determine the energetic efficiency across the plant–animal interface and transference across the food webs (Brett & Müller-avarra, 1997). Herbivores convert the short-chain PUFA (<20 carbons) and α-linolenic acid (18:3ω3) to long-chain PUFA (≥20 carbons), for example, eicosapentaenoic (EPA, 20:5ω3), arachidonic acid (ARA, 20:4ω6) and docosahexaenoic (DHA, 22:6ω3) acids, albeit with different efficiency (Brett & Müller-Navarra, 1997). FAs play a vital role in human life, i.e., EPA and DHA are precursors for eicosanoids with anti-inflammatory, antithrombotic and vasodilatory activities, related to the prevention of diseases such as dyslipidemia and hypertension (Hernández-Becerra et al., 2014).

Anthropogenic activities cause environmental pollution in the aquatic habitats altering the lipids and fatty acid compositions (Rocchetta et al., 2012; 2014). The lipid composition can influence growth, reproduction, defence/detoxification systems and the physiology of organisms (Parrish et al., 2000; Rocchetta et al., 2014). Metals, metal ion and sewage discharges, containing high levels of faecal coliform bacteria can damage lipids by oxidative processes (Sabatini et al., 2011; Rocchetta et al., 2012). Pinto et al., (2020) found the presence for Giardia cysts in 79.2% samples analysed in Ubatuba (Itagua) beach, showing the discharge of untreated sewage. Just 40% of municipalities have sewage disposal systems, through which only 40% of the total sewage volume is treated in Brazil (SNIS 2014). The highest frequencies of gastroenteritis cases occur during the summertime when the number of tourists increase in the Sao Paulo coast (Brazil), and, consequently, there are more sewage discharges into the sea (Sato et al.

2005). Thus, one of our hypotheses is that FA profiles are a good marker for the response of the tissue to the environmental changes caused by contamination. All of these changes can generate imbalances in energy transfer within food chains. Thus, a better comprehension on the factors that can control these interactions is essential to ecological studies (Kelly & Scheibling, 2012).

Phytoplankton are the base of pelagic food webs, these microalgae can be contribute to benthic food webs, in some cases the characteristics of FAs in benthic organisms indicates a trophic connection between pelagic and benthic food webs (Dalsgaard et al., 2003; Parrish et al., 2009). Benthic food webs also rely on food sources from macroalgae, vascular plants (such as mangle trees and seagrass), and bacteria (Kharlamenko et al., 2001; Dalsgaard et al., 2003). In benthic food webs, the shrimps are an important primary consumer, responsible for energy transfer to other trophic levels (Abarca-Arenas et al., 2007; Nagata et al., 2015).

Recent studies revealed that the seabob shrimp *Xiphopenaeus kroyeri* (Heller, 1862), are not the only species in the studied sites, but part of a species complex (Kerkhove et al. 2019a, b; Carvalho-Batista et al. 2019). In Cananéia, Carvalho-Batista et al. (2019) recently registered the occurrence of two species of this complex through morphological (SEM) and genetic (Multigene) techniques, *X. kroyeri* and *Xiphopenaeus dincao* nov. sp., but the only morphologic character to separate these species is the petasma and *appendix masculina*, for this reason in our study we will use the terminology *Xiphopenaeus* spp.

As a first consumer, shrimp *X. kroyeri* feed on detritus, benthic microalgae, sedimentary organic matter, plant material and small benthic animals, being an opportunistic omnivor (Dall et al., 1990; Willems et al., 2016). This shrimp is a node, transferring the energy from these food resources to the next trophic level (Abarca-Arenas et al., 2007; Nagata et al., 2015; Willems et al., 2016). *Xiphopenaeus* spp. is the most abundant shrimp in in the south and southeast of Brazil (Castilho et al., 2015; Boss et al., 2016) - in the state of São Paulo, 3044 tons of this shrimp were landed in 2019 (Instituto de Pesca, 2019). The spawning and reproduction of *X. kroyeri* occurs in inshore areas (Nakagaki & Negreiros-Fransozo, 1998; Costa et al., 2011; Castilho et al., 2015). In this sense, it is likely that there is no habitat partitioning along the ontogeny of this shrimp. Thus, another hypothesis is that the FA profile in the seabob shrimp may vary among sampled sites, but not between benthic life phases (juvenile and adult).

In short, *X. kroyeri* has an economic value to fisheries, besides being a food

resource to the human population living near the coast, in the Southeastern Brazilian littoral. Additionally, it is a crucial link in the trophic web. In this sense, we carried out this study in two different sites: 1) Cananéia (CA), a well-preserved site with reserves of mangroves nearby (Mendonça et al., 2010); 2) Ubatuba (UBA), a highly urbanized and developed site, with anthropization from tourism (Mantelatto et al., 1999; 2016; Melo & Furlan, 2017; Pinto et al., 2020). Artisanal fishing is the main source of subsistence for countless families in these both areas. Considering the features of both sites, we analysed if the fatty acids (FA) profile in *Xiphopenaeus* spp. reflect environmental differences between these coastal areas.

Material and methods

Samples were collected in the subtropical Southern part of the Brazilian coast (São Paulo State): Cananéia and Ubatuba. These sampling sites were chosen to test the hypotheses of this study as they are influenced differently by the anthropic factors like urbanization, anthropogenic residuals, deforestation, tourism and fishing exploitation (Mendonça et al., 2010; Mantelatto et al., 2016; Batista & Harari, 2017;).

Study areas

Cananéia (São Paulo, Brazil 25º 04' S 47º 50' W)

Cananéia (CA) represents a remarkably diverse environment area with high floral and faunal diversity (Diegues, 1987). The mangrove ecosystem has been recognised worldwide as the third-largest productive in the South Atlantic, because it has well-conserved environmental resources (Mendonça et al., 2010). In 1993, the Atlantic Forest biome in CA was designated as a Biosphere Reserve and in 1999 was entitled as a World Natural Heritage Site (UNESCO, 1999; 2005). According to CETESB (2018), the quality of water was good and the evaluation of the sediment toxicology in this site indicated great quality, respectively, because of low tourism activities, low contaminant output and a small population. However, these coastal sites are undergoing urbanization and growth of the human population, all of which could generate pollutant disposal in the environment. Cananéia is characterised by intense fishing activities, practiced by many families for subsistence (Mendonça & Katsuragawa, 2001). This site has an estimated population of 12 thousand people and an economy based in the agriculture, livestock and

fishing (IBGE 2019). However, during the tourism time (summer) the population could grow go to 150 thousand people (Becegato, 2007).

Ubatuba (São Paulo, Brazil 23° 27´S, 45° 02´W)

Due to its proximity to Serra do Mar, UBA has as an indented coast without any large rivers flowing into the sea. In the area, there are many islands protecting the coast area and, consequently, creating many microhabitats which help the protection of marine species (Negreiros-Fransozo et al., 1991). Ubatuba has an estimated population of 91 thousand people and the economy is mainly based on agriculture, fishing and tourism (IBGE, 2019).

