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JOSÉ DIEGO BARBOSA DA SILVA

ECOLOGIA TRÓFICA E DISTRIBUIÇÃO ESPAÇO-TEMPORAL DAS ESPÉCIES *POMADASYS RAMOSUS* (POEY, 1860) E *HAEMULOPSIS CORVINAEFORMIS* (STEINDACHNER, 1868) (HAEMULIDAE) AO LONGO DO GRADIENTE DE VARIAÇÃO NO ESTUÁRIO DO RIO GOIANA (PE/PB)

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Dissertação apresentada à Universidade Federal de Pernambuco como pré-requisito do programa de Pós-Graduação em Oceanografia para obtenção do título de Mestre em Oceanografia.

Área de concentração: Oceanografia
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Orientador: Prof. Dr. Mario Barletta
Coorientador: Dr. André Ricardo de Araújo Lima

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RESUMO

O estudo discute como a flutuação das condições ambientais de um ecossistema transicional reflete na utilização do habitat, na ecologia alimentar e na contaminação por microplásticos, das espécies *Pomadasys ramosus* e *Haemulopsis corvinaeformis* (Haemulidae), durante seu ciclo de vida, levando em consideração os aspectos espaciais e sazonais. As espécies foram capturados no canal principal do estuário do Rio Goiana, considerado uma área de proteção ambiental, a reserva extrativista Resex Acaú-Goiana. O estuário é dividido, de acordo com a geomorfologia e o gradiente de salinidade em estuários superior, intermediário e inferior. Foram realizados 6 arrastos de fundo mensais, totalizando 216 arrastos. A partir dos dados, as espécies foram classificada como estuarino-dependentes. A variabilidade na densidade dos Haemulidae foi influenciada pelas diferenças entre as áreas ($P < 0,05$), enquanto que a biomassa foi influenciada por diferenças entre as áreas, as estações e as fases ontogenéticas ($P < 0,01$). A presença de adultos e subadultos de *P. ramosus* exclusivamente nas áreas superior e intermediária do estuário está associada, principalmente a estação chuvosa, quando a salinidade teve as menores concentrações. Por outro lado, adultos e sub-adultos de *H. corvinaeformis* habitaram exclusivamente o estuário inferior durante a estação de seca, quando a salinidade aumenta no estuário. Entretanto, detectar as áreas de berçário para estas espécies ainda é difícil, uma vez que poucos espécimes juvenis foram capturadas. Juvenis de *P. ramosus* são zooplânctívoros, se alimentando principalmente de copépodas Calanoida (FO = 60% e $\%I_{RI}$ = 79%). Sub-adultos e adultos são zoobentívoros, se alimentando principalmente poliquetas (FO e $\%I_{RI} > 50$ a 100%). Juvenis de *H. corvinaeformis* não foram capturados no canal principal, mas os sub-adultos e adultos também apresentaram hábitos zoobentívoros, se alimentando principalmente de *Anomalocardia flexuosa* (FO = 71,4% a 100% e I_{RI} = 71,1% a 90%), *Mytella falcata* (FO = 41% a 100% e I_{RI} = 3,8% a 34%) e poliquetas (FO = 41% a 100% e I_{RI} = 14,4% a 16,6%). Durante o final da seca, *A. flexuosa* e *M. falcata* tiveram suas maiores médias de ingestão em número e peso por adultos ($P < 0,01$), enquanto que poliqueta foi o item mais ingerido em peso por sub-adutlos ($P < 0,01$). Dentre as espécies avaliadas, 25% dos juvenis, 75,3% dos sub-adultos e 85,7% dos adultos de *P. ramosus* estavam contaminados, enquanto que 68,7% dos sub-adultos e 46,4% dos adultos de *H. corvinaeformis* apresentaram ingestão de microfilamentos. Para *H. corvinaeformis*, a maior ingestão média de microfilamentos, que ocorreu na fase adulta,coincidiu com o pico de ingestão de *A. flexuosa*, no estuário inferior, duraente o final da estação seca ($P < 0,01$). Além disso, tal contaminação pode ser atribuída ao período em que estas fases mudam para uma

dieta mais diversa e iniciam estratégias de forrageamento mais complexas em invertebrados bentônicos. Dessa forma, a contaminação por microplasticos deve considerar hábitos espécie-específicos, uma vez que a ingestão de microplásticos é independente dos padrões de distribuição e da guilda trófica das espécies.

Palavras-chave: Haemulidae. Ecoclina de salinidade. Estuário tropical. Ingestão de microplasticos. Ontogenia. Conservação.

ABSTRACT

This study describes how the fluctuation of environmental conditions of a transitional ecosystem reflects habitat use, feeding ecology and even the microplastic contamination of two species *Pomadasys ramosus* and *Haemulopsis corvinaeformis* (Family: Haemulidae), during their life cycle, taking into account the seasonal and spatial aspects. The species were captured in the main channel of the Goiana Estuary, characterized as a marine protected area (Resex Acaú-Goiana). The ecosystem is divided into upper, middle and lower portions, according to its geomorphology and salinity gradient.Six monthly bottom trawls were made totalling 216 trawls. Regarding habitat use, these species were classified as estuarine-dependent. Differences in Haemulidae densities were caused by spatial variability ($P < 0.05$). Spatial, seasonal and ontogenetic factors were responsible for biomass differences ($P < 0.01$). The occurrence of adults and sub-adults of *P. ramosus*, exclusively in the upper and middle estuary is mainly related to the rainy season, when salinity reaches the lowest values in those habitats. On the other hand, adults and sub-adults of *H. corvinaeformis* inhabit exclusively the lower estuary, during the dry seasons, when salinity increases in the estuary. However, identification of nursery ground for both species is difficult, since only a few juveniles were sampled in the main channel. Juveniles of *P. ramosus* are zooplanktivorous, feeding mainly on Calanoid copepods (FO = 60% and $\%I_{RI}$ = 79%). Sub-adults and adults are zoobenthivores, preying mostly on polychaetes (FO and $\%I_{RI}$ > 50 to 100%). *H. corvinaeformis* juveniles do not occur in the main channel, but sub-adults and adults classified as zoobenthivores, feeding mainly on *Anomalocardia flexuosa* (FO = 71.4% to 100% and I_{RI} = 71.1% to 90%), *Mytella falcata* (FO = 41% to 100% and I_{RI} = 3.8% to 34%) and polychaeta (FO = 41% to 100% and I_{RI} = 14.4% to 16.6%). During the late dry season, *A. flexuosa* and *M. falcata* had highest ingestion rates in number and weight by the adults of *H. corvinaeformis* ($P < 0.01$), while polychaeta was the most important food item in weight for sub-adults ($P < 0.01$). Regarding the ontogenetic of *P. ramosus*, 25% of juveniles, 73.3% of sub-adults and 85.7% of adults were contaminated by microplastic filaments, while 68.7% of sub-adults and 46.4% of adults of *H. corvinaeformis* ingested microplastic filaments. For *H. corvinaeformes*, the highest mean contamination microplastic filaments occurred in the adult phase and coincided with the peak of ingestion of *A. flexuosa*, in the lower estuary, during the late dry season ($P < 0.01$). Moreover, the increase in contamination rates might be associeted to dietary shifts, when species change to a more diverece diet, using complex strategies to forage on benthic

invertebrates. Thus, studies on microplastic contamination might consider specie speciffic behaviours, once the contamination rates are highly influenced by distribution patterns and the tophic guild of species.

Keywords: Haemulidae. Sanility ecocline. Tropical estuary. Microplastic ingestion. Ontogeny. Conservation.

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SUMÁRIO

1 INTRODUÇÃO

(A presente dissertação é composta por dois documentos principais. O primeiro documento se refere à fundamentação teórica e justificativa do estudo, incluindo seus objetivos, hipóteses, materiais e métodos. O segundo documento se refere a um artigo científico produzido como resultado final do curso de mestrado do Programa de Pós-Graduação em Oceanografia - UFPE, que foi submetido (MS: ENVPOL_2018_530) ao periódico "Environmental Pollution", com fator de impacto 5,099. O artigo é intitulado "Use of Resources and Microplastic Contamination throughout the Life Cycle of Grunts (Haemulidae) in a Tropical Estuary").

Estuários são definidos como o encontro de águas continentais com as águas costeiras marinhas, produzindo uma diversidade de habitats biológiocos e abióticos altamente dinâmicos e controlados por escalas temporais de curto e longo prazo. A conexão entre esses ecossistemas permite o movimento de uma diversidade de peixes marinhos, estuarinos e de água doce de acordo com suas capacidades fisiológicas de suportar mudanças de salinidade, formando diversas assembleias de peixes ao logo do canal principal do estuário. A alta produtividade estuarina e seus diversos habitats são atrativos para que peixes realizem diversas atividades biológicas como alimentação, proteção, reprodução recrutamento e berçarios (Blaber, 2000; Barletta-Bergan et al., 2002*a,b*; Barletta et al., 2003; 2005., Dantas et al., 2010; Ramos et al.; 2014; 2016; Ferreira et al., 2016).

Um exemplo desses ecossistemas é o estuário do Rio Goiana, que fornece uma diversidade de serviços ecossistêmicos (fluxo de energia, abrigo, alimento natural) para as espécies de peixes completarem seu ciclo de vida, eu por sua vez são importantes meios de subsistência para as famílias que vivem em seu entorno (Barletta & Costa, 2009). Entretanto, impactos de origem antrópica vêm sendo observados ao longo de sua extensão, como o desmatamento para plantio de cana-de-açúcar, perda de manguezais para criação de camarão, dragagem do canal principal (Costa & Barletta., 2016), contaminação por metais (Barletta et al., 2012), poluentes orgânicos (Arruda-Santos et al., 2018) e resíduos sólidos (Lima et al., 2014). Essas atividades ameaçam a diversidade biológica, a produção pesqueira e a preservação dos valores culturais das comunidades da região (Barletta & Costa, 2009; Barletta et al., 2010; Blaber & Barletta, 2016; Barletta et al., 2016).

A fauna de peixes que vivem em ecossistemas estuarinos tropicais pode ser fortemente influenciada pela variação sazonal das variáveis ambientais e do gradiente de salinidade, causando mudanças nos padrões de distribuição e abundância das assembleias de peixes

nesses habitats aquáticos (Barletta et al., 2005; 2008; Barletta & Blaber, 2007; Blaber, 2000; Dantas et al., 2012*a*). Nos trópicos, os padrões sazonais na precipitação são responsáveis por mudanças fisico-químicas nos diferentes habitats do estuário, formando uma ecoclina sazonal que se movimenta ao longo do gradiente de salinidade e define os padrões de distribuição de uma espécie ao longo do seu ciclo de vida (Dantas et al., 2010; 2012*a*; Lima et al., 2015; Ramos et al., 2014; 2016). Assim, cada fase ontogenética de uma espécie utiliza diferentes habitats do estuário de acordo com sua necessidade fisiológica e uso dos recursos alimentares, respondendo de forma diferente a estas variações ambientais (Dantas et al., 2012*a*; 2015; Lima et al., 2014).

Além da relevância ecológica para a compreensão da biologia da espécie, o estudo da ecologia alimentar colabora com o desenvolvimento de estratégias para o manejo sustentável dos sítios de alimentação das espécies e fornece subsídios para a conservação dos mesmos, para que exista uma exploração racional dos recursos pesqueiros marinhos de valor comercial e de subsistência (Gasalla & Soares, 2001; Hahn & Delariva, 2003; Sousa et al., 2013). A maior parte das espécies da ictiofauna de ecossistemas estuarinos apresentam grande plasticidade na dieta (Lowe McConell,1999). Além disso, predadores alteram suas presas à medida que crescem e mudam de habitat, influenciados pelas diferenças sazonais e espaciais na disponibilidade de alimento (Lowe McConell,1999).

Trabalhos sobre ecologia trófica de peixes vêm destacando a ingestão de contaminantes ambientais como fragmentos de plástico (Possatto et al., 2011; Dantas et al., 2012*b*; Ramos et al., 2012; 2016; Ferreira et al., 2016). Em estuários, esses fragmentos tem origem nas ações antrópicas como o descarte impróprio de resíduos plásticos durante atividade de recreação/turismo, resíduos domésticos e agro-industriais, e principalmente pelo desgaste dos aparelhos utilizados na pesca local. Os fragmentos são principalmente provenientes da bacia do rio e da região costeira adjacente ao estuário (Lima et al., 2014). Durante seu tempo de residência nos ecossistemas, esses detritos sofrem fragmentações e tornam-se microplásticos (< 5 mm) (Barnes et al., 2009). No estuário do Rio Goiana foi identificado que a densidade de microplásticos representou metade da densidade de larvas de peixes e foi comparável com a densidade de ovos de peixes, indicando um alto nível de contaminação local (Lima et al., 2014). Estes fragmentos estão presentes em todos os habitats do estuário, tanto em massas d'água de superfície e de fundo, compartilhando os mesmos habitats que a ictiofauna estuarina durante todo ciclo sazonal (Lima et al., 2014; 2015).

Neste mesmo estuário, a ingestão de microfilamentos plásticos foi observada em 20% dos bagres da família Ariidae (*Cathorops spixii*, *Cathorops agassizii*, *Sciades herzbergii*) (Possatto et al., 2011), e em 13% das carapebas da família Gerreidae (*Diapterus rhombeus*, *Eugerres brasilianus*, *Eucinostomus melanopterus*) (Ramos et al., 2012). Nas pescadas da família Sciaenidae, foi registrada uma contaminação em 8% do gênero *Stellifer* (*Stellifer brasiliensis*, *S. stellifer*) (Dantas et al., 2012*b*) e 64% para a espécie *Cynoscion acoupa* (Ferreira et al., 2016). Segundo os autores, a ingestão de plástico pode resultar em bloqueio intestinal, causando morte por fome, assimilação de poluentes orgânicos persistentes e metais traços (Possatto et al., 2011; Dantas et al., 2012*a*, 2015; Ramos et al., 2012; Ferreira et al., 2016). Essas concentrações de contaminantes interferem na ecologia alimentar e consequentemente na pesca artesanal que é fortemente desenvolvida nas comunidades tradicionais do entorno do estuário do Rio Goiana (Barletta & Costa, 2009). Atualmente, é as espécies de peixes que habitam nesse ecossistema estuários estão contaminadas por microplásticos.

Dentre essas espécies estão incluídas as da família Haemulidae, como *Pomadasys ramosus* (Poey, 1860) and *Haemulopsis corvinaeformis* (Steindachner, 1868) (Figura 1). Ambas são espécies de importância econômica e de subsistência, distribuídas desde o Caribe até a costa Atlântica subtropical, sendo comuns em áreas estuarinas (Araújo et al., 1998; Chaves & Corrêa, 2000; Froese & Pauly 2017; Hackradt, et al., 2009; Menezes & Figueiredo, 1980; Santana et al., 2013). Nesse sentido, as espécies de peixes da Família Haemulidae são encontradas nos oceanos Atlântico, Índico e Pacífico, habitam principalmente fundos lamosos e ambientes turvos, sendo encontrados em ambientes marinhos e estuarinos, e raramente em água doce (Carpenter, 2002). Estudos sobre a ecologia e distribuição de *P. ramosus* e *H. corvinaeformis* na costa Atlântica são escassos. Neste sentido, conhecer melhor suas ecologia alimentar e os padrões de distribuição são fundamentais para fornecer informações sobre seu papel trófico e funcional dessas espécies no escossistema estuarino.

No sentido de ampliar o conhecimento sobre estudos estuarinos em ecologia de peixes e suas interações com fatores abióticos (salinidade, oxigênio dissolvido, temperatura, transparência da água e precipitação), incluindo sua contaminação por microplásticos, este estudo surge como uma ferramenta para avaliar como esses compartimentos interagem ao longo de escalas temporais e sazonais canal principal do estuário do Rio Goiana. Assim, será possível descrever a população desses indivíduos em termos de estrutura ecológica e uso dos recursos disponíveis.

Apesar da área de estudo ser considerada uma reserva extrativista desde 2007, ainda não existe um plano de manejo para a região. Levando em consideração que diversos estudos sobre ecologia e os impactos antropogênicos vem aumentando numa região de constante ocupação e ação antrópica, ainda é importante enfatizar a importância da identificação do papel desses habitats para a ontogenia das espécies presentes no local. O estudo irá adicionar dados que contribuam para a aplicação de medidas de manejo voltadas á preservação desses habitats, bem como propor medidas para proteger essas espécies durante seu período dentro do ecossistema.

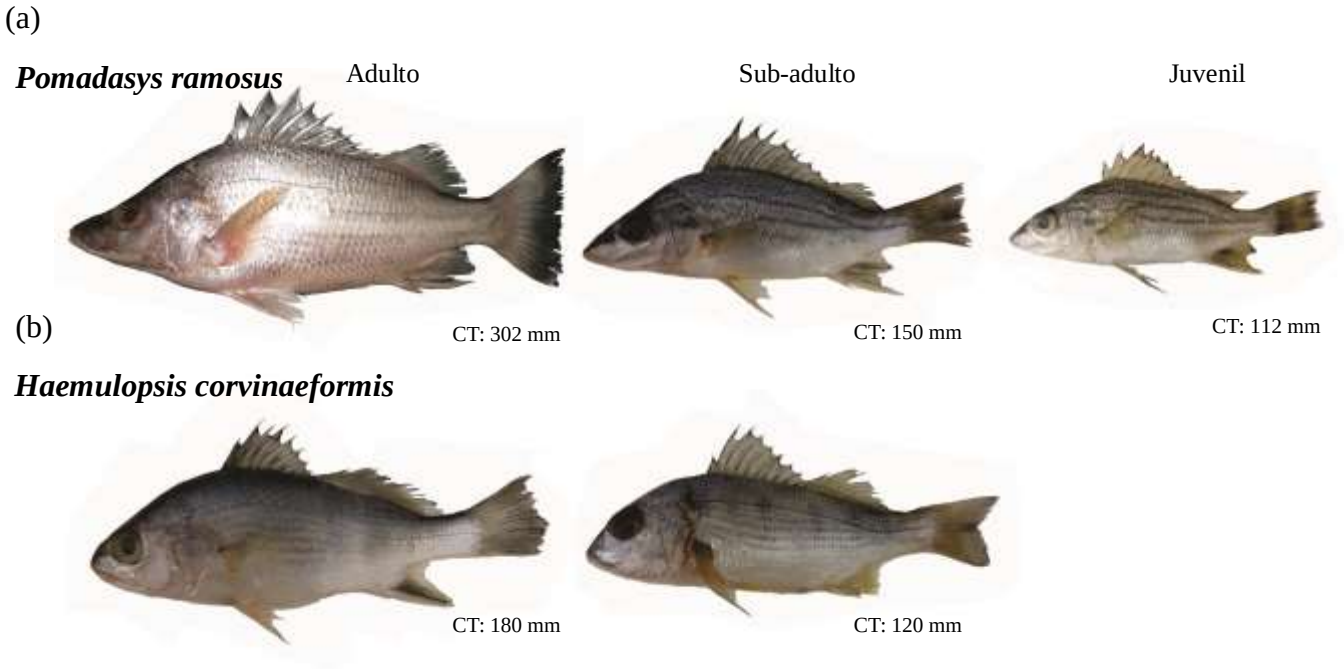

Figura 1- Fases ontogenéticas das espécies da família Haemulidae. (a) *Pomadasys ramosus*; (b) *Haemulopsis corvinaeformis*, CT: Comprimento total. Fonte: LEGECE.

2 OBJETIVOS E HIPÓTESES

2.1 Objetivo geral

Avaliar a distribuição, a ecologia trófica e a dinâmica de ingestão de microfilamentos (diferentes cores) das diferentes fases ontogenéticas das espécies *P. ramosus e H. corinaeformis* (juvenil, sub-adulto e adulto) nos diferentes habitats (superior, intermediário e

inferior) do estuário do Rio Goiana ao longo do ciclo sazonal (início e final da chuva; início e final da seca).

2.2 Objetivos específicos

- Avaliar as variações espaciais e temporais na distribuição e abundância das diferentes fases ontogenéticas de *P. ramosus* e de *H. corvinaeformis* no canal principal do estuário do Rio Goiana;
- Identificar os principais itens alimentares na dieta das diferentes fases ontogenéticas das espécies e suas variações em escalas temporais e espaciais, incluindo a frequência de ocorrência e a importância relativa de cada item;
- Avaliar a influencia dos fatores espaciais e temporais na dinâmica de ingestão de microfilamentos pelas diferentes fases ontogenéticas de *P. ramosus* e *H. corvinaeformis* no canal principal do estuário e suas relações com a ecologia das espécies.
- Avaliar a influência da variabilidade ambiental (salinidade, temperatura da água, oxigênio dissolvido, precipitação e transparência da água) nos padrões de uso dos recursos estuarinos pelas espécies.

2.3 Hipóteses

1*)* H_0*:* As diferentes fases ontogenéticas das duas espécies de Haemulidae (*P. ramosus* e *H. corvinaeformis*) se distribuem de forma homogênea nos diferentes habitats do canal principal do estuário do Rio Goiana, independente da sazonalidade local.

2) H_0*:* Não há variação na dieta (número e peso dos intens) e na ingestão de microfilamentos (número) em diferentes cores pelas diferentes fases ontogenéticas das espécies em relação aos fatores espaciais e temporais no canal principal do estuário do Rio Goiana.

3 MATERIAIS E MÉTODOS

3.1 Descrição da área de estudo

O ecossistema estuarino do Rio Goiana possui um canal principal de 17 km de comprimento e uma área de 4.700 ha, incluindo o canal principal e a floresta de manguezal circundante (Barletta & Costa, 2009). Em 2007, o estuário do Rio Goiana tornou-se a Resex Acaú-Goiana, uma unidade de conservação federal classificada como reserva extrativista. Ela está localizada no Nordeste do Brasil (7°31'S; 34°53'W), na fronteira entre os estados de Pernambuco (Goiana) e Paraíba (Caaporã e Pitimbu) (Barletta & Costa, 2009) (Figura 2). A área de estudo foi dividida em três porções de acordo com o gradiente de salinidade e a geomorfologia do canal principal, em estuário superior (salinidade entre 0 e 5), intermediário (salinidade entre 5 e 20) e inferior (salinidade entre 20 e 35) (Barletta & Costa, 2009).

