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TÍTULO DA TESE: Brioprospecção de compostos antioxidantes na fauna acompanhante da pesca demersal

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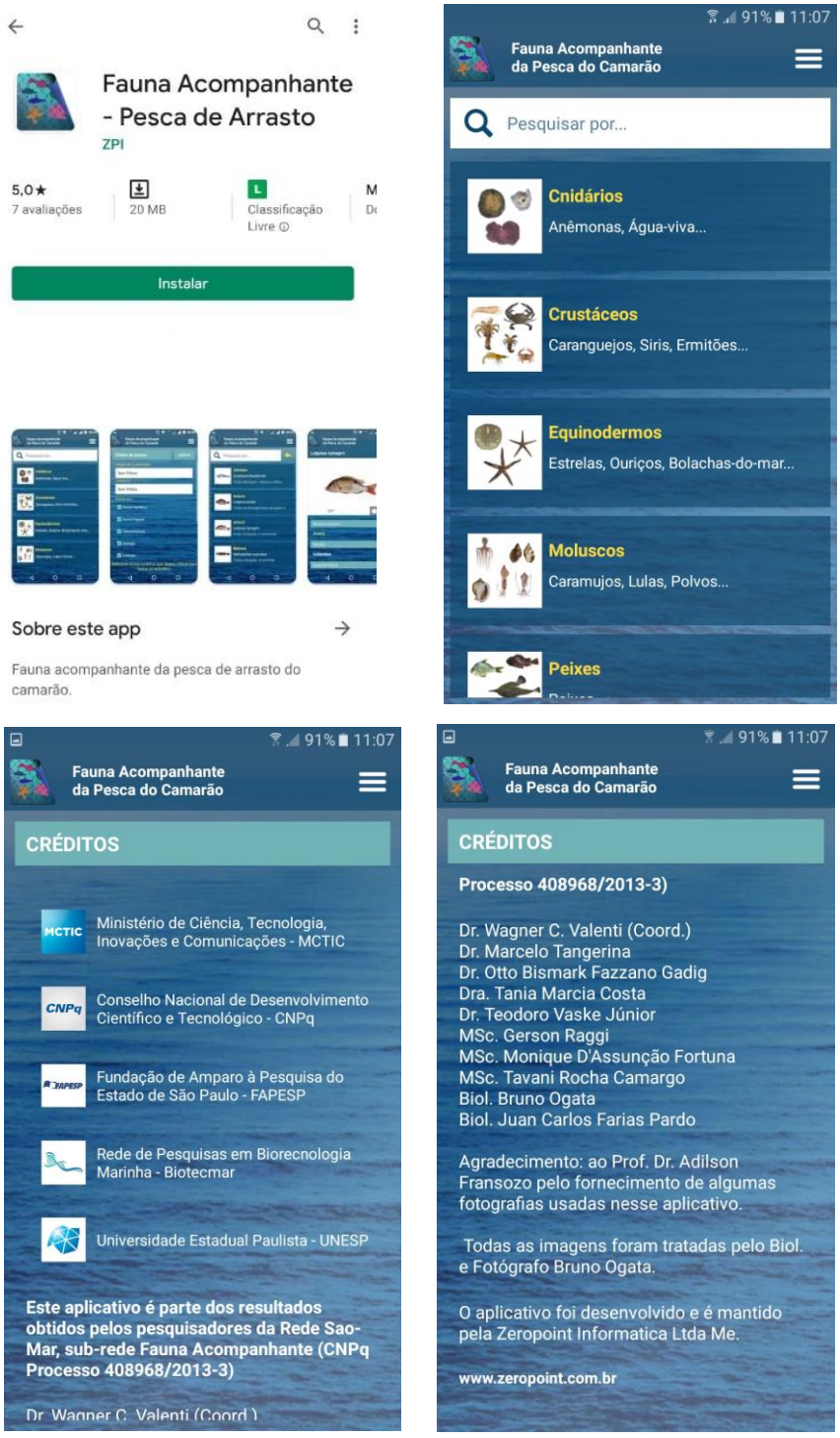
Sumário

Apresentação da Tese

Esta tese insere-se dentro da rede de pesquisa em Biotecnologia Marinha "Rede Sao-Mar", instituída pelo MCTI/CNPq (Processo #408968/2013-3) envolvendo a Universidade Federal do Rio Grande (FURG), Instituto de Biociências do câmpus do Litoral Paulista, São Vicente-SP, Instituto de Estudos Avançados do Mar - IEAMar, o Centro de Aquicultura - CAUNESP e a Universidade Federal de Santa Catarina - UFSC. O projeto "Fauna Acompanhante: um Universo Químico a ser Explorado", vinculado à Rede SAO-MAR, tem como objetivos investigar quimicamente as espécies da fauna acompanhante da pesca do camarão no litoral paulista e avaliar/estudar o potencial das moléculas encontradas na fauna acompanhante. Em 2018, dentro do projeto, foi desenvolvido o aplicativo para celular, plataforma Androide, denominado "Fauna Acompanhante" (Registrado no INPI sob o nº. BR512019001402-2). O objetivo do aplicativo é possibilitar que pescadores, técnicos e a sociedade em geral conheçam as principais espécies da fauna acompanhante da pesca de arrasto do camarão. Seu acesso pode ser feito por meio dos seguintes passos: abra o aplicativo da Google Play Store no dispositivo Android. Utilize a caixa de pesquisa rápida e coloque as palavras "fauna acompanhante". O nome do aplicativo deverá ser visualizado, bem como o ícone do mesmo e uma caixa verde com a palavra "Instalar". Ao clicar em "Instalar", aparecerá um ícone na barra de notificações do smartphone Android informando o início do download. Após ser instalado, o aplicativo poderá ser aberto diretamente da barra de notificações ou na gaveta de aplicativos do aparelho. As espécies que são capturadas acidentalmente estão separadas de acordo com o filo e subfilo. Ao clicar nas espécies é possível visualizar informações sobre o nome científico, a família a que pertence e a região de ocorrência.

Figura 1. Layout do aplicativo Fauna Acompanhante.



A temática da presente tese é de interesse mundial e atualmente inúmeros países utilizam a pesca de arrasto em plataformas continentais, sendo que a ação continuada desta pesca contribui para a diminuição da biodiversidade marinha. Assim, a extração de biomoléculas pode ser uma destinação sustentável para o aproveitamento do *bycatch*,

possibilitando a diminuição da pesca extrativista e gerando renda adicional aos pescadores por meio da venda tanto dos camarões quanto dos organismos atualmente rejeitados. Dessa forma, os objetivos principais da presente tese foram investigar a ocorrência de biomoléculas antioxidantes em hidrolisados obtidos dos animais da fauna acompanhante da pesca demersal e testar a hipótese de que a microencapsulação retém a atividade antioxidante dos hidrolisados. Foram escolhidos os quatro animais mais abundantes da fauna acompanhante no litoral de São Paulo: os peixes *Paralonchurus brasilensis* (maria luiza) e *Micropogonias furnieri* (corvina); o siri *Callinectes ornatus* (siri azul) e o caranguejo *Hepatus pudibundus* (siri-baú). Os trabalhos foram realizados com o apoio do Prof. Dr. José Monserrat, da Universidade Federal do Rio Grande - FURG, do Prof. Dr. Willian Fernando Zambuzzi, da UNESP câmpus de Botucatu, e do Prof. Dr. Adem Gharsallaoui da Universidade de Lyon, na França.

Resumo

Nos últimos anos, a fauna acompanhante da pesca camaroeira vem sendo alvo de estudos por ser um dos fatores do grande impacto ambiental causado pela pesca de arrasto. Em contrapartida, esse rejeito de pesca pode ter propriedades funcionais e bioativas, como peptídeos antioxidantes, que poderiam agregar valor a esse rejeito e se tornar um produto de interesse para as indústrias alimentícias. Assim, o presente estudo visou investigar a atividade antioxidante dos hidrolisados proteicos obtidos dos animais mais abundantes da fauna acompanhante e microencapsular esses hidrolisados para agregar valor à este material normalmente descartado. Os resultados demonstram que a hidrólise enzimática, utilizando as enzimas comerciais Alcalase 2.4 L® e Protamex®, é um método eficiente para liberar peptídeos de interesse econômico nas duas espécies mais abundantes de peixes (*Micropogonias furnieri* e *Paralonchurus brasilensis*) e nas duas mais abundantes de crustáceos (*Callinectes ornatus* e *Hepatus pudibundus*). A hidrólise liberou peptídeos com atividade antioxidante em todas as amostras analisadas, submetidas às duas enzimas testadas. A coacervação complexa e subsequente microencapsulação por *spray-drying* mostrou-se eficiente para proteger a atividade antioxidante desses hidrolisados proteicos. Assim, os resultados fornecem evidências para o potencial uso dos hidrolisados das espécies analisadas como ingrediente funcional ou nutracêutico na indústria alimentícia.

Palavras-chave: bycatch, hidrolisados proteicos, pesca de arrasto, sustentabilidade.

Abstract

In recent years, the bycatch of shrimp fishing has been the subject of studies as it is one of the factors of the great environmental impact caused by trawling. However, this bycatch can have functional and bioactive properties, such as antioxidant peptides, which could add value to this reject and become a product of interest to the food industries. Thus, the present study aimed to investigate the antioxidant activity of protein hydrolysates obtained from the most abundant animals of the bycatch and to microencapsulate these hydrolysates to add value to this normally discarded material. The results demonstrate that enzymatic hydrolysis, using the commercial enzymes Alcalase 2.4 L® and Protamex®, is an efficient method to release peptides of economic interest in the two most abundant species of fish (*Micropogonias furnieri* and *Paralonchurus brasilensis*) and in the two most abundant crustaceans (*Callinectes ornatus* and *Hepatus pudibundus*). Hydrolysis released peptides with antioxidant activity in all samples analyzed, submitted to the two enzymes tested. Complex coacervation and subsequent microencapsulation by spray-drying proved to be efficient to protect the antioxidant activity of these protein hydrolysates. Thus, the results provide evidence for the potential use of hydrolysates of the analyzed species as a functional or nutraceutical ingredient in the food industry.

Keywords: bycatch, protein hydrolysates, trawling, sustainability.

Introdução Geral

O Brasil é um dos países mais ricos em biodiversidade do mundo, porém o uso adequado dos recursos naturais para gerar benefícios sociais e econômicos ainda é incipiente. No início da década de 2010, cerca de 3,5 milhões de pessoas estavam envolvidas na pesca e aquicultura no país, o que implica na adoção de políticas para exploração racional da biodiversidade aquática (FAO, 2013). Apesar disso, a pesca tem sido praticada da mesma maneira que nos países com baixa diversidade, uma vez que a forma de captura é rudimentar e de pequena seletividade (Graça-Lopes et al., 2002a, b; Severino-Rodrigues et al., 2002, 2007; Castilho et al., 2008; Medeiros et al., 2013; Babcock et al., 2018). A pesca de arrasto demersal causa um enorme impacto ambiental, pois a baixa seletividade dos petrechos leva ao descarte de grande número de organismos capturados acidentalmente denominados fauna acompanhante ou *bycatch* (Graça-Lopes et al., 2002a, b; Severino-Rodrigues et al., 2002, 2007; Robert et al., 2007; Garcia et al., 2016; Orbesen et al., 2017; Varisco et al., 2017; Anjos et al., 2018; Ruibal Núñez et al., 2018).

O termo fauna acompanhante pode ser definido como o grupo de organismos capturados com a espécie-alvo, que não são comercializados, e são devolvidos ao mar ou rejeitados (Graça-Lopes et al., 2002a, b; Hall, 2000; Severino-Rodrigues et al., 2002, 2007; Fonseca et al., 2005; Kumar & Deepthi, 2006; Orbesen et al., 2017; Varisco et al., 2017; Gray & Kennelly, 2018; Ruibal Núñez et al., 2018). A maioria da fauna acompanhante é devolvida ao mar morta ou com poucas chances de sobrevivência (Severino-Rodrigues et al., 2002, 2007). Os debates públicos buscando potenciais soluções para este problema foram iniciados no início da década de 1990 (Conolly, 1986; Clucas, 1997), devido a captura de golfinhos em redes de atum e tartarugas em redes de arrasto de camarão (Castilho et al., 2008; Yang et al., 2015; Serena et al., 2016).

A pesca do camarão captura enorme quantidade de fauna acompanhante, sendo provavelmente a que causa maior impacto (Graça-Lopes et al., 2002a, b; Severino-Rodrigues et al., 2002, 2007; Castilho et al., 2015; Hannah et al., 2015; Yang et al., 2015; Pajuelo et al., 2018). Isso provoca uma ampla destruição da região bentônica. No entanto, deve-se levar em consideração os aspectos sociais e econômicos desse tipo de pesca. No litoral do Estado de São Paulo, por exemplo, os aspectos econômicos da captura de espécies não-alvo não estão associados à perda de valor econômico, mas sim aos custos dessas capturas indesejáveis, como o desgaste do barco e a contratação de mão-de-obra para ajudar na triagem dos animais que vem na rede (comunicação pessoal com pescadores do litoral norte do Estado de São Paulo). Os aspectos sociais, em contrapartida, vêm da busca constante de soluções para o *bycatch*, pois proibir ou embargar a pesca de arrasto pode acarretar em sérios prejuízos aos pescadores, cuja a principal fonte de renda é o camarão. Assim, o equilíbrio entre os impactos ambientais, sociais e aspectos econômicos tem sido um desafio constante da pesca de arrasto demersal há várias décadas.

Pesquisas sobre a diversidade das espécies da fauna acompanhante (Severino-Rodrigues et al., 2002, 2007; Robert et al., 2007; Burgess et al., 2018; Pajuelo et al., 2018), bem como o número de indivíduos capturados (Graça-Lopes et al., 2002a; Severino-Rodrigues et al., 2007; Ruibal Núñez et al., 2018), a manutenção dos estoques e a exploração com novos petrechos (Graham, 2003; Campos & Fonseca et al., 2004; Fonseca et al., 2005; Medeiros et al., 2013) vêm sendo realizadas. Porém, ainda não foi encontrado um destino adequado para o *bycatch*. Neste contexto, a enorme biodiversidade encontrada na fauna acompanhante oferece um vasto cenário para a extração de moléculas e compostos químicos que podem ser utilizados para diversos fins (Donia & Hamann, 2003; Chakraborty & Ghosh, 2010).

A fauna acompanhante pode ser uma fonte de produtos naturais e contém elevado teor de proteínas, aminoácidos, antioxidantes e outros compostos que podem ser transformados em produtos com valor de mercado, como alimentos para animais (Chalamaiah et al., 2012). Para isso, técnicas, como a hidrólise enzimática, têm sido desenvolvidas a fim de extrair peptídeos de interesse comercial (Chalamaiah et al., 2012; Halim et al., 2016; Zamora-Sillero et al., 2018a; Altınelataman et al., 2019). Na hidrólise enzimática, várias enzimas proteolíticas têm sido usadas para produzir hidrolisados de proteínas de peixe (Fish Protein Hydrolysates - FPH) a partir de resíduos da filetagem, como a pele, músculo, vísceras e outros subprodutos (Kristinsson & Rasco, 2000; Chalamaiah et al., 2012; Centenaro et al., 2014; Zamora-Sillero et al., 2018a, b; Altınelataman et al., 2019). As enzimas clivam as ligações peptídicas das proteínas formando peptídeos menores, que podem ter função biológica, agregando valor a esses subprodutos, que geralmente são descartados (Kristinsson & Rasco, 2000; Chalamaiah et al., 2012; Zamora-Sillero et al., 2018a, b; Altınelataman et al., 2019). Estudos prévios demonstraram que os FPH possuem compostos bioativos, nutrientes essenciais e são potenciais fontes de peptídeos antioxidantes, sendo que estes peptídeos possuem um grande valor comercial (Kristinsson & Rasco, 2000; Da Frota Jr et al., 2009; Bougatef et al., 2010; Chalamaiah et al., 2012; Halim et al., 2016; Zamora-Sillero et al., 2018a, b; Altınelataman et al., 2019).

A extração de peptídeos com ação antioxidante a partir de animais marinhos é de interesse comercial. Essas substâncias inibem o estresse oxidativo causado pelo excesso de espécies reativas de oxigênio (ERO), produzidas pelos organismos aeróbicos (Guedes et al., 2013; Perdicaris et al., 2013; Shebis et al., 2013). A formação de ERO ocorre devido aos processos bioquímicos do próprio organismo, como a fosforilação oxidativa, porém o excesso de ERO pode comprometer a fisiologia e as defesas imunológicas dos

organismos (Hemes-Lima, 2004; Soto-Otero et al., 2008). Diante disso, as substâncias antioxidantes podem ser acrescentadas na formulação de remédios ou aplicadas como nutracêuticos na alimentação de espécies com interesse para a aquicultura marinha ou de água doce (Rangel et al., 2001; Binsan et al., 2008; Da Frota Jr et al., 2009), pois a ingestão de uma dieta rica em antioxidantes pode melhorar a resposta de um organismo ao estresse oxidativo, auxiliando de maneira mais eficiente o funcionamento do metabolismo (Krinsky, 1989; Arnao et al., 2001; Binsan et al., 2008; Soto-Otero et al., 2008; Carocho & Ferreira, 2013). Apesar das inúmeras vantagens dos antioxidantes, estes e outros compostos bioativos, como as proteínas, são biopolímeros susceptíveis aos efeitos das condições do meio (Zamora-Sillero et al., 2018a). Dessa forma, para usar esses compostos na indústria alimentícia é necessário utilizar processos que protejam as suas propriedades bioativas e evitem perdas nutricionais (Zamora-Sillero et al., 2018a). Processos como a microencapsulação permitiram a ampliação do uso desses compostos na indústria de alimentos, farmacêutica e química (Gharsallaoui et al., 2007).

A microencapsulação é uma técnica que forma microcápsulas por meio do recobrimento de uma substância de interesse por um material que a isole total ou parcialmente do ambiente (Gharsallaoui et al., 2007). A microencapsulação tem por objetivo proteger um composto e modular a sua liberação (Gharsallaoui et al., 2007). Os processos de microencapsulação são aplicados na proteção de substâncias sensíveis à temperatura, oxirredução, microrganismos entre outras ações indesejáveis (Gharsallaoui et al., 2007). A formação das microcápsulas ocorre por métodos físicos (*spray-drying*, extrusão, co-cristalização), químicos (polimerização interfacial, inclusão molecular) e físico-químicos (coacervação simples ou complexa) (Gharsallaoui et al., 2007). Entre estas metodologias, a coacervação complexa possui vantagens pela sua elevada eficiência de encapsulação (acima de 99%), facilidade de liberação controlada de seu conteúdo e

pelas condições brandas de temperatura no processamento (Gharsallaoui et al., 2007; Amara et al., 2017; Wang et al., 2019). Esta método envolve a combinação de duas dispersões hidrocolóides de cargas opostas, por meio de interação eletrostática, resultando na formação de microcápsulas (Gharsallaoui et al., 2007; Gharsallaoui et al., 2012). A finalidade da coacervação complexa é melhorar a estabilidade e estrutura dos polímeros complexos (Amara et al., 2017; Wang et al., 2019). Outro método amplamente utilizado é o *spray-drying*, devido ao baixo custo e facilidade de obtenção dos equipamentos necessários para a encapsulação (Gharsallaoui et al., 2007; Gharsallaoui et al., 2012). O processo de *spray-drying* seca um material fluido formando partículas secas (pó) pela aspersão desse fluído em um meio de secagem aquecido (Gharsallaoui et al., 2007; Gharsallaoui et al., 2012). A produção de microcápsulas associando diferentes processos é usualmente empregada para aumentar a estabilidade e melhorar a aplicabilidade dos compostos (Gharsallaoui et al., 2007; Gharsallaoui et al., 2012). Microcápsulas formadas por processos de coacervação e inclusão molecular podem ser desidratadas por *spray-drying*, visando melhorar as propriedades das mesmas (Gharsallaoui et al., 2007; Gharsallaoui et al., 2012). Assim, os processos de microencapsulação podem ser uma solução para proteger compostos bioativos, como os antioxidantes (Gharsallaoui et al., 2012).

O principal fator limitante para uso de moléculas biologicamente ativas extraída dos organismos que compõem a biodiversidade é a necessidade de grande quantidade de material. O rejeito da pesca é uma fonte abundante de matéria prima que pode atender as necessidades de escala da indústria. Os organismos presentes no rejeito podem ser ricos em peptídeos antioxidantes. A hidrólise enzimática poderia garantir uma extração sustentável destas biomoléculas, e oferecer um destino economicamente viável para parte das espécies da fauna acompanhante. Dessa forma, iniciou-se a busca de moléculas com

atividade biológica nas quatro espécies mais abundantes na pesca do camarão em São Paulo, sendo duas de peixes (*Micropogonias furnieri* e *Paralonchurus brasilensis*) e duas de crustáceos (*Callinectes ornatus* e *Hepatus pudibundus*). Portanto, a presente tese visa estudar a produção de hidrolisados proteicos a partir dessas espécies, a bioprospecção de peptídeos com capacidade antioxidante nesses hidrolisados e a microencapsulação dos hidrolisados. As informações obtidas irão fornecer subsídios para gerar tecnologia para agregar valor a esses animais que são descartados. O presente estudo está dividido em quatro capítulos: no primeiro realizou-se uma revisão bibliográfica sobre a fauna acompanhante e a pesca de arrasto no Brasil; no segundo avaliou-se a capacidade antioxidante e a viabilidade biológica dos hidrolisados proteicos obtidos dos animais mais abundantes da fauna acompanhante; no terceiro e no quarto investigou-se o efeito da complexação e da microencapsulação na atividade antioxidante dos hidrolisados proteicos.

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Capítulo 1

An overview of trawling and its bycatch in Brazil

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Abstract

Trawling fisheries are a cause of mortality of invertebrates, fish, sea turtles, marine mammals, and birds. In addition to the loss of biodiversity, the trawling can cause resuspension of sediments and alterations in seafloor morphology. Nevertheless, banning trawl fishing in developing countries, like Brazil, can cause a major social and economic problem as countless people depend on it for their livelihoods. In this way, measures to make this type of fishing more sustainable are a challenge. Besides the efforts to delineate the scenario of artisanal and industrial trawling in Brazil, it is still necessary a change in the model and management of trawling. The introduction of new technologies (as Bycatch Reduction Devices), together with more efficient management strategies based on a transparent and participatory decision-making processes are essential to make this sector a more economically and environmentally sustainable activity. The preservation of these habitats in the form of fishing exclusion areas is essential for sustainability, however, the closure of fishing areas can affect the income of many fishing families. Thus, it is important to enabling these fishermen to have other livelihood alternatives. In this sense, the creation of cooperatives that provide new skills for fishermen and their families is an option to improve income during the closure of the fisheries areas. The balance between the economic, social and environmental aspects is not easy to solve, however, it is essential to search for solutions and to this, is necessary to involve government, conservation management agencies, scientists, industry representatives, and fishing communities.

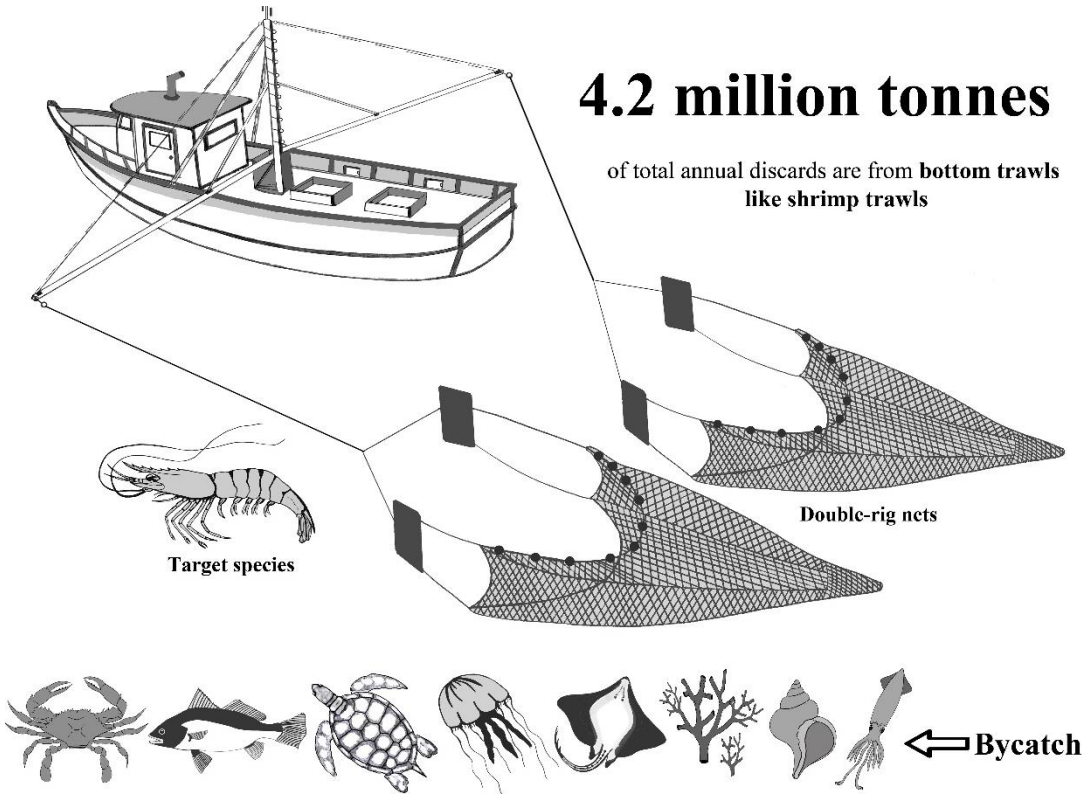
Keywords: fisheries, bottom trawl, economic impacts, social impacts

1. Introduction

The discards in fisheries have been the subject of many studies since the beginning of the decade of 90, where the first public debates were initiated due to the capture of dolphins in tuna nets and turtles in shrimp trawls (Hall, 1996; Clucas, 1997; Severino-Rodrigues et al., 2002, 2007; Yang et al., 2015; Serena et al., 2016; Burgess et al., 2018). The term bycatch can be defined as the group of organisms caught with the target species, which are not marketed, and are returned to the sea or rejected (Hall et al., 2000; Graça-Lopes et. al., 2002; Severino-Rodrigues et al., 2002; 2007; Fonseca et al., 2005; Kumar & Deepthi, 2006; Obersen et al., 2017; Varisco et al., 2017; Ruibal Núñez et al., 2018). Also, the term can be used to refer to the part of the catch which is not the main target of the fishing effort (Alverson et al., 1994; Clucas, 1997; Kumar & Deepthi, 2006; Ruibal Núñez et al., 2018).

Millions of tons of fish, invertebrates, turtles, seabirds and mammals die in fishing nets designed to catch other species (Hall, 1996; Clucas, 1997; Yang et al., 2015; Serena et al., 2016; Ruibal Núñez et al., 2018). In the past decade, shrimp trawling had the highest catch rates for non-target species, accounting for more than one-third of the world's bycatch (Hall et al., 2000). In addition to the loss of biodiversity, other environmental impacts may be attributed to trawling such as resuspension of sediments, alterations in seafloor morphology and increase of bottom water turbidity (Thrush & Dayton, 2002; Pusceddu et al., 2014). It is estimated that the organic carbon removed daily by trawling in the north-western Mediterranean region accounts for 60-100% of the input stream and such impact is causing the degradation and desertification of the seabed (Pusceddu et al., 2014). In addition, bottom trawling has become an important factor in the evolution of the deep-sea landscape, and the morphology of the upper continental slope could be altered by intensive trawling (Puig et al., 2012).

Figure 1. According to FAO (2019), the annual discards from global marine capture fisheries between 2010 and 2014 was 9.1 million tonnes and about 46 percent (4.2 million tonnes) of total annual discards were from bottom trawls that included shrimp trawls.



In Brazil, trawling is performed by an industrial and artisanal scale, in different environments with different technologies (Medeiros et al., 2013). Besides the great impact on the Brazilian marine ecosystems, due to the indiscriminate capture of organisms of the bycatch and the ample destruction of the benthic region, another aspect that should be taken into consideration are the social and economic aspects of this type of fishing (Diegues, 1992; Dias-Neto, 2011; Medeiros et al., 2013). In the coast of São Paulo, Santa Catarina, and Paraná states, for example, the economic aspects of the capture of non-target species are not associated only with the loss of economic value, but rather with the costs of such undesirable catches, such as boat wear and a person to help to sort and undesirable the animals that come in the net (personal communication with the fisherman of the coast of São Paulo State). The social aspects, on the other hand, come from the constant search for solutions to the bycatch, since prohibiting or seizing trawling

may lead to serious losses to fishermen, whose main source of income is shrimp. Thus, the balance between environmental, social and economic impacts is a constant challenge for demersal trawling. In this contribution, we review the impacts of trawling in Brazil, the legislation for this type of fishing, the animals that are present in the bycatch, the economic and social impacts, and suggest public policies for the sustainable of the trawling.

2. The shrimp trawling in Brazil: a brief history

Since the pre-colonial period, fishing activity is an important means of income and employment being one of the oldest cultural legacies of Brazil. It had started when several indigenous peoples established housing in the coastal areas and had fishing as a means of subsistence (Diegues, 1992). The country has many communities that depend exclusively of estuarine coastal fisheries on small-scale and related activities, being that, the artisanal fisheries is as important as industrial fisheries to the country economy (Diegues, 1992). At the beginning of the century 20th fishing activity, which was linked to small production, grew on a commercial scale an example is the sardine fishery that gave birth to the first fish industries in South-Eastern (Diegues, 1992). In the middle of 50, due to increased market demand, artisanal fishers became "professionals" and started to explore the open sea with the help of the newly introduced engines (Diegues, 1992). Thus, there was the emergence of a new social stratum "artisanal fishermen with motorized vessels that exploit the sea", and there were changes in the fish marketing system (Diegues, 1992).