In holiday periods or summer seasons UBA can receive around 300 thousand tourists with stays of 7 to 10 days, where on New Year's and Carnival holidays it can reach peaks of 800 thousand tourists (Melo & Furlan, 2017). In the summertime the number of pathogens from untreated sewage increases in this site (Burbano-Rosero et al., 2011; Rodrigues et al., 2016; Pinto et al., 2020). This excessive contingency of people causes significant anthropogenic activity with the introduction of great contribution domestic sewage, as the municipality does not have sufficient infrastructure necessary (Melo & Furlan, 2017). Additionally, oil spills, industrial residues and agriculture runoff (Mantelatto & Corrêa, 1996; Burbano-Rosero et al., 2011) has led to a progressive deterioration of the marine coastal ecosystem (Mantelatto et al., 1999; 2016). According to CETESB (2018), in Itaguá Bay, of Ubatuba municipality, the urban beaches have become compromised in terms of water quality, with sanitary risks for health.

Field sampling

The samples were collected with trawling fishing gear (mesh size: 20 mm knot-to-knot in the body and 15 mm in the cod end) in CA and UBA in São Paulo State of Brazil in September and October 2019, respectively. *Xiphopenaeus* spp. was identified according Carvalho-Batista et al. (2019). Juveniles and adults were separated according to reproductive morphology. The females were considered adult when they obtained the complete transverse ridge in the thelycum and the comma-shaped gonopore is whole, with a large quantity of setae. The males were considered adults after the union of the endopods, which forms the petasma (Fransozo et al. 2011). Based on available literature we consider adult females and males with >16 mm of carapace length (CL), that already have mature gonads (Fransozo et al. 2011; Castilho et al. 2015).

The abiotic variables related to the water column were measured using a EUREKA® multiparameter probe (Manta 2-4.0), e.g.: pH, chlorophyll (Chl), dissolved oxygen concentration (HDO), total dissolved solids (TSD), temperature (TE) and salinity (SAL). Sediment samples were obtained using a Petersen grabber and were frozen until further analysis in the laboratory. The samples were dried (70ºC for 72 h) and a 10-g subsample from each sample was ash-weighted to determine the organic matter content (OM) of the substrate.

Fatty acids analyses

In total, 80 shrimps of *Xiphopenaeus* spp. (CL size from 9 – 24.64 mm) (40 from each site) were analysed. These samples were separated in 10 samples for each group: juvenile female (Juv F), adult female (Adu F), juvenile male (Juv M) and adult male (Adu M). The samples constituted of the abdominal muscle (without the exoskeleton and bowel). This muscle tissue was frozen and later freeze-dried for 48 hours in the Centre of Stable Isotopes of Prof. Dr. Carlos Ducatti, Institute of Biosciences, São Paulo State University (UNESP). In the Marine Biology lab at Ghent University, each shrimp sample was ground individually to a fine powder and 10 mg of every sample were weighed for the analyses. Fatty acid methyl esters were prepared from the freeze-dried samples using a direct transesterification procedure with 2.5% (v:v) sulphuric acid in methanol as described by De Troch et al. (2012). The internal standard (1 µg/µL of methylnonadecanoate 19:0, Sigma-Aldrich, Bornem, Belgium) was added prior to the transesterification procedure. Fatty acid methyl esters were extracted twice with hexane.

Fatty acids composition analysis of the shrimps was carried out using a gas chromatograph (GC) (HP 7890B, Agilent Technologies, Diegem, Belgium) equipped with a flame ionization detector (FID) and connected to an Agilent 5977A Mass Selective Detector (MSD) (Agilent Technologies, Diegem, Belgium). The GC was further equipped with a PTV injector (CIS-4, Gerstel, Mülheim an der Ruhr, Germany). A 60m×0.25mm×0.20µm film thickness HP88 fused-silica capillary column (Agilent Technologies, Diegem, Belgium) was used for the gas chromatographic analysis, at a constant Helium flow rate (2 mL/min). The injected sample is split equally between the MS and FID detectors at the end of the GC column using an Agilent capillary flow technology splitter. The oven temperature was programmed as follows: at the time of sample injection the column temperature was 50°C for 2 min, then gradually increased by 10°C/min to 150°C, followed by a second increase of 2°C/min to 230°C. The injection

volume was 2 μL. The injector temperature was held at 30°C for 0.1 min and then ramped at 10°C/s to 250°C and held for 10 min. The transfer line for the column was maintained at 250°C. The quadrupole and ion source temperatures were 150 and 230°C, respectively. Mass spectra were recorded at 70 eV ionization voltages over the mass range of 50 - 550 m/z units.

Chromatogram analysis was done with the Agilent MassHunter Qualitative and Quantitative Analysis software (Agilent Technologies, Diegem, Belgium). The signal obtained with the FID detector was used to generate quantitative data of all compounds. Peaks were identified based on their retention times, compared with external standards as a reference (Supelco 37 Component FAME Mix, Sigma-Aldrich, Overijse, Belgium) and by the mass spectra obtained with the Mass Selective Detector. Quantification of fatty acid methyl esters was based on the area of the internal standard (19:0) and on the conversion of peak areas to the weight of the fatty acid by a theoretical response factor for each fatty acid (Ackman & Sipos, 1964; Wolff et al., 1995).

Shorthand FA notations of the form A:BωX were used, where A represents the number of carbon atoms, B gives the number of double bonds, and X gives the position of the double bond closest to the terminal methyl group (Guckert et al. 1985; De Troch et al., 2012).

Statistical analyses

Prior to the data analysis, some FA (mg/g dry material) found in our study were summed, based on their characteristic as an FA trophic marker (see El Sabaawi et al., 2009), to expose only one value for these FA, i.e. 15:0 (sum of 15:0 + iso15:0 + antiso15:0), 16:0 (sum of iso16:0 + 16:0), 17:0 (sum of 17:0 + iso17:0 + antiso17:0) and 18:0 (sum of 18:0 + iso18:0). The results were expressed as untransformed, absolute FA data. Data were analysed for normality and homogeneity through the Shapiro test (Sokal & Rohlf, 1987).

To test how the environmental factors of pH, Chl, HDO, TSD, TE, SAL, OM, can influence the CA and UBA sites, the environmental data was first standardized using the "descostand" function in the package "vegan" (Oksanen et al., 2011) for R (R Development Core Team 2019). In sequence, the data was then subjected to Principal Component Analysis (PCA), to identify the main factors that influence each site (Clarke & Ainsworth, 1993). Subsequently, we performed a Permutational Multivariate Analysis

of Variance (PERMANOVA), using a Euclidean method, to statistically compare the difference of the environmental factors between sites (CA and UBA).