Este estuário tem como característica principal o clima semiárido tropical, com uma

Figura 2- Estuário tropical do Rio Goiana. As demarcações (----) representam as áreas (habitats) do estuário. (●) Cidade de Goiana. Fonte: Google Earth.

estação seca que vai de setembro a fevereiro (< 50 mm $mês^{-1}$) e uma estação chuvosa que vai de março a agosto (> 400 mm $mês^{-1}$) (Barletta & Costa, 2009). Estas duas estações são subdivididas em início da seca (setembro a novembro), final da seca (dezembro a fevereiro), início da chuva (março a maio) e final da chuva (junho a agosto) (Barletta & Costa, 2009).

3.2 Coleta de dados

Os especimens analisados nesse trabalho foram coletados no canal principal do estuário do Rio Goiana, mensalmente entre dezembro de 2005 e novembro de 2006, e no fim da chuva e final da seca entre Dezembro de 2007 e Agosto de 2009, com autorização pelo Sistema de Autorização e Informação em Biodiversidade-SISBIO (licença nº: 11050-1).

Em cada porção do estuário (superior, intermediário e inferior) foram realizados 6 arrastos mensais (réplicas), perfazendo todo ciclo sazonal anual, totalizando 216 amostras (Barletta et al., 2008) (Figura 3). Após cada arrasto os peixes foram etiquetados, armazenados em gelo e conduzidos ao laboratório, onde foram fixados em formol ou armazenados em freezers. As capturas dos peixes foram feita através de arrasto com portas utilizando uma rede de 8,72 m de comprimento com malha de 35 mm no corpo e 22 mm no saco (entre nós) (tralha superior com 7,1 m e inferior com 8,5 m) (Figura 4). Para obtenção de uma amostragem representativa dos intervalos de classe dos peixes, foi utilizada uma malha de rede como sobre-saco com um tamanho de 5 mm (Figura 4).

3.3 Cálculo da densidade e biomassa

A área arrastada com a rede de arrasto com porta (Ar) foi calculada de acordo com a equação:

$$Ar = D*H* X_2$$

Onde, D corresponde a distância percorrida pela rede (m), H é o comprimento (m) da tralha superior e X_2 é a fração do comprimento da tralha superior (m), que representa a largura

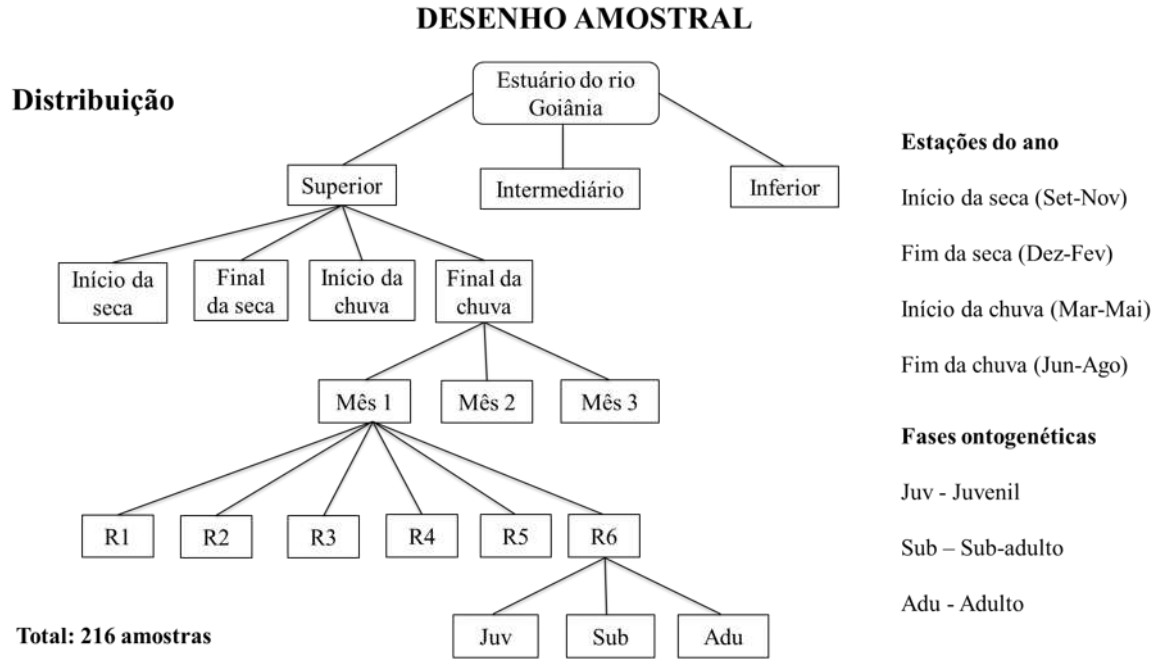

Figura 3- Desenho amostral utilizado para coletas dos espécimes de *Pomadasys ramosus* e *Haemulopsis corvinaeformis*. Fonte: LEGECE.

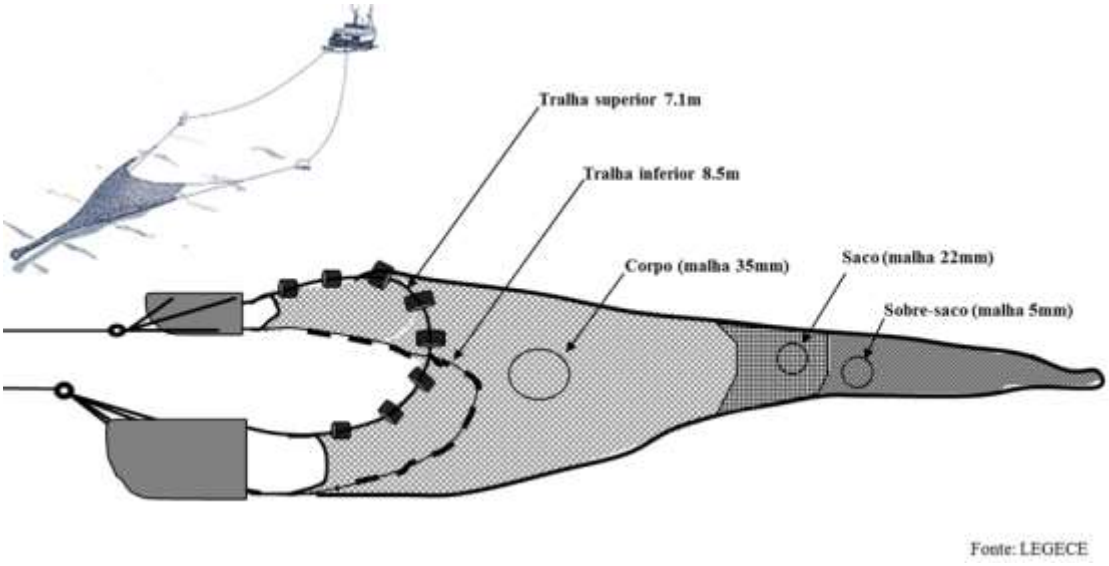

Figura 4- Arte de pesca (rede de arrasto) utilizada nas capturas das espécies em suas diferentes fases ontogenéticas no canal principal do estuário Rio Goiana. Fonte: LEGECE.

do percurso realizado pela abertura da rede. Essa abertura pode variar com a velocidade do arrasto sendo equilibrada em uma velocidade média continua de 3,5 nós h^{-1}. No entando a velocidade de arrasto foi em média de 3,5 km h^{-1}. Nessa velocidade a abertura da rede se manteve em média 0,5 m. A captura por unidade de área arrastada (CPUA) foi utilizada para calcular a densidade (ind.ha^{-2}) e a biomassa (g.ha^{-2}) dividindo o número e o peso área assastada. Nesse sentido, o cálculo de densidade e biomassa para área arrastada foi obtida utilizando a formula:

$$\text{Densidade} = \text{número/Ar (ind. ha}^{-1})$$

$$\text{Biomassa} = \text{peso (g)/Ar (g. ha}^{-1})$$

3.4 Variáveis ambientais

Antes de cada arrasto, foram coletados dados referentes às variáveis ambientais, como salinidade, temperatura da água (ºC), oxigênio dissolvido (mg/L) e saturação de oxigênio dissolvido na água (%) com multiparâmetro (Wissenschaftlich Technische Werkstätten, WTW OXI 325) e profundidade do disco de Secchi (cm). Os dados referentes à precipitação foram compilados desde 2005 da estação meteorológica mais próxima, “Curado 82900”, localizada em Recife-PE (INMET, 2006). Os dados das variáveis ambientais referentes ao canal principal do estuário foram obtidos desde 2005 pelo grupo de pesquisa do Laboratório de Ecologia e Gerenciamento de Ecossistemas Costeiros e Estuarinos (LEGECE) através dos

projetos CNPq (37384/2004-7; 474736/2004; 482921/2007-2; CT-Hidro 29/2007/ CNPq Nº 552896/2007-1 e 405818/2012-2/COAGR/PESCA) e da Fundação de Amparo à Ciência e Tecnologia de Pernambuco, FACEPE (APQ-0586-1.08/06 e APQ-0911-1.08/12).

3.5 Descrição das fases ontogenéticas

Para a classificação diferentes fases ontognéticas foram utilizados dois diferentes critérios. A partir da curva peso *vs.* comprimento foi usado para distinguir os juvenis dos subadultos a partir da equação de crescimento alométrico. O crescimento alométrico foi calculado como uma função do comprimento total (*CT*) de acordo com o modelo $Wg = \beta_0 CT^{\beta 1} + \varepsilon$, onde *Wg* (peso) é a variável dependente, *CT* a variável independente, β_0 o intercepto e β_1 o coeficiente de crescimento (Huxley, 1924). Para cada intervalo de dados que diferencia cada uma das fases, o coeficiente de crescimento do modelo de regressão foi calculado e sua robustez foi mensurada pelo calculo do r^2 (coeficiente de determinação) (Zar, 1996). No crescimento isométrico, β_1 é 3 (Van Snik *et al.*, 1997). Quando β_1 é menor que a curva isométrica, é conhecido com crescimento alométrico negativo; quando maior, crescimento alométrico positivo (Van Snik *et al.*, 1997). Desse modo, o crescimento da curva indica o momento em que os indivíduos passam a crescer mais rapidamente em comprimento do que em peso, indicando mudança no coeficiente de crescimento da curva de um padrão alométrico negativo ($\beta_1 < 3$; cresce mais rapidamente em peso do que em comprimento) para alométrico positivo ($\beta_1 > 3$; cresce mais rapidamente em comprimento do que em peso).

O comprimento de primeira maturação L_{50} é o comprimento no qual 50% dos indivíduos da população estão aptos a reproduzir, e foi utilizado para distinguir sub-adultos de adultos. Ele foi calculado através da regressão logística da relação da frequência de indivíduos adultos por classe de comprimento total (cm) (Lewis & Fontoura, 2005), conforme a equação:

$$F = 1/ (1 + e^{a + b \times L})$$

Onde, F representa a frequência de adultos em cada classe de comprimento, L é o ponto médio de cada classe de comprimento total e “a” e “b” são parâmetros da regressão (coeficiente linear e angular respectivamente). Os parâmetros “a” e “b” foram estimados através dos mínimos quadrados da função anterior linearizada:

$$- \ln [(1/F) - 1] = a + b \times L.$$

O comprimento de primeira maturação estimado pelo $L_{50} = - a/ b$, onde, a e b são parâmetros similares utilizados na equação anterior (Lewis & Fontoura, 2005).

3.6 Análise dos itens estomacais

Os peixes coletados foram identificados de acordo com Carpenter (2002) e Menezes & Figueiredo (1980). Em seguida foram triados, pesados (g). A aferidos as medidas morfométrias (comprimentos total e padrão). Em seguida, foi realizada uma incisão na cavidade abdominal dos espécimes, e suas gônadas foram removidas para avaliação e classificação dos estágios de maturação reprodutiva (Vazzoler., 1996). Os tratos digestivos de todas espécimes também foram removidos para avaliar a ecologia alimentar e a contaminação por microfilamentos. Todos esses materiais foram armazenado em formol tamponado a 4%. Um corte longitudinal nos tratos digestivos foi realizado para retirada do conteúdo estomacal e microfilamentos, que, posteriormente, foram identificados, pesados e armazenados.

A análise do conteúdo estomacal foi realizada em microscópio estereoscópico, todos os itens alimentares foram classificados até o menor nível taxonômico possível, seguindo literatura especializada (Brusca & Brusca, 2002; Ruppert et al., 2004). Já para identificação e classificação dos microplásticos (microfilamentos) foram utilizados tratamentos específicos. Os microfilamentos (< 5 mm) foram contabilizados e caracterizados por cores, em cada estômagos analisados. Para se certificar de que esses itens eram de fato microplásticos, eles foram levados à estufa a 70 °C por um período de 48 h. Os itens que murcharam ou secaram, foram descartados por serem considerados matéria orgânica natural, enquanto os itens que mantiveram suas características estruturais e cores, foram considerados microplásticos.

Para quantificação dos itens alimentares, foram utilizados os índices de frequência de ocorrência (%FO), em número (%N) e em peso (%W). A frequência de ocorrência de cada presa representa a presença ou ausência dos itens nos estômagos que foi obtida com $F_i = 100\ F_i\ F_t^{-1}$, em que o F_i é o número de estômagos contendo o item *i* e F_t é o número total de estômagos examinados (Hyslop, 1980). Na frequência em número (%N), o número total de cada item alimentar é expresso como porcentagem do número total de itens encontrados em todos os estômagos analisados por $\%N_i = 100\ N_i\ N_t^{-1}$, sendo, N_i é o número do item *i* e N_t é o número total de itens nos estômagos dos indivíduos analisados (Hyslop, 1980). A frequência em peso (%W) foi calculada com $\%W_i = 100\ W_i\ W_t^{-1}$, onde, W_i é a massa do item alimentar e W_t é a massa total de itens nos estômagos.

O índice de importância relativa ($\%I_{RI}$) une os três métodos acima citados, sendo expresso com a equação proposta por Pinkas et al. (1971): $\%I_{RI}$ = %F (%N + %W) Esse índice avalia a importância de cada item alimentar em função de sua frequência de ocorrência, e suas frequências em peso e número.

3.7 Análise estatística

Uma análise de variância fatorial (ANOVA – 4 fatores), foi usada para testar se houve diferença significativa entre a densidade e a biomassa das diferentes fases ontogenéticas (fator 1) das espécies de Haemulidae (*P. ramosus* e *H. corvinaefirmis*) (fator 2) em relação às áreas do estuário (fator 3), e as estações do ano (fator 4) (Zar, 1996). Uma ANOVA fatorial (ANOVA – 3 fatores) também aferiu se houver diferenças significativas no número e peso de cada item alimentar encontrado no conteúdo estomacal das espécies, em relação as fases ontogenéticas (fator 1), as porções do estuário (fator 2), as estações do ano (fator 3) (Zar, 1996). O teste de Levene foi utilizado para verificar a homogeneidade dos dados. Quando for encontradas diferenças significativas, o teste de Bonferroni foi utilizado para detectar as fontes de variância (nível de significância de 5%) (Quinn & Keough, 2002).

Uma análise de correspondência canônica (CCA) (CANOCO para Windows 4.5) foi realizada para detectar correlações entre as variáveis ambientais e a importância relativa dos itens alimentares (ter Braak e Smilauer, 2002). Múltiplas regressões dos mínimos quadrados foram computadas das médias ponderadas de densidades de *P. ramosus* e *H. corvinaeformis* (juvenil, subadulto e adulto), assim como a importância relativa ($\%I_{RI}$) dos itens alimentares para cada fase ontogenética, áreas do estuário e estaçõ do ano. A precipitação, temperatura da água, oxigênio dissolvido e salinidade foram variáveis independentes (ter Braak, 1986; Palmer, 1993). A partir desses procedimentos, foram produzidas variáveis ambientais como vetores. A intensidade do vetor esteve relacionada com a influência da variável ambiental na formação dos grupos compostos pelos dados de alimentação e distribuição dos espécimes.

4 USE OF RESOURCES AND MICROPLASTIC CONTAMINATION THROUGHOUT THE LIFE CYCLE OF GRUNTS (HAEMULIDAE) IN A TROPICAL ESTUARY

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ABSTRACT

The distribution, feeding ecology and microplastic contamination were assessed in different ontogenetic phases of Haemulidae species inhabiting the Goiana Estuary, over a seasonal cycle. *Pomadasys ramosus* and *Haemulopsis corvinaeformis* are estuary dependent species that use habitats with specific environmental conditions in each season. *Pomadasys ramosus* was mainly found in the upper and middle estuary during the rainy season, when salinity showed low values. *Haemulopsis corvinaeformis* was exclusively found in the lower estuary during the dry season, when salinity increases in the estuary. Juveniles of *P. ramosus* are zooplanktivores, feeding mainly on calanoid copepods. Sub-adults and adults are zoobenthivores, feeding on invertebrates associated to the bottom, mainly Polychaeta. Juveniles of *H. corvinaeformis* were not found in the main channel, but sub-adults and adults showed a zoobenthivore, feeding mainly on *Anomalocardia flexuosa* (Mollusca: Bivalvia). Dietary shifts along the life cycle and the spatio-temporal relationship between their distribution and the availability of microplastics along the estuary seem to have a strong influence in the ingestion of microplastic filaments. A proportion of 25% of juveniles, 75.3% of sub-adults and 85.7% of adults of *P. ramosus*; while 68.7% of sub-adults and 46.4% of adults of *H. corvinaeformis* were contaminated. The highest average ingestion of microplastic filaments by *P. ramosus* coincided with the peak of ingestion of Polychaeta by sub-adults in the upper estuary during the late rainy season. For *H. corvinaeformis* the high ingestion of microplastic filaments coincided with the peak of ingestion of *A. flexuosa* by adults in the lower estuary during the late dry season. Such contamination might be attributed to the time when these phases shifted to a more diverse diet and began a complex foraging on benthic invertebrates. Research on microplastic contamination must consider species-specific

behaviour, since the intake of microplastics is dependent on patterns of distribution and trophic guild within fish assemblages.

Keywords: environmental gradients, fish ontogeny, spatio-temporal distribution, feeding ecology, ingestion of microplastics

Capsule: Contamination of *Pomadasys ramosus* and *Haemulopsis corvinaeformis* with microplastics depends on the relationship between the seasonal use of estuarine habitats and dietary shifts along life cycle.

INTRODUCTION

Estuaries are transitional ecosystems where freshwater of a river connects to coastal marine waters through a salinity gradient (Barletta and Dantas, 2016). The connection between these two systems allows the displacement of a diversity of marine, estuarine and freshwater fishes, which use estuaries as feeding, protection, reproduction and recruitment (Blaber, 2000; Barletta-Bergan et al., 2002a,b; Barletta et al., 2003; 2005; 2008 Lima et al., 2014, 2015). Beyond these functions, estuaries provide a variety of habitats that are used temporarily as nursery for juveniles of many fish species according to their spatio-temporal distribution patterns (Able, 2005; Dantas et al., 2010; Elliott et al., 2007; Ferreira et al., 2016).

Estuarine reaches recognized as upper, middle and lower estuary, and their continuum forms the estuarine ecocline (Barletta et al., 2017a). Each of these habitats is an environmental unit with different abiotic characteristics. Since most estuaries are highly dynamic, seasonal variations in precipitation, especially in the tropics, are responsible for shifting physico-chemical conditions among these different habitats (Barletta et al., 2017a), moving the ecocline up and down stream, depending on river flow and tides. These seasonal variations define the distribution patterns of fish and invertebrate species along their life cycles. Thus, it is possible to define periods of reproduction, seasonal nursery habitats and the spatio-temporal use of estuarine resources (Barletta et al., 2008; Potter et al., 2013; Sheaves et al., 2015; Watanabe et al., 2014). Among these resources are food, highly available in estuaries taking into account the highly productive and complex food webs (Wolff et al., 2000; Dantas et al. 2013; Costanza et al., 2014; Ramos et al., 2014), and shelter.

Most fishes change their diets and the use of estuarine habitats according to developmental characteristics and physiological requirements (Dantas et al., 2015, Ferreira et al., 2016).

Studies regarding trophic ecology gain more attention, since ingestion of microplastics by economically and subsistence important fishes has been widely demonstrated (Bråte et al.,

2016; Jovanovic, 2017; Lusher et al., 2013; Silva-Cavalvanti *et al*., 2017). This is of special interest in estuarine systems, which are semi-enclosed environments where ingestion of microplastics is enhanced due to the high availability of this contaminant. Increased availability results from multiple source proximity, retention processes and intense access to food resources by many species (Zhao et al., 2014; Lima et al., 2014; 2015; Fok and Cheung, 2015; Lebreton et al., 2017).

The Goiana Estuary provides a diversity of environmental services and natural resources for commercial and subsistence fishes, as well as for key fish and invertebrates species (Barletta and Costa, 2009). Besides being such an important area for resident traditional fishery communities, the estuary shows signs of anthropogenic impacts and changes (Barletta et al., 2017a,b). These impacts include mangrove and Atlantic rain forest deforestation for sugar cane plantation, mining, dredging, limestone quarry and shrimp farming. Also, contamination of fish with mercury might be a concern (Barletta et al. 2012; Barletta et al., 2017a,b). Finally, contamination by solid wastes and microplastics also documented (Lima et al., 2014; 2015; 2016).

