RESSALVA

Atendendo solicitação do(a) autor(a), o texto completo desta tese será disponibilizado somente a partir de 28/08/2022.

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

BOTUCATU 2020

SÃO PAULO STATE UNIVERSITY (UNESP) INSTITUTE OF BIOSCIENCES

PhD Thesis

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

Geslaine Rafaela Lemos Gonçalves

Supervisor: Prof. Dr. Antonio Leão Castilho

Co-supervisor: Prof. Dr. Maria Lucia Negreiros-Fransozo

Thesis presented to the Institute of Biosciences from the São Paulo State University "Júlio de Mesquita Filho" – UNESP – Botucatu Campus, as part of the requirements to obtain the title of Doctor of Science, Graduate Program of Zoology - Biological Science.

Botucatu – SP 2020 FICHA CATALOGRÁFICA ELABORADA PELA SEÇÃO TÉC. AQUIS. TRATAMENTO DA INFORM. DIVISÃO TÉCNICA DE BIBLIOTECA E DOCUMENTAÇÃO - CÂMPUS DE BOTUCATU - UNESP BIBLIOTECÁRIA RESPONSÁVEL: ROSEMEIRE APARECIDA VICENTE-CRB 8/5651

Gonçalves, Geslaine Rafaela Lemos.

Ecologia e estrutura trófica do caranguejo aranha Libinia ferreirae (Decapoda: Majoidea) no litoral sul de São Paulo / Geslaine Rafaela Lemos Gonçalves. - Botucatu, 2020

Tese (doutorado) - Universidade Estadual Paulista "Júlio de Mesquita Filho", Instituto de Biociências de Botucatu

Orientador: Antonio Leão Castilho

Coorientador: Maria Lucia Negreiros-Fransozo

Capes: 20402007

1. Caranguejo - Pesca. 2. Pesca de arrastão. 3. Ácidos graxos. 4. Isótopos estáveis. 5. Simbiose.

Palavras-chave: Ácidos graxos; Isótopos estáveis; Pesca de arrasto; Simbiose; Teia trófica.

"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.

Acknowledgment

I would like to thank the São Paulo State University (UNESP), Institute of Biosciences - Botucatu Campus and the Department of Zoology for hosting me and giving me a place on the graduate program.

I am grateful to the funding agencies, who have been essential in carrying out my work since my Technical, Master and Doctorate training. Thanks to The São Paulo Research Foundation (FAPESP) for the financing that preceded my Doctorate (Biota-FAPESP # 2010 / 50188-8, Technical training, 2013 / 08517-2, Master, 2014 / 13770-1). I am grateful to FAPESP for the financing of my doctorate (2018 / 01659-0) and for the Research Internship Scholarship Abroad (BEPE, 2019 / 00105-3), which enabled the execution of my research and provided me with a refined improvement of my work and knowledge. I also thank the Coordination of Superior Level Staff Improvement (CAPES) for the scholarship granted. Thanks to funding from the European Union's Horizon 2020 research and innovation program under ASSEMBLE Plus project grant agreement No 730984. I thank the Ghent University funded by EMBRC Belgium - FWO agreement 20151029-03 and Special Research Fund of Ghent University (BOF-UGent).

My thanks to Exeter University - UK and Ghent University - Belgium for accepting me for my research internship abroad, making this process possible. Thanks to the professionals who mediated and helped this process, especially Adriana Caton - PGR Admissions Supervisor and my friend Dr. Ellie Sargent - technician from Exeter University, and Marleen Roelofs - Scientific project officer and Dr. Bruno Vlaeminck - technician, both in Ghent University.

I also thank "Instituto Chico Mendes de Conservação da Biodiversidade" (ICMBio) and the "Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis" (IBAMA) for granting permission to collect the samples.

To my supervisor Professor Dr. Antonio Leão Castilho for the trust placed in me during all these years and for believing in my work. I am grateful for the help throughout my career, throughout life-changing times, and for the strength transmitted. Thank you for sharing all your knowledge, for supervising all of my work. Thank you for giving me a chance and for believing in my potential. I leave here my admiration for you.

To my co-supervisor Professor Dr. Maria Lucia Negreiros-Fransozo for her teachings, dedication, friendship, advice and for the trust placed in me during this work.

I thank you for always being willing to help, for sharing all your knowledge, for being the example of a woman and professional, that left a mark on my life.