In this context, the shrimp trawling was among the most important activities for the fishing economy on the South-Eastern and Southern coasts of Brazil and was directed to Seabob shrimp (*Xiphopenaeus kroyeri*) and pink shrimp (*Farfantepenaeus brasiliensis*

and *Farfantepenaeus paulensis*) (Valentini et al., 1991a, b). In 1981, 15.580 tons of Seabob shrimp were fished in the Southeast and South regions (IBAMA, 1993). After 1985, the fishing of trawling in the south developed and reached more than 6.000 tons in the final of the decade of 80 (Haimovici & Mendonça, 1996). In 2005, more than 30.000 tons of shrimps were caught in Brazil (IBAMA, 2011). In this way, the fishing of the penaeid shrimp has great economic relevance nowadays, sustaining an expressive segment of the fishing sector in the Southeast and South Brazilian coast (Graça-Lopes et al., 2002; Leite & Petrere, 2006).

In these regions of the coast, the trawling of shrimps is carried out in an artisanal or industrial scale (Valentini et al., 1991a, b; Dias-Neto, 2011). Small-scale fisheries are concentrated in juvenile shrimps in coastal and estuarine areas and the fishing gear used by these fishermen varies from one region to another (Valentini et al., 1991a, b; Leite & Petrere, 2006). Thus, in Southern coast, the fisherman use different types of gear such as cast nets, trawl nets, trawling by two rowing canoes, “aviãzinho”, “saco” and “coca”, whereas in Southeastern coast the use of gears such as cast nets, trawl nets, and trawling by two rowing canoes is more popular (Valentini et al., 1991a, b; Leite & Petrere, 2006). Trawling uses trawls with single doors, with a net drawn by the stern or side of the boat, or double, with nets connected to the lateral tangs, operated simultaneously (Fig. 2) (Dias-Neto, 2011). In addition, there is a large variety of motorized boats, with vessels of different sizes, which practice trawling and allows a great fishing power (Valentini et al., 1991a, b; Dias-Neto, 2011). In this context, industrial shrimp fisheries can be classified as medium scale (vessels with length of 10 - 15 meters, operating with double drag) and large scale (vessels greater than 15 meters, with the use of fisheries that can last for weeks) (Valentini et al., 1991a, b; Dias-Neto, 2011). The shrimp fishing activity can be divided into five types: Pink shrimp caught in estuaries, enclosed bays and coastal lagoons by

artisanal fleet; Pink shrimp caught in open sea by industrial fleet; White shrimp (*Litopenaeus schmitt*) caught in estuaries, closed bays and coastal lagoons by artisanal fleet; Seabob shrimp caught in open sea, at low depth, by industrial and artisanal fleet; and "Barba-Ruça" shrimp (*Artemesia longinaris*) and "Santana" shrimp (*Pleoticus muelleri*) caught in open sea by industrial and artisanal fleet (Dias-Neto, 2011; Valentini et al., 1991a, b). These fishing are commonly carried out at night when the yield of the trawlers is higher due to the nocturnal habits of this species. During the day the fisherman use "frighteners" that are fishing gear that is used to flush the shrimps out of hiding (Valentini et al., 1991a, b; Leite & Petrere, 2006).

Despite the great economic relevance of trawling, many problems are observed and the maintenance of shrimp stocks is one of them (Fransozo et al., 2004; Leite & Petrere, 2006; Castilho et al., 2007a, b; Costa et al., 2010; Grabowski et al., 2016; Davanso et al., 2017). In 1973, there was a sharp drop in the pink shrimp landed in the Southeast and South regions, which remained stable until 1998 (Valentini et al., 1991a; Dias-Neto, 2011). However, the 5.013 tonnes of pink shrimp landed in 1972, decreased to 771.3 tonnes from 1999 to 2007 (Dias-Neto, 2011). Over time and with the decline of the fishing yield directed to the pink shrimp and Seabob shrimp, other species of shrimp became the target of the fishing, as "Barba-Ruça" shrimp and "Santana" shrimp, to supply the profitability previously obtained in these activity (Valentini et al., 1991a; Fransozo et al., 2004; Castilho et al., 2007a, b; Costa et al., 2010). Researchers have warned the state of overfishing by predicting a shrimp stock collapse, but currently, there is still a need for adequacy of the stocks management along the coast (Valentini et al., 2001; D'Incao et al., 2002; Leite & Petrere, 2006; Grabowski et al., 2016). The fishery of pink shrimp in the coast of São Paulo State, is at its maximum sustainable yield and that providence as the reduction of the fishing effort, adequate season and area closures seem to be the best

actions for the pink shrimp fishery sustainable (Leite & Petrere, 2006). To guarantee sustainable extractive, the Seabob shrimp stocks of the Brazilian coast must be treated as distinct groups (Davanso et al., 2017).

In addition to the lack of policy actions, the change in the lifestyle of the population may be influencing the differentiation of fish stocks in the Brazilian coast (Dias-Neto, 2011; Chiba et al., 2012; Davanso et al., 2017). More than 50% of the population lives in a range of up to 50 km from the coast, which implies damages to marine resources with the increase overfishing in different regions (Davanso et al., 2017). In addition, the expansion of real estate and tourism growth in the coastal regions causes impacts on fishing, creating a major social problem since industrial fishermen and artisans are harmed (Dias-Neto, 2011; Chiba et al., 2012).

Figure 2. Shrimp trawling fishing boat. Fishing boat equipped with two double rig nets. The two nets are identical and during the trawling these are used simultaneous. Detail of the illustration of the doors positioned inside the water.

In this way, it is possible to observe the enormous complexity that involves the fishing sector in Brazil. Thus, although shrimp trawling has an important socio-economic role since the beginning of 20th century in Brazil, environmental problems, such as the maintenance of stocks, are discussed until today. The conservation and proper management of the main species traded are difficult because of economic interests that overlap with environmental issues. Efforts are being made to delineate the scenario of artisanal and industrial trawling in Brazil, but it is necessary to recognize the need for a change in the model and management of trawling, for the protection of stocks and ecological systems involving the shrimp trawling in Brazil.

3. Fisheries regulations of the shrimp trawling in Brazil

Due to the great development of fisheries in Brazil in the middle of the 20th century, the sector regulation has been necessary (Diegues, 1992; Kalikoski et al., 2002; Leite & Petrere, 2006; Dias-Neto, 2011). Thus, regulation of vessel sizes, closed seasons, areas to be protected and population parameters of the species involved in this activity have been discussed and have been changed over the years (Kalikoski et al., 2002). Since the years 60, the federal government has had greater influence on the management of fishery resources with the implementation of the Superintendency of Fisheries Development - SUDEPE (decree-law 221/69) which, in addition to fiscal incentives, guided the management of resources and development of the fishing industry at the national level (Diegues, 1992; Kalikoski et al., 2002). In 1989, with the extinction of SUDEPE, the Brazilian Institute of Environment and Natural Resources (IBAMA) became responsible for the management of the fishery resources (Dias Neto, 1999; Diegues, 1992; Kalikoski et al., 2002; Dias-Neto, 2011; Leite & Petrere, 2006). In the late decade of 90, in addition to IBAMA, fisheries management became the responsibility

of the Department of Fisheries and Aquaculture - DPA of the Ministry of Agriculture, Livestock and Supply - MAPA (Kalikoski et al., 2002; Dias-Neto, 2011). In 2003, the Ministry of Fisheries and Aquaculture (MPA) was created and lasted until 2015. Currently, there is the Aquaculture and Fisheries Secretariat, which is a body linked to the MAPA, which establishes the guidelines for governmental action for the national aquaculture and fisheries policy.

Shrimp trawling was officially regulated at the end of the 1960s and, due to intensive exploitation, several measures were adopted to management, as fleet entry control, restrictions on the use of vessels, periods of operation, limitation of power and autonomy of vessels (Leite & Petrere, 2006). In 1984, an ordinance (P Sudepe N-55/1984) defined the biological parameters of the target species and established minimum catch sizes (90 mm) and trawl with minimum mesh size of 30 mm for catching pink shrimp and white shrimp. In the same year, other ordinance regulated fishing gear and the minimum mesh size in the bagger, 24 mm, and upper working length of 12 m (P Sudepe N-56/1984). Another normatives instructions regulated the criteria to permit vessels (IN Ibama 164/2007), maintained limited effort to vessels already authorized (IN Interministerial MPA 3/2011; IN Interministerial MPA/MMA 10/2011), limited the fleet to vessels with length less than 12 m and Trawl Fishing Power < 200, and prohibited port trawl fishing less than one nautical mile from shore (IN MMA 29/2004). Among the ordinance, the most controversial is the ban period of fishing (Medeiros et al., 2013). In 1983, SUDEPE recommended the first fishing ban that brought together all shrimp species of commercial interest and it started on February 1st and ending on May 31st (Leite & Petrere, 2006; Medeiros et al., 2013). In 2008, the normative instruction from Ibama (IN Ibama 189/2008) defined the closed period for all species of commercial interest, between March 1st and May 31st, in the regions between the State of Rio de Janeiro and Rio Grande do

Sul. The problem with this last normative instruction is that this regulatory measure is unified for all species of shrimp with commercial interest, and scientists and fishermen believe that grouping all traded species is inappropriate (Leite & Petrere, 2006; Medeiros et al., 2013). To *Xiphopenaeus kroyeri*, for example, studies show that the offseason periods could be started in February because besides covering the peak of recruitment of this species, it will protect a larger number of reproductive females (Heckler et al., 2013; Castilho et al., 2015; Davanso et al., 2017). In addition, it is believed that the offseason periods should be different in regions with distinct environmental characteristics (Davanso et al., 2017). In this way, differentiated offseason periods by region could result in a sustainable shrimp extraction along the Brazilian coast (Davanso et al., 2017).

Despite efforts by the Brazilian government to oversee and create measures to make the fishing industry stronger, throughout the country's development it is possible to observe a problem regarding the management of the fishery resources in the federal institutions and this can make it difficult to control the fishing fleet, production and registrations, and license emissions. The introduction of new technologies, together with more efficient management strategies based on transparent and participatory decision-making processes are essential to make this sector a more sustainable activity.

4. Understanding the impacts of fishery trawling and its bycatch

One of the biggest challenges of fishery trawling is the impact of this type of fishing on the environment making it one of the major barriers to fisheries sustainability. Moreover, the overfishing and the subsequent fish and shrimps stock decline caused by fisheries can cause on economic and social impacts. Thus, understanding these impacts is the key to the conservation of the species and to reduce fishing pressure.

4.1 Environmental impact

In the last decades, bycatch has become a major issue in global fisheries management and conservation and endangered, threatened or protected species are exposed as the fishing nets catche animals with a size outside what is authorized by laws or prohibited catch species (Castilho et al., 2008; Chaves & Silva, 2019; Zhou et al., 2019). The impacts of bycatch are a decrease of many species, or by their own death or by hungry, because some animals will not have their prey to feed (Hall et al., 2000; Chaves & Silva, 2019; Zhou et al., 2019). It is estimated that at least 20 million individuals are discarded annually throughout the world as bycatch (Gray & Kennellly, 2018). More than 665.000 thousand of marine mammals, birds and sea turtles die worldwide due to a variety of fishing nets (Cardoso et al., 2011; Burgess et al., 2018; Chaves & Silva, 2019). Recent studies showed that to reverse ongoing declines of 20 marine mammal, sea turtle, and seabird populations threatened as bycatch, it is necessary to end the overfishing of target stocks (Burgess et al., 2018; Chaves & Silva, 2019; Zhou et al., 2019). The species of seabird most commonly caught are the albatrosses, petrels, and shearwaters and the population of these species are threatened (Croxall et al., 2012; Gray & Kennellly, 2018). The Baltic Sea, for example, is globally important for wintering sea ducks, however, it is a global ‘hotspot’ for bird bycatch (Field et al., 2019). Sea turtles also interact with various fishing gears as demersal and pelagic longlines, demersal trawls, and small-scale fisheries (Gray & Kennellly, 2018). Researches showed that the mortality of the sea turtles in gillnetted is approaching 100% whereas in shrimp trawling are typically less than 33% because usually retrieved in less than 2 hours (Gilman et al., 2010; Gray & Kennellly, 2018). Marine mammals as dolphin, seals, porpoises and sea lions are entangled in gillnets and trapped in trawls and often drown and are found dead (Davis et al., 2002; Gray & Kennellly, 2018). Bycatch is the anthropogenic most threat

to common dolphins and can negative affect the population-level of these animals in European waters (Dolman & Brakes, 2018).

The charismatic species (i.e. marine megafauna such as seabirds, mammals and turtles) are not the most harmed, also there are a variety of organisms and taxon that are captured and discarded because of the lack of economic and technological interest (Clucas, 1997; Graça-Lopes et al., 2002; Castilho et al., 2008; Cattani et al., 2011; Zhou et al., 2019). The trawling, in special, catches a huge amount of bycatch and this organisms are returned to the sea dead or with little chance of survival (Clucas, 1997; Graça-Lopes et al., 2002). The trawling, also destroys the benthonic marine environment causing a major loss of biodiversity, leading to a problem of conservation especially when the endangered species are affected (Lewison et al., 2004; Soykan et al., 2008; Larsen et al., 2018; Zhou et al., 2019). The trawling destroyed areas and due to the frequency of exploitation they do not have the time required for the ecosystem to recover (Zhou et al., 2019). The impact on the species community is another trawling problem, because the accidental catch usually reduces the number of the species causing an imbalance of the population (Castilho et al., 2008; Soykan et al., 2008; Larsen et al., 2018; Zhou et al., 2019). The shrimps that are consumed by the food market live in the same environment that a lot of other species and the excessive and continuous fishing activity can decrease the abundance of these species, leading to local extinction (Graça-Lopes et al., 2002; Severino-Rodrigues et al., 2002; Branco & Verani, 2006; Cattani et al., 2011; Bochini et al., 2019; Stanski et al., 2019).

In Brazil, several studies analyzed the composition, distribution, occurrence, and abundance of the bycatch (Graça-Lopes et al., 2002; Severino-Rodrigues et al., 2002; Branco & Verani, 2006; Cattani et al., 2011; Júnior et al., 2011; Cintra et al., 2017; Bochini et al., 2019; Stanski et al., 2019). It was observed a lot of quantities of animals

captured in trawls in Brazil and researchers have already shown more than 400 species of fish and crustaceans caught as bycatch in the Brazilian coast (Table 1 and 2; Fig. 3).

Figure 3. Bycatch in the Ubatuba region in São Paulo state coast. **A.** Fisherman is separating shrimps of commercial interest from bycatch. In this situation, the animals stood long time out of the water and were returned to the sea dead or with little chance of survival. **B.** Ray captured accidentally with the shrimps.

The researchers showed a concern in the relationship between the capture of the target species (shrimps with economic value) and the animals captured accidentally (Graça-Lopes et al., 2002; Severino-Rodrigues et al., 2002; Branco & Verani, 2006; Cattani et al., 2011; Júnior et al., 2011; Cintra et al., 2017; Bochini et al., 2019; Stanski et al., 2019). On the coast of São Paulo, Brazil, 258 species belonging to mollusks, crustaceans, and other invertebrates were registered as bycatch of the red spotted shrimp (*Farfantepenaeus brasiliensis* and *F. paulensis*) and Seabob shrimp (*Xiphopenaeus kroyeri*) (Branco & Verani, 2006; Bochini et al., 2019). In the bottom-trawl fishery, which targeted to the urugavian lobster, *Metanephrops rubellus*, on the coasts from Rio de Janeiro to Santa Catarina states, 178 species of bycatch were identified (69 species of fishes, 40 of molluscs and 69 of crustaceans) (Severino-Rodrigues et al., 2007). In the artisanal double-rig trawling on the coast of Santa Catarina five species of the jellyfish in

the bycatch of seabob shrimp fisheries were observed (Schroeder et al., 2014). On the coast of Paraná, Brazil, the incidental catch of fish from the Sciaenidae family represent mostly young animals, which have not yet reached the first reproduction size and were captured probably because of the selectivity of the net used (Bernardo et al., 2011). The relation of biomass by weight of fish and shrimps in the fishing of the Seabob shrimp of the Pontal do Paraná city, Brazil, was 1: 0.57 on average, that is, for one kilogram of shrimp, 570 grams of fish were caught (Catanni et al., 2011). In addition, these authors observed a seasonal pattern in the occurrence of fish species, the greatest abundance and diversity was found in the months with higher temperatures. (Catanni et al., 2011). In the trawl fishery of Seabob shrimp in São Paulo state, Brazil, the adult crustaceans that are caught are mainly females, in this way the accidental capture of these crustaceans in the shrimp trawls can contribute to an imbalance in the community (Graça-Lopes et al., 2002; Severino-Rodrigues et al., 2002, 2007; Fonseca et al., 2005). Besides, *Callinectes ornatus* and *Hepatus pudibundus* represent 80% of the crustaceans that are caught and discarded in the trawl fishery of Seabob shrimp in São Paulo State (Graça-Lopes et al., 2002; Severino-Rodrigues et al., 2002, 2007; Fonseca et al., 2005). In the Brazilian states of Alagoas and Sergipe, 79 species of crustaceans bycatch were observed in the fishing of the Seabob shrimp (Santos et al., 2016). In Santa Catarina State and in the coast of Cananéia city (in São Paulo state) researchers observed a total of 28 and 46 species of the crustaceans bycatch, respectively (Bochini et al., 2019; Stanski et al., 2019). This information is necessary to know what species of bycatch are in Brazil and to find strategies to minimize the capture (Cintra et al., 2017; Bochini et al., 2019; Stanski et al., 2019). In addition, the biology of the species that are accidentally caught should be studied to improve the understanding of the trophic chain of species in the fishing area and the balance of exploiting ecosystems (Almeida & Branco, 2002). In this context,

long-term studies of the community of the most accidentally caught species in Brazil should be conducted to better understand the impacts of trawling on these animals.

Table 1. List of crustacean species components of the bycatch from shrimp fishery in alphabetical order and categorizing each species according to the target species.

Crustacean Bycatch in Brazil	Target Specie	Reference
Acanthilia intermedia	Urugavian lobster	Severino-Rodrigues et al. (2007)
Acantocarpus alexandri	Urugavian lobster	Severino-Rodrigues et al. (2007)
Acetes americanus	Seabob shrimp	Graça-Lopes et al. (2002)
Achelous spinimanus	Seabob shrimp	Bochini et al. (2019); Costa et al. (2018); Stanski et al. (2019)
Agononida longipes	Urugavian lobster	Severino-Rodrigues et al. (2007)
Albunea paretii	Urugavian lobster	Severino-Rodrigues et al. (2007)
Alpheus intrinsecus	Seabob shrimp	Stanski et al. (2019)
Alpheus. sp	Seabob shrimp	Bochini et al. (2019)
Anasimus latus	Pink shrimp	Cintra et al. (2017)
Apiomithrax. sp	Seabob shrimp	Bochini et al. (2019)
Arenaeus cribrarius	Seabob shrimp	Bochini et al. (2019); Costa et al. (2018); Graça-Lopes et al. (2002); Stanski et al. (2019)
Armases angustipes	Seabob shrimp	Bochini et al. (2019)
Artemesia longinaris	Seabob shrimp	Bochini et al. (2019); Costa et al. (2018); Graça-Lopes et al. (2002); Stanski et al. (2019)
Bathynomus giganteus	Urugavian lobster	Severino-Rodrigues et al. (2007)
Bathynomus miyarei	Urugavian lobster	Severino-Rodrigues et al. (2007)
Calappa gallus	Urugavian lobster	Severino-Rodrigues et al. (2007)
Calappa nitida	Pink shrimp	Cintra et al. (2017)
Calappa ocellata	Pink shrimp	Cintra et al. (2017)
Calappa sulcata	Pink shrimp, Urugavian lobster	Cintra et al. (2017); Severino-Rodrigues et al. (2007)
Callinectes bocourti	Seabob shrimp	Bochini et al. (2019)
Callinectes danae	Seabob shrimp; pink shrimp, Urugavian lobster	Bochini et al. (2019); Cintra et al., 2017; Costa et al. (2018); Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007); Stanski et al. (2019)

Crustacean Bycatch in Brazil	Target Specie	Reference
Callinectes exasperatus	Seabob shrimp	Bochini et al., 2019
Callinectes ornatus	Seabob shrimp; Pink shrimp	Bochini et al. (2019); Cintra et al. (2017); Costa et al. (2018); Graça-Lopes et al. (2002); Stanski et al. (2019)
Callinectes ornatus	Urugavian lobster	Severino-Rodrigues et al. (2007)
Callinectes sapidus	Seabob shrimp	Graça-Lopes et al. (2002); Bochini et al. (2019)
Chaceon ramosae	Urugavian lobster	Severino-Rodrigues et al. (2007)
Charybdis helleri	Seabob shrimp	Bochini et al. (2019)
Cirolana. sp	Urugavian lobster	Severino-Rodrigues et al. (2007)
Cronius ruber	Pink shrimp	Cintra et al. (2017); Costa et al. (2018)
Cryptodromiopsis antillensis	Urugavian lobster	Severino-Rodrigues et al. (2007)
Cryptodromiopsis. sp	Urugavian lobster	Severino-Rodrigues et al. (2007)
Cymothoas. sp	Urugavian lobster	Severino-Rodrigues et al. (2007)
Dardanus insignis	Seabob shrimp	Costa et al. (2018); Graça-Lopes et al. (2002); Stanski et al. (2019)
Dardanus insignis	Urugavian lobster	Severino-Rodrigues et al. (2007)
Dissodactylus crinitichelis	Seabob shrimp	Bochini et al. (2019)
Dromia erythropus	Pink shrimp	Cintra et al. (2017)
Dromia erytropus	Urugavian lobster	Severino-Rodrigues et al. (2007)
Ehipplysmata oplophoroides	Pink shrimp	Cintra et al. (2017)
Exhippolysmata oplophoroides	Seabob shrimp	Bochini et al. (2019);); Stanski et al. (2019); Graça-Lopes et al., 2002
Farfantepenaeus brasiliensis	Seabob shrimp, Urugavian lobster	Bochini et al. (2019); Costa et al. (2018); Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007); Stanski et al. (2019)
Farfantepenaeus paulensis	Seabob shrimp, Urugavian lobster	Bochini et al. (2019); Costa et al. (2018); Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007); Stanski et al. (2019)
Frevillea hirsuta	Urugavian lobster	Severino-Rodrigues et al. (2007)
Gibbesia prasinolineata	Urugavian lobster	Severino-Rodrigues et al. (2007)
Hemisquilla braziliensis	Urugavian lobster, Seabob shrimp	Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007)
Hepatus gronovii	Pink shrimp	Cintra et al., 2017
Hepatus pudibundus	Seabob shrimp; Pink shrimp; Urugavian lobster	Bochini et al. (2019); Cintra et al. (2017); Costa et al. (2018); Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007); Stanski et al. (2019

Crustacean Bycatch in Brazil	Target Specie	Reference
Hepatus scaber	Urugavian lobster; Pink shrimp	Cintra et al. (2017); Severino-Rodrigues et al. (2007)
Heterocrypta lapidea	Urugavian lobster	Severino-Rodrigues et al. (2007)
Hypoconcha arcuata	Seabob shrimp	Bochini et al. (2019)
Iliacantha liodactylus	Pink shrimp	Cintra et al. (2017)
Iliacantha sparsa	Pink shrimp	Cintra et al. (2017)
Isocheles sawayai	Seabob shrimp	Stanski et al. (2019)
Leander paulensis	Seabob shrimp	Bochini et al. (2019)
Leurociclus tuberculosus	Urugavian lobster	Severino-Rodrigues et al. (2007)
Libinia ferreirae	Seabob shrimp	Bochini et al. (2019); Graça-Lopes et al. (2002); Bochini et al., 2019; Costa et al. (2018); Stanski et al. (2019)
Libinia sp	Seabob shrimp	Graça-Lopes et al. (2002)
Libinia spinosa	Urugavian lobster	Severino-Rodrigues et al. (2007); Stanski et al. (2019)
Libinia. spinosa	Seabob shrimp	Bochini et al. (2019); Costa et al. (2018); Graça-Lopes et al. (2002)
Litopenaeus schmitti	Seabob shrimp	Bochini et al. (2019); Costa et al. (2018); Graça-Lopes et al. (2002); Stanski et al. (2019)
Livoneca redmanii Leach	Urugavian lobster	Severino-Rodrigues et al. (2007)
Loxopagurus loxochelis	Seabob shrimp	Stanski et al. (2019)
Lysiosquilla scabricauda	Pink shrimp	Cintra et al. (2017)
Menippe nodifrons	Seabob shrimp; Pink shrimp	Bochini et al. (2019); Cintra et al. (2017)
Metanephrops rubellus	Seabob shrimp	Graça-Lopes et al. (2002)
Mithrax hispidus	Pink shrimp	Cintra et al. (2017)
Munida flinti	Urugavian lobster	Severino-Rodrigues et al. (2007)
Munida forceps	Urugavian lobster	Severino-Rodrigues et al. (2007)
Munida irrasa	Urugavian lobster	Severino-Rodrigues et al. (2007)
Munida spinifrons	Urugavian lobster	Severino-Rodrigues et al. (2007)
Myropsis quinquespinosa	Urugavian lobster	Severino-Rodrigues et al. (2007)
Nematopalaemon schmitti	Seabob shrimp; Pink shrimp	Bochini et al. (2019); Cintra et al. (2017); Costa et al. (2018); Stanski et al. (2019)
Nephropsis aculeata	Urugavian lobster	Severino-Rodrigues et al. (2007)
Nerocila. sp	Urugavian lobster	Severino-Rodrigues et al. (2007)

Crustacean Bycatch in Brazil	Target Specie	Reference
Notolopas brasiliensis	Seabob shrimp	Bochini et al. (2019)
Ogyrides. sp	Seabob shrimp	Bochini et al. (2019)
Pagurus criniticornis	Urugavian lobster	Severino-Rodrigues et al. (2007)
Pagurus exilis	Seabob shrimp	Stanski et al. (2019)
Pagurus leptonyx	Seabob shrimp	Stanski et al. (2019)
Panulirus argus	Pink shrimp	Cintra et al. (2017)
Paractaea nodosa	Pink shrimp	Cintra et al. (2017)
Paradasygyius tuberculatus	Pink shrimp	Cintra et al. (2017)
Parapenaeus americanus	Seabob shrimp, Urugavian lobster	Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007)
Parasquilla meridionalis	Pink shrimp	Cintra et al. (2017)
Parribacus antarcticus	Pink shrimp	Cintra et al. (2017)
Penaeus brasiliensis	Pink shrimp	Cintra et al. (2017)
Penaeus subtilis	Pink shrimp	Cintra et al. (2017)
Periclimenes paivai	Seabob shrimp	Bochini et al. (2019); Costa et al. (2018); Stanski et al. (2019)
Persephona lichtensteinii	Seabob shrimp, Urugavian lobster	Bochini et al. (2019); Cintra et al. (2017); Costa et al. (2018); Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007); Stanski et al. (2019)
Persephona mediterranea	Seabob shrimp, Urugavian lobster, Pink shrimp	Bochini et al. (2019); Cintra et al. (2017); Costa et al. (2018); Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007); Stanski et al. (2019)
Persephona punctata	Seabob shrimp, Urugavian lobster	Bochini et al. (2019); Stanski et al. (2019); Severino-Rodrigues et al. (2007)
Petrochirus diogenes	Seabob shrimp, Urugavian lobster	Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007); Stanski et al. (2019)
Petrolisthes galathinus	Urugavian lobster	Severino-Rodrigues et al. (2007)
Planes cyaneus	Urugavian lobster	Severino-Rodrigues et al. (2007)
Platylambrus pourtalesii	Urugavian lobster	Severino-Rodrigues et al. (2007)
Platylambrus serratus	Urugavian lobster	Severino-Rodrigues et al. (2007)
Pleoticus muelleri	Seabob shrimp, Urugavian lobster	Bochini et al. (2019); Costa et al. (2018); Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007); Stanski et al. (2019)
Plesionika edwardsii	Seabob shrimp, Urugavian lobster	Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007)

Crustacean Bycatch in Brazil	Target Specie	Reference
Podochela gracilipes	Urugavian lobster	Severino-Rodrigues et al. (2007)
Politolana. sp	Urugavian lobster	Severino-Rodrigues et al. (2007)
Portunus (Anchelous) ordwayi	Pink shrimp	Cintra et al. (2017)
Portunus (Anchelous) spinicarpus	Pink shrimp	Cintra et al. (2017)
Portunus (Anchelous) spinimanus	Pink shrimp	Cintra et al. (2017)
Portunus (Portunus) rufiremus	Pink shrimp	Cintra et al. (2017)
Portunus ordwayi	Urugavian lobster	Severino-Rodrigues et al. (2007)
Portunus spinicarpus	Seabob shrimp, Urugavian lobster	Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007)
Portunus spinimanus	Seabob shrimp, Urugavian lobster	Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007)
Pseudorhombila. sp	Urugavian lobster	Severino-Rodrigues et al. (2007)
Pyromaia tuberculata	Seabob shrimp, Urugavian lobster	Bochini et al. (2019); Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007)
Ranilia constricta	Urugavian lobster	Severino-Rodrigues et al. (2007)
Raninoides loevis	Urugavian lobster	Severino-Rodrigues et al. (2007)
Rimapenaeus constrictus	Seabob shrimp; Pink shrimp	Bochini et al. (2019); Cintra et al. (2017); Costa et al. (2018); Stanski et al. (2019)
Rimapenaeus similis	Pink shrimp	Cintra et al. (2017)
Rochinia crassa	Urugavian lobster	Severino-Rodrigues et al. (2007)
Scyllarides brasiliensis	Seabob shrimp, Urugavian lobster	Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007)
Scyllarides deceptor	Urugavian lobster	Severino-Rodrigues et al. (2007)
Scyllarides delfosi	Pink shrimp	Cintra et al. (2017)
Scyllarus depressus	Urugavian lobster	Severino-Rodrigues et al. (2007)
Scyllarus chacei	Pink shrimp	Cintra et al. (2017)
Sicyonia dorsalis	Seabob shrimp; Pink shrimp, Urugavian lobster	Bochini et al. (2019); Cintra et al. (2017); Costa et al. (2018); Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007); Stanski et al. (2019)
Sicyonia parri	Urugavian lobster	Severino-Rodrigues et al. (2007)
Sicyonia stimpsoni	Pink shrimp	Cintra et al. (2017)
Sicyonia typica	Seabob shrimp, Urugavian lobster	Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007)

Crustacean Bycatch in Brazil	Target Specie	Reference
Squilla brasiliensis	Seabob shrimp, Urugavian lobster	Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007)
Squilla empusa	Pink shrimp	Cintra et al. (2017)
Squilla. sp	Seabob shrimp	Bochini et al. (2019)
Stenocionops spinosissima	urugavian lobster, Pink shrimp	Cintra et al. (2017); Severino-Rodrigues et al. (2007)
Stenorhynchus seticornis	Seabob shrimp, Pink shrimp, Urugavian lobster	Bochini et al. (2019); Cintra et al. (2017); Severino-Rodrigues et al. (2007)
Teramnonotus monodi	Seabob shrimp	Bochini et al. (2019)
Tetraxanthus rathbunae	Urugavian lobster	Severino-Rodrigues et al. (2007)
Trachynopenaeus constrictus	Seabob shrimp	Graça-Lopes et al. (2002)

Table 2. List of fish species components of the bycatch from shrimp fishery in alphabetical order and categorizing each species according to the target species.