This last problem was studied according to the seasonal distribution patterns of microplastics along the salinity ecocline of the Goiana Estuary (Lima et al., 2014). This includes their possible sources, as well as the ingestion of microplastic filaments of plastic by different phases of fishes that inhabit and/or use the estuary (Possatto et al., 2011; Dantas et al., 2012; Ramos et al., 2012; Ferreira et al., 2016; 2018). Microplastics have an average density of more than 26 fragments per m^3 in the main channel of the estuary, in surface and bottom water masses, along the whole seasonal cycle. Microplastic filaments represented more than 0.36 fragments per m^3 (Lima et al., 2014).

In the Goiana Estuary, ingestion of microplastic filaments was observed in 20% of Ariidae catfishes (*Cathorops spixii*, *Cathorops agassizii*, *Sciades herzbergii*) (Possatto et al., 2011); 13% of the Gerreidae mojarras (*Diapterus rhombeus, Eugerres brasilianus, Eucinostomus melanopterus*) (Ramos et al., 2012); 8% of drums (*Stellifer brasiliensis, S. stellifer*) (Dantas et al., 2012) and; 64% of the acoupa weakfish (*Cynoscion acoupa*) (Ferreira et al., 2016).

The presence of plastic debris in aquatic environments is a threat to fish and their predators well reported for marine ecosystems (Fossi et al., 2012; Dantas et al., 2012; Davidson and Dudas, 2016; Wright et al., 2013). Organisms that ingest plastics reduce the absorption of food, suffer from intestinal injuries, entanglement and even death (Moore, 2008;

Guebert-Bartholo et al., 2011; Wright et al., 2013). Once plastics are ingested by a fish, it can pass lower trophic level to top predators, increasing the chance of transportation of plastic debris from one habitat to another (Ferreira et al., 2016; Tosetto et al., 2017; Ferreira et al., 2018). Also, microplastics can be incorporated into fish tissues, causing damage at the cellular level, and carrying with them adsorbed organic pollutants that will then also contaminate fish products (Lusher et al., 2017).

In estuaries, hydrodynamic forces resulting from the encounter of water masses of different densities cause turbulence. When combined to wind direction and tidal influence, this environment can trap inanimate particles, such as microplastics (Lima et al., 2014). This is specially true during the driest periods, when riverflow is weak. In the rainy season, it is probable that microplastics will be more easily flushed seaward (Lima et al., 2014; Lebreton et al., 2017). Comparable size, shape, smell and densities of microplastics and living organisms will increase the chances of interaction between microplastics and biota, since it can be easily mistaken by live prey of similar characteristics by all ontogenetic phases of fishes (Galloway et al., 2017). Currently, it is asserted that all fishes are prone to be contaminated by microplastics (Ferreira et al., 2016; Silva-Cavalcanti et al., 2017; Vendel et al., 2017; Ferreira et al., 2018).

Among these species are those of the Haemulidae family. *Pomadasys ramosus* (Poey, 1860) and *Haemulopsis corvinaeformis* (Steindachner, 1868) are important commercial and subsistence fishes distributed from the Caribbean basin to the subtropical southwestern Atlantic coast (Menezes and Figueiredo, 1980; Araújo et al., 1998; Chaves and Corrêa, 2000; Hackradt, et al., 2009; Froese and Pauly 2017). Therefore, to understand how the ecological requirements of these species can determine their contamination with microplastics using spatio-temporal approaches, it is important to detect contamination patterns along their life cycle and the relationship between natural and non-natural food items. For this reason, this study aims to assess how the different ontogenetic phases (juveniles, sub-adults and adults) of two Haemulidae species (*P. ramosus* and *H. corvinaeformis*) use the different habitats (upper, middle and lower) of an estuary over a full seasonal cycle (rainy and dry seasons), regarding their distribution patterns, feeding ecology and consequent dynamics of contamination with microplastics. It also assesses the influence of seassonal fluctuations of environmental variables (salinity, rainfall, temperature, transparency and dissolved oxygen) in the patterns of estuarine uses by the ontogenetic phases of these species.

4.1 Materials and methods

4.1.1 Study area

The Goiana Estuary has a main channel 17 km long, and the total flooded area is ~4,700 ha, allowing the dominance of a mangrove forest (Barletta and Costa, 2009) (Fig. 5). The estuary is located on the northeast coast of South America (7° 31'S; 34°53'W) where climate is tropical, and the rainfall regime defines two simetrically distributed seasons: the dry season with rainfall < 50 mm month^{-1} and the rainy season with rainfall > 360 mm month^{-1} (Barletta and Costa, 2009). These two seasons can be further divided into early dry (September to November), late dry (December to February), early rainy (March to May) and late rainy seasons (June to August) (Barletta and Costa, 2009) (Fig. 6a).

The Goiana River estuary is a salt wedge estuary and the annual rainfall variability is responsible for the migration of the wedge seaward during the late rainy season; and back upstream in the driest months (Lima et al., 2015). In 2007, the estuary became a marine

protected area, classified as an extractive reserve (MPA-ER) denominated Resex Acaú-Goiana (Barletta and Costa, 2009). This ecosystem provides habitat for a diversity of animal and plant species that are important natural resources for the local traditional populations.

4.1.2 Sampling design

The estuary is divided into three distinct habitats according to the gradient of salinity

Figure 5- Goiana Estuary. The upper, middle and lower portions of the estuary are indicated by the dotted lines (-----). Fonte: Google Earth.

and its geomorphology: upper (salinity 0 - 5), middle (salinity 5 - 20) and lower estuaries (salinity >20) (Barletta and Costa, 2009) (Fig. 6b).

Specimens of *P. ramosus* and *H. corvinaeformis* were collected from the main channel using an otter-trawl net 8.2 m-long, with 35 mm mesh-size in the body and 22 mm in the cod-end (between knots) (head hope with 7.1 m and ground rope with 8.5 m). For a representative sampling of different size classes, it was used an additional cod-end cover of 5 mm. Six monthly replicates were performed in each portion of the estuary (upper, middle, lower) during 15 min, between December 2005 and November 2006, encompassing the entire seasonal cycle and totalling 216 samples to be used in the spatio-temporal distribution study. Moreover, additional sampling were performed during the late dry and late rainy seasons, from December 2007 to August 2009. This last data were used exclusively for the feeding ecology study. After each trawl, fishes were tagged, stored in ice and conducted to the laboratory.

4.1.3 Environmental variables

Data on environmental variables such as salinity, water temperature (°C), dissolved oxygen (mg L^{-1}) (Wissenschaftlich Technische Werkstätten, WTW OXI 325) and the Secchi depth (cm) were collected before each sampling in the surface and bottom. Precipitation rates were compiled from the nearest meteorological station (INMET, 2006).

4.1.4 Laboratory procedures

Fishes were identified according to Carpenter (2002) and Menezes and Figueiredo (1980). After identification, all specimens were measured (total length) and weighted. To distinguish between juveniles and sub-adults it was used the relation of curveof the weight *vs.* length relationship curve (Appendix 1, 2). Thus, juveniles were every individual below the inflexion point. To distinguish between sub-adults and adults it was used the average length at first maturation (L_{50}), the length at which 50% of the individuals of a population are ready to reproduce. The L_{50} was calculated through the logistic regression according to the equation proposed by Lewis and Fontoura (2005)

$$F = 1/(1 + e^{a + b* L}) \quad (1)$$

where, F is the frequency of adult in each length class, L is the middle point of the class at each length, “a” is the linear coefficient and “b” is the angular coefficient. The parameters “a” and “b” were estimated through the least squares of the linearized function

$$- Ln [(1/F) - 1] = a + b * L \quad (2)$$

Thus, sub-adults are every individual below the L_{50}, while adults those above it. Therefore, juveniles of *P. ramosus* are individuals < 110 mm, sub-adults are between > 110 and 300 mm, and adults are > 300 mm (Apendix 1 a, b). Juveniles of *H. corvinaeformis* are those specimens < 112 mm, sub-adults of are between (112 mm and 137 mm) and adults are > 137 mm (Appendix 2).

Juveniles of *H. corvinaeformis* and *P. ramosus* were not captured during the annual cycle of 2005/2006 and for that reason this ontogenetic phase was not included in the spatio-temporal analysis of distribution patterns. However, juveniles of *P. ramosus* were captured in the coastal areas adjacent to the estuary and were included in the feeding ecology analysis.

In total, 122 specimens of *P. ramosus* and 42 of *H. corvinaeformis* were assessed in the distribution patterns study. For the feeding ecology study, 125 stomachs of *P. ramosus* (8 juveniles, 89 sub-adults and 28 adults) and 44 of *H. corvinaeformis* (16 sub-adults and 28 adults) were examined to evaluate the spatio-temporal changes in dietary ontogenetic shifts. Stomachs were dissected under stereomicroscopy - Zeiss; STEMI, 2000-C (5 x). Natural prey were identified to the lowest possible taxonomic level, following the specialized literature (Brusca and Brusca, 2002; Ruppert et al., 2004). Non-natural items were identified and microplastics were separated. All ingested items were weighted and counted for further analysis.

To assert the presence of plastics in the gut contents, supposed particles were oven dried at 70 °C for 48 h. Withered fragments were considered natural organic matter, while those fragments without significant changes in their physical characteristics, not easily cut or broken were classified as plastics. Plastics were photographed and measured with the aid of the Axiovision LE Software. Fragments with < 5 mm were classified as microplastics, and characteristics such as colour. Microplastics were submitted to the same numerical and statistical treatment as natural food items.

The index of relative importance (%I_{RI}) was used to assess the degree of importance of each food item and microplastics to each ontogenetic phase (Pinkas et al., 1971)

$$\%I_{RI} = \%F*(\%N + \%W) \quad (3)$$

where, %F is the frequency of occurrence of item *i*, %N is the frequency in number of item *i* and %W is the frequency of item *i* in weight.

4.1.5 Statistical analyses

A factorial analysis of variance (ANOVA) was used to test whether significant differences in the density and biomass of *P. ramosus* and *H. corvinaeformis* occured according to patterns of distribution of the different ontogenetic phases (sub-adults and adults)

along estuarine habitats (upper, middle and lower) and seasons (early and late dry, early and late rainy) (Zar, 1996). Another factorial analysis of variance (three-way ANOVA) was used to test whether significant differences in diet (number and weight of prey items and microplastics) of different ontogenetic phases of *P. ramosus* (juvenile, sub-adults and adults) and *H. corvinaeformis* (sub-adults and adults) varied along estuarine habitats and seasons (Zar, 1996). The Levene test was used to check the homogeneity of the data (Quinn and Keough, 2002). These data were Box-Cox transfomed reach to normality (Box and Cox, 1964). Whenever significant differences were observed, the Bonferroni *post hoc* test was used to detect the sources of variance ($\alpha = 0.05$) (Quinn and Keough, 2002).

A canonical correspondence analysis (CANOCO 5) was performed to detect correlations between environmental variables and food preferences along the ontogeny of both species (ter Braak and Smilauer, 2002). Multiple regressions of the least squares were computed from the relative importance (%I_{RI}) of food items and (%N) microplastics for each ontogenetic phase of the two species. The %I_{RI}, ontogenetic phases, estuarine habitats and seasons were used as dependent variables. Precipitation, water temperature, dissolved Oxygen, Secchi depth and salinity were independent variables (ter Braak, 1986; Palmer, 1993). This procedures result in a triplot, where the environmental variables are as vectors.Intensity of vectors reflects the strenght of the influence of the environmental variable on the groups formed by the distribution patterns and feeding ecology.

4.2 RESULTS

4.2.1 Habitat characterization according to environmental variables

Average salinities showed high variability and were distinct in the upper of areas middle and lower, also revealing vertical stratification (Fig. 6b). During the early and late dry season (September-February), it is observed the highest values of salinity along the entire main channel, demonstrating an efficient saline intrusion, which can even reach the upper estuary when precipitation is minimal (5 to 80 mm monthly). During the early rainy season (March-May), when precipitation increases (150 to 350 mm), the salinity decreases along the main channel, responding to increased river discharge. The highest precipitation are observed in the late rainy season (June-August) (160 to 360 mm) and, therefore, the greatest river discharge is responsible to decrease the salinity to minimal values in the three areas of the estuary (Fig. 6b).

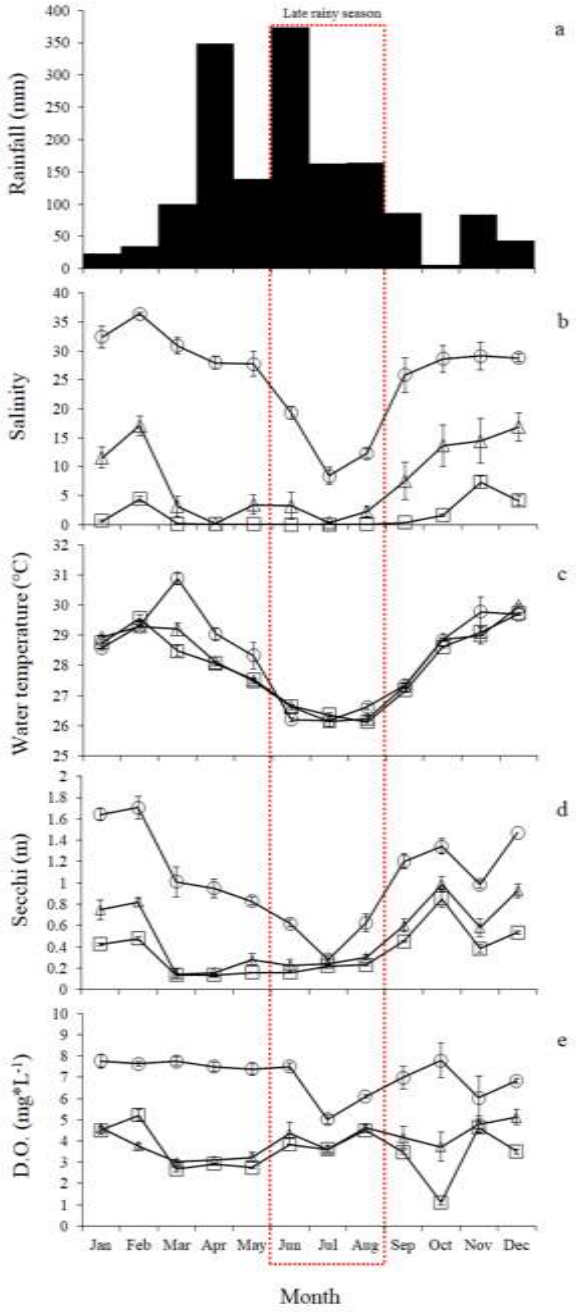

Figure 6- (a) Total monthly rainfall and averages and standard error (+S.E.) (b) salinity, (c) water temperature (°C), (d) Secchi depth and (e) dissolved Oxygen (mg L^{-1}) in the three areas (□ upper, Δ middle, ○ lower) of the Goiana Estuary from December 2005 to November 2006. The end of the rainy season are indicated by red. Fonte: Autor.

Water temperature varied similarly in the three areas of the estuary, except in the early rainy season, when temperature differed significantly among the areas (Fig. 6c). In the early rainy season, water temperature starts to decrease along the entire main channel, reaching

minimal values in the late rainy season (26 °C). In the late dry season, the average water temperature increases again in the main channel, reaching 30 °C (Fig. 6c).

The average Secchi depths were highest in the lower estuary (0.34 to 1.7 m), intermediate in the middle (0.1 to 1 m) and lowest in the upper estuary (0.1 to 0.9 m) (Fig. 6d). This demonstrates that transparency decreases from the upper estuary downstream to the lower estuary. However, during the rainy season (March-August), the average Secchi depth decreases in the three areas, emphasizing that turbidity increases along the entire main channe, when river runoff increases (Fig. 6d).

Dissolved oxygen had the highest averages in the lower estuary (5 to 8 mg L^{-1}) , with lowest averages during the late rainy season (Fig. 6e). In the upper and middle estuaries, dissolved oxygen varied between 1 and 5 mg L^{-1}, reaching lower averages during the early rainy season; and during October in the upper estuary. The greatest variability of this variable was observed during the dry season (September-February), probably due to the intrusion of saline coastal waters (Fig. 6e).

4.2.2 Spatial and temporal patterns of species distribution in the estuary

In total, 167 specimens were captured from the main channel of the Goiana River estuary, being 122 of *P. ramosus* and 42 of *H. corvinaeformis*. *Pomadasys ramosus* had a total mean density of 50 ind. ha^{-1} (sub-adult: 36 ind. ha^{-1}; adult: 14 ind. ha^{-1}) and biomass of 6.6 kg ha^{-1} (sub-adult: 2806.22 g. ha^{-1}; adult: 3775.17 g ha^{-1}), while *H. corvinaeformis* had total mean density of 21 ind. ha^{-1} (sub-adult: 9 ind. ha^{-1}; adult: 12 ind. ha^{-1}) and total mean biomass of 1 kg ha^{-1} (sub-adult: 295.34 g. ha^{-1}; adult: 746.49 g. ha^{-1}) (Appendix 3).

The variability in the density of the Haemulidae species was influenced by the differences among, areas principaly in area upper ($F=3.67$, $P < 0.05$). while biomass was influenced by the variables space (areas), time (seasons) and ontogenetic phases ($F=4.95$, $P < 0.01$) (Fig. 7 a, b, Appendix 4). Moreover, significant interactions among the factor areas *vs.* seasons *vs.* species were detected, corroborating the hypothesis that the movement patterns, density and biomass distribution of Haemulidae species along the main channel of the Goiana Estuary is influenced by the spatio-temporal variations of environmental variables salinity, dissolved oxygen, temperature, Secchi and ranifall (Fig 7 a, b, Appendix 4).

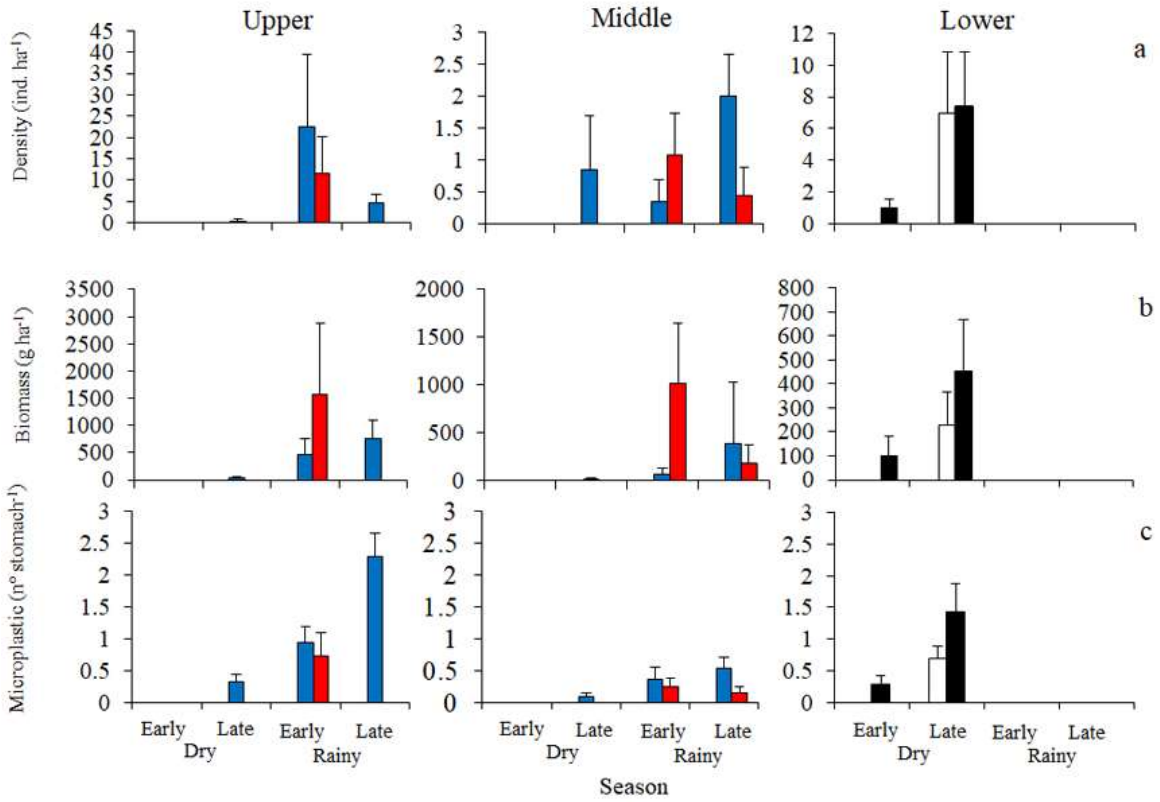

Figure 7- Average and standard error (±S.E.) density (a) biomass (b) and microplastic (c) of the different ontogenetic phases of *P. ramosus* (■ sub-adult and ■ adult) and *H. corvinaeformis* (□ sub-adult and ■ adult) along the main channel of the Goiana Estuary (upper, middle and lower), over the seasonal cycle (early and late dry seasons; and early and late rainy seasons). Fonte: Autor.

The presence of *P. ramosus* in the main channel is associated with the rainy season, when specimens were found in the upper and middle estuary (Fig. 7). Sub-adults and adults had the highest mean densities (22.6 and 11.65 ind. ha^{-1}, respectively) in the upper estuary during the early rainy season ($P < 0.05$) (Fig.3 a, b and Appendix 4). The highest values of mean biomass of adults (1561.1 g ha^{-1}) is observed in the early rainy season, and of sub-adults (759.3 g ha^{-1}) in the late rainy season, both in the upper estuary ($F=13.46$, $P < 0.05$) (Fig.7 b and Appendix 4).

The species *H. corvinaeformis* inhabited exclusively the lower estuary. Sub-adults are found during the late dry season, while adults are found during the early and late dry seasons (Fig.7 a, b, Appendix 4). However, their highest mean densities and biomasses were observed during the late dry season (Fig. 7 a, b).

4.2.3 Ontogenetic diet shifts, spatio-temporal feeding patterns and microplastic contamination

4.2.3.1 Feeding ecology and microplastics contamination of P. ramosus

The stomach contents analysis of 125 individuals of *P. ramosus* contained 18 food items (Apendix 5) and 6 items of microplastics filaments (Appendix 10). Annelids (*e.g.* Nereidae and Syllidae Polychaeta) and microplastic filaments were the most important items (Appendixes 6 a, b, c and d).

