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A ECOLOGIA DOS PREDADORES DE TOPO NA ICTIOFAUNA DO ESTUÁRIO DO RIO GOIANA

Recife
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GUILHERME VITOR BATISTA FERREIRA

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Tese apresentada ao Programa de Pós-Graduação em Oceanografia, da Universidade Federal de Pernambuco, como requisito parcial para obtenção do título de Doutor em Oceanografia.

Área de concentração: Oceanografia Biológica.

Orientador: Prof. Dr. Mário Barletta.

Dr. André Ricardo de Araújo Lima.

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RESUMO

Estuários tropicais são ecossistemas produtivos em razão do grande aporte de matéria orgânica oriundo da descarga fluvial. O ambiente estuarino tropical apresenta uma grande variabilidade sazonal, ocasionada principalmente pelo regime de chuvas, que acaba influenciando diversos fatores abióticos, que por sua vez controlam os padrões ecológicos da fauna. Dentre as diversas espécies que utilizam o ambiente estuarino, os representantes dos Camurins e as Pescadas são particularmente relevantes, em razão do seu alto valor comercial. Além dessas espécies serem alvo da atividade pesqueira, suas fases adultas usualmente ocupam o nível trófico mais alto do ambiente estuarino, sendo caracterizadas como predadores de topo. Considerando a importância deste grupo, este estudo teve como objetivo avaliar os padrões ecológicos de distribuição, alimentação e ingestão de microplásticos nos predadores de topo (*Centropomus undecimalis*, *Centropomus mexicanus*, *Centropomus pectinatus* e *Cynoscion acoupa*) do estuário do rio Goiana, em relação aos aspectos espaciais, sazonais e ontogenéticos. Os representantes da fase adulta das espécies estudadas usualmente são distribuídos na porção externa do estuário, principalmente no estuário inferior e na zona costeira, onde se alimentam principalmente de peixes demersais e pelágicos, utilizando camarões como uma fonte alternativa de alimento. A análise de ingestão de microplásticos identificou a fase adulta como a mais contaminada, como uma consequência direta do seu hábito alimentar. As maiores taxas de ingestão foram observadas em adultos de *C. acoupa* e *C. undecimalis* que utilizaram os hábitats externos. As espécies avaliadas realizam o processo reprodutivo na zona costeira, após a eclosão, as larvas utilizam o fluxo de maré para migrar a montante no estuário em busca de abrigo e maior disponibilidade de alimento. Os indivíduos juvenis ocupam principalmente o estuário superior, que tem um papel muito importante como principal área de berçário. As espécies utilizaram o mesmo hábitat como berçário em diferentes estações do ano, sugerindo o uso de uma estratégia para evitar competição interespecífica, principalmente em razão do hábito alimentar dessas espécies ser muito semelhante, constituído basicamente de poliquetas. De forma geral os juvenis apresentaram um hábito alimentar oportunista, evidenciado por um amplo leque alimentar, que inclui zooplâncton, invertebrados detritívoros e peixes de menores dimensões. A medida que os indivíduos juvenis se desenvolvem, eles progressivamente utilizam os hábitats mais externos do estuário. Esse processo migratório ocorre principalmente no início e no fim do período chuvoso, quando a influência do rio é maior no estuário. Os juvenis apresentaram baixas taxas

de ingestão de microplástico, quando comparado com a fase adulta, o que está diretamente associado a ingestão de recursos alimentares de menor nível trófico. Os indivíduos subadultos se distribuem por todos os hábitats estuarinos avaliados, sua alimentação também é classificada como oportunista. Porém, essa fase ontogenética passa a incorporar uma maior seletividade nos recursos alimentares, apresentando uma tendência ao piscivorismo, predando peixes pelágicos no estuário inferior e na zona costeira. Os níveis de gerais de contaminação constatados nos indivíduos subadultos foram semelhantes ao dos juvenis. Porém, quando os subadultos apresentaram uma ingestão de presas com maior nível trófico, seus níveis de contaminação foram superiores ao dos juvenis.

Palavras-chave: Estuário tropical. Distribuição espacial. Ecologia alimentar. Ingestão de microplásticos. Ecoclina estuarina.

ABSTRACT

Tropical estuaries are highly productive ecosystems due to the great input of organic matter provided by the river flow. Moreover, tropical estuaries are highly variable regarding seasonality, which is mostly influenced by rainfall, affecting many abiotic features that in turn rule the ecological patterns of wildlife. Among the great variety of species that inhabit the estuarine environment, the snooks (*Centropomus* spp.) and acoupa weakfish (*Cynoscion* sp.) are particularly relevant because of their commercial value. This group is one of the main target species of the fishery activity, and when in the adult phase they usually have the highest trophic level within estuarine community (top predators) and control the entire food web. Assuming the relevance of this group, this study aimed to evaluate the patterns of distribution, feeding and microplastic ingestion of the top predators (*Centropomus undecimalis*, *Centropomus mexicanus*, *Centropomus pectinatus* and *Cynoscion acoupa*) of the Goiana Estuary, regarding the spatial, seasonal and ontogenetic variability. The specimens belonging to the adult phase of the species usually inhabit the outer habitats of the estuary, mostly the lower estuary and the coastal zone, where they feed on demersal and pelagic fishes, and prey on shrimps as a complementary resource. The analysis of microplastic ingestion identified the adult phase as the most contaminated, as a directed consequence of their feeding habit. The highest ingestion rates were observed in *C. acoupa* and *C. undecimalis* that inhabited the outer habitats. The studied species spawn in the coastal zone, and larvae use the tidal flow to migrate towards the upper estuary in search of shelter and feeding grounds. The juveniles inhabit mostly the upper estuary that is nursery ground for the studied species. The species used the same habitat as a nursery ground, but in different seasonal periods, suggesting a behavioural adaptation to avoid competition. Their feeding habit is very similar, with all species relying on Polychaeta. Overall, the juveniles are classified as opportunistic, preying on a wide variety of resources, including zooplankton, detritivorous invertebrates and even small fishes. When the juveniles develop, they gradually use the outer estuarine habitats. This migratory process occurs mainly during the early rainy and late rainy seasons, when river have a higher influence on the middle and lower estuaries. Juveniles have lower rates of microplastic contamination when compared to the adult phase, which is associated with the ingestion of prey of lower trophic levels. The sub-adults use all estuarine habitats, their feeding habit is also asserted as opportunistic. However, this ontogenetic phase shifts their diet towards a narrow spectrum, preying on pelagic fishes, mostly in the lower estuary and the coastal zone. Overall, the contamination rates of sub-adults were similar to those of juveniles.

However, when the sub-adults fed on prey of higher trophic levels, their contamination rates were higher than the observed for juveniles.

Keywords: Tropical estuary. Spatial distribution. Feeding ecology. Microplastic ingestion. Estuarine ecocline.

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1 INTRODUÇÃO

O ecossistema estuarino é responsável por desempenhar um importante papel na estruturação ecológica, social e econômica dos ambientes costeiros (COSTANZA et al., 2014; ODUM, 1984). Do ponto de vista ambiental, a grande relevância dos estuários é o resultado das suas características de ambiente transicional, estabelecendo uma conexão entre os ecossistemas marinhos e continentais. Este processo é responsável por um forte gradiente nos parâmetros físico-químicos, que apresentam uma grande variabilidade sazonal (BARLETTA; LIMA, 2019; BURTON, 1976), resultando na formação de uma ecoclina, caracterizada pela sucessão de espécies que ocorrem ao longo desse gradiente ambiental (BARLETTA et al., 2005).

Desta forma, os estuários são capazes de sustentar uma grande produção biológica, viabilizando uma ampla ocorrência da comunidade aquática, sobretudo da ictiofauna (BLABER; BREWER; SALINI, 1989; LIMA; FERREIRA; BARLETTA, 2019). Caracterizando esse ecossistema como fundamental para esse grupo ecológico, por fornecer locais ideais para alimentação, abrigo, reprodução, crescimento e principalmente habitats berçário, utilizados pelas espécies para completar seu ciclo de vida (DANTAS et al., 2012). Hábitats berçário são caracterizados por apresentarem uma contribuição acima da média de indivíduos juvenis que recrutam para a população adulta, quando comparado aos demais hábitats do ecossistema (BECK et al., 2001).

A assembleia de peixes estuarina é definida como um conjunto de diferentes espécies que ocorrem de forma simpátrica, utilizando os recursos disponíveis de um ecossistema de diversas formas. Dentre os principais grupos, estão os que ocupam o estuário permanentemente, como no caso das espécies estuarinas residentes, ou de forma temporária, como as espécies estuarinas dependentes, que utilizam esse ecossistema principalmente nos estágios iniciais, em busca de locais de alimentação e abrigo dos predadores marinhos (ELLIOTT et al., 2007). Dentre as diversas interações que ocorrem na ictiofauna, a predação é uma das mais importantes (TAYLOR, 2005), sendo responsável por influenciar toda a cadeia trófica de um ecossistema, desta forma as espécies designadas como predadores de topo, exercem uma função crucial em seus ecossistemas, controlando toda a teia trófica local (CARPENTER; KITCHELL; HODGSON, 1985).

As principais espécies predadoras de topo dos ecossistemas estuarinos do Atlântico ocidental possuem um grande porte e usualmente compõem espécies da família Centropomidae e Sciaenidae (CERVIGÓN et al., 1993; CONTENTE; STEFANONI; GADIG,

2009). A família Centropomidae contém 12 espécies pertencentes a um único gênero e são conhecidos popularmente como robalos ou camurins. No caso da família Sciaenidae, os principais representantes dos níveis tróficos mais elevados pertencem ao gênero *Cynoscion*, composto por 24 espécies denominadas de pescadas (ORRELL, 2002).

Essas espécies (robalos e pescadas) possuem um hábito demersal e são comumente encontradas em substratos lamosos de ambientes estuarinos, lagunas e nas zonas costeiras adjacentes (ORRELL, 2002). Sua distribuição ocorre ao longo das áreas mais externas do estuário, durante a fase adulta e utilizam as regiões estuarinas internas e áreas com maior complexidade estrutural em busca de proteção nas fases iniciais de vida (CERVIGÓN; ALCALÁ, 1991; DANTAS; BARLETTA, 2016; STEVENS; BLEWETT; POULAKIS, 2007). Sua alimentação é baseada em um hábito de forrageamento noturno, predando principalmente peixes e crustáceos (ADAMS; WOLFE; LAYMAN, 2009; CONTENTE; STEFANONI; GADIG, 2009; FERREIRA et al., 2016).