Analysis of variance (ANOVA) was applied, to find if there is a difference in total fatty acids (TF); Saturated FA (SFA); Monosaturated FA (MFA), Polyunsaturated FA (PUFA), ω3 FA, ω6 FA, PUFA/SFA, ω3/ω6 between the 80 shrimps samples (40 from UBA and 40 from CA) in function of sampling site (CA and UBA), sex and age. The analyses were performed using the "car", "arm", "lsmeans" and "multcompView" packages in the R software (R Development Core Team, 2008).

Multivariate analysis of the FA data was conducted by non-metric multidimensional scaling (nMDS) based on Bray-Curtis similarity. Next, ANOSIM (analysis of similarities) was used to assess the similarities of FA composition between the sampling site, sex and age (juvenile and adult). To complement ANOSIM, differences in FAs composition between sites, sex and age were also analysed through "adonis" functions in PERMANOVA, using 9,999 permutations. Unlike ANOSIM, PERMANOVA can determine whether the interactions between diets, tissues, and maturity status were significant. Percentages of similarity (SIMPER) were calculated for untransformed, absolute FA data to determine the main FAs contributing to any differences. These analyses were performed using "ggplot2" and "vegan" packages in the R software (R Development Core Team, 2008).

To detect a possible connection among the variables CL, pH, Chl, HDO, TSD, TE, SAL, OM with the different FAs in shrimp from both sites, we used Redundancy Analysis (RDA). RDA produces final coordination scores that sum up the linear relationship between the explanatory and the response variables. The analysis relevance was verified by a Monte Carlo permutation test performed on 9999 random permutations using the "vegan" package and plotted by "ggplot2" in the R software (R Development Core Team, 2008).

Results

The PCA Axis 1 explained 73.3% of the variation, while Axis 2 explained 18.1% and the scores plotted (Axis 1 and 2) showed a separation between CA and UBA sites (PERMANOVA: $df = 1$, $F = 60.61$, $P = 0.001$) (Figure 1), regarding environmental factors. In CA, the Chl and HDO showed a significant positive correlation with this site,

while OM, Sal, TDS and TE were the predominating factors correlated positively with UBA site (Figure 1).

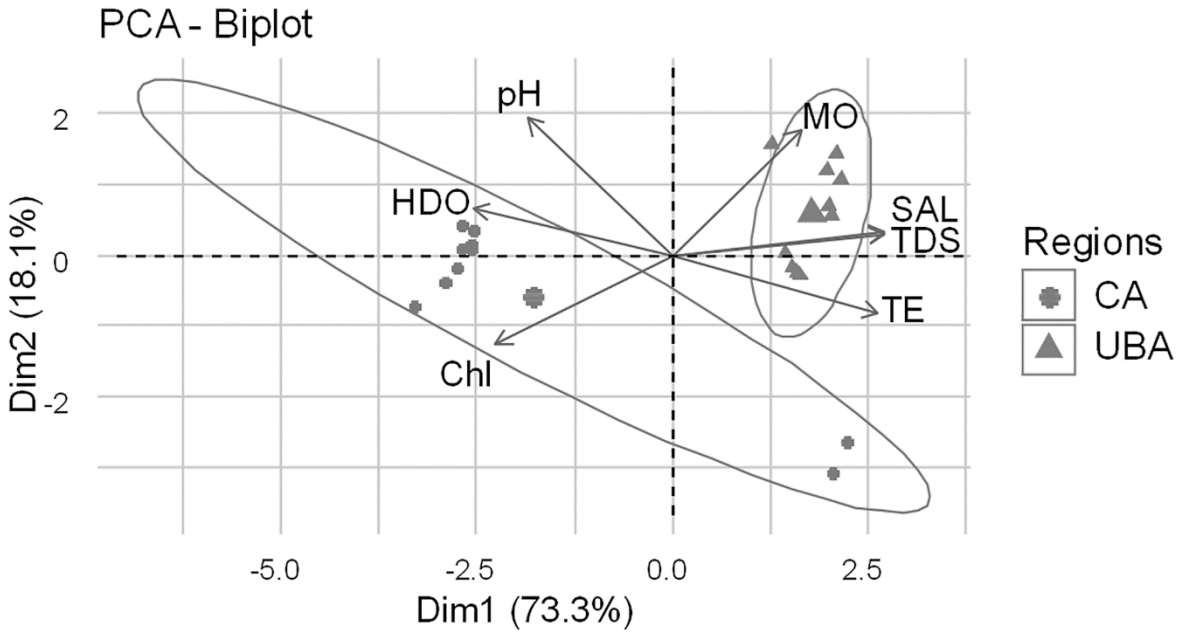


Figure 1. Principal Components Analysis (PCA) results showing the variation of the environmental factors from Cananéia and Ubatuba sites, State of São Paulo, Brazil (Axis 1 and 2 of PCA represented by Dim1 and Dim2, respectively). Axis 1 explained 73.3% and Axis 2 explained 18.1% of the variation.

In total, we found 24 different FAs for *Xiphopenaeus* spp., which were determined and quantified. The most abundant FAs in shrimps in both sites were 16:0, 18:0, 18:1ω9, 20:4ω6 (ARA), 20:5ω3 (EPA) and 22:6ω3 (DHA). In UBA only the FA 17:0, ARA and 22:5ω6 were higher than in CA (Fig 2). The table 1 shows the difference in FAs between sites, sex and age.

Table 1. *Xiphopenaeus* spp., average (± standard deviation - SD) of absolute fatty acids (FA) correlations in shrimp, from Cananéia and Ubatuba sites. DW = Dry Material, Juv F = juvenile female, Adu F = adult female, Juv M = juvenile male, Adu M = adult male, SFA = Saturated FA, MFA = Monosaturated FA, PUFA = Polyunsaturated FA. ARA: arachidonic acid, DHA: docosahexaenoic acid, EPA: eicosapentaenoic acid.