Fish Bycatch in Brazil	Target Specie	Reference
Achirus declivis	Seabob shrimp	Catanni et al. (2011); Sedrez et al. (2013)
Achirus lineatus	Seabob shrimp	Branco & Verani (2006); Catanni et al. (2011); Sedrez et al. (2013)
Aetobatus narinari	Urugavian lobster	Severino-Rodrigues et al. (2007)
Anchoa marinii	Seabob shrimp	Haimovici & Mendonça (1996)
Anchoviella lepidentostole	Seabob shrimp	Sedrez et al. (2013)
Alutera monocerus	Seabob shrimp	Graça-Lopes et al. (2002)
Anchoa filifera	Seabob shrimp	Catanni et al. (2011); Graça-Lopes et al. (2002)
Anchoa lyolepsis	Seabob shrimp	Graça-Lopes et al. (2002)
Anchoa spinifer	Penaeid shrimps	Branco & Verani (2006); Catanni et al. (2011); Lira et al. (2019)
Anchova tricolor	Seabob shrimp	Graça-Lopes et al. (2002)
Anchoviella brevirostris	Seabob shrimp	Graça-Lopes et al. (2002)

Fish Bycatch in Brazil	Target Specie	Reference
Anisotremus virginicus	Seabob shrimp	Graça-Lopes et al. (2002)
Antigonia capros	Seabob shrimp	Graça-Lopes et al. (2002)
Apionichthys unicolor	Seabob shrimp	Graça-Lopes et al. (2002)
Ariosoma sp	Urugavian lobster	Severino-Rodrigues et al. (2007)
Aspistor luniscutis	Penaeid shrimps	Catanni et al. (2011); Lira et al. (2019)
Astrocopus sexpinosus	Seabob shrimp	Graça-Lopes et al. (2002); Haimovici & Mendonça (1996)
Astrocopus y-graecum	Seabob shrimp	Graça-Lopes et al. (2002)
Atlantoraja castelnaui	Urugavian lobster	Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007)
Atlantoraja cyclophora	Seabob shrimp, Urugavian lobster	Graça-Lopes et al. (2002); Sedrez et al. (2013); Severino-Rodrigues et al. (2007)
Atlantoraja platina	Urugavian lobster	Severino-Rodrigues et al. (2007)
Bagre bagre	Seabob shrimp	Catanni et al. (2011); Graça-Lopes et al. (2002)
Bagre marinus	Penaeid shrimps	Lira et al. (2019)
Bairdiella ronchus	Seabob shrimp	Bernardo et al. (2011); Catanni et al. (2011); Graça-Lopes et al. (2002)
Balistes capriscus	Urugavian lobster	Graça-Lopes et al. (2002); Haimovici & Mendonça (1996); Severino-Rodrigues et al. (2007)
Balistes vetula	Seabob shrimp	Branco & Verani (2006)
Bassanago albescens	Urugavian lobster	Severino-Rodrigues et al. (2007)
Bembrops heterurus	Seabob shrimp	Graça-Lopes et al. (2002)
Benthodesmus elongates	Urugavian lobster	Severino-Rodrigues et al. (2007)
Brevoortia pectinata	Seabob shrimp	Graça-Lopes et al. (2002); Haimovici & Mendonça (1996)
Caranx crysos	Seabob shrimp	Branco & Verani (2006); Graça-Lopes et al. (2002)
Carax hippos	Seabob shrimp	Graça-Lopes et al. (2002)
Carax ruber	Seabob shrimp	Graça-Lopes et al. (2002)
Carcharhinus brevipinna	Seabob shrimp	Graça-Lopes et al. (2002)
Carcharhinus limbatus	Seabob shrimp	Graça-Lopes et al. (2002)
Carcharhinus plumbeus	Seabob shrimp	Graça-Lopes et al. (2002)

Fish Bycatch in Brazil	Target Specie	Reference
Cathorops spixii	Seabob shrimp	Branco & Verani (2006); Catanni et al. (2011)
Catathiridium declives	Seabob shrimp	Graça-Lopes et al. (2002)
Catathiridium garmani	Seabob shrimp	Graça-Lopes et al. (2002)
Caulolatilus chrysops	Urugavian lobster	Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007)
Cetengraulis edentulous	Penaeid shrimps, Seabob shrimp	Branco & Verani (2006); Catanni et al. (2011); Lira et al. (2019)
Centropomus paralellus	Seabob shrimp	Branco & Verani (2006); Graça-Lopes et al. (2002)
Chaetodipterus faber	Penaeid shrimps, Urugavian lobster	Catanni et al. (2011); Graça-Lopes et al. (2002); Lira et al. (2019); Severino-Rodrigues et al. (2007)
Cheilodactylus bergi	Urugavian lobster	Severino-Rodrigues et al. (2007)
Chilomycterus spinosus	Seabob shrimp	Branco & Verani (2006); Catanni et al. (2011); Graça-Lopes et al. (2002); Haimovici & Mendonça. (1996)
Chilomycterus spinosus spinosus	Seabob shrimp	Catanni et al. (2011)
Chirocentrodon bleekerianus	Seabob shrimp	Branco & Verani (2006); Catanni et al. (2011); Graça-Lopes et al. (2002); Sedrez et al. 2013
Chloroscombrus chrysurus	Penaeid shrimps	Branco & Verani (2006); Lira et al. (2019)
Citharichthys arenaceus	Seabob shrimp	Graça-Lopes et al. (2002)
Citharichthys macrops	Seabob shrimp	Lira et al. (2019)
Citharichthys spilopterus	Penaeid shrimps	Branco & Verani (2006); Catanni et al. (2011); Lira et al., 2019; Sedrez et al., 2013
Conger orbignianus	*Pleoticus muelleri*, *Artemesia longinaris*, Seabob shrimp, Urugavian lobster	Branco & Verani (2006); Graça-Lopes et al. (2002); Haimovici & Mendonça. (1996); Sedrez et al. (2013); Severino-Rodrigues et al. (2007)
Conodon nobilis	Penaeid shrimps	Branco & Verani (2006); Catanni et al. (2011); Graça-Lopes et al. (2002); Lira et al. (2019)
Cookelus boops	Seabob shrimp	Graça-Lopes et al. (2002)
Corniger spinosus	Seabob shrimp	Graça-Lopes et al. (2002)
Coryphaena hippurus	Urugavian lobster	Severino-Rodrigues et al. (2007)
Ctenosciaena gracilicirrhus	Seabob shrimp	Bernardo et al. (2011); Catanni et al. (2011); Graça-Lopes et al. (2002); Sedrez et al. (2013)
Cyclichthys spinosus	Seabob shrimp	Branco & Verani (2006); Sedrez et al. (2013)

Fish Bycatch in Brazil	Target Specie	Reference
Cyclopsetta chittendeni	Seabob shrimp	Sedrez et al. (2013)
Cyclopsetta decussata	Seabob shrimp	Sedrez et al. (2013)
Cynoponticus savanna	Seabob shrimp	Graça-Lopes et al. (2002)
Cynoscion acoupa	Seabob shrimp	Bernardo et al. (2011); Catanni et al. (2011); Graça-Lopes et al. (2002);
Cynoscion guatucupa	*Pleoticus muelleri, Artemesia longinaris* Urugavian lobster,	Haimovici & Mendonça. (1996); Severino-Rodrigues et al. (2007)
Cynoscion jamaicensis	Seabob shrimp, Urugavian lobster	Bernardo et al. (2011); Catanni et al. (2011); Graça-Lopes et al. (2002); Haimovici & Mendonça (1996); Sedrez et al. (2013); Severino-Rodrigues et al. (2007)
Cynoscion striatus	Urugavian lobster	Severino-Rodrigues et al. (2007)
Cynoscion leiarchus	Seabob shrimp	Bernardo et al. (2011); Branco & Verani (2006); Catanni et al. (2011); Graça-Lopes et al. (2002)
Cynoscion microlepidotus	Seabob shrimp	Bernardo et al. (2011); Catanni et al. (2011); Graça-Lopes et al. (2002)
Cynoscion striatus	*Pleoticus muelleri, Artemesia longinaris*	Haimovici & Mendonça (1996)
Cynoscion virescens	Penaeid shrimps	Bernardo et al. (2011); Catanni et al. (2011); Graça-Lopes et al. (2002); Lira et al. (2019)
Dactylopterus volitans	Seabob shrimp	Catanni et al. (2011); Graça-Lopes et al. (2002); Haimovici & Mendonça (1996)
Dasyatis centroura	Seabob shrimp	Graça-Lopes et al. (2002)
Dasyatis guttata	Seabob shrimp	Graça-Lopes et al. (2002)
Dasyatis say	Seabob shrimp	Graça-Lopes et al. (2002)
Decapterus punctatus	Urugavian lobster	Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007)
Diapterus auratus	Penaeid shrimps	Lira et al. (2019)
Diapterus rhombeus	Penaeid shrimps, Seabob shrimp	Branco & Verani (2006); Graça-Lopes et al. (2002); Lira et al. (2019); Sedrez et al. (2013)
Diplectrum formosum	Seabob shrimp	Graça-Lopes et al. (2002)
Diplectrum radiale	Seabob shrimp	Branco & Verani (2006); Sedrez et al. (2013)
Diplodus argenteus	Seabob shrimp	Graça-Lopes et al. (2002)

Fish Bycatch in Brazil	Target Specie	Reference
Dules auriga	*Pleoticus muelleri; Artemesia longinaris*	Graça-Lopes et al. (2002); Haimovici & Mendonça (1996); Sedrez et al. (2013)
Engraulis anchoita	*Pleoticus muelleri; Artemesia longinaris*	Haimovici & Mendonça (1996)
Epinephelus flavolimbatus	Seabob shrimp, Urugavian lobster	Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007)
Epinephelus marginatus	Seabob shrimp, Urugavian lobster	Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007)
Epinephelus niveatus	Urugavian lobster	Severino-Rodrigues et al. (2007)
Etropus crossotus	Seabob shrimp	Branco & Verani (2006); Catanni et al. (2011); Graça-Lopes et al. (2002); Haimovici & Mendonça (1996); Sedrez et al. (2013)
Etropus intermedius	Seabob shrimp	Graça-Lopes et al. (2002)
Etropus longimanus	Seabob shrimp	Graça-Lopes et al. (2002); Sedrez et al. (2013)
Eucinostomus argenteus	Seabob shrimp	Graça-Lopes et al. (2002)
Eucinostomus gula	Penaeid shrimps	Branco & Verani (2006); Catanni et al (2011); Graça-Lopes et al. (2002); Lira et al. (2019); Sedrez et al. (2013)
Eucinostomus melanopterus	Seabob shrimp	Branco & Verani (2006); Sedrez et al. (2013)
Eugomphodus Taurus	Seabob shrimp	Graça-Lopes et al. (2002)
Evoxymetopon taeniatus	Urugavian lobster	Severino-Rodrigues et al. (2007)
Fistularia petimba	Penaeid shrimps	Graça-Lopes et al. (2002); Haimovici & Mendonça (1996); Sedrez et al. (2013)
Fistularia tabacaria	Seabob shrimp	Graça-Lopes et al. (2002)
Genidens barbus	Seabob shrimp, Urugavian lobster	Branco & Verani (2006); Catanni et al. (2011); Sedrez et al. (2013); Severino-Rodrigues et al. (2007)
Genidens geniden	Penaeid shrimps	Branco & Verani (2006); Catanni et al. (2011); Graça-Lopes et al. (2002); Haimovici & Mendonça (1996); Sedrez et al. (2013)
Genyatremus luteus	Penaeid shrimps	Catanni et al. (2011); Lira et al. (2019)
Genypterus brasiliensis	Penaeid shrimps, Urugavian lobster	Graça-Lopes et al. (2002); Haimovici & Mendonça (1996); Severino-Rodrigues et al. (2007)
Gymnachirus nudus	Penaeid shrimps	Haimovici & Mendonça.,1996; Graça-Lopes et al. (2002)

Fish Bycatch in Brazil	Target Specie	Reference
Gymnothorax ocellatus	Seabob shrimp	Branco & Verani (2006); Catanni et al. (2011); Graça-Lopes et al. (2002); Sedrez et al. (2013)
Gymnura altavela	Seabob shrimp, Urugavian lobster	Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007)
Haemulon aurolineatum	Penaeid shrimps	Lira et al., 2019; Graça-Lopes et al. (2002)
Haemulon plumieri	Penaeid shrimps	Lira et al., 2019; Graça-Lopes et al. (2002)
Haemulon steindachneri	Penaeid shrimps	Lira et al., 2019; Graça-Lopes et al. (2002)
Haemulopsis corvinaeformis	Penaeid shrimps	Lira et al. (2019)
Harengula clupeola	Penaeid shrimps	Branco & Verani (2006); Catanni et al. (2011); Graça-Lopes et al. (2002); Lira et al. (2019)
Helicolenus dactylopterus	Urugavian lobster	Severino-Rodrigues et al. (2007)
Hemicaranx amblyrhynchus	Seabob shrimp	Catanni et al. (2011)
Holocentrus ascencionis	Seabob shrimp	Graça-Lopes et al. (2002)
Hypocampus sp.	Penaeid shrimps	Haimovici & Mendonça (1996)
Hippocampus erectus	Seabob shrimp	Branco & Verani (2006); Graça-Lopes et al. (2002)
Hippocampus reidi	Seabob shrimp	Branco & Verani (2006); Graça-Lopes et al. (2002)
Isopisthus parvipinnis	Penaeid shrimps	Bernardo et al. (2011); Branco & Verani (2006) Catanni et al., 2011; Graça-Lopes et al. (2002); Lira et al. (2019); Sedrez et al. (2013)
Isurus oxyrinchus	Seabob shrimp	Graça-Lopes et al. (2002)
Larimus breviceps	Penaeid shrimps	Bernardo et al. (2011); Branco & Verani (2006); Catanni et al., 2011; Lira et al. (2019); Sedrez et al. (2013)
Lagocephalus laevigatus	Seabob shrimp	Branco & Verani (2006); Catanni et al. (2011); Sedrez et al. (2013); Graça-Lopes et al. (2002)
Lagocephalus sphenoides	Seabob shrimp	Graça-Lopes et al. (2002)
Larimus breviceps	Seabob shrimp	Graça-Lopes et al. (2002)
Lophius gastrophysus	Penaeid shrimps	Graça-Lopes et al. (2002); Haimovici & Mendonça (1996); Severino-Rodrigues et al. (2007)
Lophius gastrophysys	Seabob shrimp	Graça-Lopes et al., 2002
Lutjanus analis	Urugavian lobster	Severino-Rodrigues et al. (2007)

Fish Bycatch in Brazil	Target Specie	Reference
Lopholatilus villarii	Seabob shrimp, Urugavian lobster	Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007)
Lutjanus synagris	Penaeid shrimps	Lira et al. (2019)
Lutjanus purpureus	Urugavian lobster	Severino-Rodrigues et al. (2007)
Lycengraulis grossidens	Penaeid shrimps	Branco & Verani (2006); Catanni et al. (2011); Haimovici & Mendonça (1996); Lira et al. (2019); Sedrez et al. (2013)
Macrodon ancylodon	Penaeid shrimps	Bernardo et al. (2011); Branco & Verani (2006); Catanni et al. (2011); Graça-Lopes et al. (2002); Haimovici & Mendonça (1996); Lira et al. (2019)
Macrodon atricauda	Seabob shrimp	Sedrez et al. (2013)
Macrorhamphosus scolopa	Seabob shrimp	Graça-Lopes et al. (2002)
Menticirrhus americanus	Penaeid shrimps	Bernardo et al. (2011); Branco & Verani (2006); Catanni et al. (2011); Graça-Lopes et al. (2002); Lira et al. (2019); Sedrez et al. (2013)
Menticirrhus littorallis	Seabob shrimp	Graça-Lopes et al. (2002)
Merluccius hubbsi	Urugavian lobster	Haimovici & Mendonça (1996); Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007)
Menticirrhus littoralis	Penaeid shrimps	Branco & Verani (2006); Haimovici & Mendonça (1996); Sedrez et al. (2013)
Micropogonias furnieri	Penaeid shrimps, Urugavian lobster	Bernardo et al. (2011); Branco & Verani (2006) Catanni et al. (2011); Graça-Lopes et al. (2002); Haimovici & Mendonça (1996); Lira et al. (2019); Sedrez et al. (2013); Severino-Rodrigues et al. (2007)
Mugil sp.	*Pleoticus muelleri; Artemesia longinaris*	Haimovici & Mendonça (1996)
Mullus argentinae	*Pleoticus muelleri; Artemesia longinaris, Urugavian lobster*	Haimovici & Mendonça (1996); Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007)
Mustelus canis	*Pleoticus muelleri; Artemesia longinaris,* Urugavian lobster	Haimovici & Mendonça (1996); Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007)
Mustelus fasciatus	*Pleoticus muelleri; Artemesia longinaris*	Haimovici & Mendonça (1996)
Mustelus schmitti	*Pleoticus muelleri; Artemesia longinaris,* Urugavian lobster	Haimovici & Mendonça (1996); Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007)
Monacanthus ciliates	Seabob shrimp	Graça-Lopes et al. (2002)

Fish Bycatch in Brazil	Target Specie	Reference
Myliobatis sp	*Pleoticus muelleri; Artemesia longinaris*	Haimovici & Mendonça (1996); Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007)
Narcine brasiliensis	*Pleoticus muelleri*, *Artemesia longinaris*, Seabob shrimp	Branco & Verani (2006); Catanni et al. (2011); Graça-Lopes et al. (2002); Haimovici & Mendonça (1996)
Nebris microps	Penaeid shrimps	Bernardo et al. (2011); Catanni et al. (2011); Graça-Lopes et al. (2002); Lira et al. (2019)
Netuma barba	Seabob shrimp	Graça-Lopes et al. (2002)
Netuma planifrons	*Pleoticus muelleri; Artemesia longinaris*	Haimovici & Mendonça (1996)
Notarius grandicassis	Seabob shrimp	Graça-Lopes et al. (2002)
Notorynchus pectorosus	*Pleoticus muelleri; Artemesia longinaris*	Haimovici & Mendonça (1996)
Ocyurus chrysurus	*Urugavian lobster*	Severino-Rodrigues et al. (2007)
Odontaspis Taurus	*Pleoticus muelleri; Artemesia longinaris*	Haimovici & Mendonça (1996)
Odontognathus mucronatus	Penaeid shrimps	Lira et al. (2019)
Ogcocephalus vespertilio	*Pleoticus muelleri; Artemesia longinaris*, Seabob shrimp	Branco & Verani (2006); Graça-Lopes et al. (2002); Haimovici & Mendonça (1996); Severino-Rodrigues et al. (2007)
Oligoplites saliens	Seabob shrimp	Catanni et al. (2011); Graça-Lopes et al. (2002)
Oligoplites saurus	Seabob shrimp	Branco & Verani (2006); Catanni et al. (2011); Sedrez et al. (2013); Graça-Lopes et al. (2002)
Oncopterus darwini	Penaeid shrimps	Haimovici & Mendonça (1996)
Ophichthus gomesii	Seabob shrimp	Branco & Verani (2006); Catanni et al. (2011); Graça-Lopes et al. (2002); Sedrez et al. (2013)
Ophioscion sp	Penaeid shrimps	Lira et al. (2019)
Ophidion holbrokii	Seabob shrimp	Graça-Lopes et al. (2002)
Ophioscion punctatissimus	Penaeid shrimps	Bernardo et al. (2011); Graça-Lopes et al. (2002); Lira et al. (2019)
Ophychtus gomesii	Penaeid shrimps	Haimovici & Mendonça (1996)

Fish Bycatch in Brazil	Target Specie	Reference
Opisthonema oglinum	Penaeid shrimps	Branco & Verani (2006); Graça-Lopes et al. (2002); Lira et al. (2019)
Orthopristes ruber	Penaeid shrimps	Branco & Verani (2006); Graça-Lopes et al. (2002); Haimovici & Mendonça (1996); Sedrez et al. (2013)
Pagrus pagrus	Penaeid shrimps, Urugavian lobster	Graça-Lopes et al. (2002); Haimovici & Mendonça (1996); Severino-Rodrigues et al. (2007)
Paralichthys orbignyanus	Penaeid shrimps	Catanni et al. (2011); Haimovici & Mendonça (1996)
Paralonchurus brasiliensis	Penaeid shrimps	Branco & Verani (2006); Bernardo et al. (2011); Catanni et al. (2011); Graça-Lopes et al. (2002); Lira et al. (2019); Sedrez et al. (2013)
Paralichthys isósceles	Penaeid shrimps Urugavian lobster	Graça-Lopes et al. (2002); Haimovici & Mendonça (1996); Severino-Rodrigues et al. (2007)
Paralichthys orbignyanus	Penaeid shrimps	Haimovici & Mendonça (1996)
Paralichthys patagonicus	Penaeid shrimps, Urugavian lobster	Graça-Lopes et al. (2002); Haimovici & Mendonça (1996); Sedrez et al. (2013); Severino-Rodrigues et al. (2007)
Paralichtys triocellatus	Urugavian lobster	Severino-Rodrigues et al. (2007)
Paranthias furcifer	Seabob shrimp	Graça-Lopes et al. (2002)
Parona signata	Penaeid shrimps	Graça-Lopes et al. (2002); Haimovici & Mendonça (1996)
Pellona harroweri	Penaeid shrimps	Branco & Verani (2006); Catanni et al. (2011); Graça-Lopes et al. (2002); Lira et al. (2019); Sedrez et al. (2013)
Pempheris schomburgkii	Penaeid shrimps	Lira et al. (2019)
Peprilus paru	Penaeid shrimps, Urugavian lobster	Branco & Verani (2006); Catanni et al. (2011); Graça-Lopes et al. (2002); Haimovici & Mendonça (1996); Sedrez et al. (2013); Severino-Rodrigues et al. (2007)
Percophis brasiliensis	Penaeid shrimps, Urugavian lobster	Graça-Lopes et al. (2002); Haimovici & Mendonça (1996); Severino-Rodrigues et al. (2007)
Peristedion altipinne	Seabob shrimp	Graça-Lopes et al. (2002)
Pinguipes brasilianus	Seabob shrimp	Graça-Lopes et al. (2002)

Fish Bycatch in Brazil	Target Specie	Reference
Polymixia lowei	Urugavian lobster	Severino-Rodrigues et al. (2007)
Polyprion americanus	Penaeid shrimps, Urugavian lobster	Haimovici & Mendonça (1996); Severino-Rodrigues et al. (2007)
Polydactylus virginicus	Penaeid shrimps	Catanni et al. (2011); Lira et al. (2019); Sedrez et al. (2013)
Pomacanthus paru	Seabob shrimp	Branco & Verani (2006); Sedrez et al. (2013)
Pomadasys corvinaeformis	Seabob shrimp	Branco & Verani (2006); Catanni et al. (2011); Graça-Lopes et al. (2002); Sedrez et al. (2013)
Pomadasys croco	Seabob shrimp	Graça-Lopes et al. (2002)
Pomatomus saltatrix	Seabob shrimp	Catanni et al. (2011); Graça-Lopes et al. (2002); Haimovici & Mendonça (1996)
Porichthys porosissimus	Seabob shrimp, Urugavian lobster	Branco & Verani (2006); Catanni et al. (2011); Graça-Lopes et al. (2002); Haimovici & Mendonça (1996); Sedrez et al. (2013); Severino-Rodrigues et al. (2007)
Priacanthus arenatus	Urugavian lobster	Severino-Rodrigues et al. (2007)
Prionotus nudigula	Penaeid shrimps, Urugavian lobster	Graça-Lopes et al. (2002); Haimovici & Mendonça (1996); Severino-Rodrigues et al. (2007)
Prionotus punctatus	Penaeid shrimps, Urugavian lobster	Branco & Verani (2006); Catanni et al. (2011); Graça-Lopes et al. (2002); Sedrez et al. (2013); Severino-Rodrigues et al. (2007)
Priacanthus arenatus	Penaeid shrimps	Graça-Lopes et al. (2002); Haimovici & Mendonça (1996)
Psammobatis extenta	Penaeid shrimps	Haimovici & Mendonça (1996)
Psammobatis glandissimilis	Seabob shrimp	Graça-Lopes et al. (2002)
Pseudopeneus maculate	Seabob shrimp	Graça-Lopes et al. (2002)
Pseudopercis numida	Seabob shrimp, Urugavian lobster	Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007)
Pseudopercis semifasciata	Seabob shrimp, Urugavian lobster	Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007)
Raja castelnaui	Penaeid shrimps	Haimovici & Mendonça (1996)
Raneya fluminensis	Penaeid shrimps	Haimovici & Mendonça (1996)
Rhinobatus horkelli	Penaeid shrimps, Urugavian lobster	Graça-Lopes et al. (2002); Haimovici & Mendonça (1996); Severino-Rodrigues et al. (2007)

Fish Bycatch in Brazil	Target Specie	Reference
Rhinobatos percellens	Seabob shrimp	Branco & Verani (2006); Catanni et al. (2011); Graça-Lopes et al. (2002)
Rhinoptera bonasus	Seabob shrimp	Graça-Lopes et al. (2002); Sedrez et al. (2013)
Rhizoprionodon lalandii	Seabob shrimp	Graça-Lopes et al. (2002)
Rhizoprionodon porosus	Seabob shrimp	Graça-Lopes et al. (2002)
Rhomboplistes aurorubens	Seabob shrimp, Urugavian lobster	Sedrez et al. (2013); Severino-Rodrigues et al. (2007)
Rioraja agassizi	Seabob shrimp	Graça-Lopes et al. (2002); Sedrez et al. (2013);
Rioraja platana	Seabob shrimp	Graça-Lopes et al. (2002)
Rypticus randalli	Seabob shrimp	Catanni et al. (2011)
Sardinella brasiliensis	Penaeid shrimps	Branco & Verani (2006); Graça-Lopes et al. (2002); Haimovici & Mendonça (1996); Sedrez et al. (2013)
Sardinella janeiro	Seabob shrimp	Catanni et al. (2011)
Saurida brasiliensis	Seabob shrimp	Graça-Lopes et al. (2002)
Saurida caribbea	Seabob shrimp	Graça-Lopes et al. (2002)
Sciadeichthys luniscutis	Seabob shrimp, Urugavian lobster	Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007)
Sciaenoides bergi	Penaeid shrimps	Haimovici & Mendonça (1996)
Scorpaena isthmensis	Seabob shrimp, Urugavian lobster	Sedrez et al. (2013); Severino-Rodrigues et al. (2007)
Scorpaena plumieri	Seabob shrimp	Graça-Lopes et al. (2002)
Selene brownii	Penaeid shrimps	Lira et al. (2019)
Selene setapinnis	Seabob shrimp	Branco & Verani (2006) Catanni et al. (2011); Graça-Lopes et al. (2002); Haimovici & Mendonça (1996); Sedrez et al. (2013)
Selene vomer	Penaeid shrimps, Urugavian lobster	Branco & Verani (2006); Catanni et al. (2011); Graça-Lopes et al. (2002); Lira et al. (2019); Sedrez et al. (2013); Severino-Rodrigues et al. (2007)
Seriola lalandi	Urugavian lobster	Severino-Rodrigues et al. (2007)
Sphoeroides pachygaster	Urugavian lobster	Severino-Rodrigues et al. (2007)
Sphoeroides greeleyi	Seabob shrimp	Sedrez et al. (2013)

Fish Bycatch in Brazil	Target Specie	Reference
Sphoeroides testudineus	Seabob shrimp	Branco & Verani (2006); Catanni et al. (2011); Sedrez et al. (2013)
Sphyraena guachancho	Penaeid shrimps	Graça-Lopes et al. (2002); Lira et al. (2019)
Sphyraena tome	Penaeid shrimps	Graça-Lopes et al. (2002)
Sphyrna lewini	Seabob shrimp	Graça-Lopes et al. (2002)
Sphyrna sp	Penaeid shrimps	Haimovici & Mendonça (1996)
Sphyrna zygaena	Seabob shrimp	Graça-Lopes et al. (2002)
Squatina argentina	Seabob shrimp	Graça-Lopes et al. (2002)
Squatina oculta	Seabob shrimp	Graça-Lopes et al. (2002)
Squatina Guggenheim	Seabob shrimp	Graça-Lopes et al. (2002)
Squatina squatina	Penaeid shrimps	Haimovici & Mendonça (1996)
Squalus sp	Penaeid shrimps	Haimovici & Mendonça (1996)
Stellifer brasiliensis	Penaeid shrimps	Bernardo et al. (2011); Catanni et al. (2011); Graça-Lopes et al. (2002); Lira et al. (2019); Sedrez et al. (2013)
Stellifer microps	Penaeid shrimps	Lira et al. (2019)
Stellifer rastrifer	Seabob shrimp	Bernardo et al. (2011); Catanni et al. (2011); Graça-Lopes et al. (2002); Sedrez et al. (2013)
Stellifer stellifer	Seabob shrimp	Bernardo et al. (2011); Catanni et al. (2011); Graça-Lopes et al. (2002); Sedrez et al. (2013)
Stephanolepis hispidus	Seabob shrimp	Branco & Verani (2006); Graça-Lopes et al. (2002); Sedrez et al. (2013)
Stephanolepis setifer	Penaeid shrimps	Haimovici & Mendonça (1996)
Stromateus brasiliensis	Penaeid shrimps	Haimovici & Mendonça (1996)
Syacium micrurum	Seabob shrimp	Graça-Lopes et al. (2002); Sedrez et al. (2013)
Syacium papillosum	Seabob shrimp	Catanni et al. (2011); Graça-Lopes et al. (2002); Haimovici & Mendonça (1996)
Sygnathus folletti	Seabob shrimp	Graça-Lopes et al. (2002)
Symphurus jenynsi	Penaeid shrimps	Haimovici & Mendonça (1996)
Symphurus plagusia	Penaeid shrimps	Branco & Verani (2006); Graça-Lopes et al. (2002); Haimovici & Mendonça (1996); Lira et al. (2019)