O ecossistema estuarino é crucial para o processo reprodutivo e o recrutamento larval dessas espécies. De forma geral, as espécies da família Centropomidae apresentam um comportamento hermafrodita protândrico, no qual as gônadas inicialmente sofrem o processo de maturação no sexo masculino e posteriormente, caso haja necessidade, em virtude de uma baixa densidade de fêmeas, os indivíduos são capazes de se converterem ao sexo feminino, provocando uma diferença entre a proporção de machos e fêmeas entre os hábitats (PERERA-GARCÍA et al., 2011). No caso do gênero *Cynoscion*, que não apresenta o mesmo comportamento hermafrodita, o processo reprodutivo também ocorre na zona costeira adjacente ao estuário, posteriormente as fases larvais entram no ecossistema estuarino utilizando o fluxo de maré (LIMA; BARLETTA; COSTA, 2015).

Além da grande importância ecológica, estes grupos (Centropomidae e *Cynoscion*) também apresentam uma ampla relevância econômica, principalmente nos estuários tropicais e nas zonas costeiras adjacentes. Os principais representantes desses grupos são encontrados em uma área que se estende do Golfo do México ao sul do Brasil. Em muitos casos, sendo inclusive as espécies alvo da atividade pesqueira local (BARLETTA; COSTA, 2009; NIETO-NAVARRO et al., 2010), registrando uma produção média anual de 4 mil (*Centropomus sp.*) e 20 mil (*Cynoscion acoupa*) toneladas no Brasil (IBAMA, 2007).

Em razão da forte pressão exercida nas espécies de importância econômica, principalmente pela atividade pesqueira e pela perda de habitats essenciais para as espécies completarem o seu ciclo de vida nos ecossistemas estuarinos, suas populações vêm sofrendo um rápido declínio global (BARLETTA; LIMA, 2019; MYERS; WORM, 2003). Além disso,

toda a ictiofauna (*eg*. Trichiuridae, Ariidae, Gerreidae), sobretudo as espécies de maior nível trófico (Sciaenidae), estão altamente suscetíveis ao processo de contaminação por resíduos da atividade antrópica nos estuários, como metais pesados e fragmentos de plástico (DANTAS; BARLETTA; COSTA, 2012; FERREIRA et al., 2016; POSSATTO et al., 2011; RAMOS; BARLETTA; COSTA, 2012; SILVA et al., 2018).

Produtos manufaturados a partir de polímeros de hidrocarbonetos, popularmente denominados como plásticos, possuem uma grande relevância na dinâmica econômica global (ANDRADY; NEAL, 2009), principalmente por apresentarem uma ampla versatilidade de uso, uma grande durabilidade, baixo peso molecular e baixo custo de produção (DERRAIK, 2002). Entretanto, uma considerável parcela dos produtos que utilizam plásticos não são propriamente descartados após o uso, resultando na entrada desses resíduos em ambientes aquáticos (BARLETTA; LIMA; COSTA, 2019; FENDALL; SEWELL, 2009).

No ambiente aquático os plásticos são expostos a radiação ultravioleta, a hidrodinâmica dos corpos d'água e à ação da alça microbiana, e consequentemente têm sua estrutura física e química degradada, resultando em uma fragmentação em menores partículas (GEWERT; PLASSMANN; MACLEOD, 2015). As partículas menores que 5mm são denominadas como microplásticos (ARTHUR; BAKER; BAMFORD, 2009) e são o tipo mais comum de partículas antropogênicas em ambientes marinhos (THOMPSON, 2004), em alguns casos sua densidade pode apresentar a mesma ordem de grandeza que a do ictioplâncton (LIMA et al., 2016). Em decorrência da grande disponibilidade, pequena dimensão e semelhança (visual e olfativa) com organismos aquáticos, os microplásticos são altamente suscetíveis à serem ingeridos pela comunidade estuarina (STEER et al., 2017; SUN et al., 2017).

Desta forma, estudos que avaliam a ecologia e o grau de exposição dessas espécies às pressões antrópicas, são de grande urgência. Levando em consideração essas informações, a hipótese avaliada nesse estudo é que a variabilidade sazonal da ecoclina estuarina influencia na dinâmica ecológica e na contaminação por microplástico ao longo do ciclo de vida dos predadores de topo do estuário do Rio Goiana.

1.1 OBJETIVOS

Avaliar a influência da variabilidade sazonal e especial do estuário do Rio Goiana na dinâmica de utilização dos recuros estuarinos e na contaminação por microplásticos ao longo da ontogenia dos predadores de topo.

1.1.1 Objetivo geral

O presente estudo tem como objetivo descrever a influência do ciclo de vida e dos fatores ambientais na ecologia dos predadores de topo da assembleia de peixes estuarina do Rio Goiana (*Centropomus undecimalis, Centropomus mexicanus*, *Centropomus pectinatus* e *Cynoscion acoupa*). Além de estabelecer uma associação entre a dinâmica de contaminação de microplásticos e os padrões ambientais, e ecológicos das espécies avaliadas.

1.1.2 Objetivos específicos

- Classificar as espécies avaliadas de acordo com a sua ontogenia em juvenis, subadultos e adultos;
- investigar como a distribuição espacial e sazonal dos predadores de topo, ao longo do estuário está atribuída ao seu ciclo de vida e as flutuações das condições ambientais da área de estudo;
- identificar o eventual uso dos hábitats do estuário do Rio Goiana como área de berçário pelas espécies estudadas;
- descrever a variação na matriz alimentar em função do desenvolvimento ontogenético das espécies e da variação sazonal e espacial no estuário do Rio Goiana;
- avaliar os padrões de contaminação por microplásticos em relação a variabilidade ambiental do ecossistema;
- identificar a influência dos aspectos ecológicos e ontogenéticos na ingestão de microplásticos pelas espécies avaliadas.

2 METODOLOGIA

Metodologia aplicada com o intuito de responder as hipóteses elaboradas no estudo.

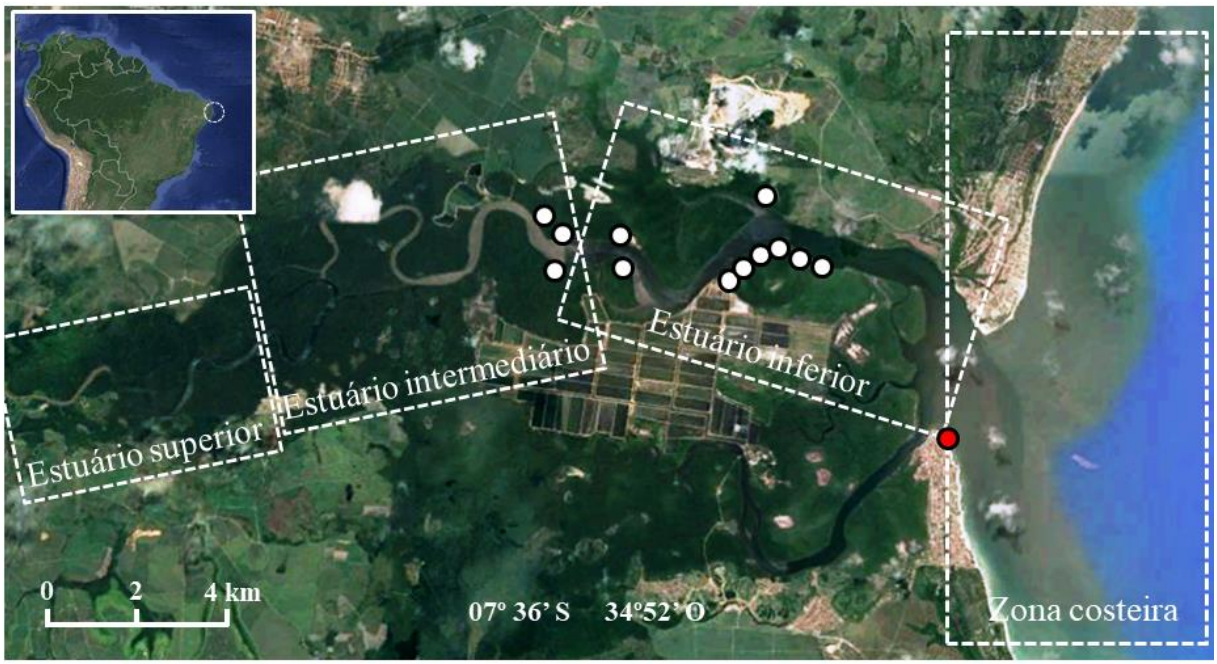
2.1 ÁREA DE ESTUDO

O estuário do Rio Goiana está situado no extremo leste da América do Sul, especificamente na região nordeste do Brasil, entre os estados de Pernambuco e Paraíba (Fig. 1). O ecossistema estuarino possui uma área total de 4700 ha e apresenta uma grande diversidade de habitats costeiros, como o canal principal do rio, a planície de maré, a floresta de mangue que circunda todo o estuário, praias arenosas localizadas na foz do estuário, além da região costeira altamente influenciada pela descarga do rio que também engloba prados de capim marinho e recifes de arenito (BARLETTA; COSTA, 2009). No ano de 2000, foi criada uma unidade de conservação do tipo extrativista dentro da região estuarina do Rio Goiana (Resex Acaú-Goiana), que tem como objetivo desenvolver o uso sustentável dos recursos naturais do ambiente pelas populações tradicionais.

A principal atividade econômica exercida pela população que ocupa as margens do estuário é a pesca artesanal, que explora principalmente a ictiofauna pertencente às famílias Centropomidae, Sciaenidae, Carangidae, Mugilidae, Hemiramphidae e Lutjanidae, além de crustáceos como ostras, caranguejos e lagostas. Diversas artes de pesca são utilizadas no estuário (*eg.* tarrafa, rede de espera, rede de arrasto, curral, covo e mergulho livre com arpão) e a frota pesqueira é composta principalmente de embarcações rústicas movidas à força eólica e pequenos barcos motorizados (GUEBERT-BARTHOLO et al., 2011).

O clima da região é classificado como tropical, com uma temperatura média do ar de 27ºC, apresentando uma pequena amplitude de 2ºC. De acordo com as características pluviométricas, a sazonalidade deste ambiente apresenta quatro estações; o início do período chuvoso (entre março e maio), o fim do período chuvoso (junho a agosto), o início da estiagem (setembro a novembro) e o fim da estiagem dezembro a fevereiro) (BARLETTA; COSTA, 2009).