FA	Cananéia – SP				Ubatuba – SP			
mg/g DW	Juv F	Adu F	Juv M	Adu M	Juv F	Adu F	Juv M	Adu M
14:0	0.29 ± 0.05	0.36 ± 0.08	0.24 ± 0.06	0.24 ± 0.04	0.18 ± 0.05	0.15 ± 0.02	0.18 ± 0.05	0.17 ± 0.03
15:0	0.35 ± 0.09	0.31 ± 0.07	0.29 ± 0.05	0.3 ± 0.06	0.23 ± 0.05	0.27 ± 0.07	0.27 ± 0.08	0.26 ± 0.06
16:0	5.01 ± 0.42	5 ± 0.47	4.74 ± 0.67	4.87 ± 0.42	3.76 ± 0.43	3.68 ± 0.39	3.63 ± 0.59	3.81 ± 0.72
16:1ω7	1.12 ± 0.13	1.26 ± 0.19	0.98 ± 0.2	1.08 ± 0.12	0.7 ± 0.12	0.82 ± 0.15	0.78 ± 0.21	0.76 ± 0.16
17:0	0.77 ± 0.13	0.75 ± 0.09	0.8 ± 0.12	0.82 ± 0.12	0.72 ± 0.17	0.91 ± 0.22	0.82 ± 0.16	0.82 ± 0.22
17:1ω8	0.18 ± 0.02	0.23 ± 0.04	0.17 ± 0.04	0.24 ± 0.04	0.14 ± 0.1	0.23 ± 0.08	0.2 ± 0.07	0.19 ± 0.06
18:0	3 ± 0.35	2.83 ± 0.17	2.78 ± 0.32	2.87 ± 0.36	2.27 ± 0.32	2.59 ± 0.33	2.39 ± 0.32	2.6 ± 0.45
18:1ω9	1.78 ± 0.15	2.09 ± 0.26	1.59 ± 0.31	1.93 ± 0.19	1.13 ± 0.19	1.5 ± 0.31	1.2 ± 0.25	1.49 ± 0.25
18:1ω7	0.98 ± 0.19	0.94 ± 0.14	0.94 ± 0.2	0.97 ± 0.15	0.64 ± 0.08	0.74 ± 0.09	0.68 ± 0.13	0.68 ± 0.14
18:2ω6	0.26 ± 0.05	0.27 ± 0.04	0.29 ± 0.08	0.31 ± 0.03	0.28 ± 0.06	0.28 ± 0.06	0.27 ± 0.05	0.31 ± 0.05
18:3ω3	0.14 ± 0.02	0.14 ± 0.04	0.13 ± 0.03	0.13 ± 0.03	0.1 ± 0.03	0.08 ± 0.02	0.09 ± 0.03	0.11 ± 0.02
18:4ω3	0.08 ± 0.03	0.07 ± 0.03	0.06 ± 0.02	0.04 ± 0.01	0.05 ± 0.02	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
19:1ω10	0.08 ± 0.02	0.08 ± 0.02	0.08 ± 0.01	0.08 ± 0.01	0.05 ± 0.02	0.06 ± 0.02	0.05 ± 0.02	0.04 ± 0.02
20:0	0.12 ± 0.02	0.14 ± 0.01	0.14 ± 0.03	0.14 ± 0.02	0.11 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.14 ± 0.02
20:1ω11	0.05 ± 0.01	0.07 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.03	0.07 ± 0.02	0.03 ± 0.02	0.04 ± 0.02
20:1ω9	0.13 ± 0.02	0.14 ± 0.02	0.12 ± 0.02	0.11 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.02	0.07 ± 0.02
20:1ω7	0.08 ± 0.02	0.08 ± 0.02	0.07 ± 0.02	0.06 ± 0.02	0.03 ± 0.01	0.06 ± 0.02	0.03 ± 0.01	0.04 ± 0.01
20:2ω6	0.18 ± 0.04	0.2 ± 0.02	0.16 ± 0.04	0.15 ± 0.03	0.12 ± 0.02	0.14 ± 0.02	0.12 ± 0.02	0.14 ± 0.03
20:4ω6 ARA	1.18 ± 0.12	1.27 ± 0.17	1.23 ± 0.19	1.41 ± 0.23	1.33 ± 0.34	1.7 ± 0.33	1.63 ± 0.33	1.79 ± 0.44
20:5ω3	3.16 ± 0.35	3.11 ± 0.27	2.94 ± 0.38	3.1 ± 0.42	2.23 ± 0.31	2.48 ± 0.34	2.26 ± 0.32	2.47 ± 0.39
22:0	0.19 ± 0.04	0.25 ± 0.03	0.21 ± 0.05	0.25 ± 0.04	0.16 ± 0.02	0.16 ± 0.02	0.17 ± 0.02	0.22 ± 0.04
22:5ω6	0.23 ± 0.03	0.24 ± 0.03	0.23 ± 0.05	0.22 ± 0.04	0.26 ± 0.04	0.27 ± 0.05	0.26 ± 0.06	0.23 ± 0.08
22:5ω3 EPA	0.26 ± 0.06	0.22 ± 0.02	0.18 ± 0.03	0.19 ± 0.03	0.17 ± 0.12	0.29 ± 0.15	0.17 ± 0.06	0.18 ± 0.09
22:6ω3 DHA	5.47 ± 0.48	5.03 ± 0.47	5.18 ± 0.65	4.75 ± 0.45	4.28 ± 0.43	4.02 ± 0.28	3.92 ± 0.51	4.01 ± 0.68

The ANOVA test showed differences between the sites in terms of TFA, SFA, MFA, PUFA, PUFA/SFA, ω3, ω6, ω3/ω6. For the factor of age, there were significant

differences for MFA and the ω3/ω6 ratio. We did not find any differences in FA between the sexes (Table 2).

Table 2. *Xiphopenaeus* spp. ANOVA test between the sites (Cananéia and Ubatuba), sex (Sex) and age (Age, juvenile and adult) in terms of total lipids = TFA, SFA = Saturated FA, MFA = Monosaturated FA, PUFA = Polyunsaturated FA, ω3, ω6, ω3/ω6. Significant differences are given by asterisk at 0.05: *p < 0.05; **p < 0.01 and ***p < 0.001 using the ANOVA test (Tukey), df =79.

FA	Site		Sex		Age	
	F	p	F	P	F	P
TFA	69.37	0.00***	0.86	0.35	2.80	0.09
SFA	67.76	0.00***	0.20	0.65	1.57	0.21
MFA	112.51	0.00***	2.66	0.10	18.71	0.00***
PUFA	53.94	0.00***	0.80	0.37	0.84	0.36
PUFA/SF	14.93	0.00***	1.63	0.20	1.15	0.28
ω3	14.93	0.00***	1.63	0.20	1.15	0.28
ω6	104.07	0.00***	3.96	0.05	0.27	0.60
ω3/ω6	16.60	0.00***	3.09	0.08	8.22	0.00**