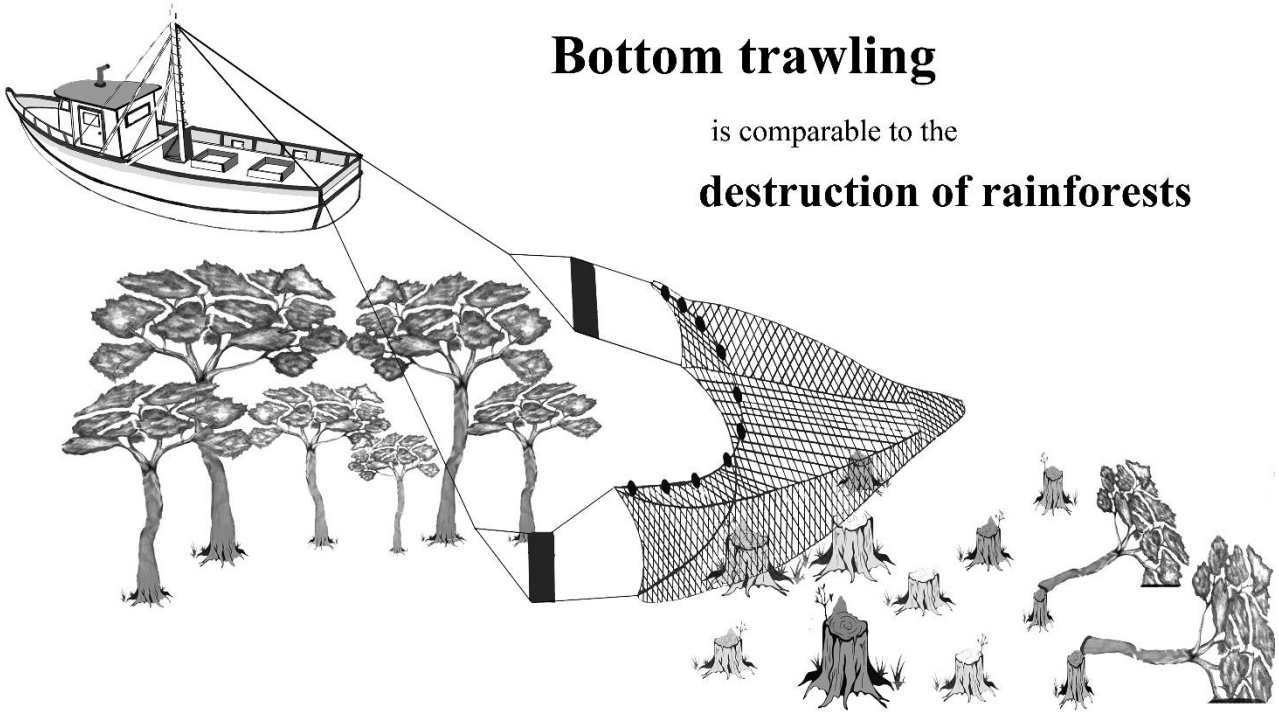
Fish Bycatch in Brazil	Target Specie	Reference
Symphurus tessellatus	Penaeid shrimps	Branco & Verani (2006); Catanni et al. (2011); Lira et al. (2019); Sedrez et al. (2013)
Sympterigia acuta	Penaeid shrimps	Haimovici & Mendonça (1996)
Sympterigia bonapartei	Penaeid shrimps	Haimovici & Mendonça (1996)
Synodus foetens	Seabob shrimp	Graça-Lopes et al. (2002)
Synodus intermedium	Seabob shrimp	Graça-Lopes et al. (2002)
Thalassophryne montevidensis	Seabob shrimp	Graça-Lopes et al. (2002)
Thyrsitops lepidopoides	Urugavian lobster	Severino-Rodrigues et al. (2007)
Trachinocephalus myops	Seabob shrimp	Graça-Lopes et al. (2002)
Trachinotus carolinus	Seabob shrimp	Graça-Lopes et al. (2002)
Trachinotus falcatus	Seabob shrimp	Branco & Verani (2006)
Trachinotus marginatus	Penaeid shrimps	Haimovici & Mendonça (1996)
Trachurus lathami	Penaeid shrimps, Urugavian lobster	Graça-Lopes et al. (2002); Haimovici & Mendonça (1996); Severino-Rodrigues et al. (2007)
Trichiurus lepturus	Penaeid shrimps, Urugavian lobster	Branco & Verani (2006); Catanni et al. (2011); Graça-Lopes et al. (2002); Haimovici & Mendonça (1996); Lira et al. (2019); Sedrez et al. (2013); Severino-Rodrigues et al. (2007)
Trinectes microphthalmus	Penaeid shrimps	Catanni et al. (2011); Graça-Lopes et al. (2002); Haimovici & Mendonça (1996)
Trinectes paulistanus	Penaeid shrimps	Catanni et al. (2011); Lira et al. (2019)
Tyrsytops lepidopoides	Seabob shrimp	Haimovici & Mendonça (1996)
Umbrina canosai	Penaeid shrimps	Graça-Lopes et al. (2002); Haimovici & Mendonça (1996); Severino-Rodrigues et al. (2007)
Umbrina coroides	Seabob shrimp	Bernardo et al. (2011); Severino-Rodrigues et al. (2007)
Upeneus parvus	Seabob shrimp, Urugavian lobster	Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007)
Urophycis brasiliensis	Seabob shrimp	Branco & Verani (2006); Catanni et al. (2011); Graça-Lopes et al. (2002); Haimovici & Mendonça (1996); Sedrez et al. (2013)
Urophycis cirrata	Urugavian lobster	Severino-Rodrigues et al. (2007)
Urophycis mystaceus	Seabob shrimp	Graça-Lopes et al. (2002)

Fish Bycatch in Brazil	Target Specie	Reference
Verecundum rasile	Penaeid shrimps	Haimovici & Mendonça (1996)
Zapteryx brevirostris	Penaeid shrimps	Catanni et al. (2011); Haimovici & Mendonça (1996); Sedrez et al. (2013)
Zenopsis conchifer	Seabob shrimp, Urugavian lobster	Graça-Lopes et al. (2002); Severino-Rodrigues et al. (2007)
Zeus faber	Seabob shrimp	Graça-Lopes et al. (2002)

The animals that are captured as bycatch are dumped in the ocean and it is causing a problem of waste because millions of tons of protein are unduly discarded (Hall, 2000). In addition, the trawling represents a major threat to the deep seafloor ecosystem at the global scale, because of the devastation it causes to marine habitats and because it is the least selective type of fishing (Pusceddo et al., 2014; Zhou et al., 2019). Bottom trawling effect in the seafloor is comparable to the destruction of rainforests (Pusceddo et al., 2014; Zhou et al., 2019). The untraveled areas and chronically trawled areas in the north-western Mediterranean Sea were compared and was observed significant decreases in organic matter content, slower organic carbon turnover and reduced meiofauna abundance in the areas trawled (Pusceddo et al., 2014). These consequences can affect marine food chains, as many organisms rely primarily on meiofauna for nutrients and energy and the damage caused by trawling means the depletion of a major food source and the destruction of cradles of many species (Pusceddo et al., 2014). The slower organic carbon turnover is because the trawling suspends this material in water and it is moved by ocean currents to even deeper regions of the ocean, away from the microorganisms that depend on it (Pusceddo et al., 2014). Studies show that what happens at 500 meters depth probably influences communities at 2.000 meters depth (Pusceddo et al., 2014; Zhou et al., 2019). In this way, the bottom trawling has a major impact on deep-sea sedimentary ecosystems, causing their degradation and a process of desertification, with

potential consequences on the biogeochemical cycles (Pusceddo et al., 2014; Zhou et al., 2019).

Figure 4. Trawling can resuspend sediments, modify seafloor morphology and increase of bottom water turbidity. Its impacts are comparable to the destruction of rainforests.



4.2 Economic and social impacts of trawling and its bycatch

In Brazil, the shrimp trawling is responsible for a high level of coastal communities employment and has a socioeconomic relevance (Júnior et al., 2011; Chavez & Silva, 2019). However, the economic consequence of this type of fisheries is not just associated with good impacts, because some costs are not calculated. Often the animals that are captured can rip the nets, break the cables and due to the low selectivity of the trawl (Clucas, 1997; Hall et al., 2000). The “not alive” bycatch as stone and trash can also damage the boats, obliterating the mesh of the net and increasing the boat wear bringing

a lot of expenses to the fishermen (personal communication with fishermen of the coast of São Paulo State). Animal mortality is associated with biodiversity and protein loss, and the bycatch capture, together with the organic and inorganic trash, is an aggravating factor in shrimp fishing (Chavez & Silva, 2019). In addition, the accidental captured of the no target species has an additional cost due to the higher fuel consumption and the employment of extra crew members (Clucas, 1997). Such high energy dependence has strong social impacts due to the volatility of the oil price (Medeiros et al., 2013). Also, the extra crew is necessary to remove the unwanted catch from the target species (Hall et al., 2000). The fishers alone cannot separate quickly the species from bycatch and long times that the animals stayed on-deck or in the sea can reduce the quality and value of the target species (Clucas, 1997; Hall et al., 2000; Júnior et al., 2011; Chavez & Silva, 2019). These situations can add costs to trawling fisheries (Hall et al., 2000). To the fishers the regulations and limitations on the use of resources are a problem and, to compensate for the temporal or spatial closures during the ban period, the fishers increase the level of effort in open areas or seasons (Hall et al., 2000). The environmental problems also can affect the economics of the trawling fisheries as the overfishing can cause a stock collapse and fishers can be fishing less (Davanso et al., 2017). Besides that, the capture of the organisms of bycatch gives fishers a bad public image and this is one of the social impacts (Hall et al., 2000). In many developing countries large numbers of people depend on shrimp fisheries to own subsistence and the prohibition can impact the small scale fisherfolk, thus it is necessary to search alternative income-generating activities for these people (Clucas, 1997; Hall et al., 2000; Júnior et al., 2011; Chavez & Silva, 2019). In addition the fishers have fishing as a source of income, the national economy of less developed countries may depend on exportations of high-value marine products such as shrimp (Clucas, 1997; Hall et al., 2000).

The animals captured accidentally in the fisheries are always mentioned as the major impact of fishing, however, understanding the economic and social impacts is fundamental to the development of a sustainable fishing system. Gear modifications and more selective fishing methods can help reduce this waste. Another action that should be considered is to choose the closure areas including target species and non-target species areas and for this it should be considered the degree of incidence of bycatch (Hall et al., 2000). The preservation of these habitats in the form of fishing exclusion areas is another act that is essential for sustainability. The closure of fishing areas can affect the income of many fishing families affecting the livelihood of such people and because of this, it is important to enabling these fishermen to have other livelihood alternatives. The creation of cooperatives that provide new skills for fishermen and their families is an alternative to improve income during the closure of the areas of the fisheries. The balance between the economic, social and environmental aspects is not easy to solve, however, it is essential to search for solutions and to do so it is necessary to involve government, conservation management agencies, scientists, industry representatives, and fishing communities to share information about the bycatch. Besides that, scientists and fishery industries need to maintain close cooperation with fishers in regional and global scale in order to develop practical solutions and regulations to become the fishery trawling an even more sustainable activity.

5. Solutions to reduce the bycatch

The trawling is an activity linked to many environmental problems, however, banning this activity can have a major impact on the lives of many families that depend on the fishing to subsistence (Hall et al., 2000). Thus, technological innovations have been developed to decrease bycatch and reduce the impacts of this type of fishing

(Cattanni et al., 2012). One of the ways to reduce bycatch is by Bycatch Reduction Devices (BRD) that are devices inserted in fishing gear to reduce unwanted bycatch (Fonseca et al., 2005; Cox et al., 2007; Cattanni et al., 2012; Medeiros et al., 2013; Field et al., 2019). The BRD can be work through the split panels, tailored windows, and tailored square meshes to direct the catch target into the bag and escape through the top of the bagger (Cattanni et al., 2012; Medeiros et al., 2013). The BRD also can be work through tailored grilles at the bagger inlet to select the catch based on grid spacing (Cattanni et al., 2012; Medeiros et al., 2013). Some bycatch avoidance measures have been successfully implemented as appropriately designed exclusion devices that reduce pinniped bycatch in some trawlers and pingers (acoustic deterrent devices) that reduce the bycatch of some small cetacean species in gillnets (Hamilton & Baker, 2019). The addition of artificial light to the nets has also been tested to reduce the of bycatch (Hannah et al., 2015).

In countries such as the United States, Australia, and Norway, the implementation of BRD has been occurring (Cattanni et al., 2012; Medeiros et al., 2013; Jacques et al., 2019). In the Northeast Atlantic deep-water shrimp fishery the use of a Nordmøre grid is mandatory in Norway (Jacques et al., 2019). On the southeast Queensland coast the researchers recommend that the 47.6 mm square mesh codend be made mandatory in the eastern deepwater king prawn fishery as a highly effective BRD (Courtney et al., 2014).

In Brazil, research has been conducted with BRD, for example, in Parana state three Nordmøre-grids reduced significantly the total weight of unwanted brachyurids without affecting the catches of targeted seabob shrimp in penaeid canoe-trawl fishery (Silva et al., 2012). In southern of Brazil, one grid with the smallest spacing (25 mm) was efficient in reducing bycatch, excluding mature individuals from the net without loss in shrimp production (Vianna & D'Incao, 2006). An analysis of fishermen's perception of

adopting structural modifications for bycatch reduction was conducted in southern of Brazil and fishermen agree with the BRD results, but reduction of marketable bycatch has affected the acceptance of use of the BRD showing that there are many challenges in fisheries management in relation to the use of BRD (Portella & Medeiros, 2015). Although the results of the use of BRD are being positive, for pratical use management measures are necessary (Medeiros et al., 2013). For example, the use of Turtle Excluder Devices (TED) for vessels longer than 10 meters was regulated (Ibama 5/1997), but most of artisanal fishers are unaware of this obligation (Medeiros et al., 2013). Further experiments with BRD should be developed and tested in Brazil to assist implementation or modification of new fishing gears (Cox et al., 2007; Medeiros et al., 2013). Collaboration between scientists, the fishing industry and government, as well as post-implementation monitoring and incentives to fishermen are the key to BRD success (Medeiros et al., 2013).

6. What could be done with bycatch?

The bycatch is one of the biggest problems in the use of sea resources and ways of using this waste have been discussed over the years (Clucas, 1997; Chavez & Silva, 2019). In some countries, part of the biomass captured as bycatch is used for human consumption or animal feed (Clucas, 1997; Hall et al., 2000; Catanni et al., 2012). In Brazil, the landing of part of the bycatch has been used to compensate the fall in the capture of target species (Graça-Lopez et al., 2002; Catanni et al., 2012). Especially in the Southeast and South regions, the bycatch is used to compensate for the low yield in times of low shrimp production by the industrial fleet (Catanni et al., 2012). A way to utilize the huge amount of wasted protein as bycatch is making protein hydrolysates from this discarded material. The enzymatic hydrolysis can recover protein and peptides of

commercial interest adding value to these animals that are discarded (Kristinsson & Rasco, 2000; Chalamaiah et al., 2012). Previous studies demonstrated that the hydrolysates obtained from marine organisms have bioactive compounds as antioxidant peptides that can be used in the food industries, as food ingredients and nutraceuticals in animals (Chalamaiah et al., 2012; Halim et al., 2016; Zamora-Sillero et al., 2018). In this way, besides to being an abundant source of protein, the bycatch can be a source of bioactive compounds. Oceans are sources of natural products and these marine compounds can be found in animals that use these substances to modulate various biological processes and as a chemical defense against predators (Faulkner et al., 1998). For example, the Brazilian marine sponge *Polymastia janeirensis* is considered a good candidate to development of new cancer medicines (Da Frota et al., 2009). Recently, the antimicrobial protein haemocyanin isolated from the haemolymph of flower crab, *Portunus pelagicus*, was investigated and the results showed the high potential of purified haemocyanin that could be used to develop new and effective antimicrobial drugs for aquaculture purposes (Ishwarya et al., 2018).

The chemical study of bycatch organisms may reveal potential biomolecules that could be used in the pharmaceutical industry, which have enormous economic value, and which will add value to these discarded organisms. The evaluation of metabolites present in bycatch species is urgent and important because it is a source of substances with unique biological activities and properties and a source of income for fishing communities. In this context, a Brazilian project funded by the National Council for Scientific and Technological Development (CNPq) aims to identify and select constituent species of the shrimp bycatch with potential for the production of bioactive compounds. The first article of this project was published recently and indicated the presence of important and potentially bioactive compounds as sulphated glycosilated steroids and

polyhydroxysteroid in the starfish *Luidia senegalensis* present in bycatch of the shrimp fishery (Tangerina et al., 2018).

Another use for bycatch is the manufacture of jewelry with shells and scales of animals that are accidentally captured. Bio jewelry are earrings, necklaces, and bracelets that are made with seeds, leaves, straw or other natural material, and the pieces can be mixed with precious stones. As happened in many regions of Brazil, bio jewelry can be sold to tourists that are visiting the coast and this can generate additional income for fishermen and their families. Initiatives such as offering artisan courses in bio jewelry with the rejection of trawl fishing are essential to teach and to qualify those who do not know how to produce the products and how to carry out the activity. These incentives should be taken by the government, conservation institutes, coastal community cooperatives or university extension projects.

Although some alternatives to use bycatch exist, this subject is very controversial and several aspects must be taken into consideration. To utilize the bycatch, the fishermen must land all captured animals, ie the full catch of the fishery and, if they are not compensated for the extra work and additional costs, there is a chance that this activity ends and the bycatch will still being discarded at the ocean (Clucas, 1997). And if the price to be paid for bycatch is higher than the price paid for the target species, the fishermen will be encouraged to fish more, thus, bycatch value should be lower than legally fished organisms (Clucas, 1997; Hall et al., 2000). Besides that, it is necessary to add some value to bycatch and have a consumer market for these products. Therefore, there must be a balance between the fishing and the incentive given to bycatch. Also, measures must be tested to assess which incentives should be adopted and if the market is willing to pay for these products.

Figure 5. Use of the bycatch. **A.** Earrings made from fish scales. The Bio jewelry can add value to the material that is discarded. **B.** Protein hydrolysates obtained from most abundant fishes from bycatch in the trawling in São Paulo coast. The hydrolysates are sources of essential amino acids and may contain biomolecules of commercial interest.

7. Final considerations

The shrimp trawling is an important socio-economic activity in Brazil and is necessary to recognize the need for a change in the current model of management. Throughout the country's development, it is possible to observe a problem regarding the management of the fishery resources in the federal institutions making the fishing fleet, production, and registrations, and license emissions control difficult. Because of that, more efficient management strategies based on transparent and participatory decision-making processes are essential to the sector. Besides that, the animals captured accidentally are the major impact of fishing, and it is necessary to understand the economic and social impacts of this to develop a sustainable fishing system. Gear modifications and more selective fishing methods can help reduce this waste.

Additionally, despite the potential of using bycatch, this is a very controversial question and several aspects must be taken into consideration. It is essential to search for solutions that involve government, conservation management agencies, scientists, industry representatives, and fishing communities to share information about the bycatch. Scientists and fishery industries need to maintain close cooperation with fishers in a regional and global scale in order to develop practical solutions and regulations to turn the fishery trawling an even more sustainable activity.

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Capítulo 2

Discovery of the biological activity in the protein hydrolysate obtained from bycatch of shrimp trawl fisheries

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Abstract

The bycatch of the shrimp trawl fisheries may have species with functional and bioactive properties, such as antioxidant peptides, which could add value to this reject. Thus, the present study aimed to investigate the antioxidant activity of the protein hydrolysates obtained from the most abundant animals from bycatch, i.e., the swiming crab *Callinectes ornatus*, the crab *Hepatus pudibundus* and the fishes *Micropogonias furnieri* and *Paralonchurus brasilensis.* The animals were collected between 2016 and 2018 by double-rig in a commercial shrimp trawling in Ubatuba-SP. The samples were hydrolyzed using the enzymes Alcalase 2.4 L® ou Protamex®. Subsequently, the *in vitro* antioxidant capacity against peroxyl radicals, DPPH radicals and sulfhydryl groups was investigated. The hydrolysates obtained from the four species showed antioxidant activity. No significant differences were observed in the antioxidant activity against DPPH radicals with the hydrolysates obtained with the two enzymes and between the species. All hydrolysates reduced peroxyl radicals, however, the crustaceans showed higher antioxidant capacity than the fishes. The two hydrolysates of crustaceans have higher concentrations of sulfhydryl groups, than hydrolyzed fish. The enzymatic hydrolysis is an efficient technique that allows the release of peptides with antioxidant activity of the four most abundant species of the bycatch, with potential use in the food industry.

Keywords: Hydrolysis, DPPH, ACAP, Alcalase, Protamex.

1. Introduction

In past decades, shrimp trawl fisheries have been studied because of a large number of species caught accidentally, called bycatch (Hall et al., 2000; Orbesen et al., 2017; Varisco et al., 2017; Ruibal Núñez et al., 2018; Fauconnet et al., 2019). The bycatch is rejected and has no commercial value, thus a large proportion of the animals are returned to the sea, dead or with little chance of survival (Hall et al., 2000; Gilman, 2011; Yang et al., 2015; Serena et al., 2016; Varisco et al., 2017; Burgess et al., 2018; Gray & Kennelly, 2018). This waste of a wide variety of organisms, and overexploitation of fishery resources, causes widespread destruction of the benthic environment and a huge impact on marine biodiversity (Hall et al., 2000; Willems et al., 2016; Wakefield et al., 2017; Burgess et al., 2018; Ruibal Núñez et al., 2018; Field et al., 2019). Research about the diversity of species in the bycatch (Severino-Rodrigues et al., 2002; 2007; Robert et al., 2007; Burgess et al., 2018), number of individuals caught (Graça-Lopes et al., 2002; Severino-Rodrigues et al., 2007; Ruibal Núñez et al., 2018), the exploitation of new resources and Bycatch Reduce Device (Graham et al., 2003; Campos & Fonseca et al., 2004; Fonseca et al., 2005; Medeiros et al., 2013; Bielli et al., 2020) have been conducted, but a destination for bycatch has not yet been found.

The bycatch contains a high amount of protein, amino acids, oils and others materials that can be processed and used as raw material for producing animal feed (Chalamaiah et al., 2012; Zamora-Sillero et al., 2018a; Sarteshnizi et al., 2019; Ashaolu, 2020). Techniques such as enzymatic hydrolysis have been developed to produce hydrolysates from fish by-products to recovery protein and peptides of commercial interest (Chalamaiah et al., 2012; Halim et al., 2016; Khiari & Mason, 2018; Zamora-Sillero et al., 2018a). Thus, the enzymatic production of hydrolysates can be an efficient technique to be applied to species of the bycatch to add value to them.

The fish protein hydrolysates (FPH) has essential nutrients and are potential sources of antioxidant peptides (Kristinsson & Rasco, 2000; Bougatef et al., 2010; Chalamaiah et al., 2012; Halim et al., 2016; Bashir et al., 2018; Zamora-Sillero et al., 2018a, b). According to Chalamaiah et al. (2012), hydrolysis of fish proteins using proteolytic enzymes is a widely applied process for the production of antioxidative peptides. This antioxidative peptides from FPH are molecules with easy absorption, high activity and low cost (Kristinsson & Rasco, 2000; Bougatef et al., 2010; Chalamaiah et al., 2012; Halim et al., 2016; Bashir et al., 2018; Latorres et al., 2018; Zamora-Sillero et al., 2018a, b; Abuine et al., 2019; Sarteshnizi et al., 2019). Additionally, not only in hydrolysates derived from fish have antioxidant properties (Sila & Bougatef, 2016). Recently, Latorres et al. (2018) showed that the protein hydrolysates from white shrimp have antioxidant properties that can be used in the formulation of functional foods.

Besides of the antioxidants molecules, several bioactive compounds are released in the hydrolysis of the fish byproducts, that are called bioactive peptides (Zamora-Sillero et al., 2018a; Abuine et al., 2019). Residues from Nile tilapia (*Oreochromis niloticus*) submitted to enzymatic hydrolysis, showed antimicrobial activity against *Bacillus megaterium* and *Edwardsiella tarda* (Robert et al., 2015). More recently, antibacterial activity was detected in an enzymatic hydrolysate generated by processing rainbow trout by-products (Wald et al. 2016). Thus, the bioactive properties of FPH are utilized for the production of value-added products that can be used as a functional ingredient in the food industry (Abuine et al., 2019).

In this way, the techniques to produce protein hydrolysates can be applied to the species caught accidentally in the shrimp trawl. The animals of the bycatch can have functional and bioactive properties, such as antioxidant peptides, which could add value to this reject. Thus, some species of the bycatch can generate products of interest to the

food industries, as food ingredients and nutraceuticals in preparing animal diets. In this context, the aim of the present work was to evaluate the antioxidant activity of protein hydrolysates prepared from the four most abundant animals from the bycatch of shrimp fisheries in Brazil. They are the swimming crab *Callinectes ornatus*, the crab *Hepatus pudibundus* and the fishes *Micropogonias furnieri* and *Paralonchurus brasilensis*. The information obtained can subsidize the development of scaled process to extract these active molecules, which would add value to these discarded animals.

2. Materials and Methods

2.1 *Raw material*

The animals (Figure 1) were collected in February 2017, September 2017 and February 2018 in the region of Ubatuba, state of São Paulo, Brazil, by double-rig trawl, a commercial shrimp trawling. Five collection points were selected: I - 23° 26' 791" LS 45° 00' 843" LW; II - 23° 25' 502" LS 45° 01' 028" LW; III - 23° 26' 598" LS 45° 01' 584" LW; IV - 23° 27' 158" LS 45° 01' 764" LW; V - 23° 27' 180" LS 45° 01' 900"LW (Figure 2). The muscle and skin and the muscles with exoskeleton of the animals were blended and packaged in sealed 100 g plastic bags and transported in cool boxes with dry ice to the Aquaculture Center (CAUNESP), Campus of Jaboticabal, where they were kept in the freezer at -20°C until analysis.

2.2 *Determination of bromatological parameters and enzymatic hydrolysis*

Before the start of hydrolysis process, the proximate chemical composition of the muscles were analyzed. The moisture, ash, lipid and protein were determined according to the AOAC methods (AOAC, 2005). The moisture determination was obtained by keeping the samples at 105°C for 5 h. The ash content was obtained by pyrolysis of the

sample at 550 °C for 5 h. The lipid content was determined using the Soxhlet method. The crude protein content was determined by Kjeldahl method using the nitrogen analyzer LECO FP-428 in the Laboratory of Animal Nutrition at School of Agrarian and Veterinary Sciences of UNESP, Campus of Jaboticabal. All analyzes were performed in triplicate.

Figure 1. Animals more abundant from bycatch in São Paulo, used in the present study. **A**, *Callinectes ornatus*. **B**, *Hepatus pudibundus*. **C**, *Micropogonias furnieri*. **D**, *Paralonchurus brasiliensis*.