Figura 1 - Estuário do Rio Goiana, situado na região nordeste do Brasil entre os estados de Pernambuco e Paraíba



Fonte: O Autor, 2015.
Os pontos marcados por ○ representam a entrada dos canais de maré e ● indica a estação meteorológica.

2.2 MÉTODOS AMOSTRAIS

Para averiguar como ocorrem os padrões de movimentação e alimentação dos predadores de topo do Estuário do Rio Goiana, foram avaliados diversos ecossistemas, para que se possa ter um entendimento de como o ambiente estuarino atua no ciclo de vida dessas espécies. Entre os ambientes estudados estão o canal principal do rio (que compreende diferentes porções do estuário), os canais de maré e a região costeira adjacente a foz do rio (Fig. 1).

A coleta da ictiofauna e das variáveis abióticas deste estudo tem sido realizada desde 2005, através de diversos projetos de pesquisa (Projeto FACEPE Nº: APQ-0586-1.08/06, APQ-0911-1.08/12; Projeto Universal CNPq Nº: 37384/2004-7, 474736/2004 e 482921/2007-2, CT-Hidro 29/2007/CNPq Nº: 552896/2007-1, 405818/2012-2/COAGR/PESCA), realizados com o suporte de uma autorização ambiental para atividades com finalidade cientifica (SISBIO nº 11050-1).

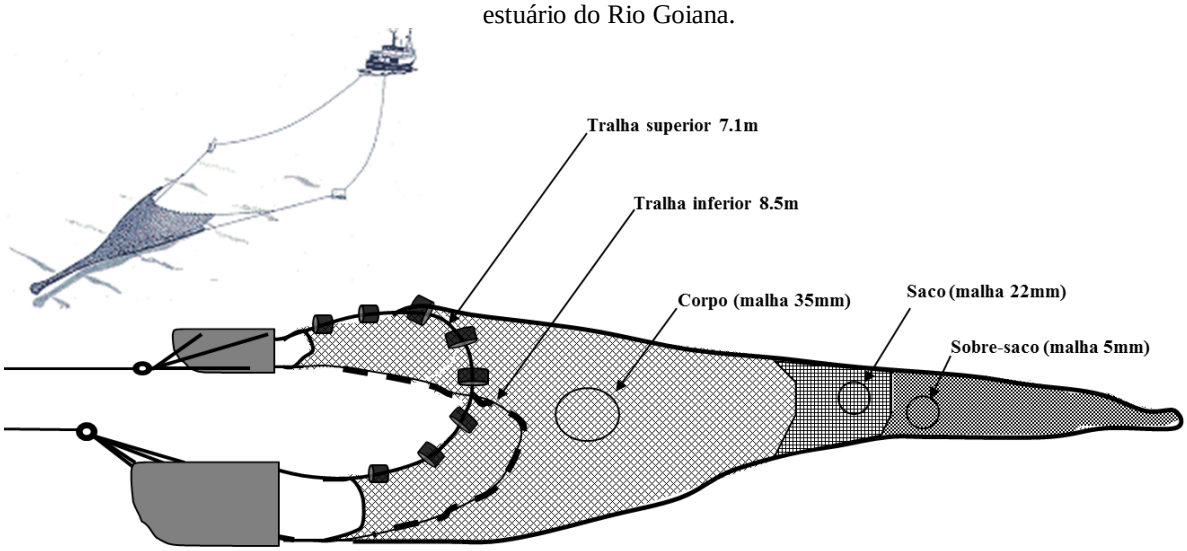
a) Canal principal

O canal principal do estuário foi dividido de acordo com sua morfologia e salinidade em: estuário superior (salinidade < 5; largura 5 - 9m; profundidade média 4,5m), intermediário (salinidade 5 – 20; largura 3 – 37m; profundidade média 4,7m) e inferior

(salinidade > 20; largura 14 – 61m; profundidade média 4,1m) (BARLETTA; COSTA, 2009). Seis réplicas foram realizadas mensalmente em cada área do estuário entre dezembro de 2005 e novembro de 2006. Adicionalmente, foram realizadas amostragens complementares (seis réplicas) no fim do período chuvoso e no fim do período de estiagem entre 2006 e 2009.

As amostragens foram realizadas por uma rede de arrasto de fundo com portas, com malha de 35mm no corpo da rede, 22mm no saco e 5mm no sobre saco (Fig. 2). Os arrastos foram realizados durante a maré de quadratura, por um período de 5 min, em uma profundidade média de 5 metros (Fig. 2) (BARLETTA; COSTA, 2009).

Figura 2 – Rede de arrasto com portas, utilizada para amostragem da ictiofauna no canal principal do estuário do Rio Goiana.

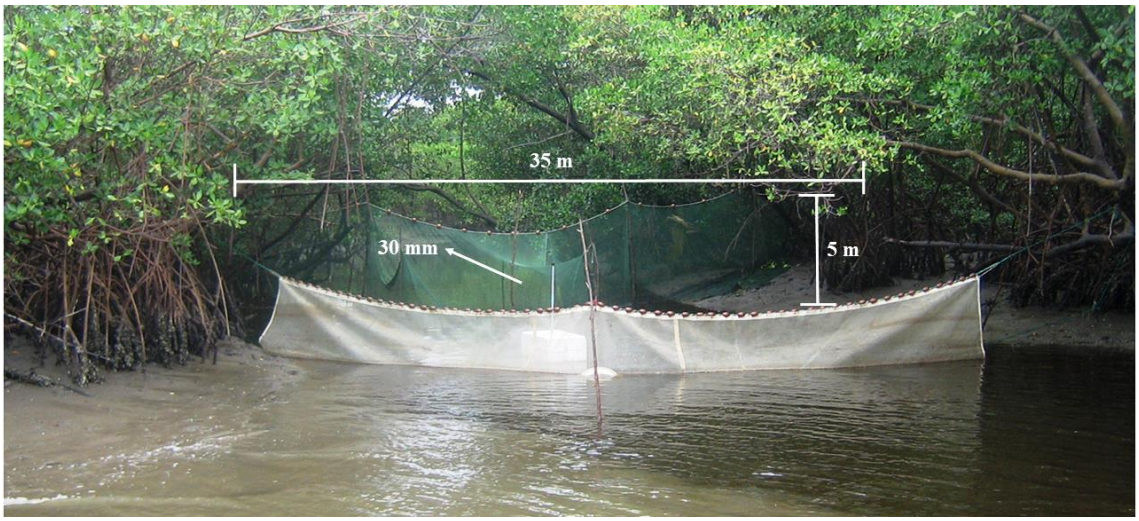


Fonte: LEFECE, 2015.

b) Canais de maré

Para avaliar a importância do hábitat de manguezal, foram realizadas amostragens nos canais de maré localizados no estuário inferior entre os meses de abril e maio de 2008 (três replicas). Foi utilizada uma rede de tapagem de 35m de comprimento e 5m de altura (malha de 10mm), que foi fixada na entrada dos canais de maré durante a preamar e posterirormente retirada na baixa-mar (RAMOS et al., 2011) (Fig. 3). As informações provenientes dos canais de maré foram utilizadas somente no estudo da ecologia alimentar e contaminação por microplástico nas espécies.

Figura 3 - Rede de tapagem, utilizada para amostragem da ictiofauna nos canais de maré do estuário do Rio Goiana.



Fonte: LEFECE, 2015.

c) Zona costeira

Para complementar os dados utilizados no estudo da ecologia alimentar e na contaminação por microplásticos foram incluídos espécimes provenientes da zona costeira do estuário do Rio Goiana. Esses indivíduos foram obtidos diretamente no entreposto pesqueiro local, onde foram obtidas informações das espécies capturadas (número e peso), arte de pesca, local e data da captura, essas informações foram adquiridas entre os anos de 2013 e 2017.

d) Parâmetros ambientais

Simultaneamente ao processo de captura da ictiofauna, também foram obtidos diversos parâmetros abióticos da água de superfície e de fundo, como temperatura (Cº), salinidade (Salinometer WTW LF 197), oxigênio dissolvido (mg/L) (Oximeter WTW Oxi 340) e transparência (Disco de Secchi - cm).

Os dados meteorológicos (*eg.* temperatura do ar, pressão atmosférica, pluviometria, período de insolação, direção e velocidade do vento) foram coletados *in situ* por uma estação meteorológica situada na área de estudo (Fig. 1). Os parâmetros físico-químicos referentes a massa d'água da zona costeira também foram coletados *in situ* por uma boia oceanográfica (Fig. 1). Adicionalmente, uma série história de dados de pluviometria foi compilada do Instituto Nacional de Metrologia (INMET, 2014).

2.3 PROCEDIMENTOS LABORATORIAIS

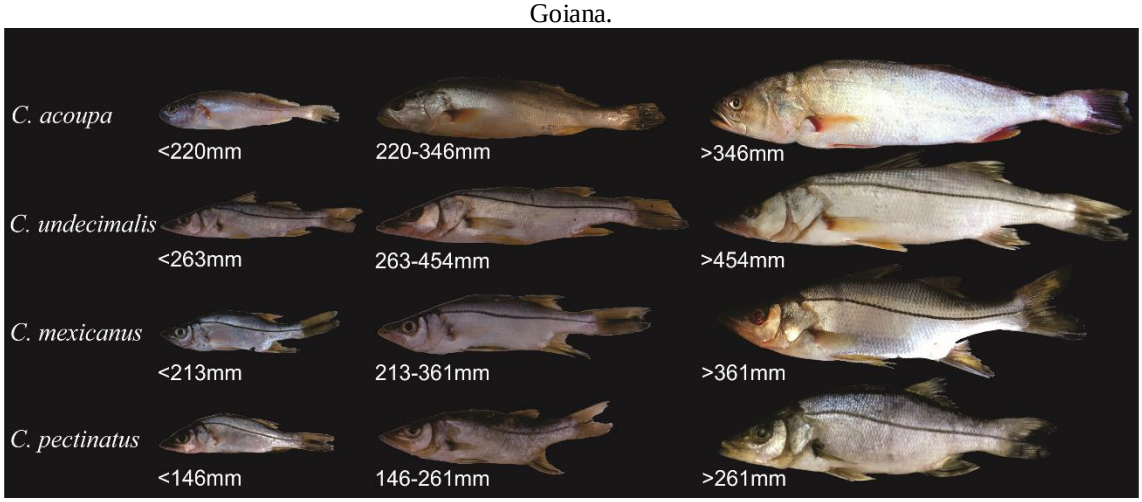
Após a coleta os exemplares foram etiquetados, congelados e transferidos para um banco de amostras. Em laboratório os indivíduos foram descongelados à temperatura ambiente, triados e identificados, em seguida foram tomadas diversas medidas morfométricas

(mm) e o peso total (g) foi aferido. Os peixes foram eviscerados e o trato digestivo (estômago e intestino) foi removido para análise. Posteriormente, os espécimes foram divididos em diferentes classes de tamanho, de acordo com suas fases ontogenéticas.

a) Fases ontogenéticas

O tamanho da transformação, obtido na literatura, foi utilizado para distinguir os espécimes pertencentes à fase larval (não foram analisados no estudo) das demais fases. O ponto de inflexão da curva de peso *vs.* comprimento, foi utilizado para distinguir os indivíduos juvenis dos subadultos e o comprimento médio da primeira maturação (L_{50}), para separar indivíduos subadultos dos adultos (Fig. 4). O comprimento médio da primeira maturação foi obtido através da análise macroscópica das gônadas (VAZZOLER, 1996).