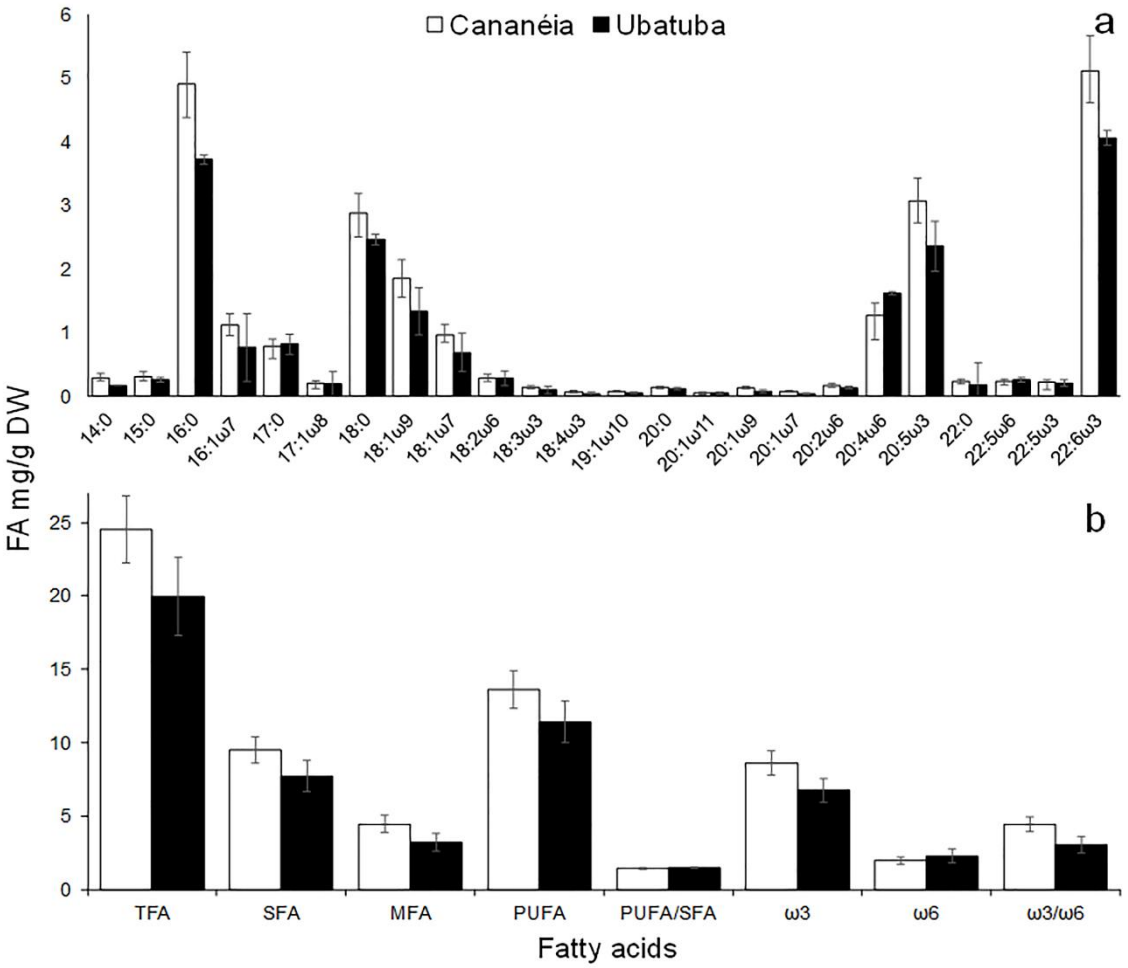


Figure 2. Fatty acids variation in shrimp *Xiphopenaeus* spp. from Cananéia and Ubatuba, São Paulo, Brazil. a) Average (±SD) of absolute FA concentrations (in mg/g DW) found in the shrimp; b) showed the FAs grouped according to the similarity of FA types: Saturated FA (SFA), Monosaturated FA (MFA), Polyunsaturated FA (PUFA), ω3 FA, ω6 FA, PUFA/SFA, ω3/ω6. Unit of measurement mg/g DW (dry material).

The non-metric multidimensional scaling (nMDS) applied to the FA profiles of the shrimps in relation to the two sites (Fig. 3) showed significant separation among the FA profiles of shrimps (ANOSIM, R: 0.63, p = 0.001), but not between the sex (ANOSIM, R: 0.004, p = 0.27) and age (ANOSIM, R: 0.034, p = 0.06). According to the PERMANOVA test, the FA of the shrimps was different between sites and age only (Table 3). SIMPER showed that the average dissimilarity between sites is 14% and the FAs, which together were responsible by 73% of this difference between the sites (CA and UBA), were 16:0, 22:6ω3, 20:5ω3, 18:1ω9, 18:0 and 20:4ω6.

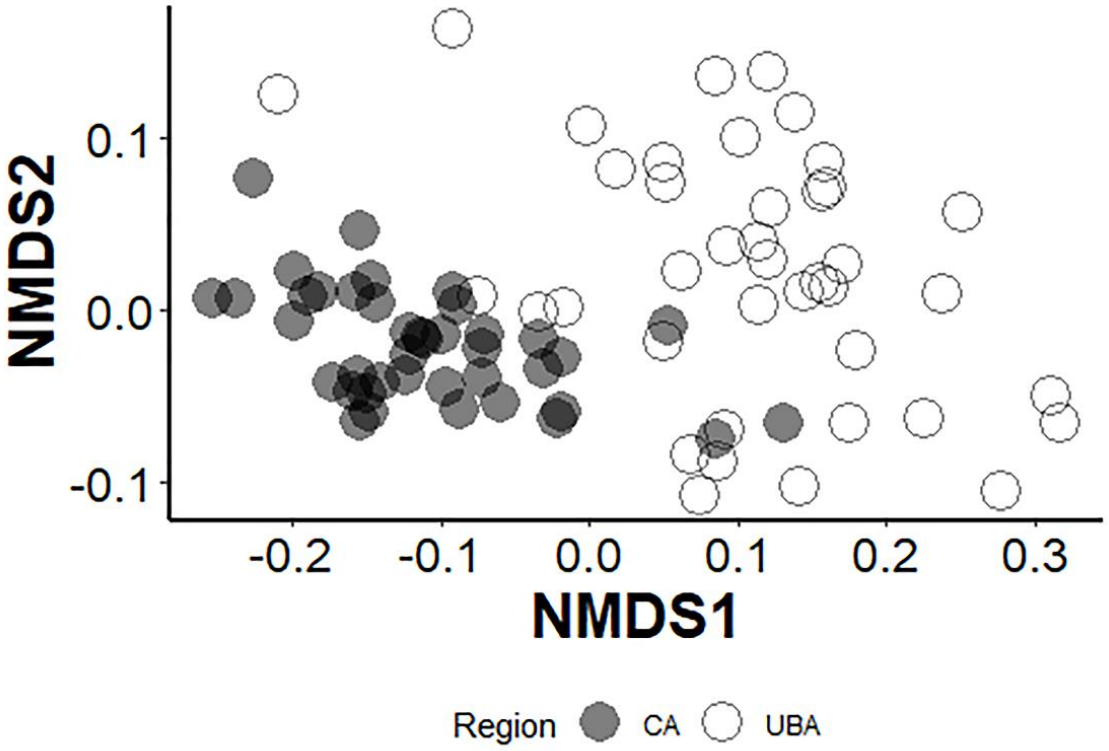


Figure 3. Non-metric multidimensional scaling (Bray-Curtis similarity) on untransformed, absolute FA concentrations of *Xiphopenaeus* spp., nMDS plots for the analysis between the sites = Cananéia and Ubatuba.