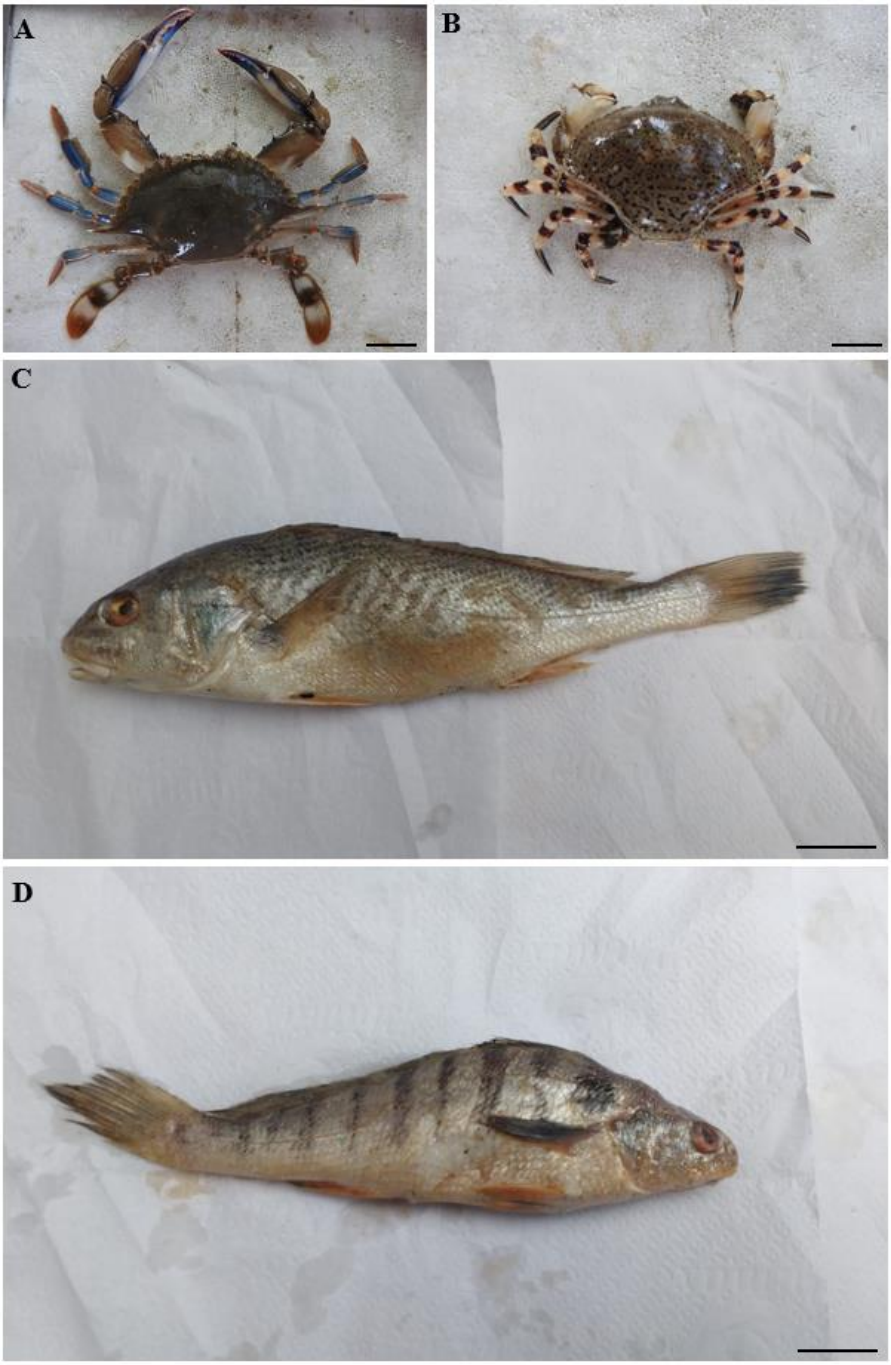
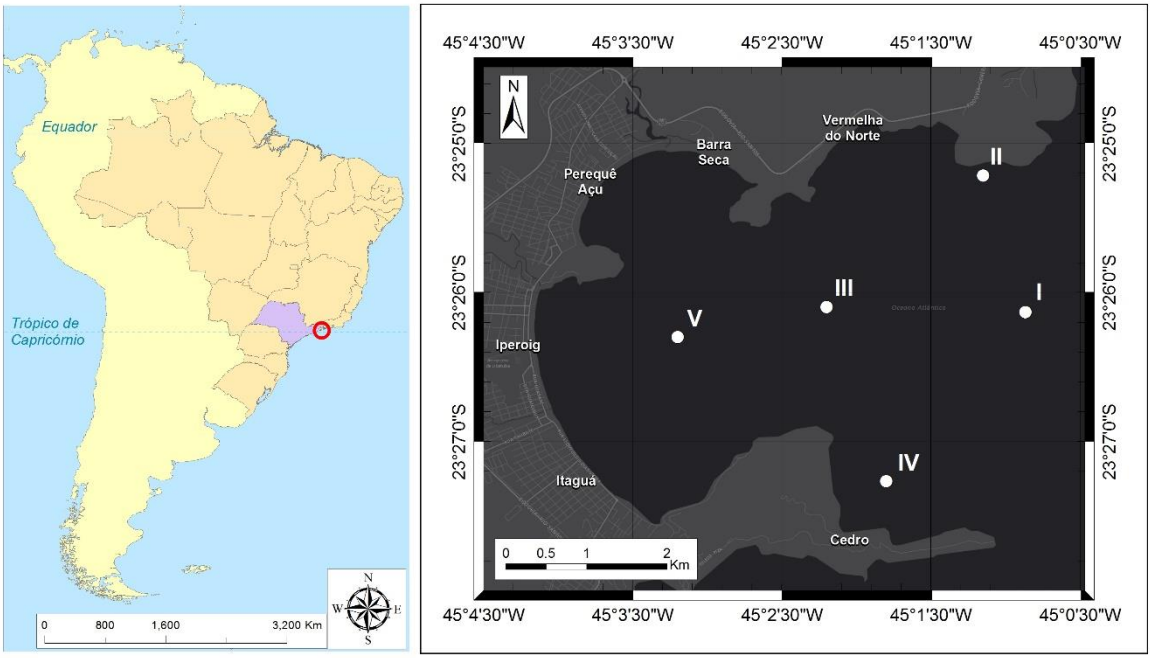


Figure 2. Map of the region of study with the location of the points sampled: I - 23° 26' 791" LS 45° 00' 843" LW; II - 23° 25' 502" LS 45° 01' 028" LW; III - 23° 26' 598" LS 45° 01' 584" LW; IV - 23° 27' 158" LS 45° 01' 764" LW; V - 23° 27' 180" LS 45° 01' 900"LW.



The hydrolyzes were initiated with sub-samples of 100 g that were thawed in the refrigerator at 4°C for 24 h. The sub-samples were homogenized in a blender, with distilled water, following by heating at 80°C for 20 minutes to inactivate the endogenous enzymes. Subsequently, the hydrolysis was performed, using enzyme Alcalase 2.4 L® (Novozymes, Bagsvaerd, Denmark) or enzyme Protamex® (Sigma Aldrich, MO, USA). The enzyme-substrate ratio was set at 2% (w/w) for both enzymes to determine the maximum degree of hydrolysis for each enzyme. The hydrolysis using the enzyme Protamex® was conducted at pH 7 and 50°C, whereas the hydrolysis using the enzyme Alcalase 2.4 L® was conducted at pH 8 and 50°C and the pH value adjustments were carried out with 0.10 mol/L NaOH. The degree of hydrolysis (DH) was monitored throughout the process and was defined according to the formula below:

$$GH\ (\%) = [(B \times N_b) / MP] \times (1 / \alpha) \times (1/h_{tot}) \times 100$$

In which h_{tot} is the number of peptide bonds (mole equiv / kg) for fish (8.6 mole/kg); *B* is the volume of the base consumed during the hydrolysis to maintain the pH constant (mL); *Nb* is the NaOH concentration in mol/L; MP is the mass of protein (grams, determined in N x factor of Kjeldahl); and α is the degree of dissociation. The degree of dissociation α is calculated according to equation:

$$\alpha = (10^{pH-pK}) / (1+ 10^{pH-pK})$$

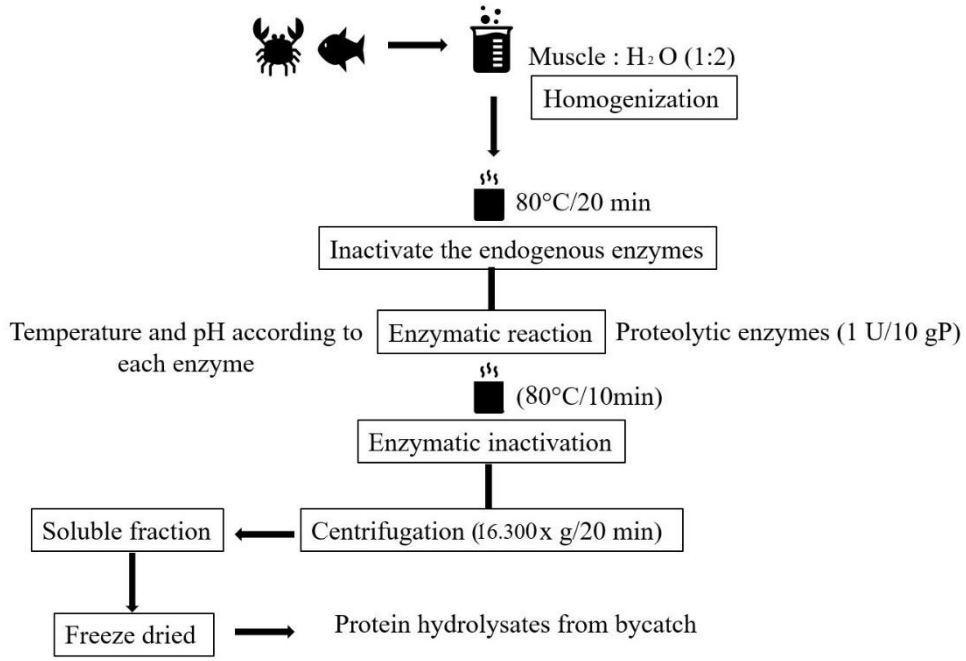
Where the *pH* is the values at which the hydrolysis process was conducted, and the *pK* is estimated by the equation below:

$$pK = 7.8 + [(298 – T) / (298 \text{ x } T)] \text{ x } 2400$$

according Beychok & Steinhart (1964, apud Kristinsson & Rasco, 2000). In which T is the temperature expressed in Kelvin at which the hydrolysis was performed.

The hydrolyzes had a duration of 4 to 5 h and the sub-samples were heating at 80°C for 20 minutes to stop the hydrolysis. Then, sub-samples were centrifuged at 16.300 x g for 20 min ate -4 ºC and supernatants were frozen at -80°C and subsequently freeze dried and stored at -20°C until analysis was performed (Figure 3).

Figure 3. General schedule of the enzymatic hydrolysis process of the muscle of the animals.



2.3 *In vitro determination of antioxidant activity*

Initially, the freeze-dried hydrolysates were weighed on a precision scale (Mettler Toledo MS-TS). Then, they were dissolved in a 0.1 mol/L citrate phosphate buffer (pH 7), supplemented with 0.3% (v/v) Triton X-100 to reach a concentration of 4 mg protein/mL. After dilution, the three protocols described below were performed.

2.3.1 *1.1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity*

The DPPH (1.1-diphenyl-2-picrylhydrazyl) method was described by Nicklisch & Waite (2014) and Zamora-Sillero et al. (2018b) and adapted in the present work. An aliquot of the hydrolysates (190 μL) of each sample was mixed with 10 μL of a prepared solution of DPPH at 2 mmol/L in methanol. The reduction of the DPPH was determined by measurements of absorbance at 490 nm wave-length on a spectrophotometer with a microplate reader (ELx808, Biotek Instruments Inc., Winooski, Vermont). A control was run in the same way using the citrate-phosphate buffer (pH 7.0) supplemented with 0.3% (v/v) Triton X and adding methanol instead of the DPPH solution. The DPPH radical scavenging activity was calculated according to the following equation:

$$\text{DPPH inhibition (\%)} = ((A_{Control} - A_{Sample}) / A_{Control}) \times 100$$

In which, A*control* is the absorbance of control and A*sample* is the difference between the absorbance of the sample and the absorbance of the sample with methanol instead of DPPH solution.

2.3.2 *Antioxidant capacity against peroxyl radicals (ACAP)*

The antioxidant capacity against peroxyl radicals (ACAP) method was performed according to Amado et al. (2009) and Zamora-Sillero et al. (2018b) with some modifications. The samples were placed in triplicate on a 96 well plate. The substrate (H2DCF-DA) was added to the pools for with and without 2.2′-azobis (2 methylpropionamidine) dihydrochloride (ABAP) readings. The reading was performed on a microplate reader (Vitor 2, Perkin Elmer, USA) at 5 min intervals for 60 min at 37°C. ACAP was determined by differences in fluorescence values obtained with and without ABAP areas. The results were expressed considering the inverse of the relative area between the area with ABAP and the difference between the with and without ABAP. The smaller is the relative area calculated, the greater is the antioxidant capacity of the sample and vice versa. The relative area was calculated as follows in the equation:

$$(ROS_{areaABAP} - ROS_{areabackground}) / ROS_{areabackground}$$

2.3.3 Determination of sulfydhryl groups

For determination of the sulfhydryl groups, 100 μL of hydrolysates were placed in triplicate in a 96 well plate. Subsequently, 200 μL of 0.4 mol/L Tris-Base buffer (pH 8.9) and 10 μL of the DTNB reagent (5.5-dithiobis-2-nitrobenzoic acid) were added. Subsequently, the samples were incubated for 15 min at room temperature. After the reaction, the absorbance of the mixture was measured at 405 nm wave-length in a spectrophotometer (ELx808, Biotek Instruments Inc., Winooski, Vermont). The concentration of sulfhydryl groups was determined by the following reaction:

$$[\text{Groups SH}] = 3*(ABS_{SH} - ABS_{Br})*10^{6}*0.10/1000/13.100/4$$

In which, ABS_{SH} is the absorbance of sample and ABS_{Br} is the absorbance of the solution without sample.

2.4 *Statistical analysis*

All data were expressed as the mean ± standard error (SE). After removal of the discrepant values, the residues met the assumptions of homoscedasticity and normality, as verified by the boxcox and Cramer-von Mises tests, respectively. The analysis of variance (ANOVA) and the Tukey test at the mean significance level ($p < 0.05$) were performed using the agricolae package (Mendiburu, 2017) in software R (Core Team, 2017).

3. Results and Discussion

3.1 *Animals*

The amount of individuals of each species and the minimum and maximum weight of the animals captured are shown in Table 1. In relation to crustaceans, it can be observed that *H. pudibundus* had the highest number of individuals captured. In the three field trips small and sexual immature crustaceans were captured. According to Graça-Lopes et al. (2002a), *C. ornatus* and *H. pudibundus* are regularly occurring species representing 80% of the crustaceans that are caught in the trawl fishery of Seabob shrimp in the shallow waters of the coast of São Paulo. Thus, the populations of these species are likely to be unbalanced due to accidental catch (Graça-Lopes et al., 2002a, b; Severino-Rodrigues et al., 2002, 2007; Fonseca et al., 2005).

Table 1. Amount of individuals collected in the three collects.

Species	Number of individuals	Weight (g) min/max
Callinectes ornatus	449	5/76
Hepatus pudibundus	667	7/86
Micropogonias furnieri	370	22/210
Paralonchurus brasiliensis	601	4/160

The fish *P. brasiliensis* had higher nu1mber of individuals captured than *M. furnieri*. The species of the Sciaenidae family *P. brasiliensis*, *Stellifer rastrifer*, *Selene setapinnis* represent 80% of the total catch in the composition of ichthyofauna of the bycatch from artisanal shrimp trawls (Catanni et al., 2011). In the present study, the fishes captured were mostly young specimens, which have not yet reached the first reproduction size. This also was observed in other studies with fishes from bycatch (Graça-Lopes et al. 2002a; Amorim et al., 2016).

The minimum and maximum weight of the fishes rejected in the ichthyofauna bycatch in São Paulo also was reported by Graça-Lopes et al. (2002a) and was similar to what we found of *M. furnieri* in this study, however, was higher than observed in *P. brasiliensis*. Despite the economic importance, *M. furnieri* is only marketed with the minimum weight of the 900 g. In the bycatch, the individuals are generally smaller and, thus, are discarded (Amorim et al., 2016). Thus, the accidental captured of fishes and crustaceans in the shrimp trawls can contribute to an imbalance in the community due to the fishery of the immature individuals (Jennings et al., 2001).

3.2 *Determination of bromatological parameters*

Before the hydrolysis process, it is important to characterize the raw material since large lipid contents can affect the taste of the hydrolysates (Amorim et al., 2016). Moreover, the knowledge of animal nutrient content is important to evaluate the quality of these foods (Ogawa & Maia, 1999; Amorim et al., 2016). Thus, the proximal composition of the samples of each species was determined (Table 2). The mean moisture content observed in the samples of the four species ranged from 76.0% to 79.7%. *Callinectes ornatus*, *M. furnieri* and *P. brasiliensis* showed very close means of crude protein (17.2%, 16.7% and 16.9%, respectively) and no statistical differences were observed between these species.

Table 2. Proximate chemical composition muscles and skin of the fish and muscles with exoskeleton of the crabs from the bycatch.

Species	M (%)	P (%)	L (%)	A (%)
Callinectes ornatus	76.0 ± 0.5^{b}	17.2 ± 0.7^{a}	0.5 ± 0.0^{ab}	5.0 ± 1.2^{a}
Hepatus pudibundus	79.4 ± 1.5^{a}	9.9 ± 1.0^{b}	0.2 ± 0.0^{c}	7.1 ± 1.7^{a}
Micropogonias furnieri	78.8 ± 0.0^{a}	16.7 ± 0.1^{a}	0.5 ± 0.0^{a}	1.8 ± 0.5^{b}
Paralonchurus brasiliensis	79.7 ± 0.7^{a}	16.9 ± 0.5^{a}	0.3 ± 0.2^{bc}	2.0 ± 0.7^{b}

[1] Values are expressed as the average and standard error. Values in columns marked with the same letters do not differ significantly ($p < 0.05$). M = moisture; P = protein content; L= lipid content; A= ash content.

The mean variation range of the ethereal extract was 0.2% to 0.5%. The average of the ash content ranged from 1.8% to 7.1%, being the lowest and the highest values found in *M. furnieri* and *H. pudibundus*, respectively. The crustaceans showed the highest averages of ash content and the fishes presented the smallest. No significant difference (*p*

<0.05) in the values of ash content was observed between both crustaceans and both fishes species.

In general, the centesimal composition of the crustaceans was similar to those found in the literature (Skonberg & Perkins, 2002; Chiou & Huang, 2003; Gökoðlu & Yerlikaya, 2003; Nazck et al., 2004). The moisture of the crab meat can range from 73% to 83% and similar values were found in the present study (Gökoðlu & Yerlikaya, 2003; Nazck et al., 2004). The results of the lipids were also similar to those observed in the other researches, in which they ranged from 0.2% to 1.8% (Skonberg & Perkins, 2002; Chiou & Huang, 2003). Besides that, the results of proteins obtained in *C. ornatus* were similar to what was reported to species of the Portunidae family: *Callinectes sapidus* and *Portunus pelagicus* (Gökoðlu & Yerlikaya, 2003).

The crab *H. pudibundus* presented lower mean of the protein content than *C. ornatus*. This variations between the crustaceans can occur according to the distribution and origin of organisms, eating habits, time of year and age of the animals (Canli & Atli, 2003). In addition, the quality of the water also is a factor that exerts influence in the variations of the centesimal composition (Gökoðlu & Yerlikaya, 2003). Thus, it is suggested that the differences observed between the crustaceans species, in the present study, can be due to the age of the animals.

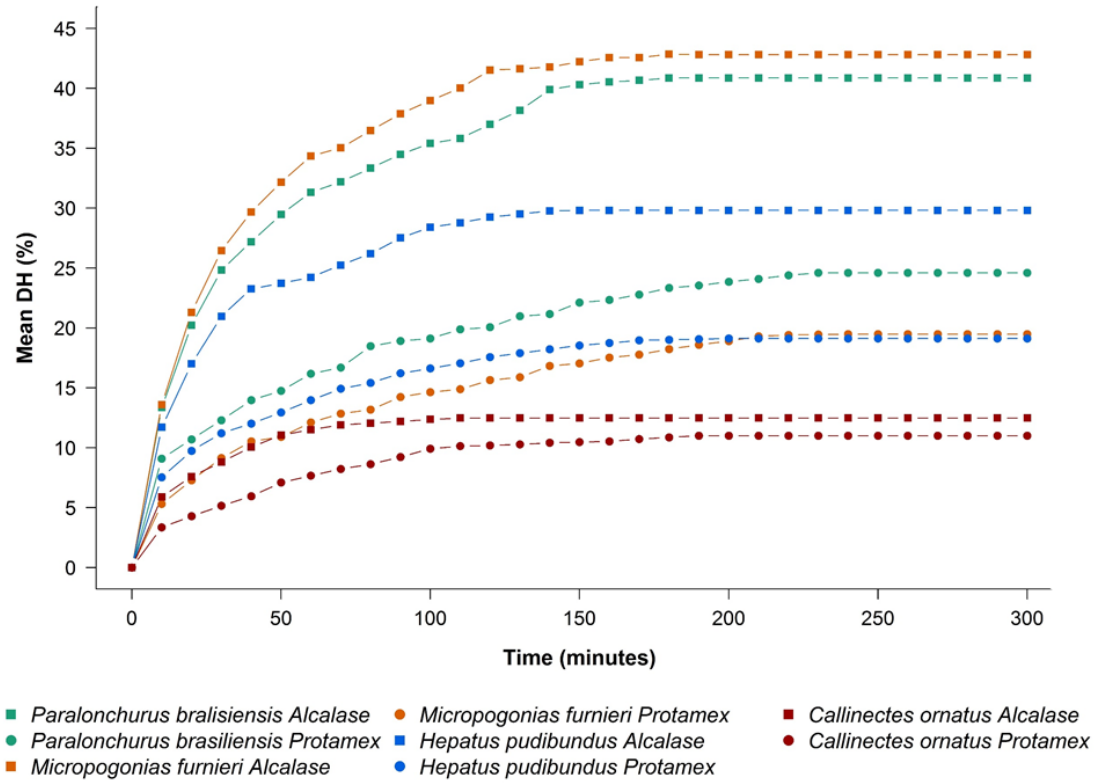
The body centesimal composition was similar between the two species of fish. According to literature, the moisture for different species of fish ranges from 60% to 90%, the lipids and ash from 0.9% to 7.7% and 1% to 2%, respectively (Silva et al., 1994; Ogawa & Maia, 1999; Vila Nova et al., 2005; Furlong et al., 2006; Martins et al., 2009; Silva et al., 2014; Amorim et al., 2016). The results of the centesimal composition of *M. furnieri* was similar to what was found in previous studies for the same species (Bonacina & Queiroz et al., 2007; Centenaro et al., 2008; Amorim et al., 2016). The protein content

of both species indicates an intermediate concentration between 15% and 20%, which allows to classify species in the class of lean fish, which includes species that present at least 16.4% of protein and 0.5% of lipids (Jacquot, 1961).

3.3 *Enzymatic hydrolysis*

In the present study, the hydrolysis was characterized by a high rate of hydrolysis during the first 100 min in all samples (Figure 4). Thereafter, a slower rate of hydrolysis was found after 150 minutes until a steady-state phase (Figure 4).

Figure 4. Enzymatic hydrolysis in the samples of the species and the maximum degree hydrolysis (DH) for each enzyme.



The degree of hydrolysis (DH) curves obtained for each species are typical of the enzymatic hydrolysis because the cleavage of the peptide bonds is rapid in the initial

stage, following a stationary phase because of the cleavage of less susceptible bonds (Chalamaiah et al., 2012; Zamora-Sillero et al., 2018a, b; Abuine et al., 2019). The DH curves obtained in the present study was similar to those observed in the hydrolysis of different protein sources, as reported by some authors (Guérard et al., 2001; Chalamaiah et al., 2012; Silva et al., 2014; Egerton et al., 2018; Zamora-Sillero et al., 2018b).

On average, the enzyme Alcalase 2.4 L® conditioned higher DH values than the enzyme Protamex® (Table 3). The DH for *C. ornatus* did not show significant difference when Alcalase 2.4 L® (12.4 % ± 0.5) or Protamex® (11.0 % ± 1.0) were used. Whereas, the DH of *H. pudibundus* was superior to *C. ornatus* using both enzymes and was higher when using the enzyme Alcalase 2.4 L® (32.0 % ± 1.8).

Table 3. Descriptive analysis of the DH at 300 minutes of the samples under the action of the enzymes Alcalase 2.4 L® and Protamex®.

Species	Enzyme	
	Protamex®	Alcalase 2.4 L®
	Average (EP)	Average (EP)
Callinectes ornatus	11.0 (1.0)^{d}	12.4 (0.5)^{d}
Hepatus pudibundus	19.1 (1.4)^{c}	32.0 (1.9)^{b}
Micropogonias furnieri	19.5 (0.7)^{c}	42.8 (1.3)^{a}
Paralonchurus brasiliensis	24.6 (1.3)^{c}	40.8 (2.4)^{a}

The fishes showed higher DH than the crustaceans. *Paralonchurus brasiliensis* and *M. furnieri* treated with the enzyme Alcalase 2.4 L® had a higher degree of hydrolysis (40.9 % ± 2.4 and 42.8 % ± 1.3, respectively) than the muscle treated with Protamex®

(24.6 % ± 0.6 and 19.5 % ± 0.7, respectively). No statistical differences were observed between the fish species when hydrolyzed with the enzyme Alcalase 2.4 L® or Protamex®.

The DH is a parameter to characterize the protein hydrolysates and is defined as the percentage of peptide bonds cleaved during the hydrolysis (Kristinsson & Rasco, 2000; Halim et al., 2016; Egerton et al., 2018). In this way, higher DH implies that more peptides were produced in the hydrolysis, that which will result in an increase in the possibility to recover the protein to be used as a food additive (Zamora-Sillero et al., 2018a). Vioque et al. (2000) classified products depending on the degree of hydrolysis as partially hydrolyzed (DH < 10%) and highly hydrolyzed (DH > 10%). Thus, the products obtained in this study are considered highly hydrolyzed and can be used as nutritional supplements and as nutraceuticals.

The differences between the activity of the enzymes Alcalase 2.4 L® and Protamex® found in this study could be because Alcalase 2.4 L® is an endopeptidase that hydrolyzes the peptide bonds of the protein molecules at random to produce relatively large peptides (Clemente, 2000). Others studies reported the difference between enzymes activities. Martins et al. (2009) showed that the DH in the muscles of *M. furnieri* was higher using Alcalase 2.4 L® (37%) than enzyme Flavourzyme (18%). Da Rocha et al. (2018) evaluated the effect of protein hydrolysate properties and the shelf-life improvement of flounder fillets and showed that the Alcalase 2.4 L® hydrolysate was more effective than the Protamex® hydrolysate. Blue whiting fish were hydrolysates with commercial enzymes and was observed that Protamex® conditioned lower final DH than Alcalase 2.4 L® (Egerton et al., 2018). In common carp byproducts, the enzyme Alcalase 2.4 L® showed higher substrate affinity leading to higher DH than Protamex® enzyme (Zamora-Sillero et al., 2018b).

The enzymatic hydrolysis is the best process to hydrolyze fish products, such as skin, muscle and viscera without losing nutritional value (Abuine et al., 2019) leaving residual organic solvents or toxic chemicals in the hydrolysates (Chalamaiah et al., 2012). This method is preferred in the food industries (Zamora-Sillero et al., 2018a, b; Abuine et al., 2019). The hydrolysis process depends on the temperature, pH, time, enzyme-substrate ratio and protein substrate concentration (Adler-Nissen 1984; Chalamaiah et al., 2012; Zamora-Sillero et al., 2018a; Abuine et al., 2019). Furthermore, the specificity of the enzyme affects the composition of free amino acid, the size, the amount, and peptides which can influence the bioactivity of the obtained hydrolysate (Zamora-Sillero et al., 2018a).

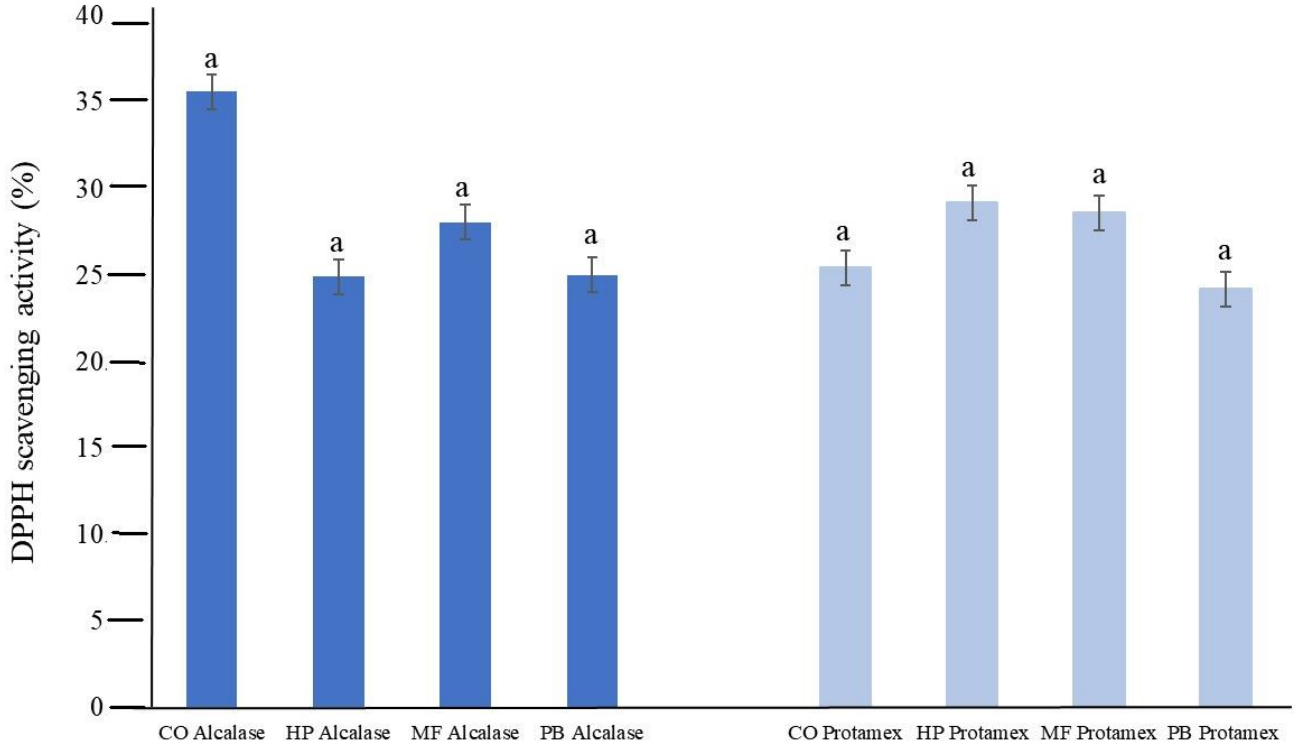
The hydrolysis of the four species suggests that the high DH observed with enzyme Alcalase 2.4 L® can be due to the affinity of the enzyme to the samples of the muscles/skin and muscles/exoskeleton. It can be inferred that Alcalase 2.4 L® and Protamex® have different proteolytic affinities for the samples and, therefore, Alcalase 2.4 L® is more efficient than Protamex®. Thus, the use of the Alcalase 2.4 L® enzyme in the hydrolysis process is recommended for these four species of the bycatch.