Figura 4 – Fases ontogenéticas (juvenil, subadulto e adulto) dos predadores de topo do estuário do rio Goiana.



Fonte: O Autor, 2019.

b) Análise de dados

Para a análise da distribuição espacial das espécies, foram utilizadas informações de densidade (ind./m²) e biomassa dos indivíduos (peso/m²) de cada unidade amostral. Para o estudo da ecologia alimentar, os exemplares foram eviscerados e o conteúdo alimentar foi extraído do estômago e do intestino, e identificado até o menor nível taxonômico possível, com o auxílio da literatura especializada (RUPPERT; FOX; BARNES, 2004). Em seguida o conteúdo foi lavado com água destilada, seco e pesado em balança analítica. Quando partículas de plásticos foram observadas no conteúdo estomacal dos peixes, elas foram armazenadas em placas de Petri cobertas e secas na estufa a 70ºC por 48h, posteriormente foram fotografadas e avaliadas quanto a sua forma, cor e comprimento.

O Índice de Importância Relativa (PINKAS; OLIPHANT; IVERSON, 1971) foi calculado com o intuito de ser utilizado em uma técnica de ordenação multivariada. O domínio do I_{RI} varia de 0 a 20.000 e o índice consiste da seguinte equação:

$$I_{RI} = \%F_i * (\%N_i + \%P_i)$$

No qual, F_i é o valor referente à frequência de ocorrência dos itens alimentares, N_i representa a frequência numérica dos itens e P_i a porcentagem do peso de cada item alimentar (HYSLOP, 1980).

c) Análises estatísticas

Com o intuito de alcançar a normalidade dos dados, foi utilizado o método de transformação Box-Cox (BOX; COX, 1964). Em seguida, foi aplicado o teste de Levene, para testar a homocedasticidade dos tratamentos e o teste de Kolmogorov-Smirnov, para avaliar se os dados pertencem a uma distribuição normal (UNDERWOOD, 1997). Todos os testes estatísticos foram realizados com um intervalo de confiança superior a 95%.

Posteriormente a análise de variância (ANOVA) foi utilizada para testar se a densidade e a biomassa total dos indivíduos, em suas diferentes fases ontogenéticas apresentaram diferenças significativas em relação aos fatores temporais (estações do ano) e espaciais (diferentes habitats amostrados). Quando os resultados das análises determinaram diferenças significativas, foram realizados testes à *posteriori* (Bonferroni) para definir as fontes de variância (QUINN; KEOUGH, 2002). A análise canônica de correspondência (CCA) foi realizada para constatar possíveis interações ecológicas entre a densidade dos indivíduos e os parâmetros ambientais de cada um dos hábitats avaliados (PALMER, 1993).

As análises estatísticas citadas à cima, ANOVA e CCA, e seus respectivos processos, também foram executadas para os dados obtidos no estudo da ecologia alimentar e ingestão de microplásticos. A análise de variância foi utilizada para testar se o número e o peso dos itens ingeridos por cada espécie, assim como os microplásticos ingeridos apresentaram diferenças significativas em relação aos fatores temporais, espaciais e ontogenéticos. A análise canônica de correspondência foi realizada para identificar possíveis interações ecológicas entre os valores de I_{IR} dos itens ingeridos por cada espécie e os parâmetros ambientais dos hábitats avaliados (Palmer, 1993).

3 ESTRUTURA DA TESE

De acordo como os objetivos e os resultados obtidos ao longo da realização do estudo, a tese de doutorado foi dividida em três artigos. Os artigos foram publicados em periódicos científicos e seguem as normas de publicação dos respectivos periódicos.

Artigo 1: Use of estuarine resources by top predator fishes. How do ecological patterns affect rates of contamination by microplastics?
Artigo publicado na revista Science of the Total Environment (ISSN: 0048-9697) (Impact factor: 5.589). Este estudo avaliou os padrões de distribuição espacial, ecologia alimentar e contaminação por microplástico em relação a variabilidade espaço-temporal das espécies *C. undecimalis* e *C. mexicanus* ao longo do seu ciclo de vida.

Artigo 2: Dynamics of marine debris ingestion by profitable fishes along the estuarine ecocline.
Artigo publicado na revista Scientific Reports (ISSN 2045-2322) (Impact factor: 4.011). Este estudo avaliou os padrões de ingestão de microplástico em relação as diferentes cores e comprimentos de partículas nas espécies *C. undecimalis, C. mexicanus* e *C. pectinaus* de acordo com a variabilidade espaço-temporal e ontogenética.

Artigo 3: High intake rates of microplastics in a Western Atlantic predatory fish, and insights of a direct fishery effect.
Artigo publicado na revista Environmental Pollution (ISSN 00269-7491) (Impact factor: 5.714). Este estudo avaliou os padrões de ingestão de microplástico em relação as diferentes cores e comprimentos de partículas na espécie *C. acoupa* de acordo com a variabilidade espaço-temporal e ontogenética.

4 ARTIGO 1 - USE OF ESTUARINE RESOURCES BY TOP PREDATOR FISHES. HOW DO ECOLOGICAL PATTERNS AFFECT RATES OF CONTAMINATION BY MICROPLASTICS?

Artigo publicado na revista Science of the Total Environment.

Use of estuarine resources by top predator fishes. How do ecological patterns affect rates of contamination by microplastics?

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ABSTRACT

This study assessed the seasonal patterns of habitat utilization, feeding ecology and microplastic contamination in different ontogenetic phases of sympatric snooks (*Centropomus undecimalis* and *C. mexicanus*) inhabiting a tropical estuary. More than 50% of snooks, in all ontogenetic phases, ingested microplastics (1.5 ±0.1 and 1.4 ±0.1 particles ind.$^{-1}$). Juveniles migrated to nursery grounds in the upper estuary, during the early dry (*C. undecimalis* 6.5 ±2.8 ind.$^{-1}$) ($p < 0.01$) and early rainy seasons (*C. mexicanus* 4.1 ±1.9 ind.$^{-1}$). There, they fed mostly on invertebrates (Polychaeta) ($p < 0.01$), and became contaminated by microplastics (*C. undecimalis*: 0.8 ±0.4 particles ind.$^{-1}$; *C. mexicanus*: 1.7 ±0.5 particles ind.$^{-1}$). Sub-adults of both species forage principally in the estuarine habitats after shifting their diet from invertebrates (shrimps) in the upper reaches (1806.4 ±1729.6 mg ind.$^{-1}$) to pelagic fishes (*R. bahiensis*) in seaward habitats (2507.7 ±1758.4 mg ind.$^{-1}$). During feeding continues the contamination by microplastics (3.1 ±0.8 part. ind.$^{-1}$). Adults use the adjacent coastal as feeding and spawning grounds during the rainy season. In this phase, snooks are mostly piscivorous (*R. bahiensis*: up to 5303.8 ±3213.4 mg ind.$^{-1}$), but also ingest penaeid shrimp as complementary item (up to 175.9 ±156.7 mg ind.$^{-1}$). Microplastics contamination rates increased towards the adult phase, with maximum contamination coinciding with peaks of fish ingestion, suggesting trophic transfer of microplastics. The lower estuary and adjacent coastal zone were important contamination sites, especially during the rainy season (up to 3.1 ±0.8 part. ind^{-1}) ($p < 0.01$), when fishery activities is intense and river basin runoff increases.

Consequently, the availability of microplastics is higher during this time of year in the lower portion of the estuary. Snooks had similar prey preferences, but the use of different habitats along the life cycle of each species avoids overlaps in estuarine use and minimizes competition.

Keywords: Life cycle. Marine pollution. Synthetic fibers. Estuarine ecocline. Habitat use. Feeding ecology.

INTRODUCTION

Estuaries are important ecosystems for providing a variety of ecological and economic services (BECK et al., 2001; COSTANZA et al., 2014). These ecosystem are characterized by strong environmental gradients, caused mainly by the mixture of river discharges and oceanic water intrusion (LIMA; BARLETTA; COSTA, 2015). The balance of these forcings provides a sharp variation in the oceanographic parameters along a salinity gradient, within a relatively small and semi-enclosed area, resulting on a diversity of habitats with a great complexity of natural factors and anthropic disturbances, such as environmental contaminants (LIU et al., 2018; REIS et al., 2016). Moreover, estuarine gradients are highly susceptible to seasonal variations (WATANABE et al., 2014) that impact the patterns of habitat use by fish assemblages, and the availability of contaminants (BARLETTA et al., 2005; LUO et al., 2014).

Estuarine fishes are also exposed to other threats as habitat loss, changes in hydrodynamics and poor water quality that might affect ecological services (BARLETTA; LIMA; COSTA, 2019; BLABER et al., 2000; WENGER et al., 2017), and prevents earlier phases to reach reproduction age (PERERA-GARCÍA et al., 2011). During time spent in estuaries, fishes are vulnerable, inclusive to contamination by microplastics while feeding (BROWNE et al., 2008; ROCHMAN et al., 2013). This threat is of emerging concern, since the estuarine habitats are likely to be contaminated with microplastics (LEBRETON et al., 2017), and thus, every fish species are probably being affected by this type of contamination (LUSHER; HOLLMAN; MANDOZA-HILL, 2017). Furthermore, top predators might be especially susceptible to a higher contamination with microplastics, not only through direct ingestion, but also due to trophic transfer, when a prey previously contaminated with microplastics is ingested (AU et al., 2017; FERREIRA et al., 2016; NELMS et al., 2018).