To Professor Dr. Stuart Bearhop for accepting me as his student during my internship abroad, thanks for the chance to improve my work and my English. I am grateful for the countless discussions and for encouraging discoveries. Thank you for taking me to the field, for the opportunity to learn about geese collection techniques, which brought me closer to the team, creating more than collaborations but friendships. I really appreciate all the support that you gave me.

Professor Marleen De Troch, I thank you for being interested in my work and for providing me with a new possibility of learning, partnership and friendship. Thank you for teaching me and giving me such solid support that it made everything lighter and simpler throughout the process. I thank you for the conversations, laughter and support. I really appreciated every moment spent with you.

Professors Stuart and De Troch, you have no idea of the dimension of changes in my life that the opportunity to work with you brought, the Ges that went to Europe returned as a different Ges, with better dreams, perspectives and strength. Words are not enough to thank you for the opportunities I had with you. I leave here my great admiration for both.

To Professor Dr. Felipe W Amorim for his help in the development of my work through analysis, interpretation and discussion.

I am grateful to the Stable Isotopes Center (CIE), Institute of Biosciences, São Paulo State University (UNESP) for the stable isotope analysis, and Professor Dr. Vladimir Eliodoro Costa for his assistance in interpreting the analysis and developing the work. I also thank the technician Cibele Regina de Souza Kruliski for her help and patience.

To the Department of Zoology of the Biosciences Institute, UNESP Botucatu campus, for providing good employees who have helped us countless times, thank you: Flávio da Silva, Silvio Almeida, and in particular, Hamilton A. Rodrigues and Roseli Aguiar de Oliveira for their competence and willingness to help.

To the Graduate Program of Zoology – Biological Science, of this Institute, for the facilities granted and the employees, especially Davi Müller and the coordinator Prof. Antonio Leão Castilho.

To the dear friends of the cleaning team, especially Dona Maria for providing a better and happier environment for our work.

To Milena Regina Wolf and Aline N Sousa for their friendship, companionship for life, professionally and personally, for all the moments of relaxation and for hearing me during my moments of outburst.

To my dear laboratory friends who have helped me tirelessly in countless ways, especially Milena Wolf, Pedro V Santos, Ana Clara Denadai and Giovanna Galli, who were always willing where necessary, you were essential for the accomplishment of this work.

To Gisele Tiseo and Leandro Santos for their willingness, joy and all the help given in preparing this study.

To the Fishermen who helped in the sampling of the material for the study as: Passarinho, Cinézio, especially Mr. Aneves for his affection and simplicity.

To my boyfriend Alexander Wilkes, for his support, admiration, love and affection. Thank you for helping to improve English in my work.

My family, for being the solid foundation that made me able to overcome life's obstacles, to my parents João and Eliane who always taught me to be strong, respectful, have humility and always remember the origins. Thank you for everything you did for me to be here, now. To my dear brother Maximiliano who helped me to acquire responsibilities, which always filled me with joy and love. It was for having you as my family that I am as I am today, thank you for everything.

To the "Divine Creator & the forces of the universe", who have always guided me on the path of love and peace and who have surrounded me with wonderful and enlightened people, throughout my life.

And finally, you, the reader of this work, who chose to read it among so many others. Without you, this job would be meaningless. Thank you!

SUMMARY

Prologue	1
CHAPTER I: Ontogenetic niche specialization of the	spider crab <i>Libinia ferreirae</i>
associated with Lychnorhiza lucerna medusa	
Abstract	9
Introduction	9
Materials and Methods	11
Results	15
Discussion	24
References	28
Supplementary material	35
CHAPTER II: Trophic relationships between the c symbionts	rab <i>Libinia ferreirae</i> and its
Abstract	38
Introduction	
Materials and Methods	42
Results	44
Discussion	50
References	52
CHAPTER III: Trophic web of organisms caught fishing in southeastern of Brazil	through commercial shrimp
Abstract	59
Introduction	60
Materials and Methods	63
Results	66
Discussion	75
References	80
Supplementary material	87

CHAPTER VI: Species-specific fatty acid profiles of three consumption shrimp	
from Southeastern Brazil reveal their role in a complex local food web	
Abstract	92
Introduction	92
Materials and Methods	96
Results	98
Discussion	102
References	108
CHAPTER V: Changes in fatty acid profiles of Xiphopenaeus spp. indicates change	
in pollution levels in the Southeastern Brazilian coast	
Abstract	118
Introduction	118
Materials and Methods	121
Results	
Discussion	125
D 6	
References	131
References	131
Epilogue Epilogue	131

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 cosupervision 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 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.