Table 3. Statistical test of fatty acid profile differences as a result of site (CA and UBA), sex (female and male) and age (juvenile and adult) by two-way factorial PERMANOVA (adonis functions). Significant differences are given by asterisk at 0.05: *p < 0.05; **p < 0.01 and ***p < 0.001.

	df	MS	*F*	*P*
Site	1	0.72	64.73	0.00***
Sex	1	0.01	2.6	0.07
Age	1	0.02	4.88	0.01*
Site *vs* Sex	1	0.00	0.8	0.40
Site *vs* Age	1	0.00	1.55	0.20
Sex *vs* Age	1	0.00	0.54	0.56
Site *vs* Sex *vs* Age	1	0.00	0.92	0.36

Abiotic (pH, Chl, HDO, SAL, TE, TDS, OM) and biotic variables (CL mm) for FAs profiles in both sampling sites are presented in Fig 4. The Monte-Carlo permutation test showed that the result of this analysis is significant (Monte-Carlo, df = 8, F = 5.55, p = 0.001). Moreover, this analysis reveals that the first two axes expressed 82% of the information about what factor can influence the fatty acids, with only the first axis being significant (Monte-Carlo, df = 1, F = 36.0, p = 0.001). Axis 1 is positively correlated with

Chl (Monte-Carlo, df = 1, F = 31.7, p = 0.001), HDO (Monte-Carlo, df = 1, F = 2.92, p = 0.02) and CL (Monte-Carlo, df = 1, F = 5.06, p = 0.005), with significant differences. The variables pH, SAL, TE, TDS and OM showed a significant negative correlation with axis 1.

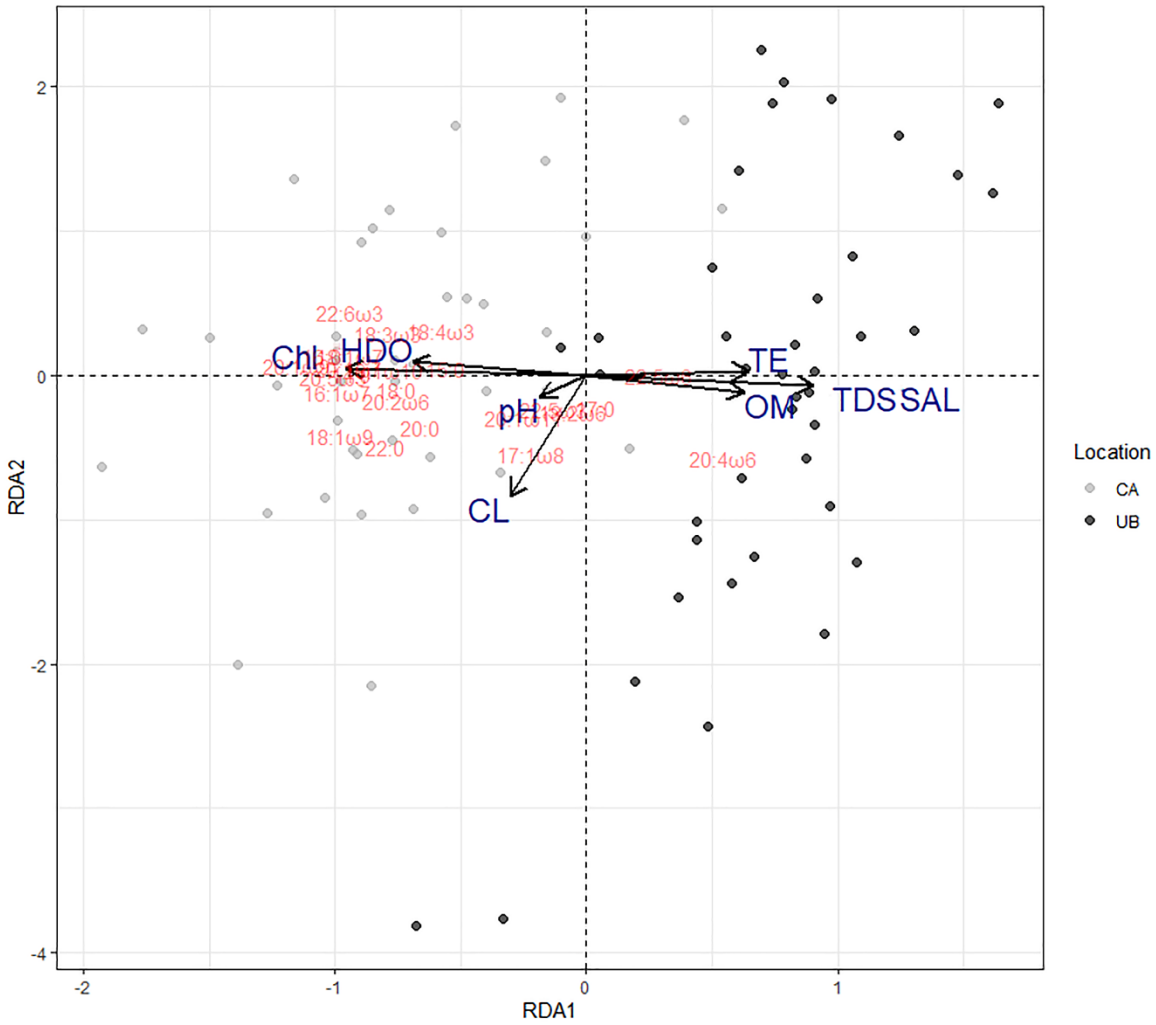


Figure 4. Redundancy analysis biplot (RDA) showing the FA profiles to the *Xiphopenaeus* spp. shrimps correlation between biotic variable CL (carapace length) and abiotic variables pH, chlorophyll (Chl), dissolved oxygen concentration (HDO), total dissolved solids (TSD), temperature (TE) and salinity (SAL), in in both sampling sites, Cananéia (CA) and Ubatuba (UBA).

Discussion

We confirmed the study's hypothesis showing how the FAs in *Xiphopenaeus* spp. are associated with the environment's features (Chl, HDO, pH and others), highlighting the lower levels of FAs in these shrimps in the UBA site. The lower concentration of PUFA in UBA, when compared to most preserved sites (CA), may occur due to the higher pollution rates in this site. Anthropic pressure from environmental pollution can decrease the abundance of fatty acids in animals living in these environments, especially essential fatty acids such as PUFAs (Bejaur et al., 2019). This interference in the accumulation of FAs such as PUFAs generates imbalances in the transfer of energy to the other trophic levels because these essential components will not be available at the other levels of the food chain.

Studying bivalves from different sites, Bejaur et al. (2019) observed that the site with the greatest pollution leads to a decrease in the percentage of PUFA. The PUFA reduction can be cause an oxidative damage response (Di Salvatore et al., 2013). Instead, Rocchetta et al. (2014) found that the increase in EPA and DHA proportions in *Dipolodon chilensis* (Gray, 1828) from the freshwater ecosystem, that could be an adaptive resilience response of environmental disturbance stress.