Plastic debris are ubiquitous in aquatic ecosystems (LUSHER; HOLLMAN; MANDOZA-HILL, 2017). They are usually introduced in the environment as a result of

untreated sewage disposal and the mistreatment of solid wastes (LUO et al., 2014). Once in the environment, plastics are weathered and fragmented into smaller pieces (GEWERT; PLASSMANN; MACLEOD, 2015). Additionally, many plastics are already manufacture in tiny dimensions (GALLOWAY; COLE; LEWIS, 2017). Those particles smaller than 5 mm are termed as microplastics, and are the most common type of marine debris (THOMPSON, 2004), being highly susceptible to be ingested by the fish (JOVANOVIĆ, 2017).

Studies regarding the ingestion of microplastics by fishes are increasing (BESSA et al., 2018; MCNEISH et al., 2018; SILVA et al., 2018). However, insights on the relationship between species-specific ecological aspects and the intake of microplastic have recently gained attention due to the assertion of contamination in commercially important fishes (FERREIRA et al., 2018). The lack of spatial and temporal replicates and the disconnection from the influence of environmental parameters on the life cycle of species,might lead to inconclusive results (UNDERWOOD; CHAPMAN; BROWNE, 2017). So, only robust experimental and sample designs will detect such processes, especially for much less abundant top predator fishes (BARLETTA; LIMA; COSTA, 2019).

Centropomidae snooks are important top predators that, as amphidromous species, use riverine, estuarine and coastal resources. They migrate from salt to fresh water responding to ecological requirements of each life phases. The complex estuarine trophic web (TRITES, 2003) is usually capable of sustaining a large biomass of these predators (BARLETTA et al., 2017a), which in turn are important estuarine resources.

In the tropical Western Atlantic coast, the snooks are represented by six species of high market value, potentially cultivated, being also important as game fishes and for the local artisanal fishery (ALVAREZ-LAJONCHÈRE; TSUZUKI, 2008; TAYLOR; WHITTINGTON; HAYMANS, 2001). Together, these species are responsible for an annual landing of ~13,000 tonnes in the Eastern coast of America (FAO, 2017). Due to the long life cycle and the intense fishery pressure on this group, it is probable that most snooks populations are under imminent, or already facing, overfishing (BARLETTA et al., 2017b; BRENNAN; WALTERS; LEBER, 2008; FROESE, 2004).

The ecology of snooks, focusing on spatial distribution (STEVENS; BLEWETT; POULAKIS, 2007), feeding ecology (CONTENTE; STEFANONI; GADIG, 2009), growth patterns (COSTA FILHO et al., 2017) and reproductive aspects (ANDRADE; SANTOS; TAYLOR, 2013) were studied. However, the spatio-temporal patterns of habitat use and feeding ecology taking into account the requirements of each ontogenetic phase of Centropomidae species are only poorly known (GILMORE; DONOHOE; COOKE, 1983).

Moreover, assessments on diverse aspects of microplastic contamination are still lacking for this group.

The present study provides information on how the ingestion of microplastics links to feeding and movement patterns through the life cycle of snooks. Based on this information this study investigates the relationship among the spatio-temporal patterns of habitat utilization, feeding ecology and microplastic contamination in the different ontogenetic phases of the common snook *Centropomus undecimalis* and the largescale fat snook *C. mexicanus* along the ecocline of the Goiana Estuary.

MATERIAL AND METHODS

Study area

The Goiana Estuary, at the tropical Western Atlantic coast, hosts a Marine Protected Area (MPA) that prioritizes the use of estuarine resources by traditional fishery folk (Fig. S1) (Barletta and Costa, 2009). The estuarine main channel is ~15 km long. It can be divided into upper, middle and lower reaches, according to the salinity gradient and section shape (Fig. 1) (Barletta and Costa, 2009). There are intertidal creeks, mostly in the lower estuary, surrounded by a mangrove forest. The shallow coastal zone adjacent to the estuary is subject to the seasonality of the river's discharge and to a meso-tidal regime (LACERDA; BARLETTA; DANTAS, 2014) (Fig. S1). Air (27 ± 2°C) and water (28.2 ± 0.1°C) temperatures are high and fairly constant throughout the year (Fig. 1). Therefore, local climate variability is driven by changes in rainfall, which defines four main seasons: early dry (Sep–Nov), late dry (Dec–Feb), early rainy (Mar–May) and late rainy (Jun–Aug) (Fig. 1) (BARLETTA; COSTA, 2009).

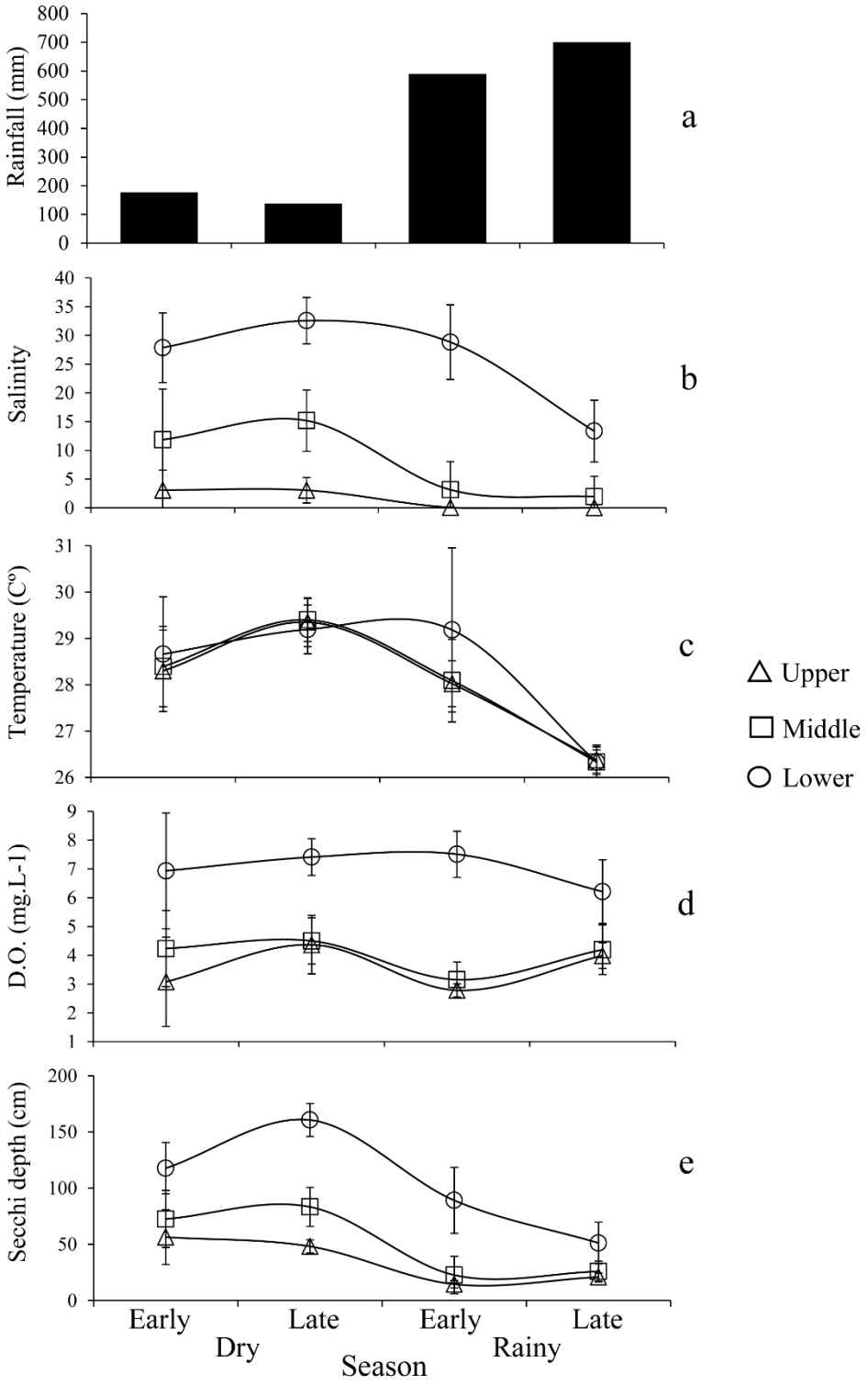

Figure 1 - Total rainfall per season (mm) (a), and seasonal average (± SE) for bottom water salinity (b), temperature (c), dissolved oxygen (d) and Secchi depth (cm) (e) according to the habitats of the Goiana Estuary.

Sampling design

To assess fishes movement patterns, six monthly replicates were performed in each area of the river main channel using an otter trawl net, between December 2005 and November 2006 (BARLETTA; LIMA, 2019; DANTAS et al., 2012) (Figs. S1, S2). The otter trawl net with different mesh size was used to ensure the capture of different ontogenetic phases of snooks (see description in Supplementary material).

For the feeding ecology study, each fish represented a replicate for a specific area, season and ontogenetic phase (Fig. S2). All fishes captured for the movement patterns study were also used in the feeding ecology and microplastic contamination analyses. However, to increase accuracy, additional samplings were performed from 2006 to 2009 in the main channel, during the late dry and late rainy seasons of each annual cycle. Additionally, twelve intertidal creeks from the lower estuary were sampled using a fyke net (10 mm mesh-size), between April and May 2008. Moreover, coastal zone samples were obtained monthly between 2013 and 2015 from the artisanal fishery fleet. All fishes collected during these additional samplings were used exclusively for the feeding ecology and microplastics contamination studies (Fig. S2).

Before biological samples were performed, environmental parameters were obtained from surface and bottom waters: salinity (Salinometer WT WLF 197), temperature, dissolved oxygen (OximeterWTW oxi 340), and Secchi depth (cm). Rainfall (mm) data were retrieved from a local weather station (Fig. S1).