Calanoid copepod was the item with highest relative importance in the diet of juveniles ($\%I_{RI}$ = ~79%), which were fed exclusively in the lower estuary in the early rainy season (Appendix 7). However, polychaet were the most important item with highest relative importance in the diet of sub-adults and adults throughout the seasonal cycle and the three areas of the estuary ($\%I_{RI}$ >50 to 100%) (Appendix 7).

The highest mean values of ingestion of Polychaeta (number and weight) were observed in sub-adults and adults in the upper estuary during the late rainy season ($F=45.6$, $P < 0.01$) (Appendix 8, Fig. 8 and Appendix 9). In addition, the ingestion of Polychaeta showed a significant interaction among area *vs.* season *vs.* ontogenetic phase ($F=4.9$, $P < 0.01$). It suggests that, with the seasonal fluctuation of the salinity gradient along the main channel of the estuary, the different ontogenetic phases of *P. ramosus* showed movement patterns between different reaches of the estuary.The most important prey was Polychaeta (Fig. 8, Appendix 8, 9). It also suggests that this species ingested microplastics available on the sediment during foraging and/or through ingestion of natural prey previously contaminated (Appendix 6).

All ontogenetic phases had specimens contaminated by microplastics, especially by blue-coloured filaments, in all areas of the estuary and throughout the entire seasonal cycle (Fig. 9). The frequency of occurrence of microplastic filaments was of 50% in juveniles inhabiting the lower estuary in the late rainy season (Appendix 5). For sub-adults and adults, the frequency of occurrence of microplastics varied between 67% and 100% in the upper and middle estuary along the seasons (Appendix 5). It suggests that, for adults and sub-adults, microplastic contamination occurs in the upper and middle portions of the estuary.

The highest fish contamination with microplastic filaments of different colours was registered in the upper estuary during the rainy season (Fig. 9, Appendix 10). Among the 6 types of microplastic filaments found in the gut contents of *P. ramosus*,blue microplastic filaments was the most important, and showed significant differences amond area, season and ontogenetic phase (Fig. 9, Appendix 10). The highest averages of ingestion of these items in

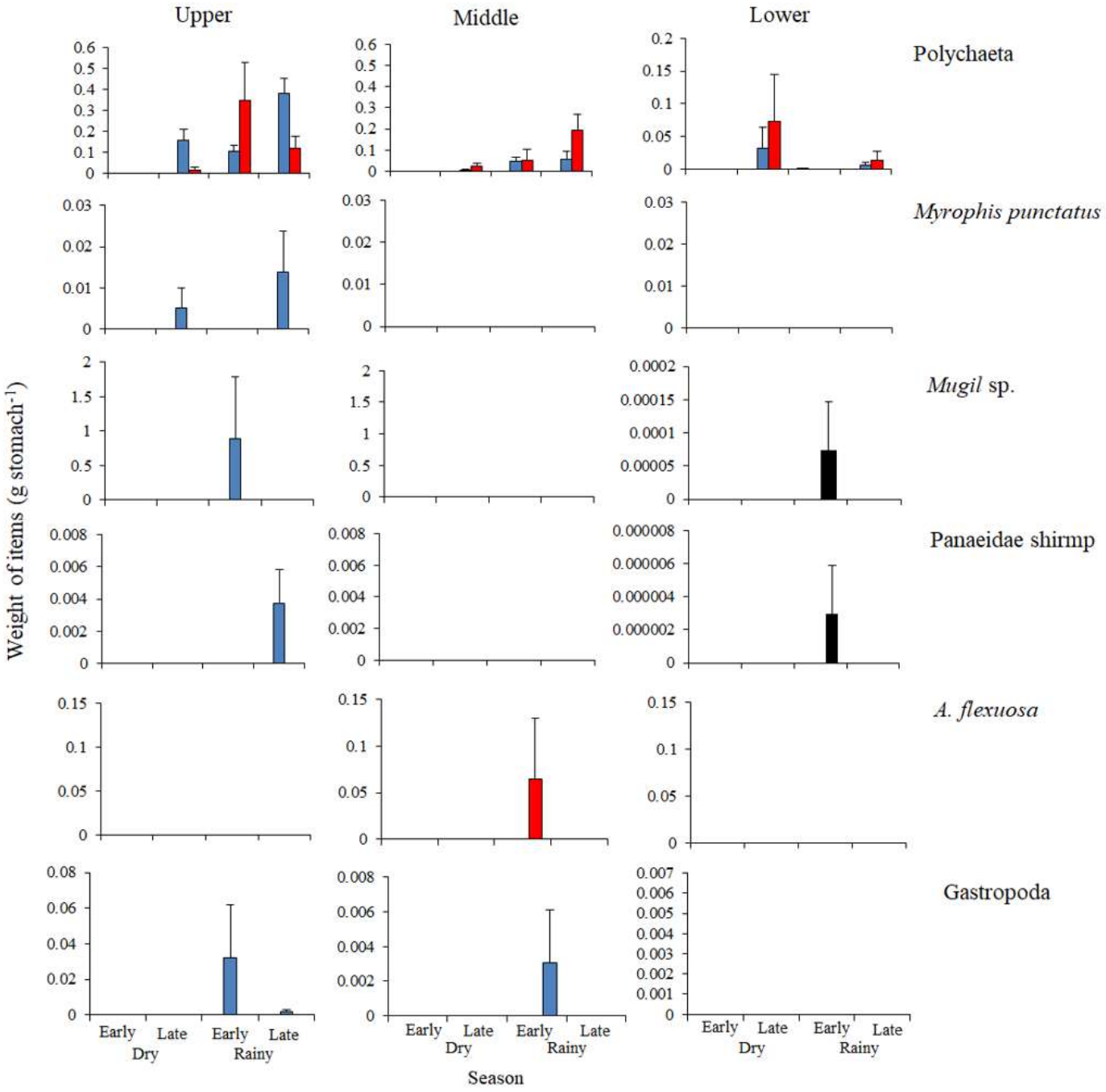

Figure 8 - Average and standard error (±S.E.) weight of prey items ingested by different ontogenetic phases of *P. ramosus* (■ Juvenile, ■ sub-adult and ■ adult) along the main channel of the Goiana Estuary (upper, middle and lower), over the seasonal cycle (early and late dry seasons; and early and late rainy seasons). Fonte: Autor.

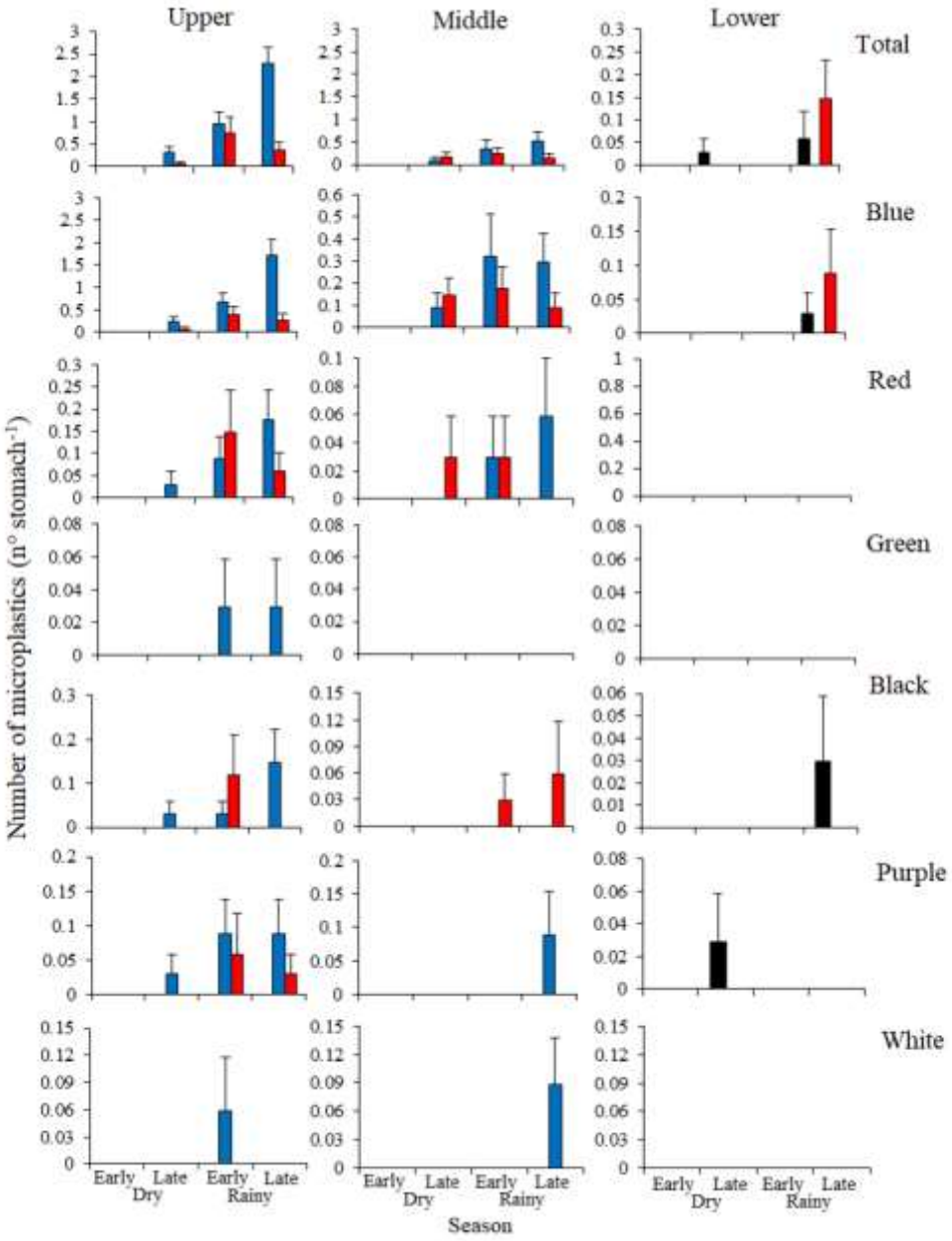

Figure 9 - Average and standard error (±S.E.) number of microplastics filaments ingested by different ontogenetic phases of *P. ramosus* (■ juvenile, ■ sub-adult and ■ adult) along the main channel of the Goiana Estuary (upper, middle and lower), over the seasonal cycle (early and late dry seasons; and early and late rainy seasons). Fonte: Autor.

number was observed in sub-adults in the upper estuary in the late rainy season ($F=31.42$, $P < 0.01$). Moreover, significant interaction among the factors area *vs.* season *vs.* ontogenetic phase was detected ($F=3.82$, $P < 0.01$) (Fig. 9 and Appendix 10). It suggests that, microplastics contamination vary in time and space, and during the *P. ramosus* growth. On the other hand, red and black microplastic filaments had high averages of ingestion by adults

in the early rainy season and by sub-adults in the late rainy season, when considering the upper estuary (Fig. 9, Appendix 10).

4.2.3.2 Feeding ecology and microplastics contamination of H. corvinaeformis

In total, 44 specimens of *H. corvinaeformis* were analysed, being 16 sub-adults and 28 adults for this stage of the work. From these, 16 food items and microplastics filaments were identified (Appendix 11; Appendix 6 e,f, g, h). During the dry season, the most important prey item for sub-adults of *H. corvinaeformis* was *Anomalocardia flexuosa* (FO = 71.4%; $\%I_{RI}$ = 71.1%) (Appendix 11, 12). Adults fed mainly on *Mytella falcata* (Mollusca: Bivalvia) and Polychaeta during early dry season (FO=100% for both items; $\%I_{RI}$ = 3.8% and 16.6 %, respectively) and late dry season (FO= 41 % for both items; $\%I_{RI}$ = 33.9 and 14.4 %, respectively). During the rainy season, adults fed on *A. flexuosa* (FO= 100%; $\%I_{RI}$ > 90%) (Appendix 11 , 12).

In the lower estuary, during the rainy season, adults of *H. corvinaeformis* fed exclusively on *A. flexuosa* (F=20.53, $P < 0.01$) (Fig. 10; Appendix 13,14). On the other hand, during the early dry season occurred the highest averages of ingestion in number and weight of Brachyuran crab (F=2.66, $P < 0.05$). During the late dry seasson, *A. flexuosa*, *M. falcata* and *Callinectes* sp. were the most important food items (F=25.28, 5.32, 2,74, $P < 0.01$) (Fig. 10; Appendixes 13, 14). The most important item ingestd by sub-adults of *H. corvieniformis* in number was Polychaeta (F=38.8, $P < 0.01$), and in weight it was *A. flexuosa* during the late dry season ($P < 0.01$) (Fig. 10; Appendixes 13, 14).

This marine species (*H. corvieniformis*) uses the lower portion of the estuary during the dry season when they are searching for food in the different habitats of this portion of the estuary. During this moment, they also ingest microplastic filaments. In total, 173 microplastics filaments were ingested by *H. corvinaeformis* (Appendix 6e, f, g and h).

Microplastic filaments had different colours (blue, read and black) and had highest averages of ingestion in the lower estuary during the late dry season ($P < 0.01$) (Fig. 11; Appendix 15). The total microplastic filaments showed the highest relative importance for adults of *H. corvinaeformis* ($\%I_{RI}$ = 20.15) in the early dry season (Appendix 11). However, the highest averages of ingestion in number of these items by adults occurred in the lower estuary in the late dry season ($P < 0.01$) (Fig. 3c and Appendix 15).

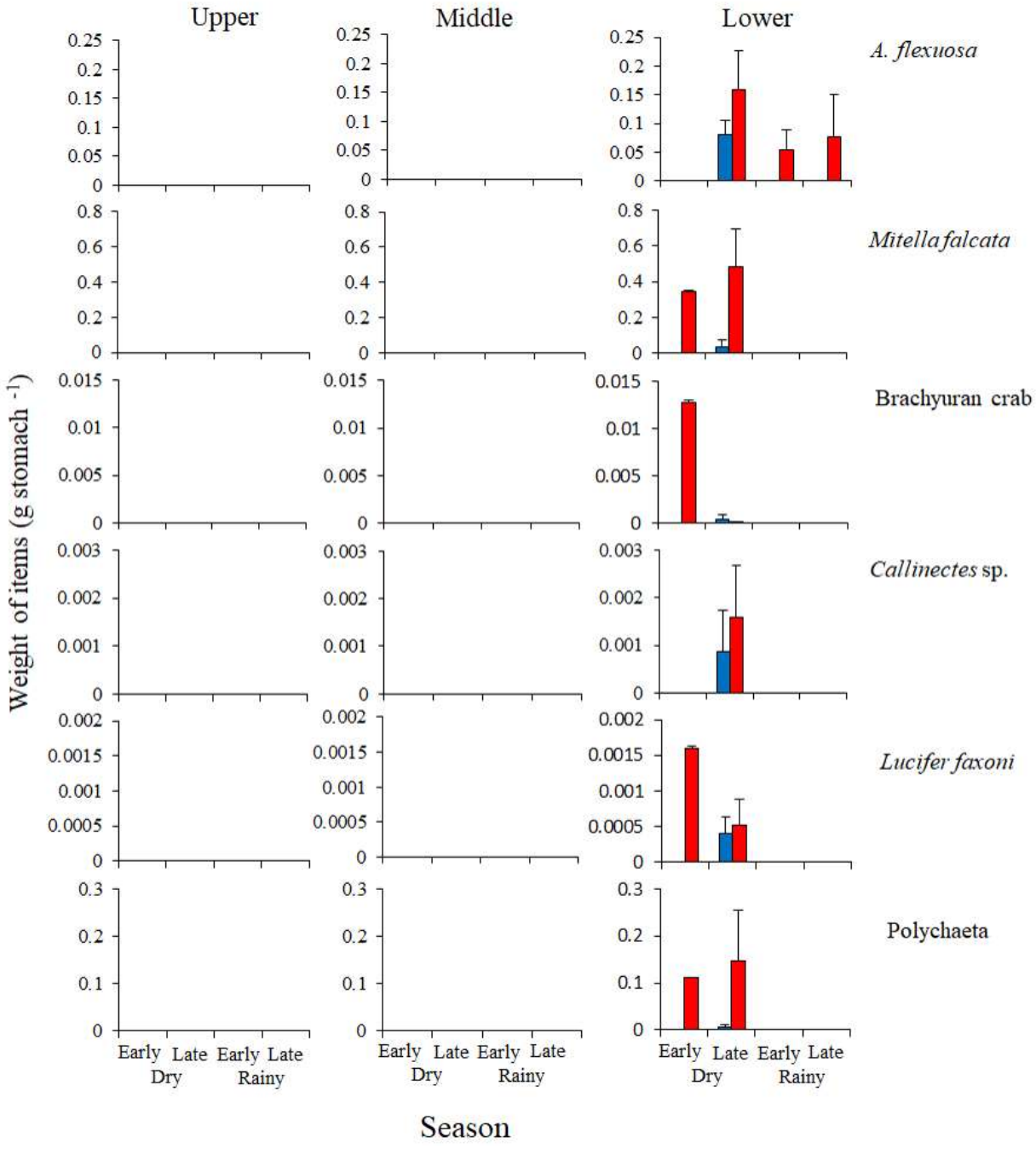

Figure 10 - Average and standard error (±S.E.) weight of prey items ingested by different ontogenetic phases of *H. corvinaeformis* (■ sub-adult and ■ adult) along the main channel of the Goiana Estuary (upper, middle and lower), over the seasonal cycle (early and late dry seasons; and early and late rainy seasons). Fonte: Autor.

4.3.3 Environmental influence on the P. ramosus and H. corvinaeformis feeding ecology and microplastic contamination

A canonical correspondence analysis (CCA) was used to assess the influence of seasonal variability of environmental factors on the distribution, feeding ecology and patterns of microplastic contamination of different ontogenetic phases of *P. ramosus* and *H. corvinaeformis* along the salinity gradient of the Goiana River Estuary (Fig. 12, Appendix 16). The first axis (Axis I) explains ~62% of data distribution and has positive correlations with salinity ($P < 0.01$) and dissolved oxygen ($P < 0.05$). Therefore, representing the salinity gradient of the estuary. The left side of Axis I represents the upper estuary and the right side represents the lower estuary (Fig. 12). Seasons are represented by the second axis (Axis II), which explains 20% of the data distribution. Axis II correlated positivelly with rainfall and negativelly with water temperature. The upper portion of the second axis represents the rainy season, and the bottom represents the dry season (Fig. 12).

Polychaeta, blue and purple microplastic filaments are located in the centre of the data ordination. It suggests that these items had high relative importance for both species throughout the seasonal cycle and areas of the estuary (Fig. 12). Black and white microplastic filaments, Gastropoda, *Mugil* sp. and *M. punctatus* correlated with sub-adults and adults of *P. ramosus* in the upper and middle estuaries, during the early and late rainy seasons, and late dry season. These items were ingested when rainfall increased, and water temperature and dissolved oxygen decreased upstream (Fig. 12 and Appendix 16).

Green and red microplastic filaments, *M. falcata*, *A. flexuosa*, Brachyuran crab, *L. faxoni* and *Callinectes* sp. correlated with sub-adults and adults of *P. ramosus* and *H. corvinaeformis* in the lower estuary, during the early and late dry seasons. These items were ingested when rainfall decreased, and water temperature and dissolved oxygen increased downstream (Fig. 12). Panaeidae shrimps correlated with juveniles and sub-adults of *P. ramosus* and adults of *H. corvinaeformis* in the lower estuary, during the late rainy season. This item was ingested when river discharge was higher (*i.e.* higher rainfall) and influenced the lower estuary (Fig. 12 and Appendix 16).

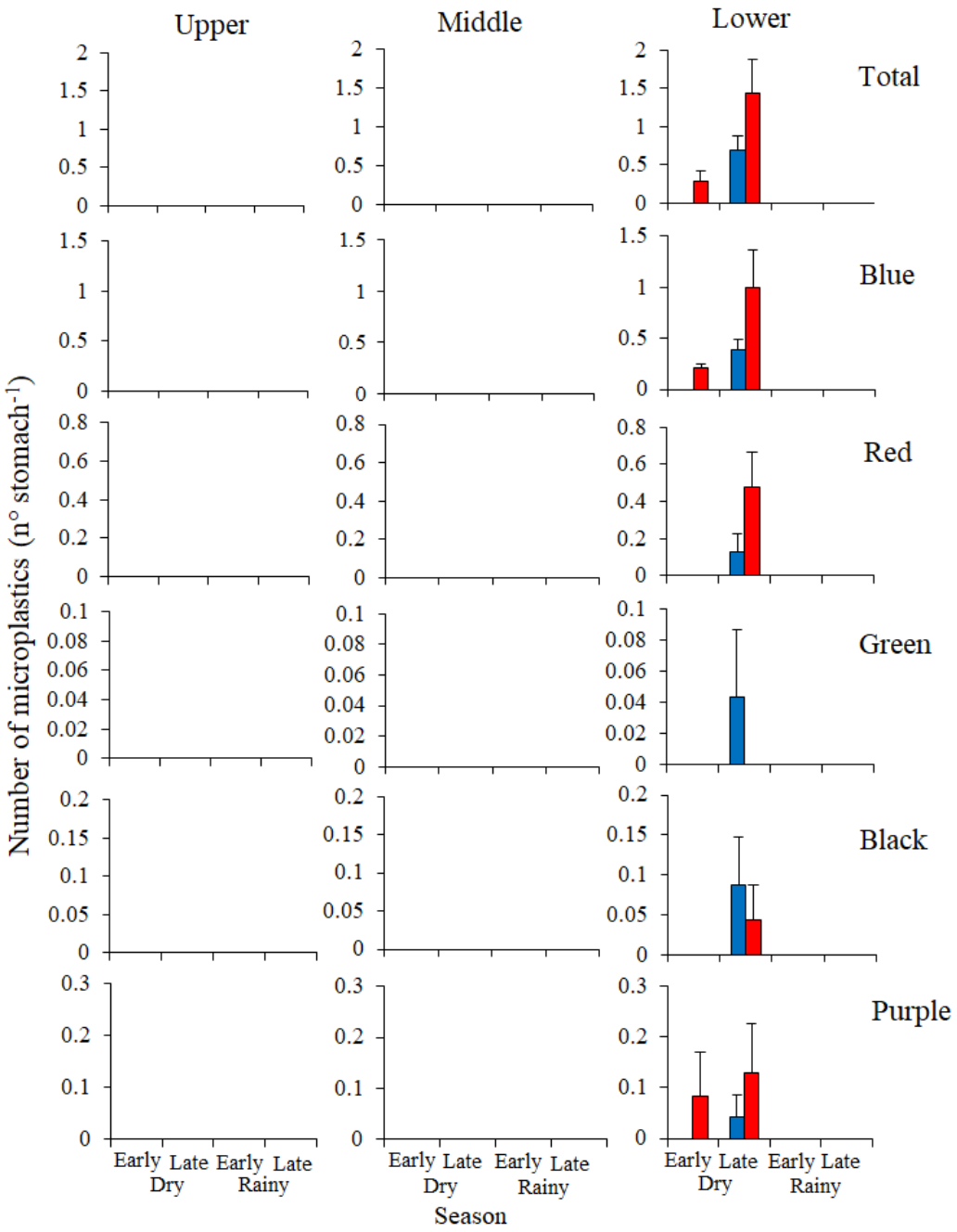

Figure 11 - Average and standard error (±S.E.) number of microplastics filaments ingested by different ontogenetic phases of *H. corvinaeformis* (■ sub-adult and ■ adult) along the main channel of the Goiana Estuary (upper, middle and lower), over the seasonal cycle (early and late dry seasons; and early and late rainy seasons). Fonte: Autor.