3.4 *In vitro antioxidant activity of hydrolysates*

The three methods used in the present study to investigated the antioxidant activity in the protein hydrolysates indicated some antioxidant activity. The ability of the hydrolysates to reduce the free radical DPPH varied between 24.2% to 35.6% (Figure 4). These results indicate that the hydrolysates of the studied species have the ability to eliminate the DPPH radical. Thus, the hydrolysates may have in their composition peptides that act as electrons donors that react with free radicals to turn them into stable molecules (Zamora-Sillero et al., 2018b). However, there were no significant differences

($p > 0.05$) between the four species and between the use of Alcalase 2.4 L® or Protamex® enzymes (Figure 5). Some studies with hydrolysates of proteins obtained from marine animals have successfully detected DPPH elimination activity using different enzymes (Klompong et al., 2007; You et al., 2010; Galla et al., 2012; García-Moreno et al., 2014; Zamora-Sillero et al., 2018b). Lee et al. (2011), investigated the antioxidant activity using DPPH in the hydrolysates of the sand eel *Hypoptychus dybowskii*, and observed that the antioxidant activity was 77.4%. Protein hydrolysates of the *Rastrelliger kanagurta* (Indian mackerel) by pepsin are potent antioxidants, capable of scavenging 46% of DPPH (Sheriff et al., 2014). Vázquez et al. (2017) investigated the antioxidant activity of the *Scyliorhinus caniculum* hydrolysates obtaining 21.4% antioxidant activity against DPPH radicals with Alcalase 2.4 L® enzyme.

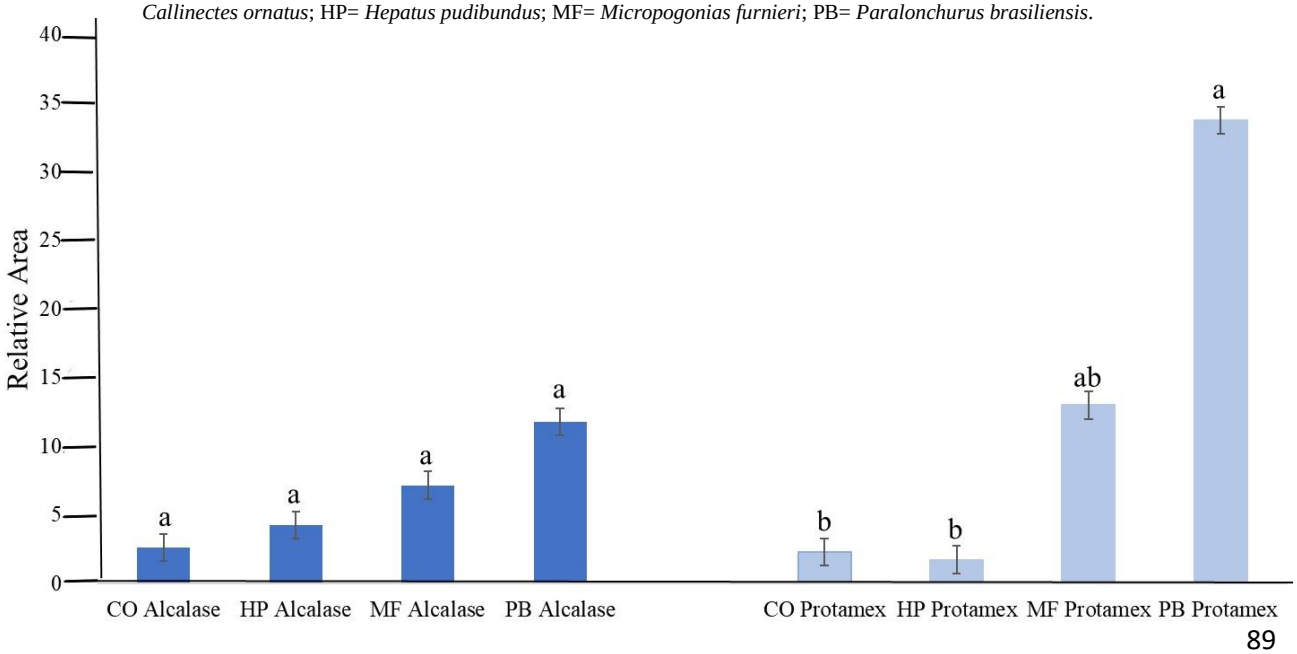
Figure 5. DPPH radical scavenging activity of protein hydrolysate by animals from bycatch using the enzymes Alcalase 2.4 L® and Protamex®. Data are expressed as mean (n=6). Identical letters indicate absence of statistical differences ($p > 0.05$). CO= *Callinectes ornatus*; HP= *Hepatus pudibundus*; MF= *Micropogonias furnieri*; PB= *Paralonchurus brasiliensis*.



Recently, Zamora-Sillero et al. (2018b), showed that the common carp byproducts hydrolyzed with the enzyme Alcalase 2.4 L® exhibited higher antioxidant activity against the DPPH radical (51.1%). The studies cited above shows that the DPPH method is very used and is efficient for the determination *in vitro* of antioxidant activity in protein hydrolysates (Zamora-Sillero et al., 2018b).

The capacity of the hydrolysates to reduce the peroxyl was described by Amado et al. (2009) and is based on the detection of ROS by fluorometry employing 2′,7′-dichlorofluorescin diacetate (H2DCF-DA) as substrate. The peroxyl radicals are generated by thermal decomposition of 2,2′-azobis dihydrochloride (ABAP) and the tissue total absorbance capacity of peroxyradical is monitored by the fluorescence signal emitted by the reaction between ROS and H2DCF-DA (Amado et al., 2009). Thus, this method measures the resistance of the sample against various forms of oxiradicals and the high relative area means low antioxidant competence and vice versa (Amado et al., 2009; Zamora-Sillero et al., 2018b). In the present study, hydrolysates of the four species have the capacity of scavenge peroxyl radicals (Figure 6).

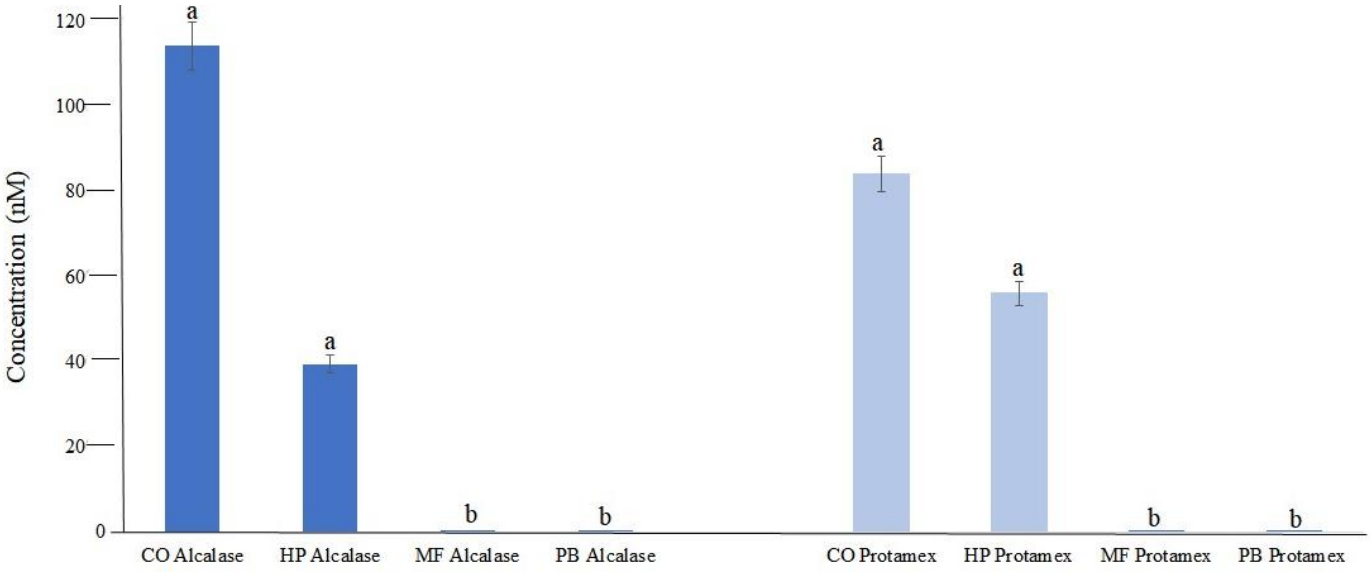
Figure 6. Total antioxidant capacity against peroxyl radicals of protein hydrolysate from bycatch animals using the enzymes Alcalase 2.4 L® and Protamex®. Data are expressed as mean (n=6). Identical letters indicate absence of statistical differences ($p > 0.05$). CO= *Callinectes ornatus*; HP= *Hepatus pudibundus*; MF= *Micropogonias furnieri*; PB= *Paralonchurus brasiliensis*.



The hydrolysates with Alcalase 2.4 L® enzyme had no significant differences ($p > 0.05$) between the species. However, with Protamex® enzyme, the hydrolysates of the fishes presented lower antioxidant capacity than crustaceans. Hydrolyzed products of shrimp shell discarded in processing plants showed similar results using Alcalase 2.4 L® enzyme (Guerard et al., 2007; Sila et al., 2014). These hydrolysates exhibited significant hydroxyl radical scavenging activity with values ranging from 2.5 to 3.9 mol of carnosine/mg protein (Ambigaipalan and Shahidi, 2017). Zamora-Sillero et al. (2018b) also detected higher total antioxidant capacity against peroxyl radicals in common carp byproducts hydrolyzed with the Alcalase 2.4 L® and Protamex® enzymes. According to this authors, peroxyl radicals are highly reactive and are involved in lipid peroxidation, causing damage to the organism. In this way, the antioxidants present in the hydrolysates obtained from the bycatch species reduce the peroxyl radical to hydro-peroxide during lipid peroxidation (Alashi et al., 2014; Zamora-Sillero et al., 2018b).

According to Flora (2009), the sulfhydryl group may act as a radical scavenger as an electron donor. In the present study, the hydrolyzed of the two species of crustaceans obtained with Alcalase 2.4 L® and Protamex® showed higher concentrations of sulfhydryl groups than the two species of fish (Figure 7). No significant differences ($p > 0.05$) between the crustaceans species and between the enzymes were observed. The hydrolysates from fish showed a sulfhydryl concentration close to zero (Figure 7). The decrease in sulfhydryl groups in the hydrolysates from fish can result in the formation of disulfide bonds through the oxidation of these sulfhydryl groups (Flora, 2009; Zamora-Sillero et al., 2018b). The results of the dosage of sulfhydryl groups were different to that found in common carp residues hydrolyzed with the enzyme Alcalase 2.4 L® and Protamex®, in which a higher concentration of sulfhydryl groups was observed in the hydrolysate with the enzyme Alcalase 2.4 L® (Zamora-Sillero et al., 2018b).

Figure 7. Total concentration of sulfhydryl (P-SH) groups present in protein hydrolysates from bycatch, using the enzymes Alcalase 2.4 L® and Protamex®. Data are expressed as mean (n=6). Identical letters indicate absence of statistical differences ($p > 0.05$). CO= *Callinectes ornatus*; HP= *Hepatus pudibundus*; MF= *Micropogonias furnieri*; PB= *Paralonchurus brasiliensis*.



The production of reactive oxygen species (ROS) is an integral part of the metabolism and observed in several physiological conditions (Aklakur, 2016). ROS have an important biological function, but when its production is exacerbated, the organism has an efficient antioxidant system that can control and restore the balance (Kristinsson & Rasco, 2000; Amado et al., 2009; Aklakur, 2016). In this way, antioxidants can be capable of retarding or inhibiting the oxidation of oxidizable substrates, and the consumption of natural antioxidants can inhibit the formation of free radicals decreasing the oxidative stress (Kristinsson & Rasco, 2000; Byun et al., 2009; Bernardini et al., 2011; He et al., 2013; Aklakur, 2016; Zamora-Sillero et al., 2018a, b; Nikoo et al., 2019). Additionality, antioxidant peptides obtained by enzymatic hydrolysis of proteins derived from marine organisms have been reported in the literature (Je et al., 2005; You et al., 2009; You et al., 2010; Lee et al., 2011; He et al., 2013; Zavareze et al., 2014; Aklakur et al., 2016; Vázquez et al., 2017; Zamora-Sillero et al., 2018b; Nikoo et al., 2019). In this study, although the enzyme Alcalase 2.4 L® presented the highest DH it did not influence

the results of antioxidant activity, because there were no significant differences between the enzymes.

4. Conclusion

The enzymatic hydrolysis of the four species more abundant in the reject of shrimp trawling fishing showed to be an efficient process to obtain hydrolyzed proteins with biological activity. This technique can add value to these discarded animals. Besides, the results indicated that the protein hydrolysates of the bycatch species have antioxidant properties. Therefore, the enzymatic hydrolysis of the bycatch animals is an effective technique that allows the release of peptides with antioxidant activity with potential to use in the food industry. The development of technology to scale up protein hydrolysis process could allows fishers to reduce the trawling area, maintaining their income, and thus, minimizing the negative impact of the shrimp fisheries on the environment.

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Capítulo 3

Microencapsulation of protein hydrolysate from shrimp bycatch: preservation of the antioxidant activity

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Abstract

The purpose of this study was to evaluate the effect of the complexation on the antioxidant activity in the protein hydrolyzed of the most abundant species of crustaceans from bycatch in Brazil. Additionally, was evaluate the effect of the complexation on the antioxidant activity before and after the microencapsulation. An increase in the ratio of Pea protein/hydrolysates complexes resulted in increased turbidity in all samples. Particle size measurements indicated that the complexes tended to form large aggregates. The DPPH radical scavenging activity of the complexes was higher than the protein hydrolysates alone, moreover, increasing levels of Pea proteins did not affect the antioxidant activity of protein hydrolysates of the two species. The complexes of the *Callinectes ornatus* was chosen for the spray-drying microencapsulation process. The results revealed that spray-drying did not have a significant effect ($p > 0.05$) on the protein hydrolysates antioxidant activity when they were complexed with Pea protein. In this way, this work suggests that the complexation with Pea proteins and subsequent microencapsulation is an efficient way to protect the biological activity of protein hydrolysates. Thus, this study provides evidence for the potential use of bycatch from shrimp fisheries as functional ingredient or nutraceuticals.

Keywords: Complex coacervation, Pea protein, DPPH, Crab, Spray-drying, Enzyme.

1. Introduction

The animals that are caught accidentally with the shrimp trawl fisheries in Brazil have no commercial value but contains a large amount of protein, amino acids, oils, and others rich materials which that can be processed into market-value products. In this context, the enzymatic hydrolysis is a technique that can aggregate value to material that is discarded in the fisheries industry (Zamora-Sillero et al., 2018). Previous studies demonstrated that protein hydrolysates derived from marine animals by-products have bioactive compounds, essential nutrients and are potential sources of antioxidant peptides (Chalamaiah et al., 2012; Zamora-Sillero et al., 2018). Recently Özogul et al. (2019), reported that several compounds when isolated from shrimp, crab, lobster, prawn, and krill by-products have functional properties, such as anti-microbial and antioxidant effects that could be used by food industries. Thus, protein hydrolysates with biological activity can aggregate value to bycatch.

The use of protein hydrolysates and/or peptides with biological activity is of great interest in the food industry because proteins are essential functional ingredients in food products (Zamora-Sillero et al., 2018). In the enzymatic hydrolyzes, the enzymes transform proteins into smaller peptides that can add value to sub-products that usually are discarded (Chalamaiah et al., 2012; Zamora-Sillero et al., 2018). Another application to the proteins hydrolysates is the formation of the complex coacervation (Eghbal et al., 2017). Proteins together with others polymers have been widely used in the food industry to enhance structure and stability of processed foods (Amara et al., 2017; Eghbal et al., 2017; Wang et al., 2019). In this context, the formation of the complex coacervation occurs between biopolymers carrying opposite charges and these electrostatic interactions can be influenced by different factors such as concentration, protein to polysaccharide ratio, ionic strength, charge distribution and pH (Amara et al., 2017). Moreover, complex

coacervation can be used for encapsulation, the formation of packaging films and production of food emulsions or gels (Eghbala & Choudhary, 2018).

Several studies described the formation of the complex coacervation and its electrostatic interactions and the use of the Pea (*Pisum sativum*) protein has received special attention for its properties of emulsifier (Gharsallaoui et al., 2012; Chen et al., 2019). Pea is an important legumin plant rich in starch and protein that has been studied in the last decade as an alternative for soy protein (Chen et al., 2019). Pea protein has good nutritional properties, low allergenicity and has been used as an emulsifier in liquid emulsions (Gharsallaoui et al., 2012; Chen et al., 2019). Reinkensmeier et al. (2015), indicate that Pea seeds can be used to prepare protein-rich intermediates for food production. Gharsallaoui et al. (2012) reported that Pea protein can be used to prepare spray-dried emulsions for the microencapsulation of oil.

In this context, the microencapsulation using Pea protein can be a solution to protect bioactive compounds (Gharsallaoui et al., 2012). The microencapsulation is a reliable technique to improve the nutrient retention in the food, preserving the stability of bioactive compounds during processing and storage, and delays the processes of degradation of functionality of the bioactive component (Gharsallaoui et al., 2012). Thus, in the present study the effect of complexation was investigated, using Pea proteins on the antioxidant properties of protein hydrolysates obtained from most abundant crustaceans from bycatch in Brazil. Moreover, the effect of the antioxidant activity before and after microencapsulation by spray-drying was evaluated to add value to the bycatch.

2. Materials and Methods

2.1 *Raw material*

The crabs *Callinectes ornatus* and *Hepatus pudibundus* are the crustaceans species the most frequently and abundant in the bycatch of shrimp fishery in Brazil. The two species were collected in February 2017, September 2017 and February 2018 in the region of Ubatuba, São Paulo, Brazil (23° 26' 791" LS 45° 00' 843" LW) by double-rig a commercial shrimp trawling. The muscles and the exoskeleton of the animals were blended and packaged in sealed 100 g plastic bags and transported in cool boxes with dry ice to the Aquaculture Center (CAUNESP, Brazil), where they were kept in the freezer at -20°C until analysis.

2.2 *Preparation of protein hydrolysates*

The hydrolyzes were initiated with samples of 100 g of muscles with exoskeleton that were thawed in the refrigerator at 4°C for 24 h. The samples were homogenized in a blender, with two volumes of distilled water (w/v), following by heating at 80°C for 20 minutes in order to inactivate the endogenous enzymes. Subsequently, the hydrolysis was started using enzyme Alcalase 2.4 L® (Novozymes, Bagsvaerd, Denmark) and enzyme Protamex® (Sigma Aldrich, MO, USA). The hydrolysis was conducted at pH 7 and 50°C to enzyme Protamex® and pH 8 and 50°C to enzyme Alcalase 2.4 L®. The enzyme-substrate ratio was set at 2% (w/w) for both enzymes to determine the maximum degree of hydrolysis for each enzyme. The degree of hydrolysis was monitored throughout the process and was defined according to Adler-Nissen (1984), where the amount of NaOH added to keep the pH constant during the hydrolysis was calculated to found the degree of hydrolysis. The hydrolyzes had a duration of four to five hours. The samples were then heated at 80°C for 20 minutes to stop the hydrolysis. Samples were centrifuged at 16.300 x g for 20 minutes. Supernatants were frozen at -80°C and subsequently freeze-dried and stored at -20°C until analysis was performed.

2.3 *Characterization of protein hydrolysate and Preparation of Pea protein/protein hydrolysates complexes*

The protein hydrolysates of the two species were evaluated to find the best conditions for the formation of complexes with Pea protein. After that, the biopolymer mixtures containing protein hydrolysates of *C. ornatus* and *H. pudibundus* (1 g/L) and Pea protein (1 g/L) were prepared by mixing different ratios (0-10) of the stock solutions with imidazole-acetate buffer at pH 3.5. The resulting suspensions were mixed for 10 min using a vortex mixer and their pH was adjusted again to 3.5.

2.4 *Zeta potential measurement*

The zeta potential (ζ-potential) of protein hydrolysates from two species of crustaceans and protein hydrolysates/Pea protein complexes were determined using a Zetasizer NanoZS90 (Malvern Instruments, Malvern, UK). The samples were diluted by 0.5% (v/v) with imidazole-acetate buffer adjusted to the suitable pH value. The mean ζ-potential (ZP) values (±SD (standard deviation)) were obtained from the instrument.

2.5 Turbidity measurement

Turbidity of protein hydrolysates/Pea protein complexes formed at various Pea protein concentrations were performed with a spectrophotometer (Jenway 7305, Villepinte, France). The absorbance was measured at 600 nm at room temperature (25 °C) against pure protein hydrolysate solutions (without Pea protein) in the imidazole-acetate buffer (5 mmol/L; pH 3.5).

2.6 *Particle size measurement*

Particle size distributions of protein hydrolysates/Pea protein complexes formed

at various Pea protein concentrations were measured by a laser diffraction instrument (Mastersizer 3000, Malvern Instruments, Malvern, UK). The complexes were stirred continuously throughout the measurement to ensure that the samples were homogeneous. The volume particle diameter (D43) was calculated from the three injections of three separate samples with three readings per sample.

2.7 *Antioxidant properties measurement*

2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity was measured on protein hydrolysates (5 g/L) dissolved in imidazole-acetate buffer (5 mmol/L, pH 3.5) and in the complexes of hydrolysates (1 g protein hydrolysates/L) / Pea protein (1 g/L). In order to determine the DPPH radical scavenging activity, the method reported by Bersuder et al. (2001) was used. Briefly, an aliquot (500 μL) of each sample was mixed with 375 μL of 99% (v/v) ethanol and 125 μL of a daily-prepared solution of DPPH (0.02% (w/v) in ethanol at 99%). The mixtures were shaken and then incubated for 60 min in dark at room temperature. The reduction of DPPH radical was determined spectrophotometrically by monitoring the decrease in absorbance at 517 nm in a spectrophotometer (Jenway 7305, Villepinte, France). DPPH radical-scavenging activity was calculated as follows in the equation:

$$\text{DPPH radical-scavenging activity (\%)} = (A_{blank} - A_{sample}) / A_{blank} \times 100$$

where A_{blank} is the absorbance of the reaction containing all reagents (except that distilled water was used instead of the sample), and A_{sample} is the absorbance in the presence of the sample. The experiment was carried out in triplicate and the results were mean values.

2.8 *Spray-drying*

The complexes formed with the samples *C. ornatus* and Pea protein prepared in the imidazole-acetate buffer (5 mmol/L; pH 2.4) containing 20 wt.% maltodextrin DE 19, as well as solutions of the same hydrolysates without Pea protein, were dried in a laboratory scale spray-drying equipped with a 0.5 mm nozzle atomizer (Mini spray-dryer B-290, BUCHI, Switzerland). Suspensions were pumped to the spray-drying at a feed rate of 0.5 L/h at room temperature and dried at an inlet temperature of 180°C and an outlet temperature of 90°C. The dried powders were collected and stored in airtight containers at 4°C. Dry powders were then weighted and mixed with imidazole/acetate buffer (5 mM, pH 2.4) to obtain reconstituted suspensions of complexes and hydrolysates with the same dry matter as before drying. After 1 h of rotation at approximately 200 rpm, samples were analyzed.

2.9 *Statistical analysis*

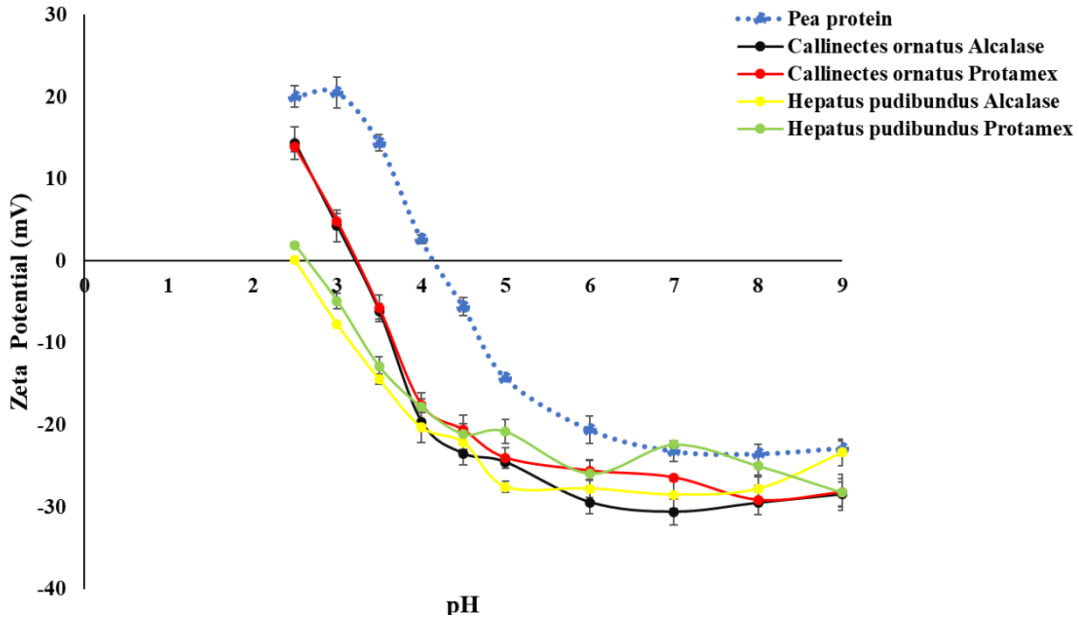
All experiments were performed using at least three freshly prepared samples. The results are the means and standard deviations that were calculated from these replicate measurements. The differences in the antioxidant activity between the samples were evaluated using a one-way analysis of variance (ANOVA), Post-hoc tests were employed using Newman-Keuls method. The significance level adopted was 5% ($\alpha = 0.05$). The ANOVA assumptions (normality by Shapiro-Wilks and variance homogeneity by Levene) were previously evaluated and tested with the same significance level (5%).

3. Results & Discussion

3.1 *Protein hydrolysates and Pea protein interactions*

ζ-potential measurements were conducted on crustacean protein hydrolysates and Pea protein, separately, to evaluate the effect of pH on their electrostatic charge. The ζ-potential was measured together pH (Figure 1). At pH 9.0 protein hydrolysates exhibit negative ζ potential, but the magnitude of the charge increased significantly below pH 5.0. The ζ-potential of protein hydrolysates increased as pH decreased (Figure 1).

Figure 1. Dependence of the ζ-potential of *Callinectes ornatus* and *Hepatus pudibundus* hydrolysates produced with Alcalase 2.4 L® or Protamex® and Pea protein on pH (5 mM imidazole-acetate buffer).



This result was observed for the two crab species. No differences in ζ potential was observed when using Alcalase 2.4 L® or Protamex® enzymes in the crustacean protein hydrolysates. Additionally, the both species presented isoelectric points ranging from 2.5 to 3.5. At pH 2.5, all protein hydrolysates presented positive ζ potential.

Despite the similar results among species, it can be observed that between the pH 3.5-2.5 the ζ potential values are higher in *C. ornatus*. This results can be due to differences in amino acid composition among the species studied. *Hepatus pudibundus*

has probably more amino acids such as glutamic acid and aspartic acid in its constitution as was found in proteins hydrolysates from krill *Euphausia superba* and in the crab *Ucides cordatus* (Zhang et al., 2002). Composition of the amino acid of the proteins hydrolysates should be determined to better understand the differences between these two species. In general, the results of the ζ potential are similar to proteins hydrolysates obtained from other marine animals. Morales-Medina et al. (2016) observed that the ζ potential of the hydrolysates obtained from sardines (*Sardina pilchardus*) and horse mackerels (*Trachurus mediterraneus*), at acidic pH, were positive. Cod protein hydrolysate at pH 2 presented ζ potential around 30 mV (Petursson et al., 2004).

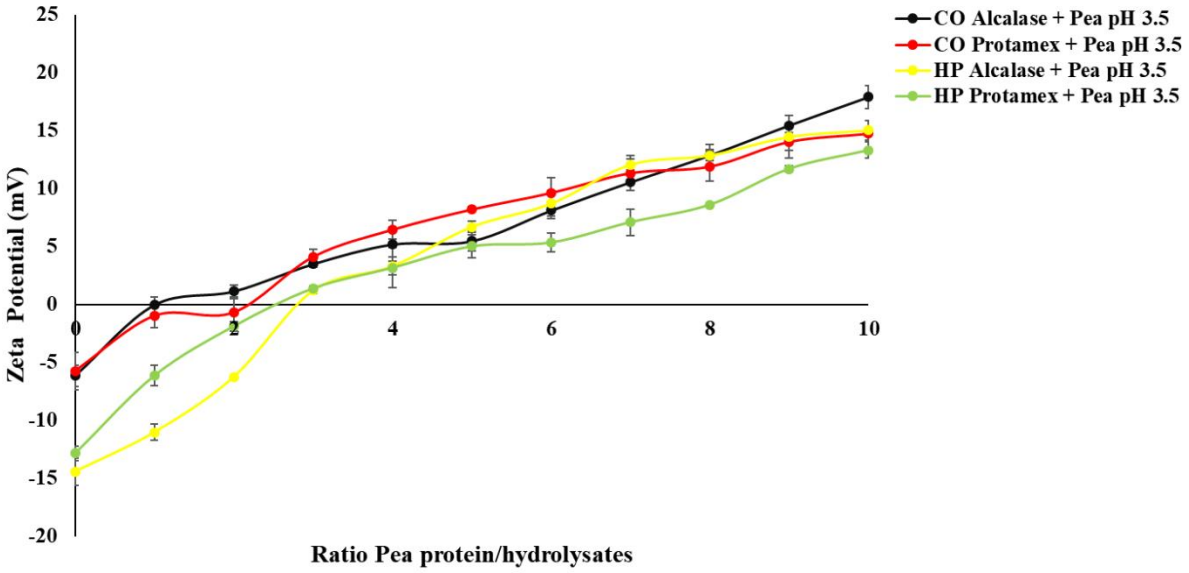
The ζ potential of Pea protein increased as the pH decreased (Figure 1). The absolute value of ζ potential increased as the pH moves away from pH 4.0, the isoelectric point of Pea protein (Gharsallaoui et al., 2009). It can observe that Pea protein in pH 3.5 had opposite charges compared with the proteins hydrolysates in the same pH (Figure 1). pH 3.5 was chosen for the formation of the complex coacervation with hydrolysates. Indeed, the difference of charges between the negative protein hydrolysates and positive Pea protein should allow the formation of electrostatic complexes. The protein hydrolysate of both species carries many negative charges at pH 3.5 favoring attractive electrostatic interactions with Pea proteins which is positively charged at this pH (Figure 1).