Laboratory procedures

Individuals were identified (MENEZES; FIGUEIREDO, 1980), weighted and morphometric and meristic measurements taken to ascertain the diagnostic characteristics of each species (CERVIGÓN et al., 1993). Gonads and digestive tracts were reserved. Gonads were analysed under stereomicroscope (Zeiss, Stemi 2000) and categorized as immature, mature, spawning and spent (VAZZOLER, 1996). These data were used to classify the ontogenetic phases of each Centropomidae species. Three ontogenetic phases were described: juveniles, sub-adults and adults (see description in Supplementary material) (Fig. S3 and Table 1).

Table 1. Ontogeny of the Centropomidae species divided by size classes.

	Juvenile	**Sub-adult**	**Adult**
C. undecimalis	< 263 mm	263 - 454 mm	> 454 mm
C. mexicanus	< 213 mm	213 - 361 mm	> 361 mm

Food items in the digestive tracts were also identified to the lowest possible taxonomic level (BOLTOVSKOY, 1999; MENEZES; FIGUEIREDO, 1980; RUPPERT; FOX; BARNES, 2004), washed in distilled water, dried in tissue paper, counted and weighted using an analytic scale (±0.001 g). The index of relative importance (I_{RI}) was calculated for the identified prey items (see description in Supplementary material). During gut content analysis, individuals were confirmed to be contaminated with microplastics. Precautionary measures were taken to avoid airborne and inter-sampling contamination of the digestive tracts contents.

Prior to the analysis, the work station was thoroughly wiped with absolute ethanol, all equipment used in the evisceration and identification of food contents were double washed with filtered distilled water, oven dried and checked for previous contamination under stereomicroscope before use. The identification of gastrointestinal contents was made in Petri dishes covered by watch glasses. In addition, 100% cotton lab coats and disposable latex gloves were used during all procedures.

Items suspected of being microplastics were visually inspected, for physical consistency, shape and brightness. Then, samples were separated in individual Petri dishes covered by a watch glass to avoid airborne contamination and oven dried in 70°C for 48h. Withered particles were considered as non-synthetic organic material and discarded. Particles which characteristics were maintained, were considered plastics (LUSHER et al., 2017). Plastics were photographed (microscope mounted camera: Canon G10) and measured (Software: Axiovision LE); and particles smaller than 5 mm, were classified as microplastics (THOMPSON, 2004).

Statistical analyses

Three-way ANOVA was performed using Statistica 12 to assess whether density and biomass of the Centropomidae species, weight of each food item and number of microplastics ingested varied among the areas, seasons and ontogenetic phases. For the feeding ecology study, only the most frequent items were considered. Whenever significant differences were observed, the post-hoc Bonferroni test ($\alpha \leq 0.05$) was used to determine the sources of variance (QUINN; KEOUGH, 2002) (see description in Supplementary material).

A Canonical Correspondence Analysis (CCA) (CANOCO 5) was performed to assess correlations among environmental variables, food items and ingested microplastics for each ontogenetic phase of the Centropomidae species (TER BRAAK; SMILAUER, 2002). The

most common items preyed by snooks and the microplastics were included in the analysis as number of items ingested. To perform the analysis, a multiple least-squares regression was computed with the site scores (derived from weighted averages of microplastics and food contents) as dependent variables, and the environmental data (salinity, precipitation, water temperature and dissolved oxygen) as independent variables. The dependent variables were analysed through a direct gradient to extract variability patterns in relation to the independent variables (Braak, 1986; Palmer, 1993) (see description in Supplementary material).

RESULTS

Environmental characterization

Environmental characteristic changed along the seasonal cycle (Fig. 1). During the early and late dry season, rainfall decrease from 177mm to 138mm, and Secchi depth from 39 to 142cm, detecting in a saline intrusion into the estuary. Salinity varied from 0.3 to 7.3 in the upper and from 7.5 to 17 in the middle estuary. In the lower estuary, salinity varied from 25.8 to 29 confirming the marine character to this habitat.

During the early rainy season, monthly total rainfall reached 589mm, leading to increased river flow and the prevalence of riverine characteristics, principally in the upper estuary (salinity 0.1 ±0.04 and Secchi depth 14 ± 1.4cm) (Fig. 1). Maximum rainfall volumes (700mm) during the late rainy season drive the major seasonal variability in this estuary. This led to a decrease in average salinities along the entire estuary (upper: 0.02 ± 0.03; middle: 1.94 ± 1.5; lower: 13.35 ± 5.5) and beyond on the adjacent coastal waters. Water temperature follow a smooth seasonal pattern. During the dry season mean water temperatures varied between 28.6 ± 1 and 29.3 ± 0.5°C in the main channel. During the rainy season, the temperature decreased to 26.2°C (Fig. 1).

Seasonal and spatial movements of snooks within the estuary

Overall, snooks had total mean density of ~1.4 ± 0.24 ind. ha^{-1} and biomass of ~720 ± 216 g ha^{-1} (Table S2). *Centropomus undecimalis* was the most abundant species with total mean density of ~0.7 ± 0.2 ind. ha^{-1} and biomass of ~574 ± 206 g ha^{-1}, followed by *C. mexicanus* (0.5 ± 0.14 ind. ha^{-1} and 130 ± 66 g ha^{-1}). Variations in density and biomass of *C. undecimalis* were explained by significant interactions among season *vs.* area *vs.* ontogenetic phase ($p < 0.01$) (Table S3 and Figs. 2 and S4). It means that, for this species, the distribution of density and biomass of each ontogenetic phase depends on the time and space. Sub-adults of *C. undecimalis* inhabited the upper and middle portions during the early dry season. Peaks

of density (2.57 ± 2.25 ind. ha^{-1}) and biomass (954.5 ± 720.5 g ha^{-1}) occurred in the lower estuary during the early rainy season.

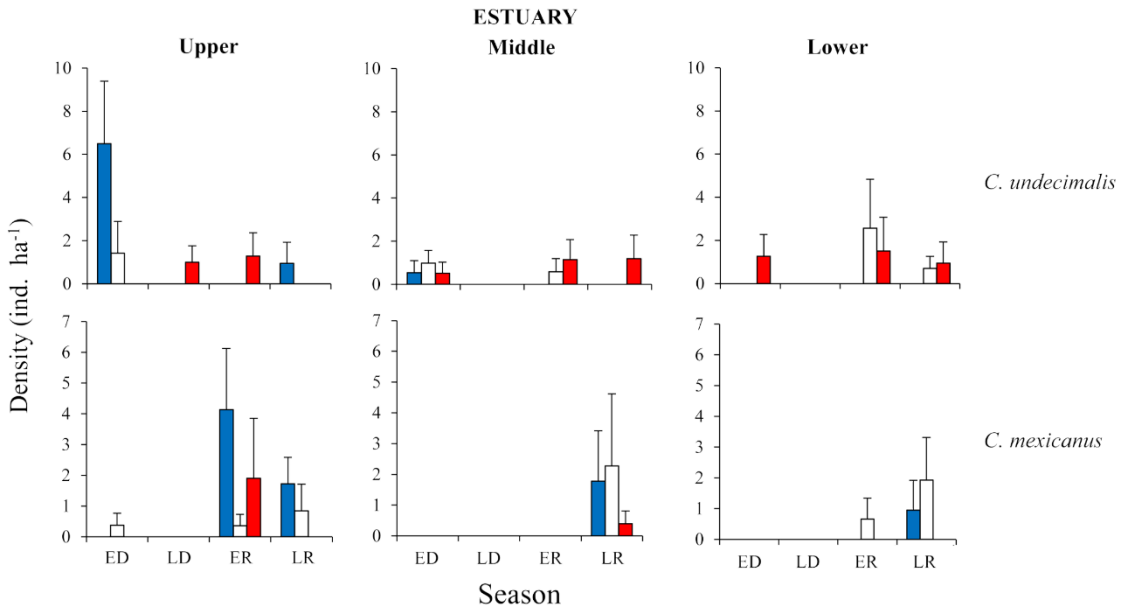

Figure 2 - Density (± SE) of Centropomidae species in the Goiana estuary, according to habitats (upper, middle and lower estuaries), seasons (ED: early dry; LD: late dry; ER: early rainy; LR: late rainy) and ontogenetic phases (juveniles ■, sub-adults □ and adults ■).

Adults occurred in the middle and lower portions during the early dry season, when peaks of biomass were registered (5324 ± 5462.3 and 3064.8 ± 2435.7 g ha^{-1}, respectively). Juveniles and Sub-adults of *C. undecimalis* inhabited the upper and middle portions of the estuary during the early dry season. However, juveniles highest density (6.49 ± 2.89 ind. ha^{-1}) and the lowest biomass (244.9±94.4 g ha^{-1}) occurred in the upper estuary, during the early dry season ($p < 0.01$), characterizing the nursery ground for this species (Table S3 and Figs. 2 and S4). On the other hand, the upper estuary served as nursery ground for *C. mexicanus* juveniles. Juveniles of this species also showed high density and low biomass in this area of the estuary, but during the early rainy season (4.13 ± 1.98 ind. ha^{-1} and 151.1 ± 106.9 g ha^{-1}). Therefore, the same portion of estuary was a nursery habitat for both species, but in different times of the year.

C. mexicanus inhabited mostly the upper estuary ($p < 0.05$) (Table S3and Figs. 2 and S4). Sub-adults during the late rainy season inhabited the middle and lower portions of the estuary, with peaks of density (2.28 ± 2.34 ind. ha^{-1}) and biomass (790.9 ± 811.54 g ha^{-1}) in the middle estuary. Adults of this species occurred in the upper estuary, during the early rainy

season (1.90 ± 1.95 ind. ha^{-1} and 1778.2 ± 1824.4 g ha^{-1}), and middle estuary during the late rainy season (0.40 ± 0.4 ind. ha^{-1} and 784.5 ± 804.9 g ha^{-1}).

Feeding ecology and microplastic contamination

In total, 48 different food items were identified in the digestive tracts of 529 individuals analysed (*C. undecimalis*: 265; *C. mexicanus*: 184), including zooplankton, zoobenthos, pelagic and demersal fishes, and microplastics (Tables S4 to S10; Figs. 3 and 4). Microplastics were highly representative in the diet of the Centropomidae species. Most (~98%) were microfilaments (1.25 ± 0.06 mm) (Fig. 5).