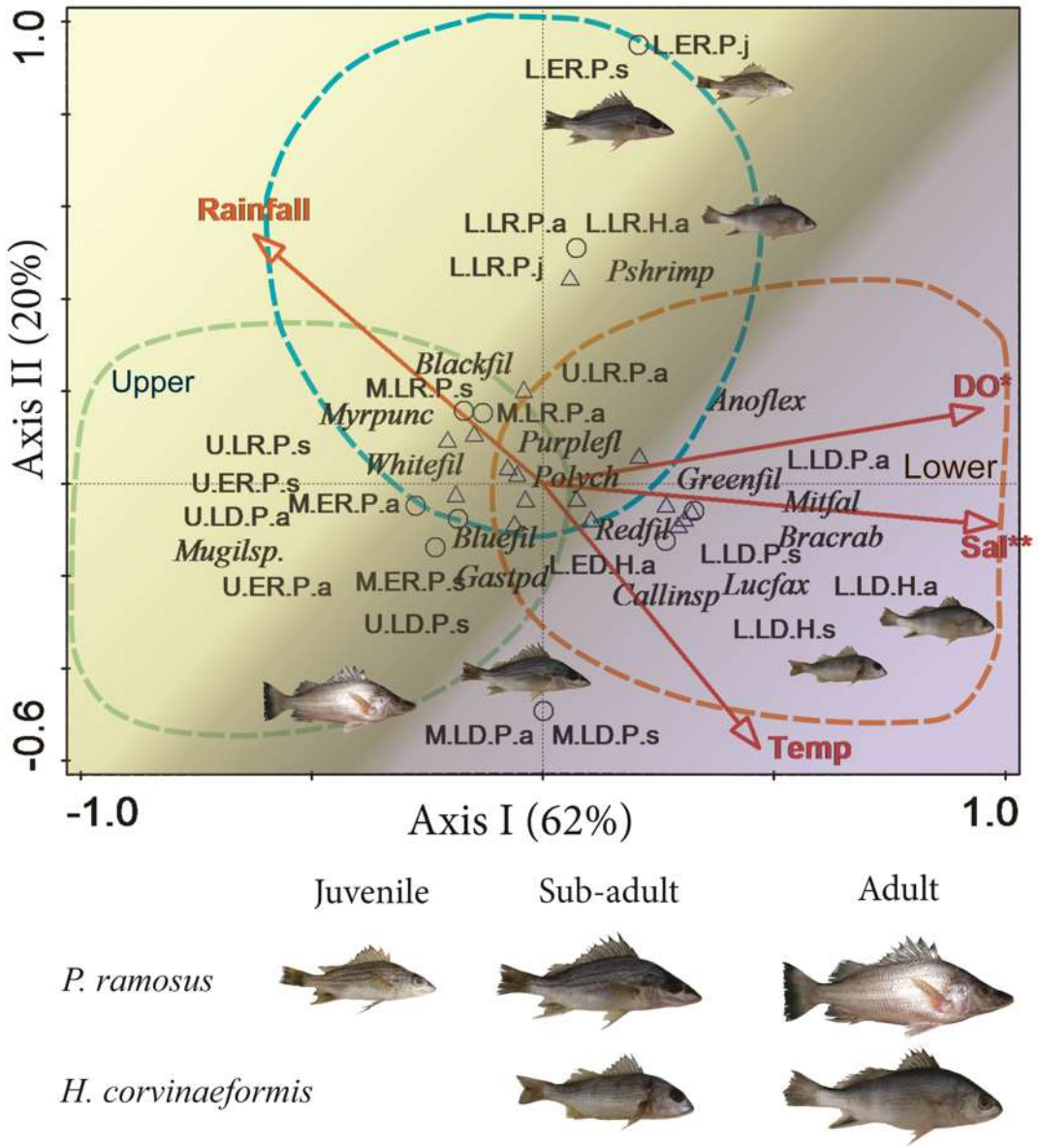

Figure 12 - Canonical correspondence analysis (CCA) triplot for the ecological correlations between the index of relative importance (%IRI) of ingested items and the environmental variables. Circles (○) represent areas (U: upper, M: middle, L: lower), seasons (ER: early rainy, LR: late rainy, ED: early dry, LD: late dry), species (P: *P. ramosus*, H: *H. corvinaeformis*) and ontogenetic phases (j: juvenile, s: sub-adult, a: adult). Triangles (Δ) represent items (%N) (*Bluefil*: blue microplastic filaments; *Redfil*: red microplastic filaments; *Geenfil*: green microplastic filaments; *Blackfil*: black microplastic filaments; *Purplefil*: purple microplastic filaments; *Whitefil*: white microplastic filaments; *Myrpunc*: *Myrophis punctatus*; Mugilsp.: *Mugil sp.*: *Polych*: *Polychaeta*; *Pshrimp*: Panaeidae shrimp: *Lucfax*: *Lucifer faxoni*; *Gastpd*: Gastropoda; *Callinssp.*: *Callinectes sp.; Bracrab*: Brachyuran crab *Mitfal*: *Mitella falcata*; *Anoflex*: *Anomalocardia flexuosa*). Fonte: Autor.

4. 3 DISCUSSION

P.ramosus and *H. corvinaeformis* are estuarine dependent fishes, whose use of resources are mainly ruled by the seasonality of the salinity ecocline along the estuary main channel. Sub-adults and adults of *P. ramosus* can withstand low salinity and dissolved oxygen and inhabit the upper and middle portions of the estuary, mainly during the rainy period, when river runoff increase and salinity showed the lowest values. Therefore, it seems that this Haemulidae species is adapted to live in riverine-estuarine conditions. These ecological requirements are emphasised in few studies along the tropical southwestern Atlantic coast (Osório et al., 2005; Sarmento-Soares et al., 2012). In fact, this species can penetrate riverine habitats (Osório et al., 2005), being found even in river basins from the southeast coast of Brazil (Benevente river) (Sarmento-Soares et al., 2012). Nevertheless, to detect the nursery habitat for this species is still a daunting task. Few juveniles (< 17 mm) are found to inhabit estuarine sandy beaches (Lacerda et al., 2014) and intertidal mangrove creeks (Ramos et al., 2011) of the Goiana Estuary, principally at the end of rainy season. Until recently, *P. ramosus* was characterized as a rare species in estuaries, with a maximum record of 8 specimen in the Paranaguá Estuarine Complex (South Brazil) (Passos et al., 2013), and as few as 1 specimen in the Acaraú River Estuary (Northeast Brazil) (Osório et al., 2005). For the Goiana Estuary, it was registered 125 specimens in the uppermost portions of the estuary, close to the river, especially in the rainy season. This supports the idea of using long-term spatial and temporal data to assess fish ecology following environmental cues (Barletta et al., 2017a). Also, these findings indicates that the ecology of this important estuarine resource must be extrapolated to the riverine environment following the concept of connectivity among habitats (Able, 2005; Sheaves and Johnston, 2008).

On the other hand, sub-adults and adults of *H. corvinaeformis* are adapted to higher values of dissolved oxygen and inhabit exclusively the lower portion of the estuary during the dry period, when water temperature increases and salinity is highest. This species never reached upstream portions, thus being considered highly dependent of the marine-estuarine connection. In estuaries from the North Brazil shelf, such as the Caeté Estuary (Marceniuk et al., 2017); and in subtropical estuaries from the southwestern Atlantic, such as Guaratuba Bay (Chaves and Corrêa, 2000) and the Paranaguá Estuarine Complex (Barletta et al., 2008), *H. corvinaeformis* is also reported to inhabit exclusively the lower portions next to the sea,

especially when salinities are higher. This species is rare in estuaries, with a register of 7,000 specimens in the Paranaguá Estuarine Complex (Passos et al., 2013), being considered a frequent species (Barletta et al., 2008). Moreover, juveniles of *H. corvinaeformis* (< 110 mm T_L) are highly adapted to live in estuarine sandy beaches associated to the mouth of the Goiana Estuary, where they are among the most abundant species in number, and appear exclusively in the dry period (Lacerda et al., 2014). Therefore, during the dry season estuarine beaches are probably the nursery ground for *H. corvinaeformis*.

The present study asserts that *P. ramosus* and *H. corvinaeformis* are adapted to live in opposite environmental conditions along the salinity gradient of the Goiana Estuary. Although having similar diets along the annual cycle, the absence of their co-existence in space and time avoid the interspecific partitioning of foraging grounds and interspecific competition for food resources. This is not a general patterns in estuaries, since interspecific competition for food resources among sympatric species belonging to the same family is common in estuaries because their feeding morphologies and functional guilds are often very similar (Cabral et al., 2002; Dantas et al., 2013, 2015; Dolbeth et al., 2008; Ramos et al., 2014; Vinagre et al., 2005). However, the co-existence of different ontogenetic phases of a species with similar diet preferences in the same habitats increases the intraspecific competition (Dantas et al., 2013, 2015; Ramos et al., 2014). Ontogenetic dietary shifts are observed along the life cycle of both Haemulidae species, and in most part, the different phases rely on the same food items, emphasizing the idea of intraspecific competition among different ontogenetic phases of a single species (Dantas et al., 2013, 2015; Davis et al., 2012; Gning et al., 2008; Ramos et al., 2014).

Juveniles of *P. ramosus* are zooplanktivores (Elliott et al., 2007), feeding mainly on calanoid copepods, amphipods and organic materials. During the sub-adult phase, this species becomes zoobenthivore, feeding mainly on Polychaeta, Gastropoda, and organic materials, but also shows signs of zooplanktivory due to the ingestion of calanoid copepods and amphipods as secondary items. In the adult phase, it becomes exclusively zoobethivore, feeding on Polychaeta and *A. flexuosa*. Although with low relative importance, juveniles and sub-adults of *P. ramosus* also ingested teleostean fishes. Sub-adults and adults of *H. corvinaeformis* are also zoobenthivores, feeding mainly on *A. flexuosa*, Polychaeta and *M. falcata*. However, the diet of adults is more diverse and includes a variety of crustaceans from the benthos (*e.g.*Brachyuran crabs and *Callinects* sp.) and microcrustaceans from the plankton (*e.g.* amphipods, isopods and *L. faxoni*). Thus, sub-adults and adults of every species have

similar dietary preferences relying on infauna invertebrates as primary prey items. Signs of resource partitioning are also observed among phases, since sub-adults of *P. ramosus* feed on zooplankton as secondary items reducing the overlap with the adult phase; and adults of *H. corvinaeformis* feed on crustaceans as secondary items reducing overlap with the sub-adult phase (Cabral et al., 2002; Platell et al., 2006). Therefore, this study corroborates the hypotheses that dietary overlap is reduced due to differences in seasonal patterns of estuarine habitats use and ingestion of different prey items by different ontogenetic phases along the salinity gradient (Dantas et al., 2013, 2015; Platell et al., 2006).

Currently, a great concern regarding fish ecology is their interaction with anthropogenic contaminants and emerging pollutants, while performing their natural tasks in the aquatic environment (Ferreira et al., 2016; 2018; Galloway et al., 2017; Lusher et al., 2017a). Microplastics ubiquitous contaminant in the estuarine environment, which amount can be comparable and sometimes surpass the amount of zooplanktonic groups (Cheung et al., 2016; Lima et al., 2014, 2015; Zhao et al., 2015). Such high and widespread availability of microplastics in estuaries increases the chances of ingestion of these particles by estuarine fish fauna, since these particles share the same habitats of their natural prey items (Dantas et al., 2012; Lima et al., 2015; Ramos et al., 2012; Ferreira et al, 2016, 2018; Vendel et al., 2017). Fishes are an important group for humans regarding their values as subsistence and economic food resources; however, when contaminated with microplastics, fishes might become a vector of harmful chemical pollutants, such as plastic additives and POPs (Santillo et al., 2017; Teuten et al., 2007). Furthermore, to understand the patterns of microplastic contamination in fishes is still difficult, especially considering species poorly studied which have complex dynamics of estuarine use, such as the haemulids *P. ramosus* and *H. corvinaeformis*.

A variety of types of microplastics can be found in estuaries (Chueng et al., 2016; Naidoo et al., 2015), however, the most frequent type ingested by the demersal fish fauna is microplastic filament, especially blue, a typical pattern reported worldwide (Boerge et al., 2010; Dantas et al., 2012; Lusher et al., 2016; Vendel et al., 2017). As expected, more than 99% of the microplastics ingested by the haemulid grunts in the Goiana Estuary were blue microplastic filaments. This is likely a result of fishing activities throughout the entire estuary and adjacent marine waters, during which poor gear operation, mainttenance and storage leads to environmental contamination (Lima et al., 2014). Microplastic filaments might be transported when there is turbulence in the water column, or sink and find their way towards

the bottom of estuaries (Lima et al., 2014). There, occurs the highest rates of accidental intake by demersal fishes foraging for epibenthos and infauna (Ferreira et al., 2016, 2018; Ramos et al., 2012). In addition, the chance of contamination with microplastic filaments seems to be enhanced by the spatio-temporal relationship between the distribution of the haemulid grunts and the availability of microplastics in the Goiana Estuary (Fig. 13). This estudy suggested the presence of *P. ramosus* in the riverine portion during the driest months and their migration towards the middle estuary during the rainy period lead to a high chance of ingestion of microplastic filaments, since this contaminant is highly available in the same areas used by *P. ramosus* during these specific seasons. This is because the river basin and its association with the continental release of solid wastes are recognized as the main source of microplastics to the estuarine environment, especially in the late rainy season, due to river runoff (Lebreton et al., 2017; Lima et al., 2014). Consequently, the highest average ingestion of blue microplastic filaments by sub-adults of *P. ramosus*, occurs in the late rainy season in the upper estuary. On the other hand, *H. corvianeformis* appear in the driest season, in the lower estuary (Fig. 13). During this season, the vertical stratification of the salinity gradient is somewhat stable and the microplastics trapped in the lower estuary cannot efficiently move toward the upstream direction due to the presence of the salt wedge in the middle estuary (Lima et al., 2014). Moreover, the highest average density of microplastic filaments in the lower estuary occurs in the late dry season (Lima et al., 2014), and coincides with the area and period when *H. corvinaeformis* is highly abundant, thus, enhancing their chance of contamination (Fig. 13).

The ingestion of microplastic filaments seems also to have a strong relation with the dietary shifts along the life cycle of these species. The average ingestion of microplastic filaments is higher in sub-adults of *P. ramosus* and in adults of *H. corvinaeformis*; and this coincides with the time when these phases shift to a more diverse diet, including higher trophic level organisms of the benthos associated to the bottom. In addition, the dynamics of ingestion of microplastic filaments have a strong relationship with the ingestion of the most frequent items by these species (Fig.13). The highest ingestion of blue microplastic filaments by sub-adults of *P. ramosus* occurred in the upper estuary, in the late rainy season, and coincided with the highest ingestion of Polychaeta. As well, the highest ingestion of blue microplastic filaments by adults of *H. corvinaeformis* occurs in the lower estuary, in the late dry season, coinciding with the highest ingestion of *A. flexuosa*. This means that the foraging preferences of an ontogenetic phase of a species can enhance the intake of microplastic filaments if these contaminants are abundant in the same habitats and season of their prefered

prey. This study emphasizes the hypotheses that the shift towards a more diverse diet in later phases of fishes lives increases their chances of contamination due to the trophic transfer of microplastics from previously contaminated prey (Eriksson and Burton, 2003; Taylor et al., 2016; Wright et al., 2013), and through direct ingestion due to more complex foraging strategies (Ferreira et al., 2016, 2018).

Although blue microplastic filaments are frequently ingested by haemulid grunts and are associated to fishing activities, microplastic filaments in other colours are also an important concern, since their availability is not so widespread and, thus, their ingestions along the estuarine gradient might indicate other probable sources of these contaminants (Ferreira et al., 2018). Currently, it is asserted that the domestic washing of clothes is a source of plastic filaments (namely fibers) when domestic effluents are released in the marine environment (Browne et al., 2011; Cesa et al., 2017), and this source is probably more signifficant near urban areas along river basins, suggesting a probable source of such diversity of colours. In the Goiana Estuary, black and white microplastic filaments are strongly associated with *P. ramosus* in the innermost reaches of the estuary (upper and middle), suggesting an upstream origin for filaments of these colours. Green and red microplastic filaments are strongly associated with both species at the lower estuary, suggesting a coastal/marine origin for filaments of these colours. However, further studies are still required to understand the sources of microplastic for this environment over several spatial and temporal scales, even using fishes as bioindicators of microplastic contamination of the whole ecosystem (Costa and Barletta, 2015; Ferreira et al., 2018; Lusher et al., 2017b).

4.4 CONCLUSION

This study evidences that the use of monthly spatial and seasonal data can be helpful when assessing the ecology of non-frequent fish species, since their presence in estuaries can be exclusively seasonal due to the preference for specific environmental conditions. It also asserts that the use of estuarine resources by dependent fish species usually lead to contamination with several types of colors pollutants, among which the most conspicuous were are microplastics. Contamination with microplastics is the main concern of this study because *P. ramosus* and *H. corvinaeformis* inhabit specific estuarine habitats during a half part of the year, and even with these patterns of estuarine use, all ontogenetic phases are prone to be contaminated with microplastics. Juveniles of *P. ramosus* and adults of *H. corvinaeformis* presented a distribution similar to the distribution patterns of microplastic

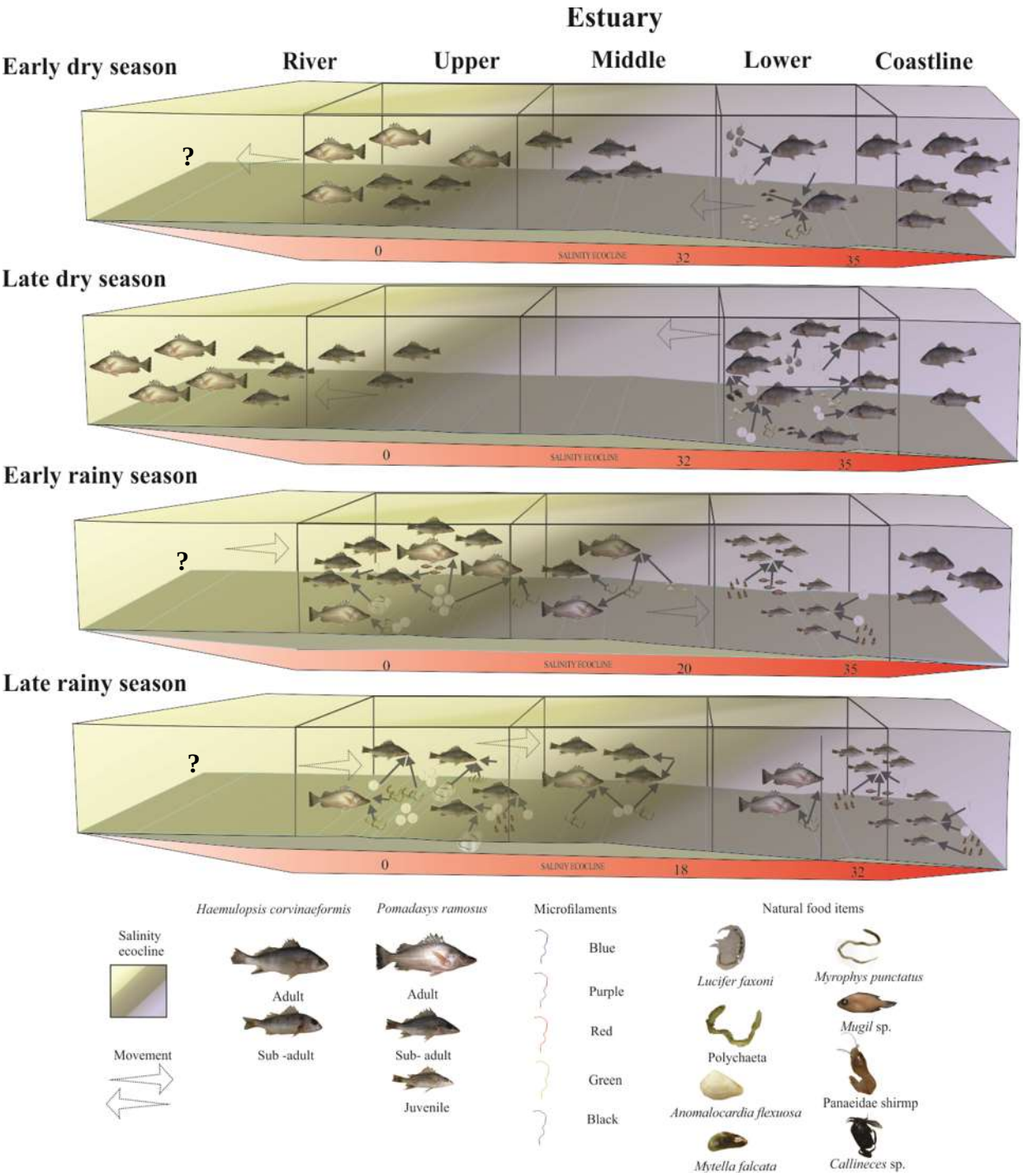

Figure 13 - Conceptual model describing the movement patterns and feeding ecology of the different ontogenetic phases of *P. ramosus* and *H. corvinaeformis* in the Goiana Estuary. Fonte: Autor.

within salt-wedge estuaries, being in higher densities when the availability of microplastics are also higher. Therefore, these ontogenetic phases were most vulnerable. In addition, the changes in diet and the onset of complex foraging strategies for benthic prey seem to have a strong influence in the dynamics of contamination by microplastics. This study also underlines that research on microplastic contamination must take into account species-specific behaviours, since patterns of distribution and trophic guilds are variable within fish assemblages and this must interfere in the intake of microplastics. Finally, this study emphasizes that the ecologic services of estuarine systems are being increasingly eroded due to intense anthropogenic interferences; and the fact that every fish resources are prone to be contaminated with microplastics and other pollutants sorbed onto these polymers may soon become a problem of human health.