3.2 *Properties of protein hydrolysates/Pea protein complexes*

Figure 2 shows the results of the ζ potential of the complexes with Pea protein and hydrolysates ratio 0-10. The ζ potential of the complexes changed from negatives values (when only the protein hydrolysates were in solution) to positive values with increasing concentrations of Pea protein. This variation occurs because at pH 3.5 the hydrolysates

and Pea protein have opposite charges, and the value of the ζ potential of Pea protein is positive. Thus, this result may be due to the increase in the amount of Pea protein, thereby forming positively charged complexes.

Figure 2. Dependence of the ζ-potential of Pea protein/hydrolysates complexes ratio when different Pea protein amounts (0-10 g/L) were added to a fixed protein hydrolysates concentration (1 g/L) (5 mM imidazole-acetate buffer pH 3.5). CO= *Callinectes ornatus*; HP= *Hepatus pudibundus*.

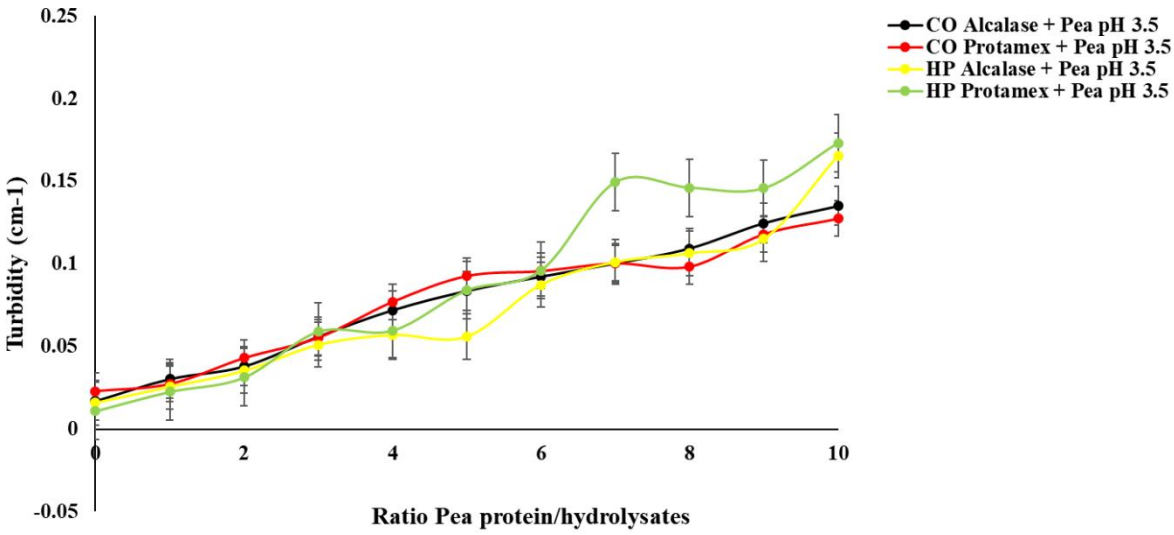


The changed of the ζ potential from negative to positive when the Pea protein concentration increased, indicates that the cationic protein molecules adsorbed to the anionic protein molecules through electrostatic attraction (Gharsallaoui et al., 2009). At Pea proteins ratio 9 the ζ potential of *H. pudibundus* Alcalase 2.4 L® hydrolysate and *C. ornatus* hydrolysate with the two enzymes is between 15 mV and 14 mV which is close to the ζ potential of Pea protein alone at the same pH. The complexes formation with the ratio Pea protein/hydrolysate 1 and 3 were smaller when using *H. pudibundus* hydrolysate

than *C. ornatus* hydrolysate. Thus, the protein hydrolysates obtained from *H. pudibundus* need more Pea protein to neutralize the ζ potential.

The increase in the Pea protein ratio resulted in increased turbidity in all samples (Figure 3). No differences were observed in turbidity when using Alcalase 2.4 L® or Protamex® enzymes with *C. ornatus*. A slight difference between these enzymes was observed with *H. pudibundus.* For both species, the turbidity peaks were reached at Pea protein ratio 10 (Figure 3).

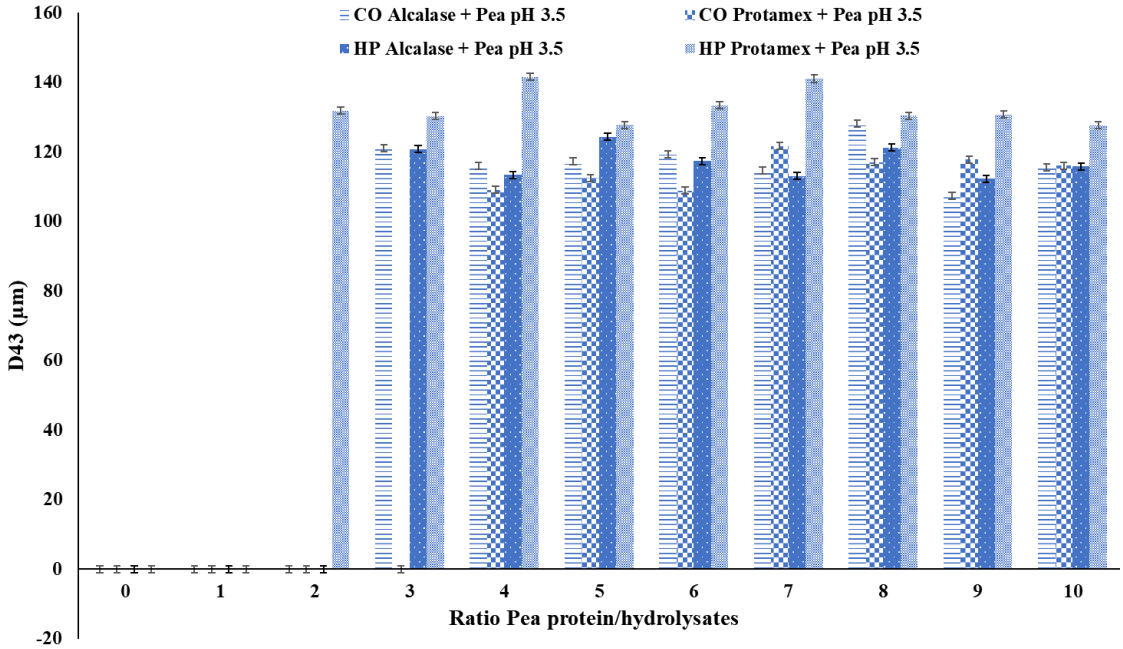
Figure 3. Turbidity of Pea protein/hydrolysates complexes ratio (at 600 nm) when different Pea protein amounts (0-10 g/L) were added to a fixed protein hydrolysates concentration (1 g/L) (5 mM imidazole-acetate buffer pH 3.5). CO= *Callinectes ornatus*; HP= *Hepatus pudibundus*.



According to Amara et al. (2017), the turbidity of the complexes increases as a result of the gradual association of individual complexes or small aggregates to form larger ones. Thus, the increase in turbidity can be caused by the formation of an increasing number of complexes and/or the formation of a larger complex (Amara et al., 2017).

Although the increase in turbidity suggests that the number and/or size of the aggregates of the complexes increases (Amara et al., 2017), the results of the particle diameter show that the size of the complexes is constant. Particle size (Figure 4) indicated that complexes tended to form larger aggregates (ranging from 112 ± 1.7 μm to 183 ± 2.8 μm). However, the particle size was relatively constant in complexes with Pea protein hydrolysates ratio from 4 to 10, indicating that they did not change in their self-association with different Pea protein concentrations.

Figure 4. Effect of on the particle size average (D43) of formed Pea protein/hydrolysates complexes when different Pea protein amounts (0-10 g/L) were added to a fixed protein hydrolysates concentration (1 g/L) (5 mM imidazoleacetate buffer pH 3.5). CO= *Callinectes ornatus*; HP= *Hepatus pudibundus*.



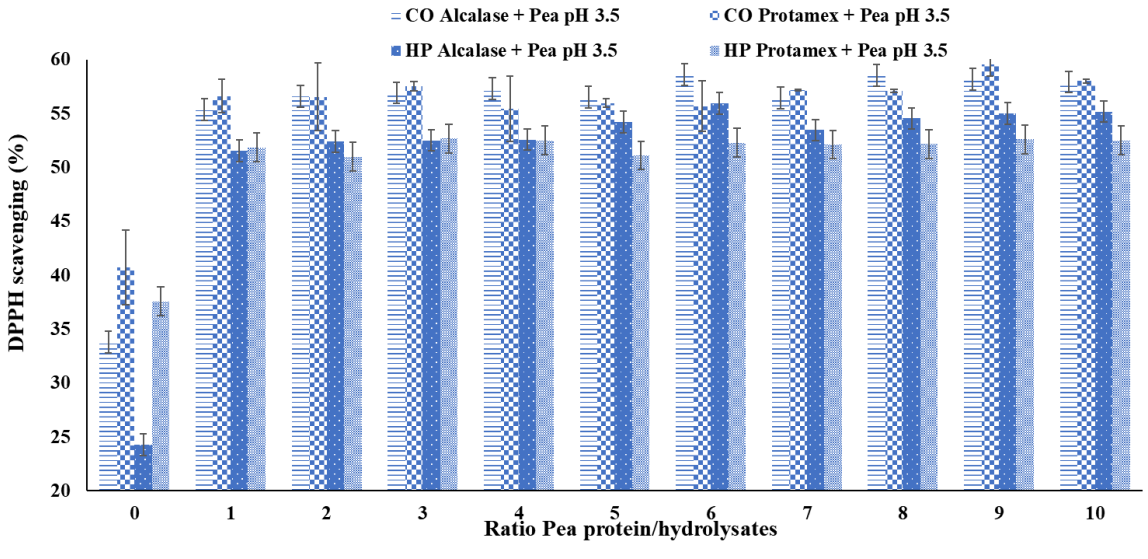
Only *H. pudibundus* Protamex® showed an increase in particle size at Pea protein hydrolysates ratio 4 and 7. This different behavior of the particle size and in turbidity in *H. pudibundus* Protamex® samples can be related to a higher proportion of low molecular

weight soluble proteins that are released with the enzyme Protamex® (Zamora-Sillero et al., 2018). However, molecular analyses of hydrolysates should be realized in order to understand this difference of particle size. Thus, based on particle size distribution analysis and turbidity, the aggregation of the complexes increased in the *H. pudibundus* Protamex® samples.

3.3 *Antioxidant properties of the protein hydrolysates and the complexes*

The DPPH was used to scavenge specific free radicals (Figure 5). The method principle is based on the DPPH reduction due to the donation of electron and or hydrogen radical from antioxidant species (eg. Peptides with sulfhydryl groups) present in protein hydrolysates (Zamora-Sillero et al., 2018). Samples of *C. ornatus* and *H. pudibundus* (ratio 0) hydrolyzed with the Protamex® enzyme presented the highest radical-scavenging activity (40.7 ± 0.4 % and 37.6 ± 1.4 %, respective) compared with *C. ornatus* and *H. pudibundus* (ratio 0) hydrolyzed with the enzyme Alcalase 2.4 L®. Several authors have reported the valuable functional properties of bioactive peptides obtained from others fish protein hydrolysates, one of them being antioxidant activity (Zamora-Sillero et al., 2018; Abuine et al., 2019).

Figure 5. DPPH radical scavenging activity of formed Pea protein/hydrolysates complexes when different Pea protein amounts (0-10 g/L) were added to a fixed protein hydrolysates concentration (1 g/L) (5 mM imidazole-acetate buffer pH 3.5). CO= *Callinectes ornatus*; HP= *Hepatus pudibundus*.



Hepatus pudibundus hydrolyzed with Alcalase 2.4 L® enzyme showed the lower antioxidant activity. A similar result was obtained by Vázquez et al. (2017) who investigated the antioxidant activity of *Scyliorhinus caniculum* hydrolysates captured as bycatch in Spain and Portugal and obtained 21.4% antioxidant activity against DPPH radicals with Alcalase 2.4 L® hydrolysate. Others studies showed significant differences in the DPPH scavenging activity between different enzymatic treatments (Fonseca et al., 2016). More recently, Zamora-Sillero et al. (2018) also detected differences of DPPH radical-scavenging activity in common carp byproducts hydrolyzed with Protamex® or Alcalase 2.4 L® enzyme.

The DPPH radical scavenging activity of the Pea protein/protein hydrolysates complexes was higher than the protein hydrolysates alone (ratio 0). Moreover, increasing levels of Pea protein did not affected the antioxidant activity of protein hydrolysates of the two species, which remained relatively constant at Pea protein/protein hydrolysates ratio from 1 to 10. Antioxidant activity was found for this Pea protein (Table 1) which could explain the increase of antioxidant activity when Pea protein was inserted with hydrolysates. The complexation of Pea protein with the hydrolysates not only added antioxidants but also The complexation of Pea protein with the hydrolysates not only added antioxidants but also improved the antioxidant activity potential of hydrolysates. The increase in the antioxidant activity potential of the hydrolysates may be associated with protection of the encapsulation process with Pea protein to the reducing sites of the antioxidant species presents in the protein hydrolysates (Table 1). The improve of antioxidant activity potential also could be due to the presence amino acids with sulfhydryl groups in Pea protein, groups that are strong reducers and consequently contribute to the increased antioxidant capacity of the Pea protein / hydrolysates complex (Sarmadi & Ismail, 2010). However, future studies on the interaction between Pea protein

and protein hydrolysates should be performed to better understand the mechanisms of antioxidant production in the complexes coacervates.

Table 1. DPPH radical scavenging activity of Protein hydrolysates, Pea protein, and complex coacervates ratio 1 and 9. The expected activity of Pea protein/Hydrolysates complex is shown[1]. CO= *Callinectes ornatus*; HP= *Hepatus pudibundus*.

	Free Hydrolysate	Complexes (ratio 1)	Ratio 1 (expected activity)	Complexes (ratio 9)
CO Alcalase 2.4 L®	33.8 ± 2.6^{b}	55.4 ± 1.7^{a}	47.7	58.2 ± 3.1^{a}
CO Protamex®	40.7 ± 6.4^{b}	56.6 ± 2.5^{a}	54.6	59.6 ± 1.1^{a}
HP Alcalase 2.4 L®	24.3 ± 4.3^{b}	51.5 ± 1.6^{a}	38.2	55 ± 2.1^{a}
HP Protamex®	37.6 ± 1.4^{b}	51.9 ± 1.6^{a}	51.5	52.6 ± 2.5^{a}
PEA proteins	13.9 ± 1.8^{c}	----------	----------	----------

[1]Values are expressed as the average and standard error; Different letters indicate significant differences determined by Tukey tests ($p < 0.05$).

3.4 *Spray-dried microcapsules containing antioxidant protein hydrolysates*

Natural antioxidants are generally unstable molecules that are sensitive to heat and light, which limits its application in the food industry (Ozkan et al., 2019). Microencapsulation is a reliable technique that can provide a solution to this problem by protecting bioactive compounds from environmental factors and maintaining their original characteristics (Gharsallaoui et al., 2012; Ozkan et al., 2019). Moreover, microencapsulation improve the nutrient retention in food, preserves the stability of bioactive compounds during processing and storage, and delays the processes of degradation and functionality of the bioactive component (Ozkan et al., 2019). Thus, microencapsulation by Spray-drying was made and the complexes formed with the *Callinectes ornatus* samples and Pea protein ratio 9 were selected due to its high

antioxidant capacity. The antioxidant activity against DPPH radical of spray-dried solutions of non-complexed hydrolysates was significantly lower after the microencapsulation (Table 2).

Table 2. DPPH radical scavenging activity of Pea protein/protein hydrolysates complex coacervates and free hydrolysates/maltodextrin before and after the spray-drying process (spray-dried and reconstituted suspension)[1]. CO= *Callinectes ornatus*.

	Before Spray-dried Hydrolysates	After Spray-dried Hydrolysates	Before Spray-dried Complexes	After Spray-dried Complexes
CO Alcalase 2.4L®	31.0 ± 1.3^{b}	18.8 ± 2.5^{c}	56.0 ± 1.4^{a}	55.4 ± 1.7^{a}
CO Protamex®	40.6 ± 1.4^{b}	17.3 ± 1.8^{c}	56.5 ± 2.5^{a}	56.7 ± 3.4^{a}

[1]Values are expressed as the average and standard error; Different letters indicate significant differences determined by Tukey tests ($p < 0.05$).

This result suggested that high temperatures of the dryer process decreased of the antioxidant activity of the hydrolysates. Mishra et al. (2014) reported similar results in the DPPH radical scavenging activity of Amla powders by increasing the drying temperature of spray drying. Araujo-Díaz et al. (2017), showed that there was a decrease in blueberry antioxidants after the spray drying process. Recently, Akbarbaglu et al. (2019) evaluated the influence of spray drying encapsulation on the retention of antioxidant properties of flaxseed protein hydrolysates by different methods and showed that the lowest antioxidants were related to DPPH radical scavenging activity. In fact, during spray-drying, peptides and/or proteins may undergo several stresses through exposure to heating which may lead to subsequent aggregation and denaturation that may result in loss of their biological activity (Amara et al., 2017). On the other hand, the antioxidant activity of the complexes formed with Pea protein and hydrolysates, after

spray-drying, were not significantly different when compared with the DPPH radical scavenging capacity of the samples before spray-drying. Thus, the spray-drying process of the complex coacervates of Pea protein/hydrolysates did not affect the antioxidant capacity significantly. The stability of the antioxidant activity of the complexes could either be attributed to the Pea protein that protected the hydrolysate against the high temperatures of the microencapsulation process.

Previous studies showed that Pea protein was an effective wall material for microencapsulation of ascorbic acid and the use for α-tocopherol microencapsulation is a promising system for application in food (Pereira et al., 2009). According to Gharsallaoui et al. (2012), the structural integrity of the Pea protein interfacial layer does not appear to be compromised by the spray-drying process when higher dextrose equivalent carbohydrates were used. Aberkane et al. (2014) evaluated the potential of pectin combination with Pea protein isolate in the microencapsulation of polyunsaturated fatty acids oil by spray drying and Pea proteins gave the best protection of the oil. Recently, Le Priol et al. (2019) compared five plant protein extracts for the microencapsulation of sunflower oil and observed that the microencapsulation remarkably improves the sunflower oil oxidative stability when using Pea protein extracts. Hence, Pea protein can be a solution to protect bioactive compounds. The results of the present study suggest that the complexation and subsequent microencapsulation by spray-drying using Pea protein is an efficient manner of protecting the biological activity of protein hydrolysates.

4. Conclusion

The complexation of Pea protein through electrostatic attraction increased the antioxidant capacity of protein hydrolysates. Thus the complexation and microencapsulation using Pea protein is an efficient way to protect the antioxidant activity

of protein hydrolysates from bycatch-crustacean. The present study provide evidence for the potential use of crabs presented in the bycatch from shrimp fisheries as functional ingredient or nutraceutics. The huge amount of swimming crabs that is discarded during shrimp fisheries worldwide is enough to provide raw material for sacale up a possible industrial production.

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Capítulo 4

Complexes coacervates for the preservation of the antioxidant activity in fish protein hydrolysates: a new use to bycatch in Brazil

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Abstract

In this study, we evaluated the effect of the complexation and microencapsulation with Pea protein on the antioxidant activity of the protein hydrolyzed from bycatch in Brazil. Pea protein and the hydrolysates have the ability to eliminate the DPPH radical and these results corroborate with the data found in the literature. The ζ-potential of the complexes change from negative to positive values by the increase in the amount of Pea protein thereby forming more positively charged complexes. An increase in the ratio of Pea protein/hydrolysates also resulted in increased turbidity in all samples. Particle size measurements indicated that the complexes tended to form large aggregates (ranged from 61.5 ± 1.7 μm to 183 ± 2.8 μm). The DPPH radical scavenging activity of the Pea protein/protein hydrolysates complexes was higher than the protein hydrolysates alone. Moreover, increasing levels of Pea protein did not affect the antioxidant activity of protein hydrolysates. The complexes of the *Paralonchurus brasiliensis* was chosen for the spray-drying microencapsulation process. The results revealed that spray-drying did not have a significant effect ($p > 0.05$) on the protein hydrolysates antioxidant activity when they were complexed with Pea protein. In this way, this work suggests that the complexation with Pea protein and subsequent microencapsulation by spray-drying is an efficient way to protect the biological activity of protein hydrolysates obtained from bycatch. Thus, this study provides evidence for the potential use of bycatch from shrimp fisheries as functional ingredient or nutraceuticals.

Keywords: Pea protein, DPPH, Microencapsulation, *Paralonchurus brasiliensis*, *Micropogonias furnieri*.

1. Introduction

The bycatch of shrimp trawl fisheries accidentally captures a large number of species that are returned to the sea dead or with little chance of survival (Fauconnet et al. 2019). The bycatch has no commercial value, but contains a large amount of protein, amino acids, oils, and others rich material that can be processed into market-value products, as animal feed (Zamora-Sillero et al. 2018; Nikoo et al. 2019). Techniques such as enzymatic hydrolysis have been developed to produce hydrolysates from fish by-products, like skin, muscle, and viscera in order to recovery protein and peptides of commercial interest (Zamora-Sillero et al. 2018; Nikoo et al. 2019). These techniques may be used to produce hydrolysates from the fish contained in the demersal fishery bycatch.

Previous studies demonstrated that the fish protein hydrolysates (FPH) has bioactive compounds, essential nutrients and are potential sources of antioxidant peptides (Zamora-Sillero et al. 2018; Abuine et al. 2019; Özogul et al. 2019). In this way, the study of biological activity in the hydrolysates obtained from discards of fisheries is important to investigate know the biochemical properties of these compounds and to aggregate value to this material that is reject (Zamora-Sillero et al. 2018; Nikoo et al. 2019; Özogul et al. 2019).

Additionally, the proteins hydrolysates can be used to the formation of the complexes coacervates that are used in the food industry to ensure the stability of processed foods (Eghbal et al. 2017; Wang et al. 2019). According to Devi et al. (2017), the complexes coacervates can be achieved in biopolymer pair of protein-polysaccharide mixtures producing electrostatic interactions. The formation of the complexes can be influenced by electrostatic interactions such pH, the concentration of the interacting components, charge distribution on the polypeptide and peptide or protein to

polysaccharide ratio (Wang et al. 2019). Moreover, the formation of complexes between proteins and biopolymers possess advantageous functional properties such as stabilization of food emulsions, micro and nano-encapsulation processes, recovery of proteins from industrial by-products and others (Amara et al. 2017; Wang et al. 2019).

In this context, the Pea (*Pisum sativum*) protein has been used to the formation of the complexation and electrostatic interactions with other polymers (Gharsallaoui et al. 2012; Burger and Zhang et al. 2019). Pea seed is rich in proteins (18-30%) and has a well-balanced amino acid profile (Chen et al. 2019). Several studies described the functionalities of the Pea protein as food ingredients in a wide range of applications (Bogahawaththa et al. 2019). And the use to Pea protein in the microencapsulation process was reported with success (Gharsallaoui et al. 2012). Gharsallaoui et al. (2012) reported that Pea protein can be used to prepare spray-dried emulsions for the microencapsulation of oil. Recently, Le Priol et al. (2019) showed that Pea protein extracts improve the sunflower oil oxidative stability in the spray-drying process.

Thus, the aim of this study is to investigate the effect of complexation using Pea protein on antioxidant activity in protein hydrolysates obtained from fish contained in the bycatch of the fishery shrimps in Brazil. Additionally, the antioxidant propriety before and after the microencapsulation by spray-drying was evaluated. Results obtained may add value to the bycatch as functional ingredient in the food industry.

2. Materials and Methods

2.1 *Raw material*

The fish were collected in February 2017, September 2017 and February 2018 in the region of Ubatuba, São Paulo, Brazil, by double-rig, a commercial shrimp trawling.

The two species of fish more abundant from bycatch were selected: *Paralonchurus brasiliensis* and *Micropogonias furnieri*. The muscles and the skin of the animals were blended and packaged in sealed 100 g plastic bags and transported in cool boxes with dry ice to the Aquaculture Center (CAUNESP, Brazil), where they were kept in the freezer at -20°C until analysis.

2.2 *Preparation of protein hydrolysates*

The hydrolyzes were initiated with samples of 100 g of muscles with skin that were thawed in the refrigerator at 4°C for 24 h. The samples were homogenized in a blender, with two volumes of distilled water (w/v), following by heating at 80°C for 20 minutes in order to inactivate the endogenous enzymes. Subsequently, the hydrolysis was started using enzyme Alcalase 2.4 L® (Novozymes, Bagsvaerd, Denmark) and enzyme Protamex® (Sigma Aldrich, MO, USA). The hydrolysis was conducted at pH 7 and 50°C to enzyme Protamex® and pH 8 and 50°C to enzyme Alcalase 2.4 L®. The enzyme-substrate ratio was set at 2% (w/w) for both enzymes to determine the maximum degree of hydrolysis for each enzyme. The hydrolyzes had a duration of four to five hours, after the incubation the samples were heating at 80°C for 20 minutes to stopped the hydrolysis. Then, samples were centrifuged at 16300 x g for 20 minutes and supernatants were frozen at -80°C and subsequently freeze dried and stored at -20°C until analysis was performed.

2.3 *Antioxidant properties measurement*

DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity was determined on protein hydrolysates (5 g/L) dissolved in imidazole-acetate buffer (5 mmol/L, pH 3.5) and in the complex coacervates of hydrolysates (1 g protein hydrolysates/L)/Pea protein (1 g/L). In order to determine the DPPH radical scavenging activity, the method reported

by Bersuder et al. (1998) was used. Briefly, an aliquot (500 μL) of each sample was mixed with 375 μL of 99% (v/v) ethanol and 125 μL of a daily-prepared solution of DPPH (0.02% (w/w) in 99% ethanol solution). The mixtures were shaken and then incubated for 60 min in dark at room temperature. The reduction of DPPH radical was determined spectrophotometrically by monitoring the decrease in absorbance at 517 nm in a spectrophotometer (Jenway 7305, Villepinte, France). DPPH radical-scavenging activity was calculated as follows in the equation:

$$\text{DPPH radical-scavenging activity (\%)} = (A_{blank} - A_{sample}) / A_{blank} \times 100$$

where A_{blank} is the absorbance of the reaction containing all reagents (except that distilled water was used instead of the sample), and A_{sample} is the absorbance in the presence of the sample. The experiment was carried out in triplicate and the results were mean values.

2.4 *Preparation of Pea protein/protein hydrolysates complexes*

Biopolymer mixtures containing protein hydrolysates of *P. brasiliensis* and *M. furnieri* (1 g/L) and Pea protein (1 g/L) were prepared by mixing different ratios (0-10) of the stock solutions with imidazole-acetate buffer at pH 3.5. The resulting suspensions were mixed for 10 min using a vortex mixer and their pH was adjusted again to pH 3.5.

2.5 *Zeta potential measurement*

The zeta potential (ζ-potential) of protein hydrolysates from two species of fish, Pea protein and Pea protein/protein hydrolysates complexes were determined using a Zetasizer NanoZS90 (Malvern Instruments, Malvern, UK). The samples were diluted by

0.5% (w/w) with imidazole-acetate buffer adjusted to the suitable pH value. The mean ζ-potential (ZP) values (±SD (standard deviation)) were obtained from the instrument.

2.6 *Turbidity measurement*

Turbidity of Pea protein/protein hydrolysates complexes were performed with a spectrophotometer (Jenway 7305, Villepinte, France). The absorbance was measured at 600 nm at room temperature (25 °C) against Pea protein/protein hydrolysates in the imidazole-acetate buffer (5 mmol/L; pH 3.5).

2.7 *Particle size measurement*

Particle size distributions of Pea protein/protein hydrolysates complexes formed at various Pea protein concentrations were measured by a laser diffraction instrument (Mastersizer 3000, Malvern Instruments, Malvern, UK). The complexes were stirred continuously throughout the measurement to ensure the homogeneity of the samples. The volume particle diameter (D43) was calculated from the three injections of three separate samples with three readings per sample.

2.8. *Spray-drying*

The complexes formed with the samples *P. brasiliensis* prepared in the imidazole-acetate buffer (5 mmol/L; pH 2.4) containing 20 % (w/w) maltodextrin DE 19, as well as solutions of the same hydrolysates without Pea protein, were dried in a laboratory scale spray-drier equipped with a 0.5 mm nozzle atomizer (Mini spray-dryer B-290, BUCHI, Switzerland). Suspensions were pumped to the spray-drier at a feed rate of 0.5 L/h at room temperature and dried at an inlet temperature of 180 °C and an outlet temperature of 90 °C. The dried powders were collected and stored in airtight containers at 4 °C. Dry

powders were then weighted and mixed with imidazole/acetate buffer (5 mmol/L, pH 2.4) to obtain reconstituted suspensions of complexes and hydrolysates with the same dry matter as before drying. After 1 h of rotation at approximately 200 rpm, samples were analyzed.

2.9 *Statistical analysis*

All experiments were performed using at least three freshly prepared samples. The results are the averages and standard deviations that were calculated from these replicate measurements. The differences in the antioxidant activity between the samples were evaluated using a one-way analysis of variance (ANOVA), post-hoc tests were employed using Newman-Keuls method. The significance level adopted was 5% ($\alpha = 0.05$). The ANOVA assumptions (normality by Shapiro-Wilks and variance homogeneity by Levene) were previously evaluated and tested with the same significance level (5%).