The ingestion of Polychaeta was significantly higher by juveniles of *C. undecimalis* inhabiting the upper estuary, during the early dry season (271 ± 448 mg ind^{-1}; $p < 0.01$) (Fig. 3 and Table S11). For *C. mexicanus*, principally juveniles and sub-adults forage on Polychaeta along the main channel during the rainy season, when significant difference was detected in the lower estuary at the beginning of this season (140 ± 62 mg ind^{-1}; $p < 0.01$) (Fig. 4 and Table S12). In addition, juveniles of this species during this time showed highest density at the upper estuary, where they feed principally on Polychaeta (4.16 ± 4.1 mg ind^{-1}) and Penaeid shrimp (17.8 ± 13.7 mg ind^{-1}). There, they also ingested microplastics (2.12 ± 0.55 part. ind^{-1}) (Fig. 5).

For sub-adults of *C. undecimalis*, Penaeid shrimp, ($\%I_{RI}$ = 8.6 to 54), and fishes, (pelagic: *A. clupeoides, C. edentulus, R. bahiensis*; and demersal: *Cathorops spixii*, *Stellifer stellifer*) ($\%I_{RI}$ = 1.7 to 45.5), were the most important prey items (Tables S3, S5, S6). Pelagic fishes, principally *R. bahiensis*, were foraged in the coastal zone during the dry and early rainy season, with highest ingestion during the late dry season in the lower estuary (1124 ± 666 mg ind^{-1}; $p < 0.01$) (Fig. 3 and Table S11).

Cathorops spixii and *S. stellifer* were preyed principally by adults during the dry season in the middle ($p < 0.05$) and in the lower portion ($p < 0.01$) of the estuary, respectively. Regarding microplastic ingestion, the middle estuary, lower estuary and coastal zone were the most important sites of contamination for all phases of *C. undecimalis* ($p < 0.01$) (Fig. 5 and Table S13). Juveniles were the ontogenetic phase with the lowest average contamination for this species. This phase ingested more microplastics in middle estuary, during the early rainy season, and in the lower estuary during the late dry season (2 part. ind^{-1}). Sub-adults and adults were contaminated by microfilaments, principally in the lower estuary and coastal zone during the early dry season ($p < 0.01$); (Fig. 5; Table S13).

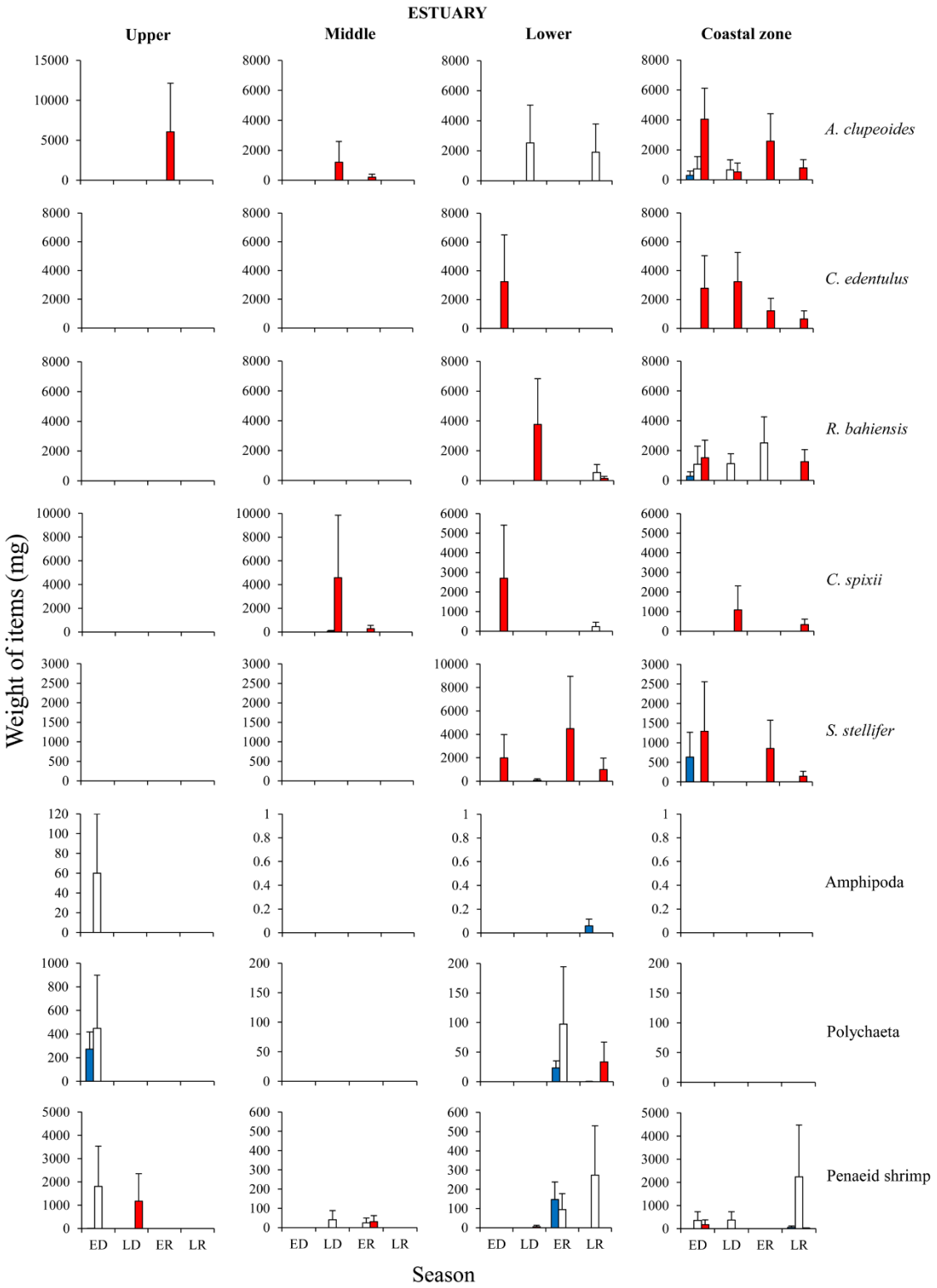

Figure 3 - Mean (± SE) weight of food items preyed by C. undecimalis in the Goiana estuary, according to habitats (upper, middle, lower estuary and coastal zone), seasons (ED: early dry; LD: late dry; ER: early rainy; LR: late rainy) and ontogenetic phases (juveniles ■, sub-adults □ and adults ■).

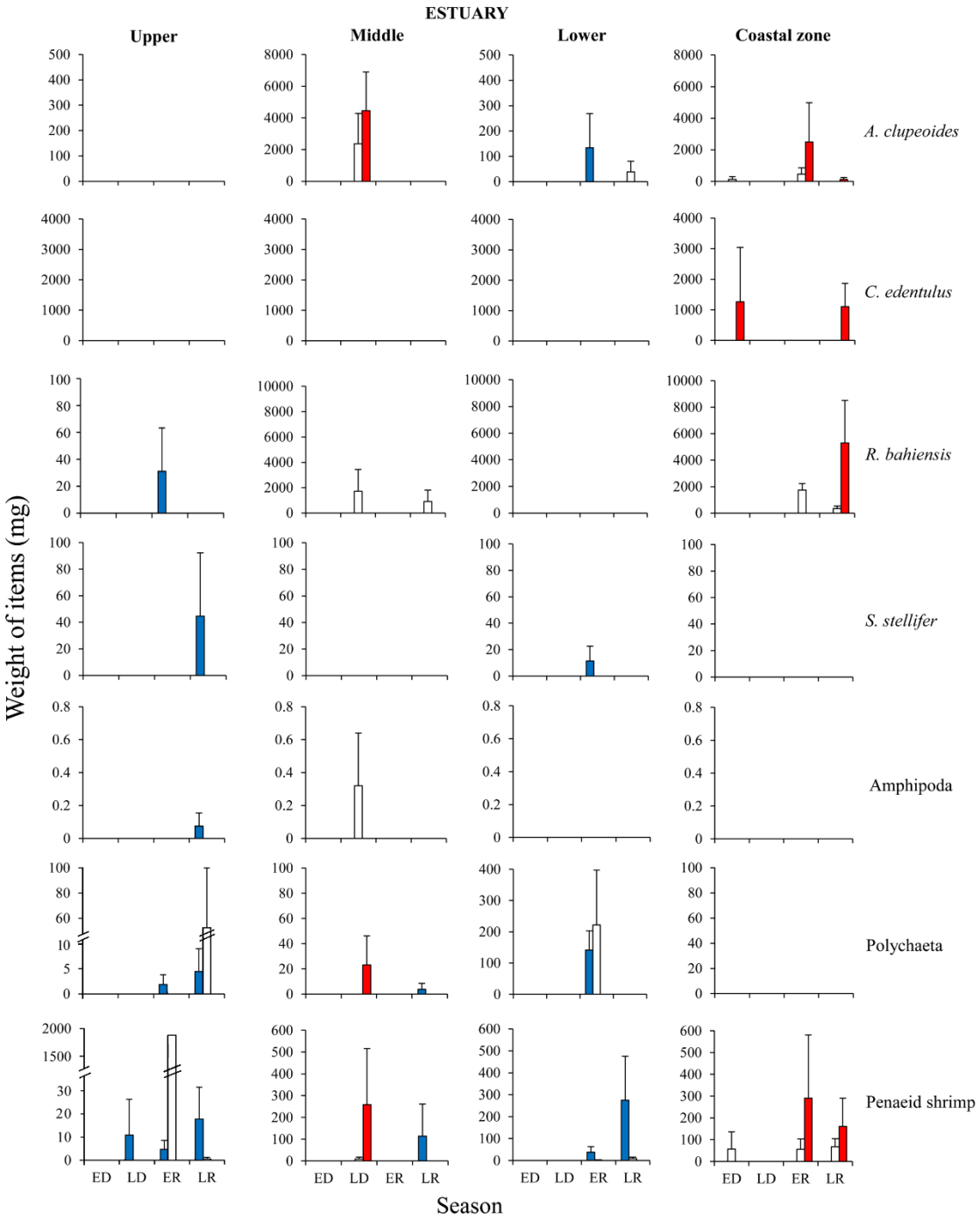

Figure 4 - Mean (± SE) weight of food items preyed by C. mexicanus in the Goiana estuary, according to habitats (upper, middle, lower estuary and coastal zone), seasons (ED: early dry; LD: late dry; ER: early rainy; LR: late rainy) and ontogenetic phases (juveniles ■, sub-adults □ and adults ■).