Acknowledgements

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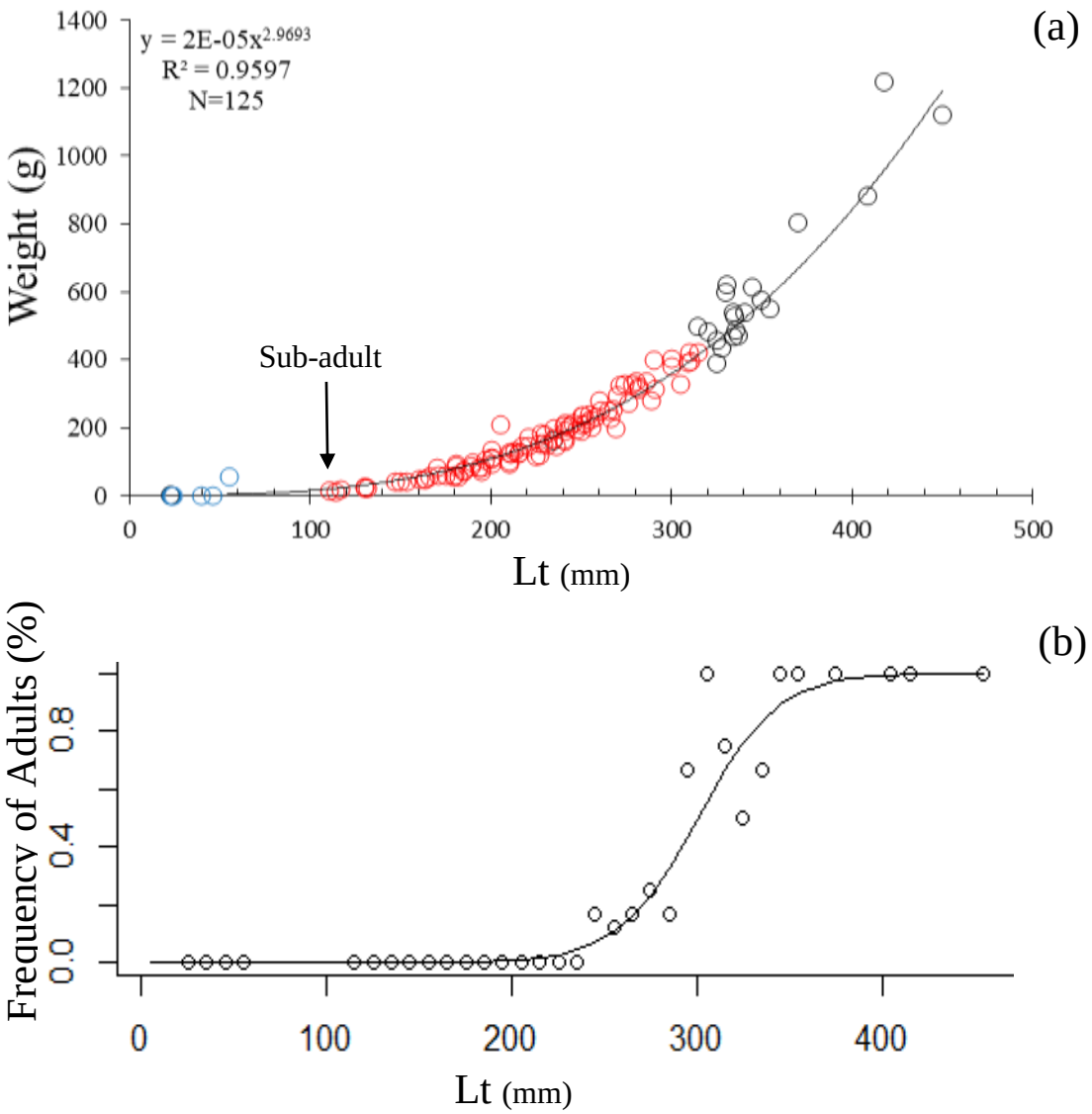
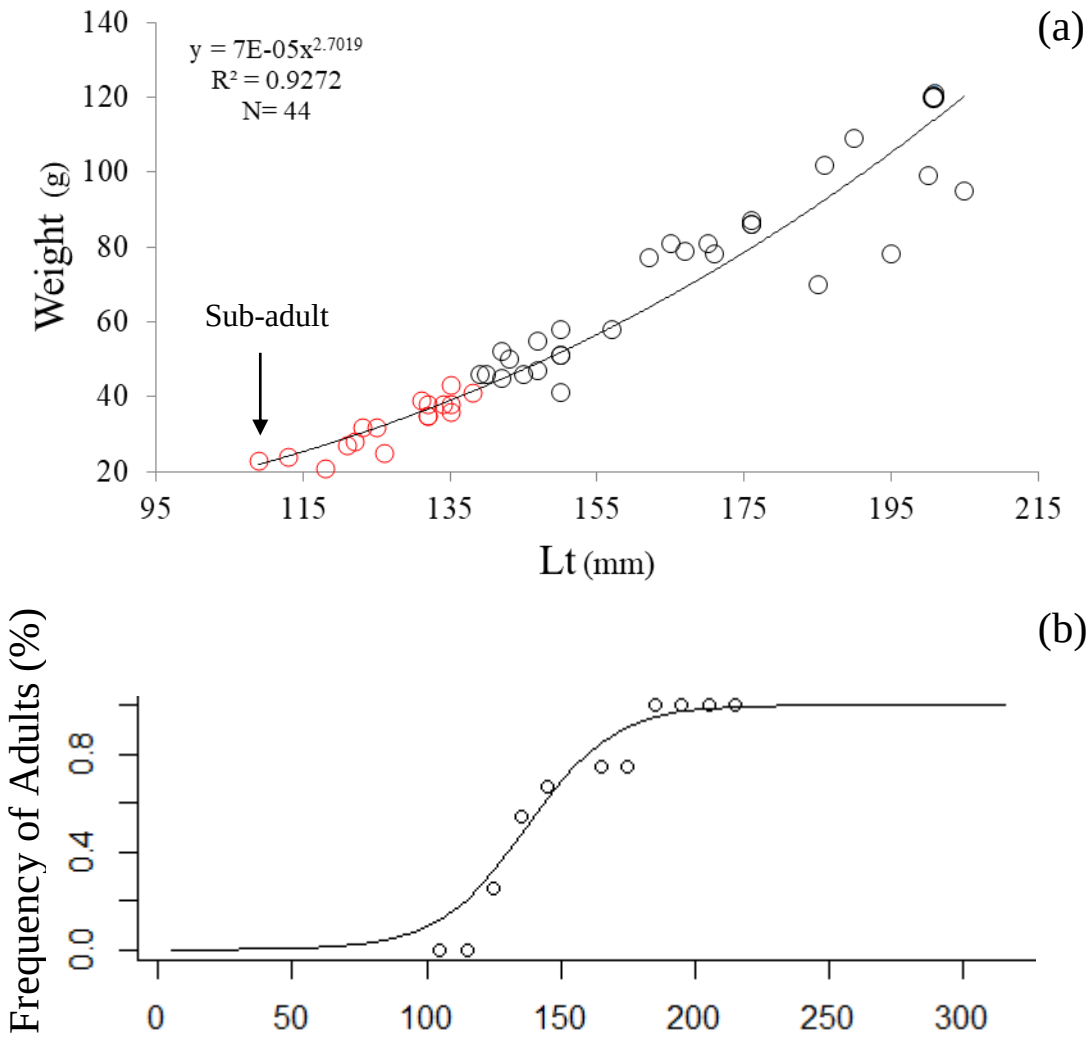
4.5 APPENDICES



Appendix 1. Inflexion point (a) of the relation curve between weight (g) *vs*. length (Lt mm) (○ juvenile, ○ sub-adult and ○ adult) and the logistic curve (b) of the frequency of adults (%) in relation to the total length (Lt mm) of *P. ramosus*.



Appendix 2. Inflexion point (a) of the relation curve between weight (g) *vs.* total length (Lt mm) (○ sub-adult and ○ adult) and the logistic curve (b) of the frequency of adults (%) in relation to the total length (Lt mm) of *H. corvinaeformis*.

Appendix 3. Density and biomass of different ontogenetic phases of *P. ramosus* and *H. corvinaeformis* (sub-adult and adult), along the main channel of the Goiana Estuary (upper, middle and lower) during different seasons (ED: early dry, LD: late dry, ER: early rainy, LR: late rainy). Total densities and biomasses are presented in bold.

				Density (indi. ha^{-1})												**Biomass (g. ha^{-1})**											
				Upper				**Middle**				**Lower**				**Upper**				**Middle**				**Lower**			
Species	**Phases**	**Density (ind.*há$^{-1}$)**	**Biomass (g.*há$^{-1}$)**	**ED**	**LD**	**ER**	**LR**	**ED**	**LD**	**ER**	**LR**	**ED**	**LD**	**ER**	**LR**	**ED**	**LD**	**ER**	**LR**	**ED**	**LD**	**ER**	**LR**	**ED**	**LD**	**ER**	**LR**
P. ramosus	Sub-adult	35.87	2806.22	-	0.4	21.18	10.3	-	1.52	0.32	2.16	-	-	-	-	-	22.82	609.38	1682.06	-	24.34	58.31	409.31	-	-	-	-
	Adult	13.84	3775.17	-	-	10.49	1.54	-	-	1.4	0.4	-	-	-	-	-	-	1405	817.76	-	-	1383.09	169.31	-	-	-	-
	Sub Total	49.71	6581.39	-	0.4	31.67	11.84	-	1.52	1.72	2.56	-	-	-	-	-	22.82	2014.38	2499.82	-	24.34	1441.4	578.62	-	-	-	-
H. corvinaeformis	Sub-adult	8.96	295.34	-	-	-	-	-	-	-	-	-	8.96	-	-	-	-	-	-	-	-	-	-	-	295.34	-	-
	Adult	12.02	746.49	-	-	-	-	-	-	-	-	1.82	10.2	-	-	-	-	-	-	-	-	-	-	122.01	624.48	-	-
	Sub Total	20.98	1041.83	-	-	-	-	-	-	-	-	1.82	19.16	-	-	-	-	-	-	-	-	-	-	122.01	919.82	-	-
	Total	**70.69**	**7623.22**	**-**	**0.4**	**31.67**	**11.84**	**-**	**3.04**	**1.72**	**2.56**	**1.82**	**19.16**	**-**	**-**	**-**	**22.82**	**2014.39**	**2499.82**	**-**	**24.34**	**1441.4**	**578.62**	**122.01**	**919.82**	**-**	**-**

Appendix 4. Summary of the ANOVA (F-values; df: degree of freedom; p-value and *post-hoc* comparisons) for the density and biomass of Haemulidae, according to the factors: species (SP1: *P. ramosus*; SP2: *H. corvinaeformis*), seasons (ED: Early dry, LD: Late dry, ER: Early rainy, LR: Late rainy), areas (U: Upper, M: Middle and L: Lower) and ontogenetic phases (Sub: sub-adult and Adu: adult). Differences among factors were determined by Bonferroni's test: not significant (ns); $p < 0.05$ (*); $p < 0.01$ (**). Bold represents the sources of variance within homogeneous groups.

	Factors	**F**	***df***	***p*-value**	***Post-hoc***
Density	Area	3.67	2	0.026	**U** M L *
	Season	2.02	3	0.109	ns
	Species	3.45	1	0.063	ns
	Phase	1.01	1	0.315	ns
	Area *vs*. Season	3.7	6	0.001	**
	Area *vs*. Phase	1.07	2	0.342	ns
	Season *vs*. Phase	0.42	3	0.741	ns
	Area *vs*. Spp	8.02	2	0.001	**
	Season *vs*. Spp	4.64	3	0.001	**
	Phase *vs*. Spp	1.54	1	0.216	ns
	Area *vs*.Season *vs*.Phase	0.29	6	0.940	ns
	Area *vs*.Season *vs*.Spp	2.39	6	0.026	*
	Area *vs*. Phase *vs* Spp	0.81	4	0.445	ns
	Season *vs*. Phase *vs* Spp	0.25	3	0.856	ns
	Area *vs*. Season *vs*. Spp *vs*. Phase	0.37	6	0.896	ns
Biomass	Area	4.95	2	0.001	**U** M L **
	Season	5.92	3	0.001	ED LD ER **LR** **
	Species	13.87	1	0.001	**SP1** SP2 **
	Phase	0.04	1	0.844	Sub Adu
	Area *vs*. Season	7.59	6	0.001	**
	Area *vs*. Phase	2.15	2	0.117	ns
	Season *vs*. Phase	2.53	3	0.056	ns
	Area *vs*. Spp	16.9	2	0.001	**
	Season *vs*. Spp	13.46	3	0.001	**
	Phase *vs*. Spp	1.34	1	0.246	ns
	Area *vs*. Season *vs*. Phase	1.11	6	0.351	ns
	Area *vs*. Season *vs*. Spp	3.8	6	0.001	**
	Area *vs*. Phase *vs* Spp	1.49	2	0.226	ns
	Season *vs*. Phase *vs* Spp	2.23	3	0.083	ns
	Area *vs*. Season *vs*. Spp *vs*. Phase	1.27	6	0.270	ns

Appendix 5. Frequency of occurrence (% FO) of food items and microplastics ingested by *Pomadasys ramosus* according to the factors: seasons (ED: Early dry, LD: Late dry, ER: Early rainy, LR: Late rainy), areas (Upper, Middle and Lower) and ontogenetic phases (juvenile, sub-adult and adult).

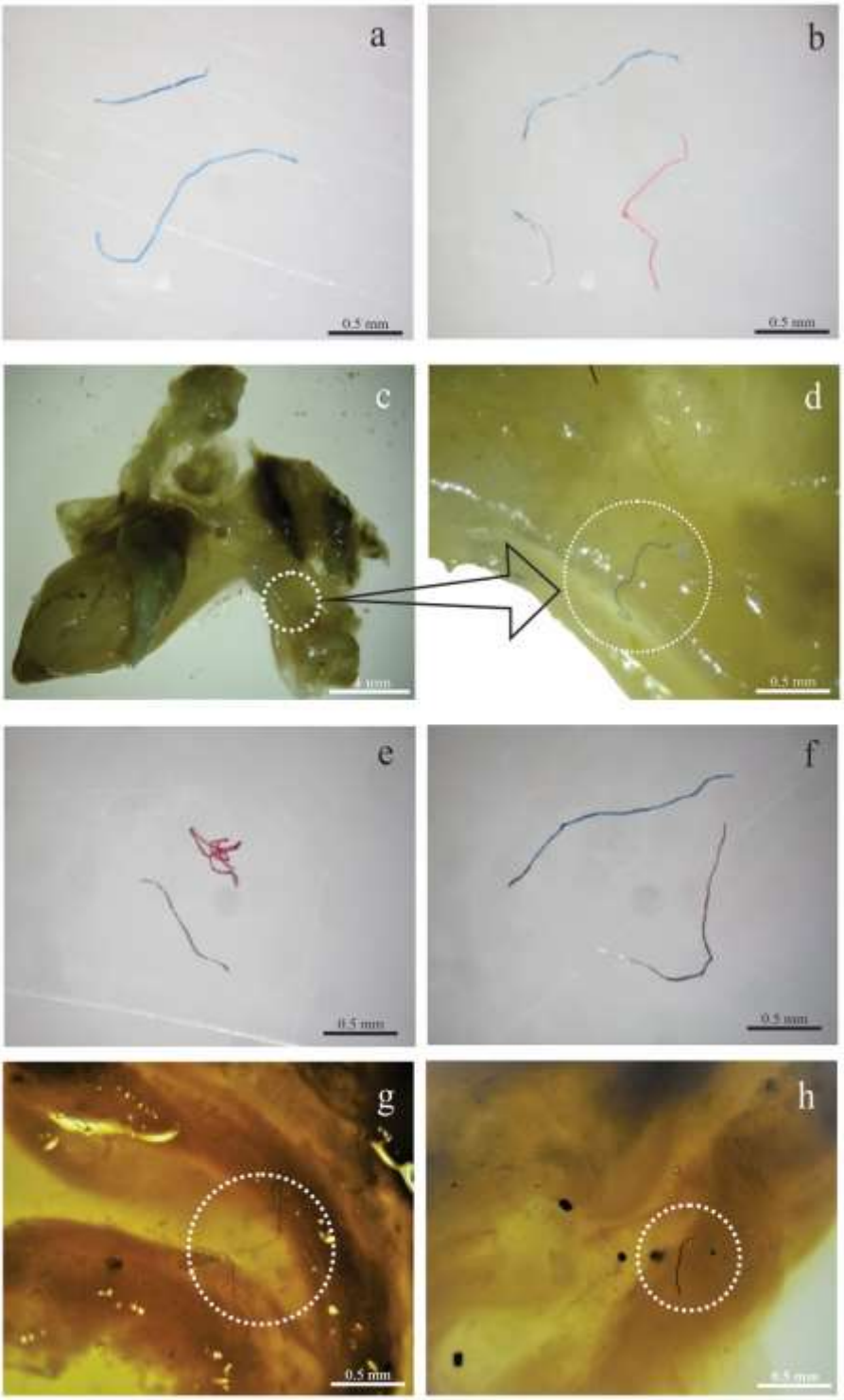
		% FO											
		Upper				Midlle				Lower			
Items ingested by *P. ramosus*		**ED**	**L D**	**ER**	**L R**	**ED**	**L D**	**ER**	**L R**	**ED**	**L D**	**ER**	**L R**
	Juvenile	-	-	-	-	-	-	-	-	-	-	-	50
Microplastic	Subadult	-	50	81.25	93.33	-	100	80	77.78	-	50	-	-
	Adult	-	100	90.01	66.67	-	100	100	100	-	50	-	50
	Juvenile	-	-	-	-	-	-	-	-	-	-	20	
Polychaeta	Subadult	-	100	100	96.67	-	100	100	100	-	100	-	50
	Adult	-	100	63.64	100	-	100	66.67	100	-	100	-	100
	Juvenile	-	-	-	-	-	-	-	-	-	-	-	-
Hyporhamphus sp.	Subadult	-	-	-	1.69	-	-	-	-	-	-	-	-
	Adult	-	-	-	-	-	-	-	-	-	-	-	-
	Juvenile	-	-	-	-	-	-	-	-	-	-	-	-
Myrophis punctatus	Subadult	-	4.37	-	1.69	-	-	-	-	-	-	-	-
	Adult	-	-	-	-	-	-	-	-	-	-	-	-
	Juvenile	-	-	-	-	-	-	-	-	-	-	20	-
Mugil sp.	Subadult	-	-	11.12	-	-	-	-	-	-	-	-	-
	Adult	-	-	-	-	-	-	-	-	-	-	-	-
	Juvenile	-	-	-	-	-	-	-	-	-	-	60	-
Calanoid copepods	Subadult	-	-	6.25	3.33	-	50	-	-	-	-	-	-
	Adult	-	-	-	-	-	-	-	-	-	50	-	-
	Juvenile	-	-	-	-	-	-	-	-	-	-	20	-
Panaeidae shrimp	Subadult	-	-	-	13.33	-	-	-	-	-	-	-	-
	Adult	-	-	-	-	-	-	-	-	-	-	-	-
	Juvenile	-	-	-	-	-	-	-	-	-	-	40	-
Amphipoda	Subadult	-	14.29	12.5	13.33	-	50	-	-	-	-	-	-
	Adult	-	-	-	-	-	-	-	-	-	-	-	-

Appendix 5. Continued.