Mensens et al. (2018) measured the effects of metal and pesticide stress (copper and atrazine) on the contribution of benthic intertidal diatom community lipid (diet quality) production. The diatom diet quantity was reduced by metal stress but not by low pesticide levels due to the presence of an atrazine tolerant. But copper stress caused diatoms to lose 40% of their lipids. Consequently, copepod consumers lost half of their lipids when feeding on diatoms grown under low and high pesticide and high metal stress.

We emphasize in this study the importance of the shrimps as an essential link in the transfer of energy between primary producers, being a first consumer they transfer energy for the secondary consumers in these sites as mentioned by Dall et al. (1990), Abarca-Arenas et al. (2007) and Nagata et al. (2015). In this case, shrimps that have low quality of food sources that come from the of primary producers (that have low HUFA) generate low biomass. This low quality will be transmitted to the others trophic levels, generate low biomass for other consumers of the food web (Brett & Müller-Navarra, 1997).

The seabob shrimp has an omnivorous diet, feeding opportunistically. An important primary consumer, this species uses food sources of organic detritus, sediment,

benthic microalgae sedimentary, organic matter and can be a predator of epibenthic animals (Dall et al., 1990; Branco & Moritz Jr., 2001; Willems et al., 2016). Branco & Moritz Jr. (2001) observed a high quantity of sediment in *X. kroyeri* stomachs; sediment can contain different food resources for this benthic crustacean, like diatoms, bacteria, some algae, OM and others.

In this study, the shrimps' FAs 16:0, 18:0, 18:1ω9, ARA, EPA and DHA from both sites were more abundant among all the FAs analysed, and these same FAs were responsible for the statistical differences between sites. The most abundant FAs in UBA were 17:00, 18:2ω6, ARA and 22:5ω3, while the remaining was higher in CA. The abundance of some FAs in this study can be a biomarker. FAs 16:0 and 18:0 originate from diatoms/green algae, in which 16:0 is desaturated to 16:1ω7 and then further desaturated to 16:4n-1, a characteristic of diatoms. Fatty acid EPA is found in food resources of dinoflagellates, diatoms, and brown and red macro-algae. Docosahexaenoic (DHA) is found in both brown and red algae, detritus and dinoflagellates. Fatty acid 18:1ω9 is found in brown and red algae, vascular plants, and dinoflagellates. These FAs, along with 16:1ω7 and 18:1ω7, are typically dominant in bacterial FA composition and are used as tracers for the contribution of hetero-trophic bacteria to sediments, suspended organic material, and animal diets (Dalsgaard et al., 2003; Kelly & Scheibling, 2012). In the UBA site, we found higher abundance of the biomarker 18:2ω6 FA than in CA. These findings represent environmental contamination, corroborated with Rocchetta et al. (2014), who presented the 18:1ω9+18:2ω7 and 18:1ω9+18:2ω6 FAs as a biomarker for urban discharges.

The difference between the sites in this study was not evidenced only by the specific FAs, but for the sum of the TFA, SFA, MFA, PUFA, ω3, ω6 and ω3/ω6. Such fact was expected because of the differences in the sites' features, which can increase or decrease the abundance of FAs. There was no difference in the FAs analysis between sex of the seabob, one could interpret this as they use the same available food resources. We found some differences between life phases (Juv and Adu) in MFA and ω3/ω6 through ANOVA, despite the ANOSIN showing no significance, however PERMANOVA showed difference in the FAs between age. This difference shown between juveniles and adults can be an indicator of traces from food resources that consume in the planktonic phase of juvenile shrimps. These results go against our hypothesis that there is no difference in the composition of FAs between juveniles and adults in the same site. Willems et al. (2016) found differences between juveniles and adults regarding isotopic

analysis of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) among *X. kroyeri* life stages in Suriname, $\delta^{15}N$ values of adult were significantly higher than both post-larvae and juveniles. This can show that they use different food resources during the development, as juveniles can have traces for FA that come from the planktonic phase.

The lower values of ω3PUFA in UBA suggests that phytoplankton/benthos biodiversity is affected by urban contamination, that decrease the proportions of diatom, green algae and dinoflagelate PUFA rich sources, as found in Sabatini et al. (2011) and Rocchetta et al. (2014). A decrease in PUFA levels could related to oxidative damage on unsaturated FA that is produced by increasing of the metal levels in the aquatic system (Di Salvatore et al., 2013). The occurrence of metals in the shrimp *X. kroyeri* from UBA site was studied by Mantelatto et al. (1999), who stated that the increase in metals in this area may be caused by an increasing human population due to the tourism. This factor is a great contributor to sources of pollution, generating accumulation of metals in this site. Considering Mantelatto's paper was published almost 20 years ago, it is important to know if these metals are higher now a day.

The higher abundance of PUFAs in CA can occur due to the contribution of nutrients which come from the mangrove, as shown by Gatune et al. (2014) in Mtwapa creek along the north coast of Kenya. Gatune's study verified an increase of ARA, EPA and DHA of *Penaeus monodon* Fabricius, 1798 in the mangrove leaf with a litter progressive decomposition and with periphytic biofilm development. The bioconversion ability of *P. monodon* producing such biofilm brings an upgrade of nutrition for the shrimp. However, the mangrove in CA displays higher quantities of OM to the sea, as is discussed by Garcia et al. (2018), who found a correlation of higher chlorophyll "a" concentration inside the estuary of CA, the OM in the sediment created higher re-suspension of phytoplanktonic cells, as well as for nutrients deposited at the bottom, increasing the algal biomass. Thus, we emphasize the importance of this ecosystem for the preservation and sustainability of the species that are dependent on the high-quality food resources that CA provides. As a result of the mangrove's influence on the food resource, the shrimps have become more nutritious, increasing the value for their consumers, including humans, as mentioned by Gatune et al. (2014).

The environmental factors Chl and HDO and biotic factor CL (mm) had different influences on the FAs composition between both sites. When Chl, HDO and CL increased, the FAs in CA increased too, but we saw inversely proportional results in UBA. The other biotic factors do not influence the difference in the FAs between the sites. The

Chl/phytoplankton was responsible for producing a large amount of oxygen in the water, as showed in Kunlasak et al. (2013). This Chl is responsible for supplying short carbon chains that consumers convert into other essential fatty acids (Brett & Müller-Navarra, 1997). Zang et al. (2011) found positive relationship with HDO and chlorophyll-a concentration, as verified in the present investigation to CA.