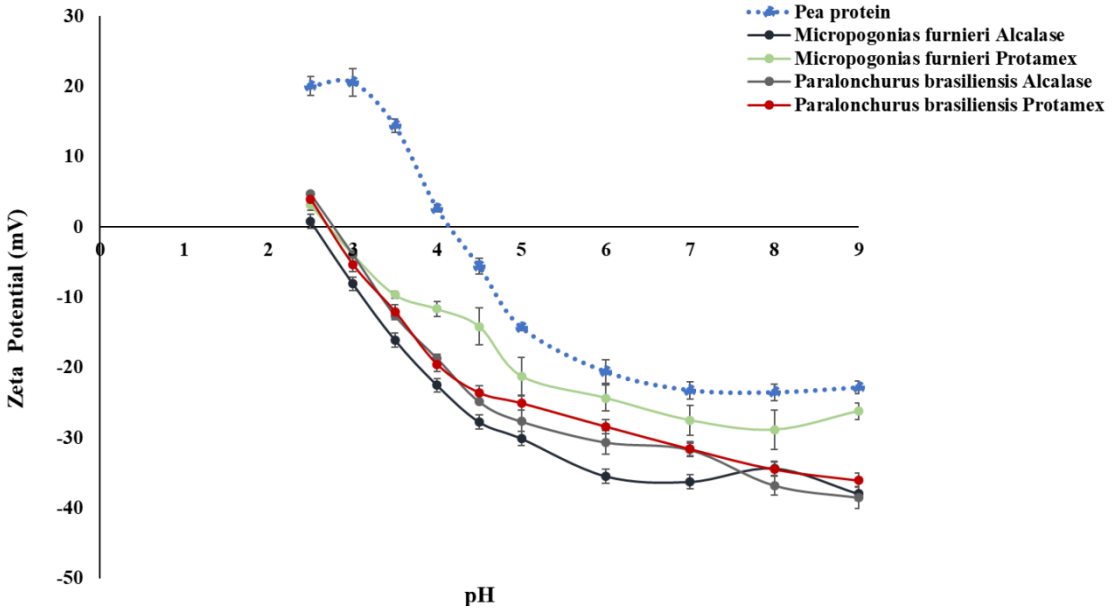
3. Results

3.1 *Determination of physical-chemical parameters of the hydrolysates and Pea protein*

The ζ-potential of the hydrolysates and Pea protein was characterized in order to find the optimum conditions for the formation of complexes. The ζ-potential was measured as a function of pH in all hydrolysates and Pea protein; it was observed that the ζ potential increases as the pH decrease (Fig. 1). The magnitude of the charge increased significantly in acidic pH (Fig. 1). Pea protein have ζ-potential positive between pH 2.5-4.0 that ranges from 2.63 ± 0.5 mV to 20.03 ± 1.0 mV. The results of the ζ-potential were similar to the two species of fish and both presented isoelectric points ranging from 2.5 to 3.5 (Fig. 1). At pH 2.5, all protein hydrolysates showed positive ζ potential.

For *P. brasiliensis*, there was no differences in ζ potential when using Alcalase 2.4 L® or Protamex® enzymes. However, *M. furnieri* hydrolyzed with the enzyme Alcalase 2.4 L® showed ζ potential close to zero in pH 2.5, which means that the charge of complexes was almost electrically neutral. These values of the ζ potential were lower compared with those obtained using the enzyme Protamex®.

Fig. 1 Dependence of the ζ-potential of *Micropogonias furnieri* and *Paralonchurus brasiliensis* hydrolysates produced with Alcalase 2.4 L® or Protamex® and Pea protein on pH (5 mM imidazole-acetate buffer).



Additionally, protein hydrolysate in pH 3.5 had opposite charges compared with the Pea protein in the same pH (Fig. 1). In this way, the pH 3.5 was chosen for the formation of the complexation, because the protein hydrolysate of both species carries many negative charges at pH 3.5 favoring attractive electrostatic interactions with Pea protein which is positively charged at this pH (Fig. 1).

Pea protein and hydrolysates have the ability to eliminate the DPPH radical (Table 1). Pea protein shows 13.9 ± 2.0 % of the antioxidant activity. *Paralonchurus brasiliensis* has more antioxidant activity (30.9 ± 3.6 %) than *M. furnieri* (26.2 ± 3.4 %). Additionally, no significant differences ($p > 0.05$) were observed between the two enzymes in both hydrolysates (Table 1).

Table 1. DPPH radical scavenging activity of Protein hydrolysates and Pea proteins[1].

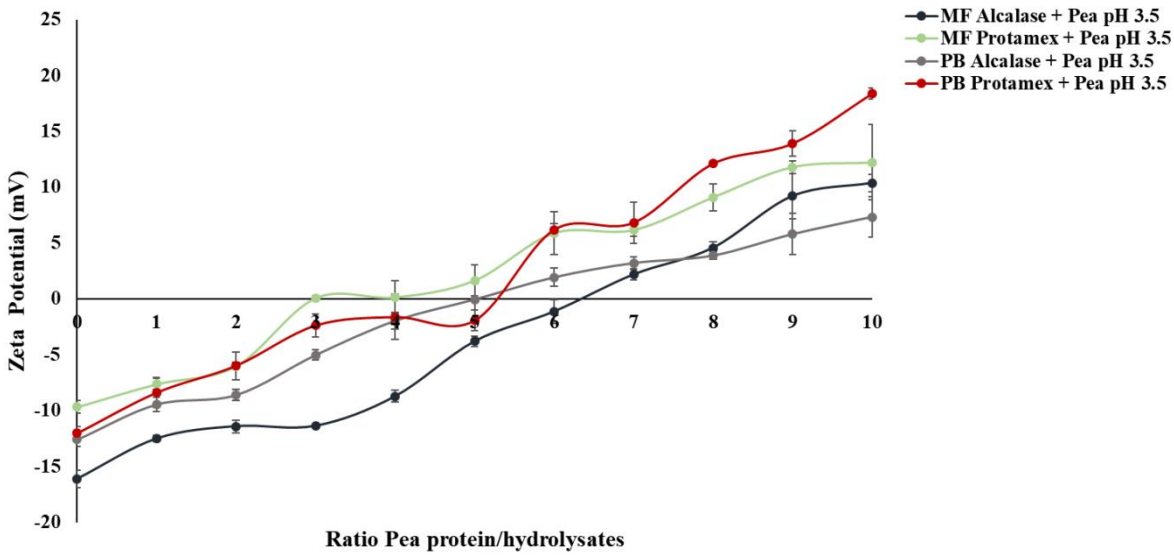
Specie	Protease	DPPH (%)
Micropogonias furnieri	Protamex®	26.1 ± 1.8[b]
Micropogonias furnieri	Alcalase 2.4 L®	26.2 ± 3.4[b]
Paralonchurus brasiliensis	Protamex®	30.8 ± 4.8[a]
Paralonchurus brasiliensis	Alcalase 2.4 L®	30.9 ± 3.6[a]
Pea proteins	----------	13.9 ± 2.0[c]

[1]Values are expressed as the average and standard error. Different letters indicate significant differences determined by Tukey tests ($p < 0,05$).

3.2 *Properties of protein hydrolysates/Pea protein complexes*

The ζ potential of the complexes with Pea protein and hydrolysates ratio 0-10 is showed in Figure 2. In the ratio 0-2, the ζ potential of the four complexes was negative ranging from -16.1 mV to -0.7 mV.

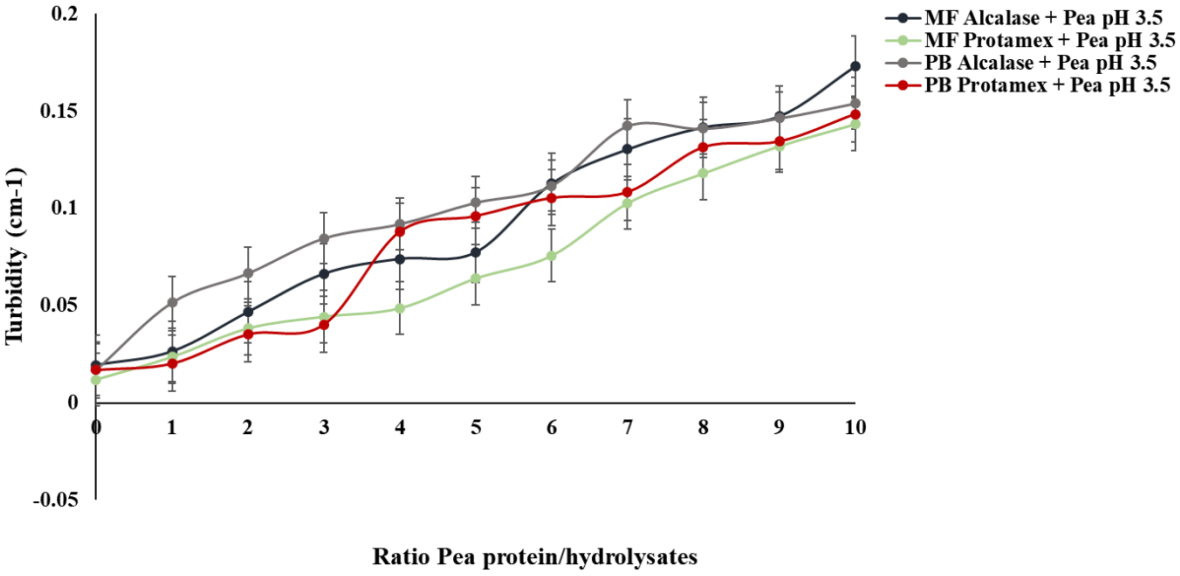
Fig. 2 Dependence of the ζ-potential of Pea protein/hydrolysates complexes ratio when different Pea proteins amounts (0-10 g/L) were added to a fixed protein hydrolysates concentration (1 g/L) (5 mM imidazole-acetate buffer pH 3.5). MF= *Micropogonias furnieri*; PB= *Paralonchurus brasiliensis.*



The complexes with *M. furnieri* Protamex® had a neutral charge in ratio 3 and 4. In almost all complexes, from the ratio 6, the ζ potential increased rapidly with ratio Pea protein increasing (Fig. 2). In this ratio, only the *M. furnieri* Alcalase 2.4 L® complex was negatively charged. In this species, the enzymes Alcalase 2.4 L® and Protamex® had close results in ratio 10 with 10.4 ± 0.5 mV and 12.2 ± 0.9 mV, respective. On the other hand, *P. brasiliensis* presented a significant difference between the enzymes in ratio 10, being that Alcalase 2.4 L® exhibited 7.3 ± 0.9 mV and Protamex® presented charge of complexes higher with 18.4 ± 0.5 mV. Additionally, at ratio 9 the ζ potential of the *P. brasiliensis* Protamex® is 13.9 ± 1.1 mV which is close to the ζ potential of Pea protein alone at the same pH (Fig. 1).

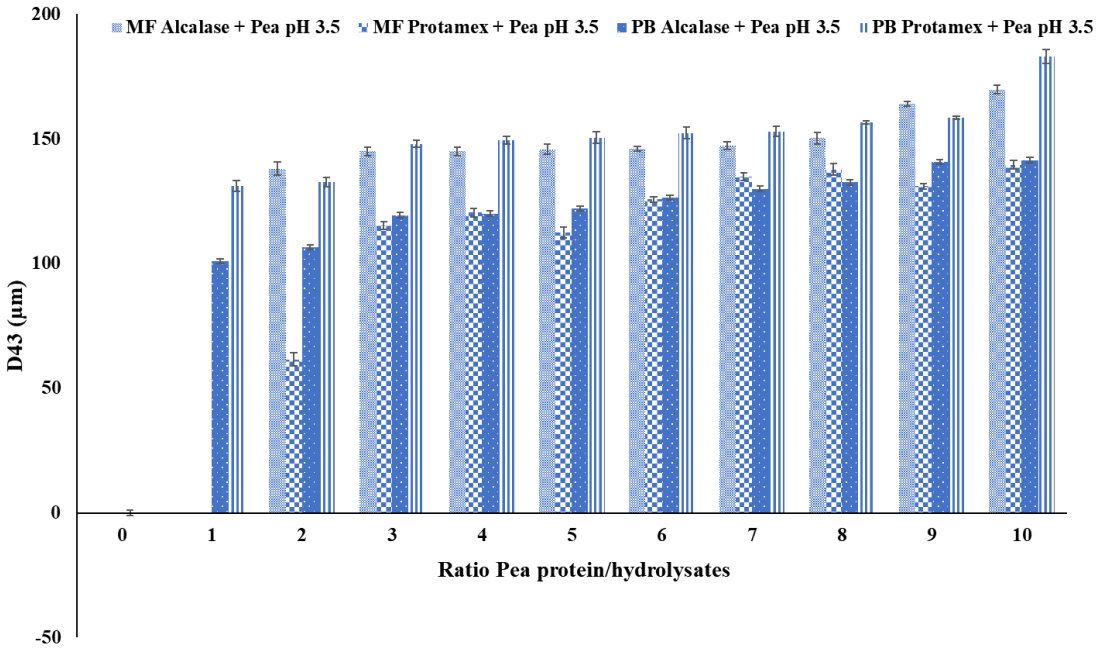
The increase in the Pea protein ratio resulted in increased turbidity in all complexes (Fig. 3). Besides that, for all complexes, the turbidity peaks were reached at Pea protein ratio 10. Thus, the addition of higher amounts of Pea protein increased the turbidity value of the complexes.

Fig. 3 Turbidity of Pea protein/hydrolysates complexes ratio (at 600 nm) when different Pea amounts (0-10 g/L) were added to a fixed protein hydrolysates concentration (1 g/L) (5 mM imidazole-acetate buffer pH 3.5). MF= *Micropogonias furnieri*; PB= *Paralonchurus brasiliensis.*



The turbidity measurements were supplemented with information about particle size. The size of the complexes also increased with the increase in the Pea protein ratio (Fig. 4). The particle size measurements ranged from 61.5 ± 1.7 µm to 183 ± 2.8 µm. However, in the ratio 7-10 of the complexes formed with *M. furnieri* Protamex® and *P. brasiliensis* Alcalase, the particle size was relatively constant.

Fig. 4 Effect of on the particle size average (D43) of formed Pea protein/hydrolysates complexes when different Pea amounts (0-10 g/L) were added to a fixed protein hydrolysates concentration (1 g/L) (5 mM imidazole-acetate buffer pH 3.5). MF= *Micropogonias furnieri*; PB= *Paralonchurus brasiliensis*.



The DPPH radical scavenging activity of the hydrolysates/Pea protein complexes was higher than the protein hydrolysates alone (concentration Pea proteins 0) (Fig.5). The antioxidant activity of complexes remained relatively constant at ratio from 1 to 10, suggesting that the ability to scavenge specific free radicals is almost the same as the increasing levels of Pea proteins. Additionally, the results expected with the addition of

the Pea protein and the obtained results with the formation of the complexes are shown in Table 2. The results showed that the fusion of the antioxidant properties of Pea protein with the hydrolysates obtained from *P. brasiliensis* not only added antioxidants, but also activated the production of more antioxidants in the hydrolysates.

Fig. 5 DPPH radical scavenging activity of formed Pea protein/hydrolysates complexes when different Pea protein amounts (0-10 g/L) were added to a fixed protein hydrolysates concentration (1 g/L) (5 mM imidazole-acetate buffer pH 3.5). MF= *Micropogonias furnieri*; PB= *Paralonchurus brasiliensis.*

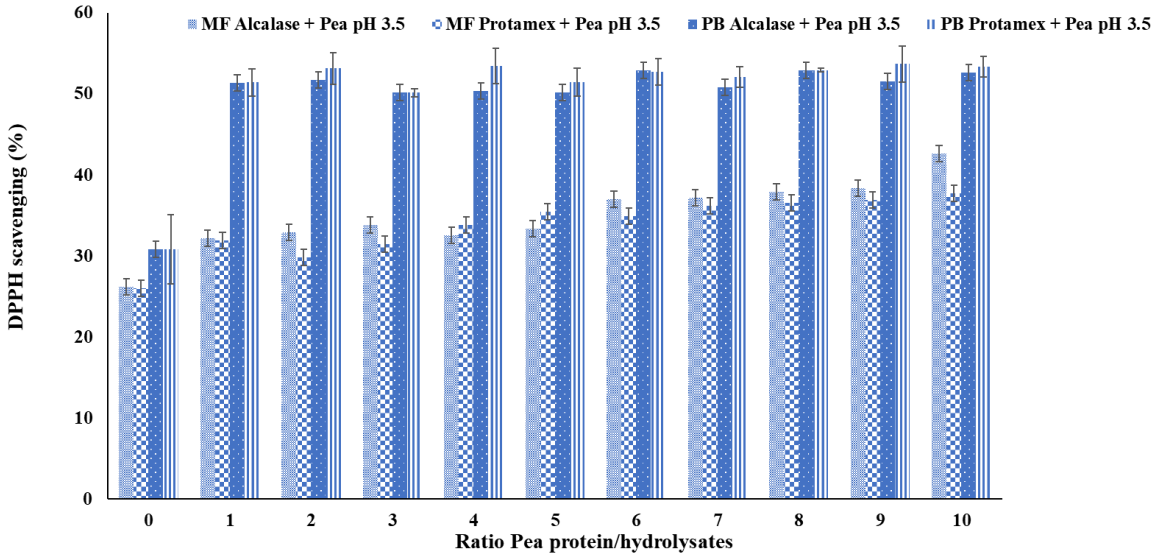


Table 2. DPPH radical scavenging activity of Protein hydrolysates, Pea proteins, and complex coacervates ratio 1 and 9. The expected activity with the join of the Pea proteins and hydrolysates is shown[1]. MF= *Micropogonias furnieri*; PB= *Paralonchurus brasiliensis*

	Ratio 1 (expected activity)	Complexes (ratio 1)	Complexes (ratio 9)
MF Alcalase	40.1	31.9 ± 1.8^{b}	39.9 ± 1.8^{b}
MF Protamex®	40	32.2 ± 3.2^{b}	38.4 ± 0.6^{b}
PB Alcalase 2.4 L®	44.8	51.4 ± 0.7^{a}	53.7 ± 1.1^{a}
PB Protamex®	44.7	51.3 ± 1.0^{a}	51.5 ± 2.3^{a}

[1]Values are expressed as the average and standard error; Different letters indicate significant differences determined by Tukey tests ($p < 0.05$).

3.3 *Spray-drying microcapsules containing antioxidant protein hydrolysates*

The complexes formed with the samples of the *P. brasiliensis* ratio 9 were selected to microencapsulation by spray-drying due to its high antioxidant capacity. The antioxidant activity against DPPH radical of spray-dried solutions of non-complexed hydrolysates was significantly lower after the microencapsulation (Table 3). On the other hand, the antioxidant activity of the complexes formed with Pea protein and hydrolysates, after spray-drying, were not significantly different when compared with the DPPH radical scavenging capacity of the samples before spray-drying. These results suggested that the high temperatures of the dryer process decrease of the antioxidant activity of the hydrolysates. However, the spray-dried process of the complex coacervates of Pea protein/hydrolysates and preserve the antioxidant capacity significantly.

Table 3. DPPH radical scavenging activity of Pea proteins/protein hydrolysates complex coacervates and free hydrolysates/maltodextrin before and after the spray-drying process (spray-dried and reconstituted suspension)[1]. PB= *Paralonchurus brasiliensis*

	Before Spray-dried Hydrolysates	After Spray-dried Hydrolysates	Before Spray-dried Complexes	After Spray-dried Complexes
PB Alcalase 2.4 L®	32.6 ± 1.5^{b}	11.4 ± 3.8^{c}	51.9 ± 5.3^{a}	55.3 ± 2.3^{a}
PB Protamex®	32.7 ± 1.4^{b}	15 ± 1.9^{c}	53.5 ± 1.3^{a}	57.8 ± 1.1^{a}

[1]Values are expressed as the average and standard error; Different letters indicate significant differences determined by Tukey tests ($p < 0,05$).

4. Discussion

The antioxidant activity found in the fish contained in the bycatch can add value to this reject and become a product of interest to the food industries, as additives and nutraceuticals. Furthermore, obtaining protein hydrolysates with biological activity from

the demersal trawl fishery can generate additional income for fishermen, which can reduce the necessity of trawling long time, decreasing the impacts caused by shrimp fishing, optimizing the fisherman's time at sea and finally reducing the damage caused to the environment and increasing the income of fishers. Thus, the results of the present study create a paradigm for future studies using the bycatch.

4.1 *Determination of physical-chemical parameters of the hydrolysates and Pea protein*

The ζ-potential of the hydrolysates of *M. furnieri* and *P. brasiliensis* are positive in acid pH and negative in neutral/basic pH. These results are similar to those observed in others marine fish. Petursson et al. (2004) investigated the ζ potential of the Cod protein hydrolysate and observed at pH 2 the ζ potential is around 30 mV. Hydrolysates obtained from sardines (*Sardina pilchardus*) and horse mackerels (*Trachurus mediterraneus*) had the ζ potential positive in acidic pH (Morales-Medina et al. 2016). Li et al. (2018) investigated the ζ-potential of the fish skin gelatin from cold water fish and observed that in the pH 6.0 the samples were negatively charged with values of -1.19 ± 0.5 mV and when in pH 3.5, the solution had a positive charge of 13.03 ± 1.6 mV.

The little difference of the ζ-potential between *M. furnieri* hydrolyzed with the enzymes Alcalase 2.4 L® or Protamex® may be due to obtaining a different amino acids composition. According to Klompong et al. (2009), the amino acid composition of fish protein hydrolysates depends on the hydrolysis conditions and enzyme source. Therefore, the amino acids portfolio of the *M. furnieri* hydrolysates should be determined to better understand the differences between the enzymes in this species. Additionally, the results obtained with Pea protein were expected, because the isoelectric point of the Pea protein is around pH 4 (Gharsallaoui et al. 2009; Burger and Zhang 2019). Thus, the absolute

value of ζ potential increased as the pH moves away from pH 4.0 and the attractive electrostatic interactions of the hydrolysates with Pea proteins was favored in pH 3.5.

Pea protein showed the antioxidant properties and these results corroborate the data found in the literature (Tamm et al. 2016). However, the hydrolysates contain a greater concentration of antioxidants than Pea proteins. Some studies with hydrolysates of proteins obtained from fish have successfully detected DPPH elimination activity using different enzymes (Zamora-Sillero et al. 2018). For instance, antioxidant activity was found in sardine by-products protein hydrolysates obtained by various proteases (Bougatef et al. 2010). Zamora-Sillero et al. (2018b) detected antioxidant capacity in common carp byproducts hydrolyzed with the enzyme Alcalase® and Protamex®. Recently, Altınelataman et al. (2019), studied the peptide profiles of the european seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) hydrolyzed with Alcalase® and identified antioxidant capacity in their peptides. In this way, the antioxidant activity is a functional property of the bioactive peptides obtained from fish protein hydrolysates and, in this study, can add value to bycatch.

4.2 *Properties of protein hydrolysates/Pea protein complexes*

The change from negative to positive complexes with the addition of the Pea proteins occurs because the hydrolysates and Pea have opposite charges in the pH 3.5. In this manner, the ζ potential of the complexes changed from negatives values (when only the protein hydrolysates were in solution) to positive values with increasing concentrations of Pea protein. According to Burger and Zhang (2019), this change of the ζ potential when used Pea proteins indicate that the cationic protein molecules adsorbed to the anionic protein molecules through electrostatic attraction. Thus, this result can be

explained by the increase in the amount of Pea proteins thereby forming more positively charged complexes.

In ratio 6, only the *M. furnieri* Alcalase 2.4 L® complex is negatively charged, which suggests that this complex needs more protons to reach a neutral state. Besides that, *P. brasiliensis* showed a significant difference between the enzymes in ratio 10. According to Chalamaiah et al. (2012), different enzymes have different proteolytic affinities in the muscle of the fish. Thus, it could be inferred that differences between the enzymes Alcalase 2.4 L® and Protamex® in *P. brasiliensis* are due to the different proteolytic affinities with the muscle in this species that can influence the formation of complexes with Pea.

The increased of the turbidity with the addition of Pea proteins indicated extensive biopolymer aggregation (Amara et al. 2017; Wang et al. 2019). The increase in turbidity could be caused by the formation of an increasing number of complexes and/or formation of a large complex (Amara et al. 2017). Besides that, the results of the particle size indicate that complexes tended to form larger aggregates with the addition of the Pea probably due to a high aggregation of individual complexes, which result in a distribution of larger particles (Amara et al. 2017). However, in the ratio 7-10 of the complexes formed with *M. furnieri* Protamex® and *P. brasiliensis* Alcalase, the particle size is relatively constant, indicating that they did not change in their self-association with these Pea proteins concentrations (Amara et al. 2017). The results of the particle size corroborate with the data obtained with turbidity. Thus, based on particle size distribution analysis and turbidity, the aggregation of the complexes increased in all complexes with Pea proteins.

The results of the antioxidant capacity of the complexes, suggest the complexes formation with *P. brasiliensis* led to better exposure of peptide bonds that led to the

release of peptides with higher antioxidant capacity when compared to complexes with *M. furnieri*. Probably, in *P. brasiliensis*, the fusion of the antioxidant properties of Pea proteins not only added antioxidants but also activated the production of more antioxidants in the hydrolysates. The mechanism through which the protein hydrolysates exert their antioxidant activity is not fully understood. However, it is known that hydrophobic amino acids can increase the potency of antioxidant peptides of the hydrolysates through the proton donation capacity, electron donation capacity and/or direct lipid radical scavengers (Chalamaiah et al. 2012; Zamora-Sillero et al. 2018; Nikoo et al. 2019; Özogul et al. 2019). A hypothesis could be that Pea proteins exposed some regions with antioxidant capacity that increased the potency of antioxidant peptides of the hydrolysates (Chalamaiah et al. 2012; Zamora-Sillero et al. 2018; Nikoo et al. 2019; Özogul et al. 2019). However, future studies on the interaction between Pea proteins and protein hydrolysates should be performed to better understand the mechanisms of antioxidant production in the complexes coacervates.

4.3 *Spray-drying microcapsules containing antioxidant protein hydrolysates*

The microencapsulation process improves the nutrient retention in the food, preserves the stability of bioactive compounds during processing and storage, and delays the processes of degradation and functionality of the bioactive component (Gharsallaoui et al. 2012; Ozkan et al. 2019). The spray-drying is a technique that is related with the atomization of a liquid into a dry powder by means of an injector including a hot drying gas stream (Ozkan et al. 2019). Among the advantages of this technique are the easy reproduction, process speed, economics and simplicity (Ozkan et al. 2019). In this manner, the spray-drying is a technique very efficient that can be used to protect the antioxidant propriety of the protein hydrolysates (Zavareze et al. 2014).

In the present study, it was observed that the high temperatures of spray-dryer process decreased the antioxidant activity. Similar results were observed in the DPPH radical scavenging activity by increasing the drying temperature of spray drying (Akbarbaglu et al. 2019). According to Amara et al. (2017), peptides and/or proteins may undergo several stresses through exposure to heating during spray-drying and this can result in subsequent aggregation and denaturation, causing possible loss of their biological activity.

On the other hand, in the results of the complexes, it can be observed that the spray-dried process did not affected the antioxidant capacity significantly. Thus, the stability of the antioxidant activity of the complexes could either be attributed to the Pea proteins. The effects of the Pea protein as an effective wall material for microencapsulation have been described in the literature (Gharsallaoui et al. 2012). Recently, Le Priol et al. (2019) observed that the microencapsulation, when using Pea protein extracts, improved the sunflower oil oxidative stability. In this manner, Pea proteins can be a solution to protect bioactive compounds against the high temperatures of the microencapsulation process as observed in the present study. Thus, the complexation and subsequent microencapsulation by spray-drying using Pea proteins is an efficient way to protect the biological activity against the high temperatures of the microencapsulation process in protein hydrolysates.

As conclusion, complexation using Pea proteins is an efficient technique that increased the turbidity, particle size and antioxidant capacity of the hydrolysates obtained from bycatch. Additionally, the microencapsulation process of the complexes using the hydrolysates of the *P. brasiliensis* did not affected the antioxidant capacity, because the Pea proteins protected the antioxidant activity of protein hydrolysates. The results provide

evidence for the potential use of fish in the bycatch from shrimp fisheries as a functional ingredient or nutraceuticals with potential in the food industry.

5. Acknowledgments

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Considerações finais

A pesca de arrasto é uma atividade de grande importância socio-econômica para o país. O equilíbrio entre os aspectos econômicos, sociais e ambientais é essencial para solucionar os problemas que ainda existem neste tipo de pesca. Estratégias de gerenciamento mais eficientes, introdução de novas tecnologias e destinação economicamente viável aos animais que são capturados acidentalmente são ações necessárias para tornar o arrasto de fundo uma atividade sustentável. Neste aspecto, os resultados do presente estudo visaram contribuir para agregar valor ao rejeito da pesca e aproveitá-lo de maneira adequada. Os resultados demonstram que a hidrólise enzimática, utilizando as enzimas comerciais Alcalase 2.4 L® e Protamex®, é um método eficiente para liberar peptídeos de interesse econômico nas duas espécies mais abundantes de peixes (*Micropogonias furnieri* e *Paralonchurus brasilensis*) e nas duas mais abundantes de crustáceos (*Callinectes ornatus* e *Hepatus pudibundus*), podendo agregar valor ao *bycatch*. A hidrólise liberou peptídeos com atividade antioxidante em todas as amostras analisadas, submetidas às duas enzimas testadas. A coacervação complexa e subsequente microencapsulação por *spray-drying* mostrou-se eficiente para proteger a atividade antioxidante desses hidrolisados proteicos. Assim, os resultados fornecem evidências para o potencial uso dos hidrolisados das espécies analisadas como ingrediente funcional ou nutracêutico na indústria alimentícia, o que poderia garantir uma extração sustentável destes antioxidantes e oferecer um destino economicamente viável para a maior fração da fauna acompanhante. Por outro lado, esses resultados abrem uma perspectiva para novos estudos a fim de verificar a existência de outras funções biológicas nos extratos das quatro espécies estudadas e em outras espécies presentes na fauna acompanhante.