		%FO											
		Upper				Midlle				Lower			
Items ingested by *P. ramosus*		**ED**	**LD**	**ER**	**LR**	**ED**	**LD**	**ER**	**LR**	**ED**	**LD**	**ER**	**LR**
	Juvenile	-	-	-	-	-	-	-	-	-	-	-	-
Isopods	Subadult	-	-	-	3.33	-	-	-	-	-	-	-	-
	Adult	-	-	-	-	-	-	-	-	-	-	-	-
	Juvenile	-	-	-	-	-	-	-	-	-	-	-	-
Ostracods	Subadult	-	-	-	-	-	-	-	-	-	-	-	-
	Adult	-	-	-	16.67	-	-	-	-	-	-	-	-
	Juvenile	-	-	-	-	-	-	-	-	-	-	-	-
Ucides cordatus	Subadult	-	7.14	-	-	-	-	-	-	-	-	-	-
	Adult	-	-	-	-	-	-	-	-	-	-	-	-
	Juvenile	-	-	-	-	-	-	-	-	-	-	-	-
Megalopa of Brachyura	Subadult	-	7.14	-	-	-	-	-	-	-	-	-	-
	Adult	-	-	-	-	-	-	-	-	-	-	-	-
	Juvenile	-	-	-	-	-	-	-	-	-	-	20	-
Unindetified Crustacea	Subadult	-	-	6.25	-	-	-	-	-	-	-	-	-
	Adult	-	-	-	-	-	-	-	-	-	-	-	-
	Juvenile	-	-	-	-	-	-	-	-	-	-	-	-
A. flexuosa	Subadult	-	-	-	-	-	-	-	-	-	-	-	-
	Adult	-	-	-	-	-	-	33.33	-	-	-	-	-
	Juvenile	-	-	-	-	-	-	-	-	-	-	-	-
Gastropods	Subadult	-	-	43.75	6.66	-	-	20	-	-	-	-	-
	Adult	-	-	-	-	-	-	-	-	-	-	-	-
	Juvenile	-	-	-	-	-	-	-	-	-	-	-	-
Molluscs shell fragment	Subadult	-	7.14	-	-	-	-	-	-	-	-	-	-
	Adult	-	-	-	-	-	-	-	-	-	-	-	-

Appendix 5. Continued.

		%FO											
		Upper				Midlle				Lower			
Items ingested by *P. ramosus*		**ED**	**LD**	**ER**	**LR**	**ED**	**LD**	**ER**	**LR**	**ED**	**LD**	**ER**	**LR**
	Juvenile	-	-	-	-	-	-	-	-	-	-	-	-
Nematoda eggs (parasite)	Subadult	-	-	13.33	3.33	-	-	-	-	-	-	-	-
	Adult	-	-	-	-	-	-	-	-	-	-	-	-
	Juvenile	-	-	-	-	-	-	-	-	-	-	-	-
Unindetified invertebrate	Subadult	-	-	-	3.33	-	-	-	-	-	-	-	-
	Adult	-	-	9.09	-	-	25	-	-	-	-	-	-
	Juvenile	-	-	-	-	-	-	-	-	-	-	20	100
Organic matter	Subadult	-	14.29	25	10	-	50	20	11.11	-	-	-	-
	Adult	-	100	45.45	33.33	-	50	-	-	-	-	-	-



Appendix 6. Different colours of microplastic filaments ingested by *P. ramosus* and *H. corvinaeformis* in the Goiana Estuary. (a) Blue and (b) red filaments ingested by *P. ramosus*. Microplastic filaments were observed in the stomach of *P. ramosus* before the removal of food contents: (c) 0.65x and (d) 5x magnified. (e) Purple and red (f) purple and blue filaments ingested by *H. corvinaeformis*. Microplastic filaments found in the stomach of *H. corvinaeformis*: (g) and (h) 5x magnified.

Appendix 7. Summary of the index of relative importance (%I_{RI}) of food items and microplastics ingested by *P. ramosus* according to the factors: season (ED-Early dry, LD- Late dry, ER-Early rainy, LR- Late rainy), area (Upper, Middle and Lower) and ontogenetic phase (juvenile, sub-adult and adult).

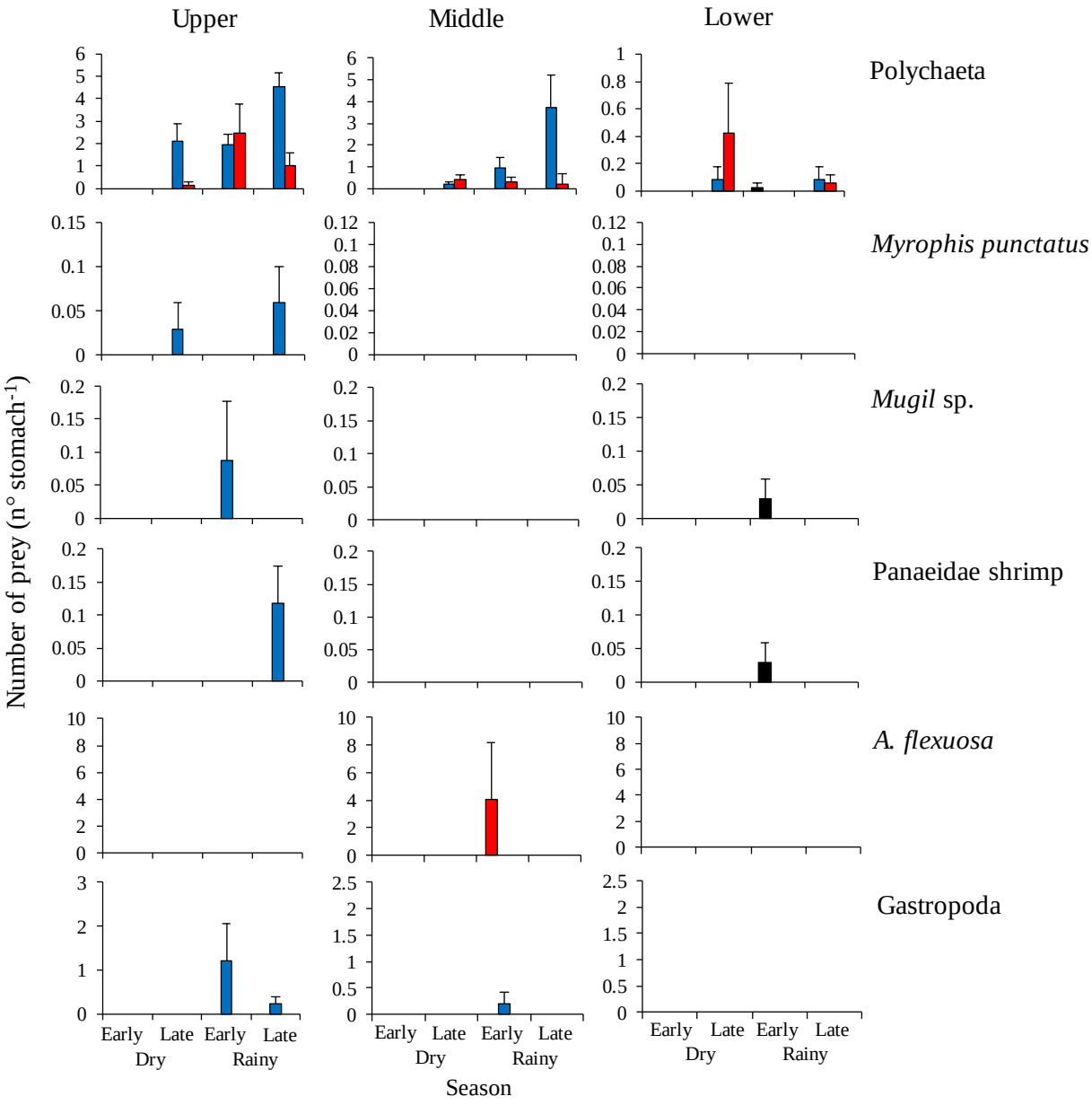
		%I_{RI}											
		Upper				**Midlle**				**Lower**			
Items ingested by *P. ramosus*		**ED**	**L D**	**ER**	**L R**	**ED**	**L D**	**ER**	**L R**	**ED**	**L D**	**ER**	**L R**
	Juvenile	-	-	-	-	-	-	-	-	-	-	-	22.08
Microplastic	Subadult	-	3.72	19.88	15.52	-	8.88	12.95	6.35	-	25.06	-	-
	Adult	-	7.72	18.01	9.34	-	7.63	2.55	14.71	-	1.47	-	16.68
	Juvenile	-	-	-	-	-	-	-	-	-	-	1.85	
Polychaeta	Subadult	-	92.53	50.85	82.86	-	61.49	81.98	93.3	-	100	-	74.94
	Adult	-	65.66	77.91	86.67	-	47.1	30.56	85.29	-	97.05	-	83.32
	Juvenile	-	-	-	-	-	-	-	-	-	-	-	-
Hyporhamphus sp.	Subadult	-	-	-	0.026	-	-	-	-	-	-	-	-
	Adult	-	-	-	-	-	-	-	-	-	-	-	-
	Juvenile	-	-	-	-	-	-	-	-	-	-	-	-
Myrophis punctatus	Subadult	-	0.19	-	0.18	-	-	-	-	-	-	-	-
	Adult	-	-	-	-	-	-	-	-	-	-	-	-
	Juvenile	-	-	-	-	-	-	-	-	-	-	2.17	-
Mugil sp.	Subadult	-	-	8.02	-	-	-	-	-	-	-	-	-
	Adult	-	-	-	-	-	-	-	-	-	-	-	-
	Juvenile	-	-	-	-	-	-	-	-	-	-	78.87	-
Calanoid copepods	Subadult	-	-	0.07	0.003	-	3.24	-	-	-	-	-	-
	Adult	-	-	-	-	-	-	-	-	-	1.48	-	-
	Juvenile	-	-	-	-	-	-	-	-	-	-	0.51	-
Panaeidae shrimp	Subadult	-	-	-	0.27	-	-	-	-	-	-	-	-
	Adult	-	-	-	-	-	-	-	-	-	-	-	-
	Juvenile	-	-	-	-	-	-	-	-	-	-	4.09	-
Amphipoda	Subadult	-	0.45	0.21	0.25	-	4.82	-	-	-	-	-	-
	Adult	-	-	-	-	-	-	-	-	-	-	-	-

Appendix 7. Continued.

		%I_{RI}											
		Upper				Midlle				Lower			
Items ingested by *P. ramosus*		**ED**	**LD**	**ER**	**LR**	**ED**	**LD**	**ER**	**LR**	**ED**	**LD**	**ER**	**LR**
	Juvenile	-	-	-	-	-	-	-	-	-	-	-	-
Isopods	Subadult	-	-	-	0.032	-	-	-	-	-	-	-	-
	Adult	-	-	-	-	-	-	-	-	-	-	-	-
	Juvenile	-	-	-	-	-	-	-	-	-	-	-	-
Ostracods	Subadult	-	-	-	-	-	-	-	-	-	-	-	-
	Adult	-	-	-	0.79	-	-	-	-	-	-	-	-
	Juvenile	-	-	-	-	-	-	-	-	-	-	-	-
Ucides cordatus	Subadult	-	0.61	-	-	-	-	-	-	-	-	-	-
	Adult	-	-	-	-	-	-	-	-	-	-	-	-
	Juvenile	-	-	-	-	-	-	-	-	-	-	-	-
Megalopa of Brachyura	Subadult	-	0.1	-	-	-	-	-	-	-	-	-	-
	Adult	-	-	-	-	-	-	-	-	-	-	-	-
	Juvenile	-	-	-	-	-	-	-	-	-	-	5.01	-
Unindetified Crustacea	Subadult	-	-	0.12	-	-	-	-	-	-		-	-
	Adult	-	-	-	-	-	-	-	-	-	-	-	-
	Juvenile	-	-	-	-	-	-	-	-	-	-	-	-
A. flexuosa	Subadult	-	-	-	-	-	-	-	-	-	-	-	-
	Adult	-	-	-	-	-	-	66.89	-	-	-	-	-
	Juvenile	-	-	-	-	-	-	-	-	-	-	-	-
Gastropoda	Subadult	-	-	16.67	0.76	-	-	4.69	-	-	-	-	-
	Adult	-	-	-	-	-	-	-	-	-	-	-	-
	Juvenile	-	-	-	-	-	-	-	-	-	-	-	-
Mollusc shell fragment	Subadult	-	1.51	-	-	-	-	-	-	-	-	-	-
	Adult	-	-	-	-	-	-	-	-	-	-	-	-

Appendix 7. Continued.

		%I_{RI}											
		Upper				**Midlle**				**Lower**			
Items ingested by *P. ramosus*		**ED**	**LD**	**ER**	**LR**	**ED**	**LD**	**ER**	**LR**	**ED**	**LD**	**ER**	**LR**
	Juvenile	-	-	-	-	-	-	-	-	-	-	-	-
Nematoda eggs (parasite)	Subadult	-	-	0.25	0.02	-	-	-	-	-	-	-	-
	Adult	-	-	-	-	-	-	-	-	-	-	-	-
	Juvenile	-	-	-	-	-	-	-	-	-	-	-	-
Unindetified invertebrate	Subadult	-	-	-	0.05	-	-	-	-	-	-	-	-
	Adult	-	-	0.17	-	-	0.74	-	-	-	-	-	-
	Juvenile	-	-	-	-	-	-	-	-	-	-	7.5	77.92
Organic matter	Subadult	-	0.89	3.93	0.023	-	21.57	0.38	0.35	-	-	-	-
	Adult	-	26.62	3.91	3.21	-	44.53	-	-	-	-	-	-



Appendix 8. Average and standard error (±S.E.) number of prey items ingested by the different ontogenetic phases of *Pomadasys ramosus* (■ juvenile, ■ sub-adult and ■ adult) along the main channel of the Goiana Estuary (upper, middle and lower), over the seasonal cycle (early and late dry seasons; and early and late rainy seasons).

Appendix 9. Summary of the ANOVA (F-values; df: degree of freedom; p-value and *post-hoc* comparisons) for the number and weight of natural prey items ingested by *Pomadasys ramosus* according to the factors: seasons (ED: Early dry, LD: Late dry, ER: Early rainy, LR: Late rainy), areas (U: Upper, M: Middle and L: Lower) and ontogenetic phases (Juv: juvenile, Sub: sub-adult and Adu: adult). Differences among factors were determined by Bonferroni's test: not significant (ns); $p < 0.05$ (*); $p < 0.01$ (**). Bold represents the sources of variance within homogeneous groups.

		Items in number				**Items in weight**			
Ingested items	**Factors**	**F**	***df***	***p*-value**	***Post-hoc***	**F**	***df***	***p*-value**	***Post-hoc***
Polychaeta	Area	26.88	2	0.001	**U** M L **	26.9	2	0.001	**U** M L **
	Season	26.77	3	0.001	ED LD ER **LR** **	23.97	3	0.001	ED LD ER **LR** **
	Phase	48.6	2	0.001	Juv **Sub** Adu **	45.6	2	0.001	Juv **Sub** Adu **
	Area *vs*. Season	4.74	6	0.001	**	5.09	6	0.001	**
	Area *vs*. Phase	12.21	4	0.001	**	7	4	0.001	**
	Season *vs*. Phase	6.35	6	0.001	**	6.17	6	0.001	**
	Area *vs*. Season *vs*. Phase	4.71	12	0.001	**	4.9	12	0.001	**
Myrophis punctatus	Area	3.21	2	0.043	**U** M L *	3.08	2	0.049	**U** M L *
	Season	1.1	3	0.349	ns	1.05	3	0.371	ns
	Phase	3.21	2	0.043	Juv **Sub** Adu *	3.08	2	0.049	Juv **Sub** Adu *
	Area *vs*. Season	1.1	6	0.363	ns	1.05	6	0.393	ns
	Area *vs*. Phase	3.21	4	0.001	**	3.08	4	0.018	*
	Season *vs*. Phase	1.1	6	0.363	ns	1.05	6	0.393	ns
	Area *vs*. Season *vs*. Phase	1.1	12	0.361	ns	1.05	12	0.404	ns
Gastropoda	Area	10.06	2	0.001	**U** M L **	0.85	2	0.427	ns
	Season	6.77	3	0.001	ED LD **ER** LR **	2.26	3	0.084	ns
	Phase	16.18	2	0.001	Juv **Sub** Adu **	2.47	2	0.086	ns
	Area *vs*. Season	3.72	6	0.001	**	0.74	6	0.617	ns
	Area *vs*. Phase	10.06	4	0.001	**	0.86	4	0.491	ns
	Season *vs*. Phase	6.76	6	0.001	**	2.26	6	0.041	*
	Area *vs*. Season *vs*. Phase	3.72	12	0.001	**	0.74	12	0.709	ns
Mugil sp.	Area	0.5	2	0.607	ns	0.99	2	0.373	ns
	Season	1.99	3	0.117	ns	1.01	3	0.389	ns

	Phase	0.5	2	0.607	ns	0.99	2	0.372	ns
	Area *vs*. Season	0.5	6	0.806	ns	0.99	6	0.432	ns
	Area *vs*. Phase	1.25	4	0.296	ns	1	4	0.408	ns
	Season *vs*. Phase	0.5	6	0.806	ns	0.99	6	0.432	ns
	Area *vs*. Season *vs*. Phase	1.25	12	0.256	ns	1	12	0.449	ns
Panaeidae shrimp	Area	0.93	2	0.397	ns	1.22	2	0.297	ns
	Season	1.18	3	0.318	ns	1.22	3	0.302	ns
	Phase	0.93	2	0.397	ns	1.22	2	0.296	ns
	Area *vs*. Season	1.95	6	0.077	ns	1.23	6	0.294	ns
	Area *vs*. Phase	2.07	4	0.087	ns	1.23	4	0.300	ns
	Season *vs*. Phase	1.95	6	0.077	ns	1.23	6	0.294	ns
	Area *vs*. Season *vs*. Phase	1.56	12	0.108	ns	1.23	12	0.269	ns
A. flexuosa	Area	1	2	0.370	ns	1	2	0.370	ns
	Season	1	3	0.395	ns	1	3	0.394	ns
	Phase	1	2	0.370	ns	1	2	0.370	ns
	Area *vs*. Season	1	6	0.427	ns	1	6	0.427	ns
	Area *vs*. Phase	1	4	0.409	ns	1	4	0.409	ns
	Season *vs*. Phase	1	6	0.428	ns	1	6	0.428	ns
	Area *vs*. Season *vs*. Phase	1	12	0.452	ns	1	12	0.452	ns

Appendix 10. Summary of the ANOVA (F-values; df: degree of freedom; p-value and *post-hoc* comparisons) for the number of microplastics ingested by *Pomadasys ramosus* according to the factors: seasons (ED: Early dry, LD: Late dry, ER: Early rainy, LR: Late rainy), areas (U: Upper, M: Middle and L: Lower) and ontogenetic phases (Juv: juvenile, Sub: sub-adult and Adu: adult). Differences among factors were determined by Bonferroni's test: not significant (ns); $p < 0.05$ (*); $p < 0.01$ (**). Bold represents the sources of variance within homogeneous groups.

		Items in number			
Ingested items	**Factors**	**F**	***df***	***p*-value**	***Post-hoc***
Total microplastics	Area	31.42	2	0.001	**U** M L **
	Season	22.68	3	0.001	ED LD ER **LR** **
	Phase	37.74	2	0.001	Juv **Sub** Adu **
	Area *vs*. Season	4.74	6	0.001	**
	Area *vs*. Phase	15.39	4	0.001	**
	Season *vs*. Phase	5.19	6	0.001	**
	Area *vs*. Season *vs*. Phase	3.82	12	0.001	**
Blue	Area	30.83	2	0.001	**U** M L **
	Season	19.71	3	0.001	ED LD ER **LR** **
	Phase	36.22	2	0.001	Juv **Sub** Adu **
	Area *vs*. Season	4.44	6	0.001	**
	Area *vs*. Phase	11.87	4	0.001	**
	Season *vs*. Phase	5.07	6	0.001	**
	Area *vs*. Season *vs*. Phase	3.71	12	0.001	**
Red	Area	8.49	2	0.001	**U** M L **
	Season	4.48	3	0.001	ED LD **ER LR** **
	Phase	6.32	2	0.001	Juv **Sub Adu** **
	Area *vs*. Season	1.80	6	0.102	ns
	Area *vs*. Phase	2.17	4	0.075	ns
	Season *vs*. Phase	1.62	6	0.146	ns
	Area *vs*. Season *vs*. Phase	1.22	12	0.273	ns
Green	Area	1.00	2	0.154	ns
	Season	1.00	3	0.536	ns
	Phase	1.00	2	0.145	ns
	Area *vs*. Season	1.00	6	0.663	ns
	Area *vs*. Phase	1.00	4	0.105	ns
	Season *vs*. Phase	1.00	6	0.663	ns
	Area *vs*. Season *vs*. Phase	1.00	12	0.756	ns
Black	Area	2.83	2	0.062	ns
	Season	2.79	3	0.042	ED LD **ER LR** *
	Phase	1.29	2	0.276	ns
	Area *vs*. Season	0.63	6	0.708	ns
	Area *vs*. Phase	3.06	4	0.019	*
	Season *vs*. Phase	1.52	6	0.174	ns
	Area *vs*. Season *vs*. Phase	1.67	12	0.078	ns
Purple	Area	1.12	2	0.018	**U** M L *
	Season	1.58	3	0.197	ns
	Phase	2.12	2	0.123	ns

	Area *vs*. Season	1.45	6	0.199	ns
	Area *vs.* Phase	2.38	4	0.054	*
	Season *vs*. Phase	1.08	6	0.378	ns
	Area *vs.* Season *vs*. Phase	0.49	12	0.915	ns
White	Area	1.12	2	0.289	ns
	Season	1.58	3	0.213	ns
	Phase	2.12	2	0.029	*
	Area *vs*. Season	1.45	6	0.038	*
	Area *vs.* Phase	2.38	4	0.292	ns
	Season *vs*. Phase	1.08	6	0.177	ns
	Area *vs.* Season *vs*. Phase	0.49	12	0.010	*

Appendix 11. Frequency of occurrence (%FO) of food items and microplastics ingested by *Haemulopsis corvinaeformis* according to the factors: seasons (ED: Early dry, LD: Late dry, ER: Early rainy, LR: Late rainy), areas (Upper, Middle and Lower) and ontogenetic phases (juvenile, sub-adult and adult).