Dissolved oxygen concentration, which here correlates with phytoplankton, is an important factor for shrimp respiration and, for the health of aquatic ecosystems and acts as key indicator in determining water quality (Kunlasak et al., 2013). Dissolved oxygen is one of the limiting factors for aquatic physiological metabolism, and its value is an indicator of aquatic growth conditions and pollution status (Bolton et al., 1978). These low environmental factors found in our study in UBA, echo the decrease in water quality in this site, probably due to the increased population, mainly through tourism, and thus a greater discharge of untreated urban waste into the ocean. According to Li et al. (2009), in the sites that were greatly affected by industrial, agricultural and domestic sewage, the decomposition of organic matter subsequently led to the depletion of oxygen. This can cause the death of countless animals and affect the chain base that is the phytoplankton, changing the entire transfer of energy through trophic levels.

The pH in our study no showed correlation, but in the RDA test it was possible to see that this parameter can influence the FAs composition in CA. Ruan et al. (2008) show that with the increase of phytoplankton biomass, the consumption of carbon dioxide caused by photosynthesis promoted an increase of the pH value, as found in our results for CA.

It was possible to observe how environmental pollution can alter phytoplankton / chlorophyll in the environment, generating a negative cascading effect on organisms. For maintaining a healthy environment, high levels of phytoplankton are required, consequently dissolving oxygen that will allow an increase in primary producers, a basis for sustaining the food chain (Zang et al., 2011; Kunlasak et al., 2013). As discussed in the Brett & Müller-Navarra (1997) work, in planktonic food webs with high HUFA content can be control the processes between plant production and herbivores, increased fisheries production. Restore places biologically altered can be done by stimulating the growth of HUFA-rich diatoms and cryptophytes.

Larger sizes (CL in mm) of shrimps showed positive correlation with the abundance of FAs from CA, this can be related to the great preservation of this site, which provides abundant good quality food resources. Distinctly UBA showed have a decrease

in energy transferred between trophic levels, related to the anthropization of this site. Other factors that can be involved with the difference in FAs between the sites are the changes in food resources and the reproduction phase for shrimps (Willems et al., 2016).

In our study, seabob shrimp were shown to be an important link in the transference of the energy between the trophic web and a good food source for the human population, as it is a source of essential fatty acids and proteins. However, it is necessary to pay attention to the environment that these animals develop in, as their potential effects may be altered by the anthropic action in the environment, decreasing their nutritional quality, as well as being bioaccumulators of other toxic components that can cause damage to health. Nevertheless, higher concentrations of EPA and DHA in *X. kroyeri* specimens indicate that this species is a good source of nutrition its importance for higher trophic levels feeding (Kerkhove, 2020). These PUFA, EPA and DHA were the most important, for health, inhibiting the transformation of the arachidonic acid to thromboxane-A2, which could activate vasoconstriction, plaque aggregation and cause arterial hypertension (Fossati, 1994).

We can suggest that exposure of *Xiphopenaeus* spp. to low water quality related by the increasing anthropic process in UBA, has negatively affected its lipid composition, altering the total lipid content. FA composition was most affected in the PUFA fraction, with a higher content of ω6. Specific markers show that diatoms, bacteria and dinoflagellate are particularly important for the energetic budget of the shrimp *Xiphopenaeus* spp.

Seabob shrimp is the one of the most abundant species in fishing (Castilho et al., 2015; Boss et al., 2016), consequently, have more accessible value, thus, is widely consumed as food. Another benefit to humans, as we saw in our study, is that this shrimp is rich in PUFA, which is essential for maintaining good health. However, as this shrimp is influenced by the quality of the environment, they can lose a large amount of PUFA in altered environments. In addition, ecologically, this shrimp is overexploited by fishing, with a cascading effect on the entire ecosystem in which it is found.

This study shows the importance of preserving the environment to maintain the high quality of organisms in the chain link (as per shrimp) and in the transfer of energy in the trophic web. As a result of reducing of fishing pressure and untreated waste discharges in the environment, there would be a higher opportunity for the restoration of this species that has such an important ecological role. This would consequently bring a high primary productivity of better quality from all the other organisms that are connected

through the trophic web. It would also improve the conservation of fishery resources and the environment. Further studies should be carried out to see how changes in FAs can affect higher trophic levels in the trophic web.

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EPILOGUE

Given all the scientific evidence demonstrated in this thesis, we can briefly conclude that:

The symbiotic relationship of the spider crab *Libinia ferreirae* with the host jellyfish is important for the development and survival of this crab, especially in the early stages of life, where the physical development of the crab and the host jellyfish is correlated over time.

In this study, two forms of symbiotic behaviour during the association of juvenile crab with the host jellyfish were demonstrated in the laboratory, one by lifting up their chelipeds and opening their pereiopods, and second, by "gliding" - being carried by the water's current. In the field, we saw that the first contact of association with the host medusa also occurs in the megalopa phase. We observed that crabs associated with jellyfish tend to compete for the host, expelling the weaker symbiotic crabs.

The relationship that *L. ferreirae* maintains with the host jellyfish shows evidence of kleptoparasitism, due to the contents of the stomach and a similar isotopic signal from the host jellyfish food resource, however this relationship should be further explored to be confirmed as kleptoparasitism. The relationship of this spider crab with its epibionts is clearly mutualism, as proposed in other studies. Evidently, these symbiotic relationships maintained by the crab *L. ferreirae* generate benefits for the crab, mainly of niche distribution between the ontogenetic phases, reducing the competition for food and space. Still, these relations bring protection for the crab and dispersion over a wider geographic area.

The trophic structure of the organisms caught by trawling was composed of 11 ecological guilds at 3 different trophic levels. The trophic web proved to be supported mainly by marine phytoplankton, where zooplankton and invertebrates were responsible for the transfer of energy between primary producers and secondary consumers. This

trophic web had some species of fish and jellyfish as top predators. Different guilds show an overlap in the ellipse area calculated in the same season of the year, showing that in the winter the species tend to compete more for food resources. Changes in some identical guilds were also evident between the winter and summer seasons regarding the ellipse calculated area, showing that some guilds have a narrowing or breadth of the food niche according to the season.

The fatty acid (FA) profile of the shrimps *Xiphopenaeus* spp., *Penaeus schmitti* and *Penaeus paulensis* were composed of the same 24 FAs, but the different species had different levels of these FAs.

Xiphopenaeus has the most distinct FA profile, due to the restricted marine life habit. The FA profile of this shrimp Genus in the study regions of Cananéia and Ubatuba were different between ages, however it was not different between sex. This shows that the young and the adults are using different food resources.

Between the studied regions, the state of environmental conservation influences the profile of FAs. The unstructured tourism in the Ubatuba region generates a large amount of untreated sewage which is discharged into the sea, likely has an influence, showed in a lower abundance of FAs, when compared with Cananéia.

The collection of studies carried out here demonstrates how species are interconnected in the environment in different ways and how important it is to conserve these organisms, as well as the environment for the survival of different species, so that they can fulfil their role in the transfer of energy along the food chain.