		%FO											
		Upper				Midlle				Lower			
Items ingested by *H. corvinaeformis*		**ED**	**LD**	**ER**	**LR**	**ED**	**LD**	**ER**	**LR**	**ED**	**LD**	**ER**	**LR**
Microplastic	Subadult	-	-	-	-	-	-	-	-	-	64.29	-	-
	Adult	-	-	-	-	-	-	-	-	100	50	-	-
A. flexuosa	Subadult	-	-	-	-	-	-	-	-	-	71.43	-	-
	Adult	-	-	-	-	-	-	-	-	-	41.67	100	100
Polychaeta	Subadult	-	-	-	-	-	-	-	-	-	42.86	-	-
	Adult	-	-	-	-	-	-	-	-	100	41.67	-	-
Mytella falcata	Subadult	-	-	-	-	-	-	-	-	-	7.14	-	-
	Adult	-	-	-	-	-	-	-	-	100	41.67	-	-
Gastropoda	Subadult	-	-	-	-	-	-	-	-	-	7.14	-	-
	Adult	-	-	-	-	-	-	-	-	-	4.16	-	-
Brachyuran crab	Subadult	-	-	-	-	-	-	-	-	-	7.14	-	-
	Adult	-	-	-	-	-	-	-	-	50	4.16	-	-
Megalopa of Brachyura	Subadult	-	-	-	-	-	-	-	-	-	-	-	-
	Adult	-	-	-	-	-	-	-	-	-	4.16	33.33	-
Callinectes sp.	Subadult	-	-	-	-	-	-	-	-	-	14.29	-	-
	Adult	-	-	-	-	-	-	-	-	33.33	8.33	-	-
Amphipoda	Subadult	-	-	-	-	-	-	-	-	-	50	-	-
	Adult	-	-	-	-	-	-	-	-	100	20.83	-	-
Lucifer faxoni	Subadult	-	-	-	-	-	-	-	-	-	35.71	-	-
	Adult	-	-	-	-	-	-	-	-	50	16.67	-	-

Appendix 11. Continued.

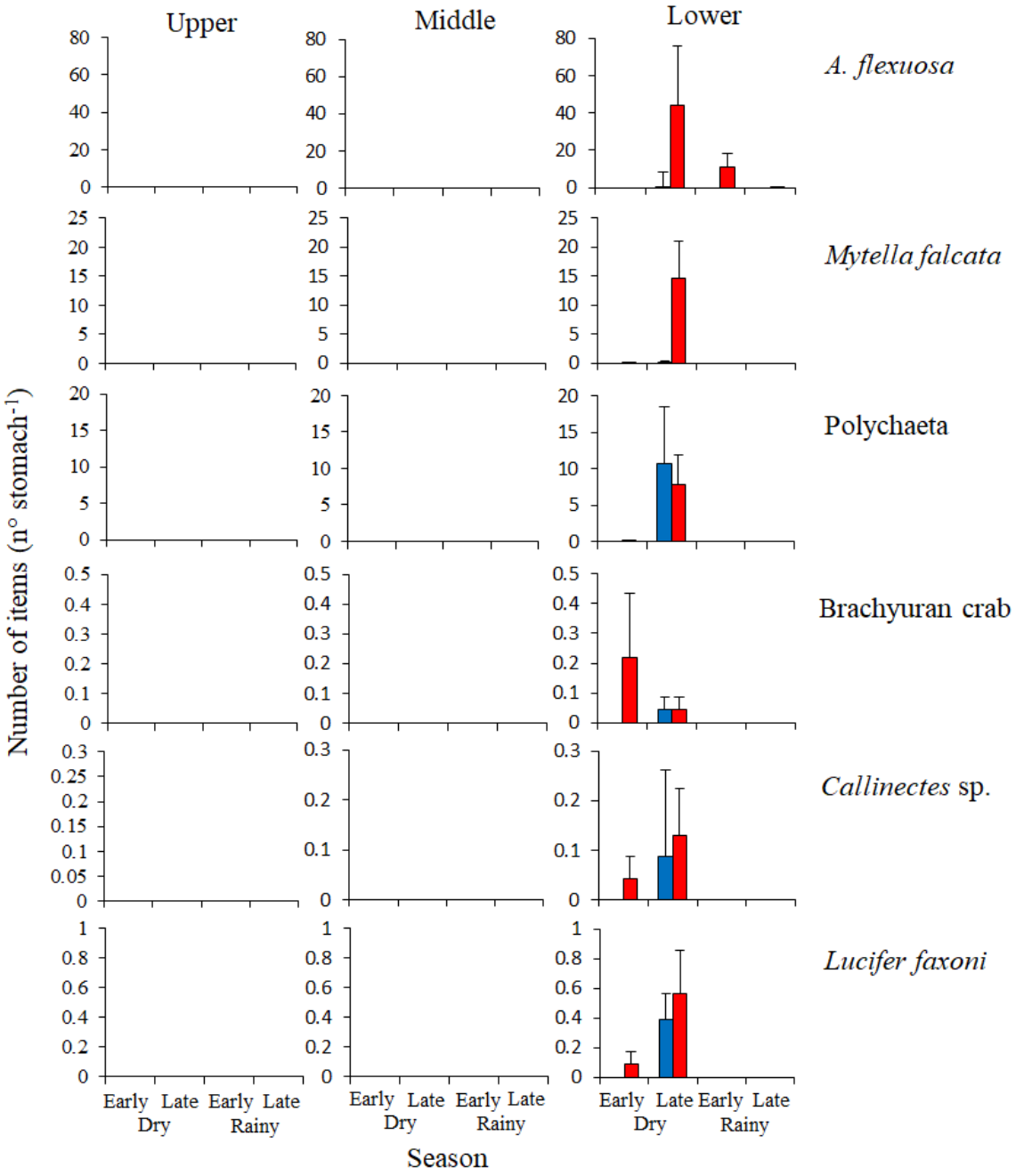
Items ingested by *H. corvinaeformis*		%FO											
		Upper				Midlle				Lower			
		ED	LD	ER	LR	ED	LD	ER	LR	ED	LD	ER	LR
Ostracods	Subadult	-	-	-	-	-	-	-	-	-	7.14	-	-
	Adult	-	-	-	-	-	-	-	-	-	-	-	-
Penaeidae shrimp	Subadult	-	-	-	-	-	-	-	-	-	-	-	-
	Adult	-	-	-	-	-	-	-	-	-	12.5	-	-
Nauplii of Cirripedia	Subadult	-	-	-	-	-	-	-	-	-	-	-	-
	Adult	-	-	-	-	-	-	-	-	-	8.33	-	-
Cifonauta	Subadult	-	-	-	-	-	-	-	-	-	7.14	-	-
	Adult	-	-	-	-	-	-	-	-	-	-	-	-
Nemtode (parasite)	Subadult	-	-	-	-	-	-	-	-	-	21.43	-	-
	Adult	-	-	-	-	-	-	-	-	-	4.17	-	100
Unidentified invertebrate	Subadult	-	-	-	-	-	-	-	-	-	21.42	-	-
	Adult	-	-	-	-	-	-	-	-	-	8.33	-	-
Organic matter	Subadult	-	-	-	-	-	-	-	-	-	35.71	-	-
	Adult	-	-	-	-	-	-	-	-	100	58.33	-	100
Isopods	Subadult	-	-	-	-	-	-	-	-	-	-	-	-
	Adult	-	-	-	-	-	-	-	-	50	-	-	-

Appendix 12. Summary of the index of relative importance (%I_{RI}) of food items and microplastics ingested by *H. corvinaeformis* according to the factors: season (ED-Early dry, LD- Late dry, ER-Early rainy, LR- Late rainy), area (Upper, Middle and Lower) and ontogenetic phase (juvenile, sub-adult and adult).

		%I_{RI}											
		Upper				**Midlle**				**Lower**			
Items ingested by *H. corvinaeformis*		**ED**	**LD**	**ER**	**LR**	**ED**	**LD**	**ER**	**LR**	**ED**	**LD**	**ER**	**LR**
Microplastic	Subadult	-	-	-	-	-	-	-	-	-	1.39	-	-
	Adult	-	-	-	-	-	-	-	-	20.15	1.11	-	-
A. flexuosa	Subadult	-	-	-	-	-	-	-	-	-	71.11	-	-
	Adult	-	-	-	-	-	-	-	-	-	45.31	98.4	89.98
Mytella falcata	Subadult	-	-	-	-	-	-	-	-	-	1.67	-	-
	Adult	-	-	-	-	-	-	-	-	3.8	33.87	-	-
Gastropoda	Subadult	-	-	-	-	-	-	-	-	-	0.56	-	-
	Adult	-	-	-	-	-	-	-	-	-	0.011	-	-
Brachyuran crab	Subadult	-	-	-	-	-	-	-	-	-	0.03	-	-
	Adult	-	-	-	-	-	-	-	-	5.82	0.01	-	-
Megalopa of brachyura	Subadult	-	-	-	-	-	-	-	-	-	-	-	-
	Adult	-	-	-	-	-	-	-	-	-	0.02	1.6	-
Callinectes sp.	Subadult	-	-	-	-	-	-	-	-	-	0.12	-	-
	Adult	-	-	-	-	-	-	-	-	2.86	0.24	-	-
Lucifer faxoni	Subadult	-	-	-	-	-	-	-	-	-	0.55	-	-
	Adult	-	-	-	-	-	-	-	-	2.04	0.18	-	-
Isopods	Subadult	-	-	-	-	-	-	-	-	-	-	-	-
	Adult	-	-	-	-	-	-	-	-	4.09	-	-	-

Appendix 12. Continued.

		%I_{RI}											
		Upper				Midlle				Lower			
Items ingested by *H. corvinaeformis*		**ED**	**LD**	**ER**	**LR**	**ED**	**LD**	**ER**	**LR**	**ED**	**LD**	**ER**	**LR**
Ostracods	Subadult	-	-	-	-	-	-	-	-	-	0.2	-	-
	Adult	-	-	-	-	-	-	-	-	-	-	-	-
Penaeidae shirmp	Subadult	-	-	-	-	-	-	-	-	-	-	-	-
	Adult	-	-	-	-	-	-	-	-	-	0.36	-	-
Nauplii of Cirripedia	Subadult	-	-	-	-	-	-	-	-	-	-	-	-
	Adult	-	-	-	-	-	-	-	-	-	0.26	-	-
Nematoda (parasite)	Subadult	-	-	-	-	-	-	-	-	-	1.23	-	-
	Adult	-	-	-	-	-	-	-	-	-	0.4	-	5.01
Polychaeta	Subadult	-	-	-	-	-	-	-	-	-	17.9	-	-
	Adult	-	-	-	-	-	-	-	-	16.64	14.38	-	-
Cifonauta	Subadult	-	-	-	-	-	-	-	-	-	0.01	-	-
	Adult	-	-	-	-	-	-	-	-	-	-	-	-
Unidentified invertebrate	Subadult	-	-	-	-	-	-	-	-	-	0.93	-	-
	Adult	-	-	-	-	-	-	-	-	-	1.31	-	-
Organic matter	Subadult	-	-	-	-	-	-	-	-	-	1.66	-	-
	Adult	-	-	-	-	-	-	-	-	12.82	2.44	-	5.01



Appendix 13. Average and standard error (±S.E.) number of prey items ingested by the different ontogenetic phases of *Haemulopsis corvinaeformis* (■ sub-adult and ■ adult) along the main channel of the Goiana Estuary (upper. middle and lower), over the seasonal cycle, (early and late dry seasons; and early and late rainy seasons).

Appendix 14. Summary of the ANOVA (F-values; df: degree of freedom; p-value and *post-hoc* comparisons) for the number and weight of prey items ingested by *Haemulopsis corvinaeformis* according to the factors: seasons (ED: Early dry, LD: Late dry, ER: Early rainy, LR: Late rainy), areas (U: Upper, M: Middle and L: Lower) and ontogenetic phases (Sub: Sub-adult and Adu: Adult). Differences among factors were determined by Bonferroni's test: not significant (ns); $p < 0.05$ (*); $p < 0.01$ (**). Bold represents the sources of variance within homogeneous groups.

		Items in number				**Items in weight**			
Ingested Items	**Factors**	**F**	***df***	***p*-value**	***Post-hoc***	**F**	***df***	***p*-value**	***Post-hoc***
A. flexuosa	Area	64.58	2	0.001	U M **L** **	21.1	2	0.001	U M **L** **
	Season	25.28	3	0.001	ED **LD** ER LR **	5.32	3	0.001	ED **LD** ER LR **
	Phase	20.53	2	0.001	Sub **Adu** **	8.32	2	0.001	Sub **Adu** **
	Area *vs*. Season	25.29	6	0.001	**	5.32	6	0.001	**
	Area *vs.* Phase	20.52	4	0.001	**	8.32	4	0.001	**
	Season *vs*. Phase	8.55	6	0.001	**	2.37	6	0.001	**
	Area *vs.* Season *vs*. Phase	8.55	12	0.001	**	2.37	12	0.001	**
Polychaeta	Area	38.08	2	0.001	U M **L** **	2.58	2	0.079	ns
	Season	22.71	3	0.001	ED **LD** ER LR **	1.88	3	0.135	ns
	Phase	9.74	2	0.001	Sub **Adu** **	1.34	2	0.264	ns
	Area *vs*. Season	22.71	6	0.001	**	1.88	6	0.087	ns
	Area *vs.* Phase	9.74	4	0.001	**	1.34	4	0.257	ns
	Season *vs*. Phase	5.89	6	0.001	**	1.38	6	0.225	ns
	Area *vs.* Season *vs*. Phase	5.89	12	0.001	**	1.38	12	0.180	ns
Mytella falcata	Area	8.07	2	0.001	U M **L** **	7.46	2	0.001	U M **L** **
	Season	3.33	3	0.021	ED **LD** ER LR *	5.42	3	0.001	ED **LD** ER LR **
	Phase	2.72	2	0.069	ns	2.79	2	0.064	ns
	Area *vs*. Season	3.33	6	0.001	**	5.43	6	0.001	**
	Area *vs.* Phase	2.73	4	0.032	*	2.79	4	0.028	*
	Season *vs*. Phase	1.53	6	0.171	ns	2.93	6	0.001	**
	Area *vs.* Season *vs*. Phase	1.54	12	0.119	ns	2.93	12	0.001	**
Brachyuran crab	Area	2.66	2	0.073	ns	1.95	2	0.145	ns

	Season	2.66	3	0.050	ns	1.95	3	0.123	ns
	Phase	2.66	2	0.73	ns	1.95	2	0.145	ns
	Area *vs.* Season	2.66	6	0.018	*	1.95	6	0.076	ns
	Area *vs.* Phase	2.66	4	0.035	*	1.95	4	0.104	ns
	Season *vs.* Phase	2.66	6	0.017	*	1.95	6	0.076	ns
	Area *vs.* Season *vs.* Phase	2.66	12	0.001	**	1.95	12	0.032	*
Lucifer faxoni	Area	11.99	2	0.001	U M **L** **	6.84	2	0.001	U M **L** **
	Season	7.91	3	0.001	ED **LD** ER LR **	5.49	3	0.001	**ED** LD ER LR **
	Phase	3.15	2	0.046	Sub **Adu** *	1.71	2	0.183	ns
	Area *vs.* Season	7.91	6	0.001	**	5.49	6	0.001	**
	Area *vs.* Phase	3.15	4	0.016	*	1.71	4	0.150	ns
	Season *vs.* Phase	2.2	6	0.046	*	1.49	6	0.186	ns
	Area *vs.* Season *vs.* Phase	2.2	12	0.014	*	1.49	12	0.135	ns
Callinectes sp.	Area	2.96	2	0.001	U M **L** **	3.66	2	0.028	U M **L** *
	Season	2.74	3	0.03	ED **LD** ER LR *	1.34	3	0.263	ns
	Phase	1.68	2	0.19	ns	1.39	2	0.251	ns
	Area *vs.* Season	2.96	6	0.001	**	1.34	6	0.243	ns
	Area *vs.* Phase	1.68	4	0.15	ns	1.39	4	0.239	ns
	Season *vs.* Phase	1.04	6	0.43	ns	0.73	6	0.625	ns
	Area *vs.* Season *vs.* Phase	1.04	12	0.42	ns	0.73	12	0.722	ns

Appendix 15. Summary of the ANOVA (F-values; df: degree of freedom; p-value and *post-hoc* comparisons) for the number of microplastics ingested by *Haemulopsis corvinaeformis* according to the factors: seasons (ED: Early dry, LD: Late dry, ER: Early rainy, LR: Late rainy), areas (U: Upper, M: Middle and L: Lower) and ontogenetic phases (Sub: sub-adult and Adu: adult). Differences among factors were determined by Bonferroni's test: not significant (ns); $p < 0.05$ (*); $p < 0.01$ (**). 00Bold represents the sources of variance within homogeneous groups.

		Items in number			
Ingested items	**Factors**	**F**	***df***	***p*-value**	***Post-hoc***
Total microplastics	Area	68.2	2	0.001	U M **L** **
	Season	42.45	3	0.001	ED **LD** ER LR **
	Phase	17.05	2	0.001	Sub **Adu** **
	Area *vs*. Season	42.45	6	0.001	**
	Area *vs.* Phase	17.05	4	0.001	**
	Season *vs*. Phase	10.61	6	0.001	**
	Area *vs.* Season *vs*. Phase	10.61	12	0.001	**
Blue	Area	62.71	2	0.001	U M **L** **
	Season	38.47	3	0.001	ED **LD** ER LR **
	Phase	15.69	2	0.001	Sub **Adu** **
	Area *vs*. Season	38.46	6	0.001	**
	Area *vs.* Phase	15.69	4	0.001	**
	Season *vs*. Phase	9.64	6	0.001	**
	Area *vs.* Season *vs*. Phase	9.64	12	0.001	**
Red	Area	12.56	2	0.001	U M **L** **
	Season	12.56	3	0.001	ED **LD** ER LR **
	Phase	3.88	2	0.001	Sub **Adu** **
	Area *vs*. Season	12.56	6	0.001	**
	Area *vs.* Phase	3.88	4	0.001	**
	Season *vs*. Phase	3.88	6	0.001	**
	Area *vs.* Season *vs*. Phase	3.88	12	0.001	**
Black	Area	3.65	2	0.028	U M **L** *
	Season	3.65	3	0.014	ED **LD** ER LR *
	Phase	1.49	2	0.228	ns
	Area *vs*. Season	3.65	6	0.002	**
	Area *vs.* Phase	1.49	4	0.205	ns
	Season *vs*. Phase	1.49	6	0.182	ns
	Area *vs.* Season *vs*. Phase	1.49	12	0.131	ns

Appendix 16. Summary of canonical correspondence analysis (CCA) analysing four environmental parameters (salinity, rainfall, water temperature and dissolved Oxygen) and the index of relative importance (%IRI) of natural prey items and microplastics ingested by the different ontogenetic phases of *P. ramosus* and *H. corvinaeformis* in the Goiana River Estuary along the seasonal cycle. * ($P < 0.05$); ** ($P < 0.01$).

	Axis 1	**Axis 2**
Eigenvalues	0.1844	0.0508
Explained variation cumulative of species-environmental variables	14.54	19.33
Pseudo-canonical	0.6934	0.6889
Explained fitted variation (%) of species-environmental variables	61.89%	82.30%

Correlation with environmental variables	**Explains (%)**	**Pseudo-*F***	***P*-value**
Salinity	14.1	3.3	0.001 **
Dissolved Oxygen (mg L^{-1})	13.4	3.1	0.01*
Rainfall (mm)	7.5	1.6	0.12
Temperature (°C)	5.5	1.2	0.25

5 CONCLUSÃO GERAL

Os hábitats do ecossistema estuarino do Rio Goiana proporcionam diversas condições espaciais e temporais para o ciclo de vida de espécies da família Hamulidae. As espécies *P. ramosus* e *H. corvinaeformis* utilizam o estuário em condições ambientais diferentes regidas pela sazonalidade local. Esses movimentos estão cicronizados com as mudanças nas variáveis ambientais que ocorrem nestas áreas ao longo do ciclo sazonal. No período chuvoso, a espécie *P. ramosus* ocupa as porções superior e intermediário do estuário, onde a salinidade é baixa. No período de seca, *H. corvinaeformis* realiza suas atividades biológicas no estuário inferior, quando a salinidade é elevada. Desse modo, as espécies se utilizam de nichos específicos e não competem por recursos, pois estão presentes em períodos e habitats distintos. Assim, esse estudo comprova que a variação do gradiente de salinidade em função da precipitação favorece a distribuição dessas espécies no estuário do Rio Goiana.

O estudo também comprova que cada fase ontogenética das espécies apresentam requerimentos alimentares distintos. Os juvenis de *P. ramosus* são zooplanktovoros se alimentando principalmente de copépodas Calanoida, enquanto os subadultos e adultos tem hábitos zoobentívoros, se alimentando de anelídeos Polychaeta, e dos bilvalves *A. flexuosa* e *M. falcata*. Além disso, foi observado que as mudanças para uma dieta mais diversa e o início de um forrageamento mais complexo em organismos bentônicos podem estar associados aos valores mais elevados de uma contaminação por filamentos de microplásticos, especialmente os de cor azul, por serem os mais abundantes.

De fato, todas as fases ontogenéticas foram contaminadas por filamentos de microplásticos. Entretanto, a principal demostração desse estudo é de que os picos de abundância das fases ontogenéticas mais contaminadas de ambas as espécies dentro do canal principal coincide com os picos de abundância de microplásticos em massas d'água de fundo. Isso sugere que, além dos hábitos alimentares bentônicos, a relação pentre a distribuição espacial e temporal dos haemulideos e dos microplásticos no estuário do Rio Goiana, pode aumentar as chances de contaminação dessas espécies.

Levando em consideração estas informações, fica claro que é necessário uma perspectiva mais atuante no manejo e conservação da Resex Acaú-Goiana, uma vez que esses contaminantes podem estar atuando como vetores de outros contaminantes ao longo da teia trófica estuarina, atingindo recursos pesqueiros importantes para as comunidades adjacentes ao estuário do Rio Goiana.

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