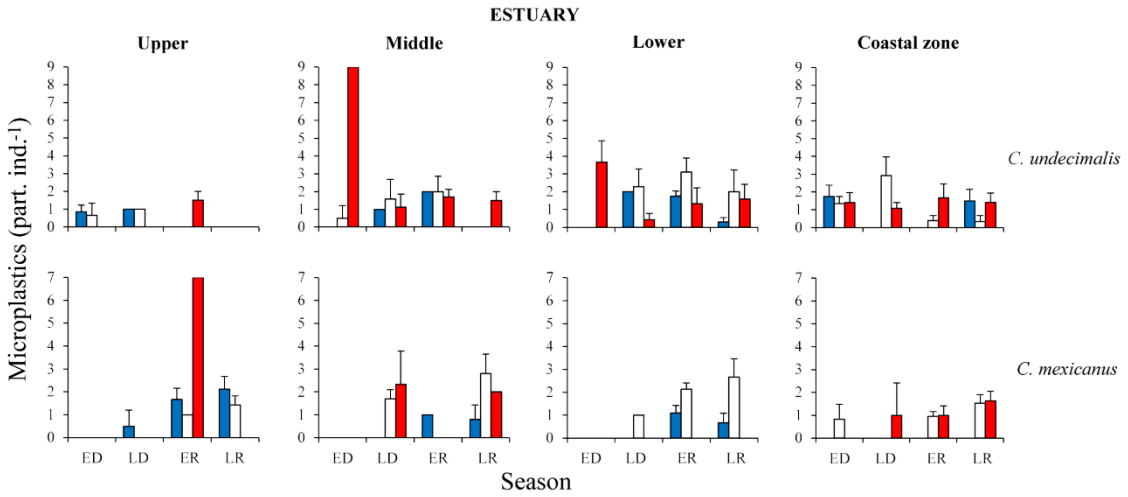

Figure 5 - Mean (± SE) number of microplastics ingested by the Centropomidae species in the Goiana estuary, according to habitats (upper, middle, lower estuary and coastal zone), seasons (ED: early dry; LD: late dry; ER: early rainy; LR: late rainy) and ontogenetic phases (juveniles ■, sub-adults □ and adults ■).

The food items with higher relative importance for sub-adults *C. mexicanus* were pelagic fishes (%I_{RI} = 8.5 to 100) (Table S7). *R. bahiensis* showed the highest ingestion at the coastal zone, during the late rainy season (1747.5 ± 493.9 mg ind^{-1}) ($p < 0.05$) (Fig. 4 and Table S12). For this species, sub-adults were the most contaminated, showing peaks of microplastics ingestion. In the middle estuary, they ingested microplastics during the late dry (1.7 ± 0.39 part. ind^{-1}) and late rainy seasons (2.8 ± 0.86 part. ind^{-1}). In the lower estuary microplastics were ingested during the early rainy (2.14 ± 0.26 part. ind^{-1}) and late rainy seasons (2.66 ± 0.79 part. ind^{-1}); and in the coastal zone, during the late rainy season (1.53±0.36 part. ind^{-1}) ($p < 0.01$) (Fig. 5 and Table S13).

The items with higher relative importance for the adults of *C. undecimalis* are Penaeid shrimp in the upper estuary (late dry season) (%I_{RI} = 100), and fishes (*A. clupeoides, R. bahiensis, Caranx latus* and *Opisthonema oglinum*) (%I_{RI} N 6.3 to 70) along the entire study area (Table S3). Fishes, independent from season, were an important food resource for adults of *C. undecimalis* along the entire estuarine ecosystem.

Pelagic fishes (*A. clupeoides, Cetengraulis edentulus, R. bahiensis*) were preyed principally on the coastal zone ($p < 0.01$) (Fig. 3 and Table S11). However, *Anchovia clupeoides* was also an important food item in the middle estuary during the late dry season (4457.5 ± 2452.3 mg ind^{-1}; $p < 0.01$).

In the coastal zone, significant differences were observed in the ingestion of C. edentulus, during the early dry and late rainy season ($p < 0.01$), and for R. bahiensis, during

the late rainy season (5303.8 ± 3213.4 mg ind^{-1}; $p < 0.01$). Demersal fishes, such as *C. spixii* had the highest ingestion in the main channel of the estuary ($p < 0.05$), and *S. stellifer* in the lower estuary and coastal zone ($p < 0.01$) (Fig. 3 and Table S11).

Adults of *C. undecimalis* also registered high microplastics contamination in the lower estuary and coastal zone. The highest value was observed in adults inhabiting the lower estuary, during the early dry season (3.66 ± 1.20 part. ind^{-1}) ($p < 0.01$) (Fig. 5 and Table S13). The adult phase also had high microplastics contamination, with a peak in the upper estuary, during the early rainy season (7 ± 0 part. ind^{-1}).

Influence of environment on ecological behaviour and microplastic contamination of snooks

The Canonical Correspondence Analysis (CCA) showed that the seasonal fluctuation of salinity and dissolved oxygen ($p < 0.05$) had a significant influence in the feeding ecology and microplastic contamination of the different ontogenetic phases of snooks along the Goiana Estuary (Fig. 6 and Table S14). The first axis (Axis I), explained 67% of the total data variability and represents the salinity gradient in the estuarine ecosystem. The positive portion of this axis represents the middle and upper portion of the estuary. Axis II (~19%) represents the seasonality in the ecosystem. The positive portion of this axis represents the rainy and the negative the dry season.

C. edentulus, S. stellifer, R. bahiensis and Penaeid shrimp positively correlated with salinity and dissolved oxygen. Adults and sub-adults of snooks forage on these food items mostly in the lower estuary and coastal zone (Fig. 6). However, *R bahiensis* and Penaeid shrimp positively correlated with rainfall, being an important resource for snooks during the rainy season (group A), and *C. edentulus* and *S. stellifer* were mostly preyed by adult snooks during the dry season (group B).

A. clupeoides (pelagic) and *C. spixii* (demersal) showed a negative correlation with rainfall, indicating that the ingestion of these prey were associated with the dry season (Fig. 6, Group B). Polychaeta was negatively correlated with salinity and dissolved oxygen. It suggests that this food resource was preyed mainly in the upper and middle estuaries. Juvenile snooks were associated with the positive portion of Axis I, indicating that Polychaeta had a higher importance for this phase, encompassing snooks from group C, during the rainy season and group D, during the dry season. Additionally, groups E and F represent nursery grounds in the upper estuary for *C. undecimalis* and *C. mexicanus*, respectively.

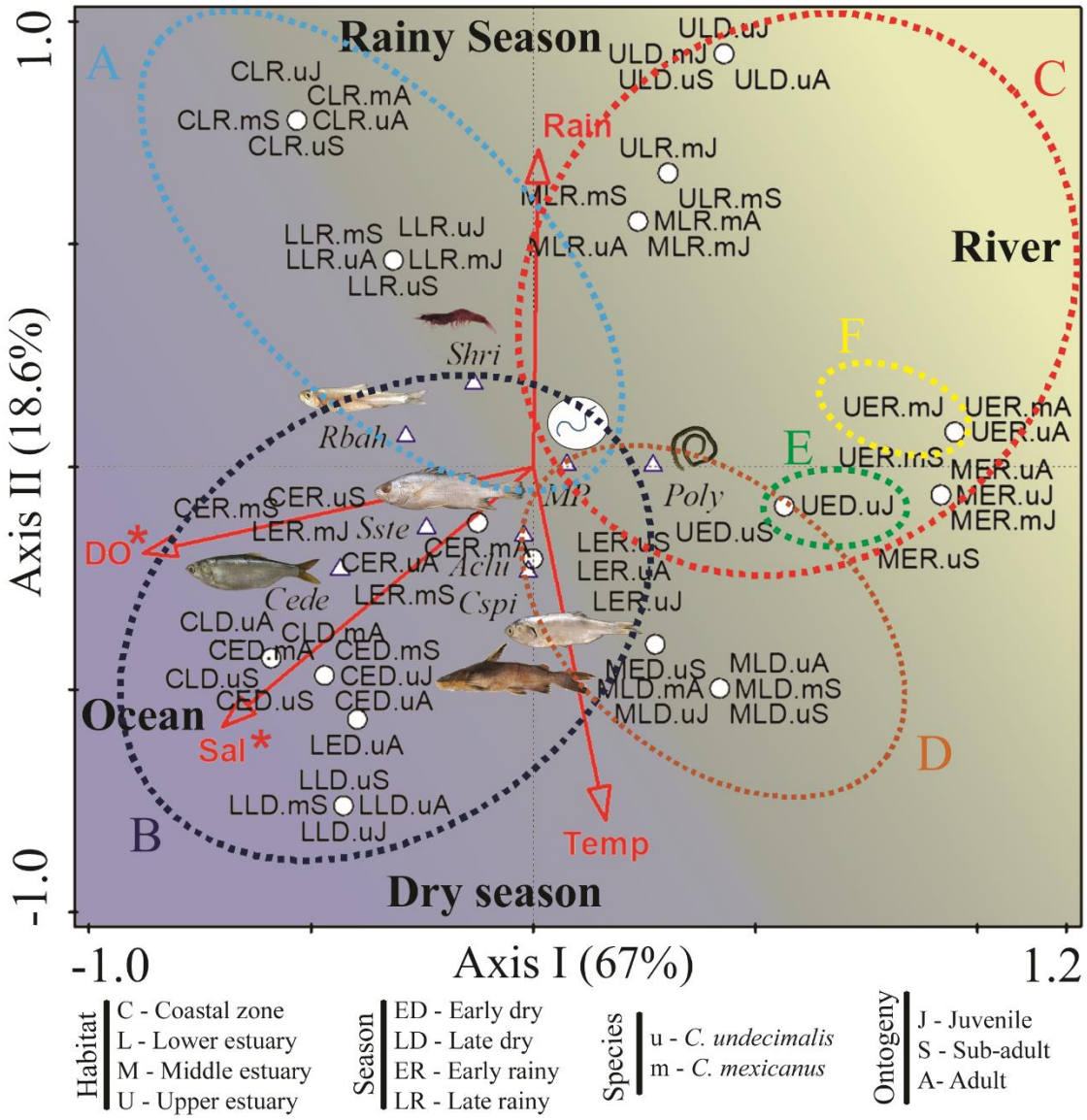

Figure 6 - Canonical Correspondence Analysis (CCA) for the correlations between the ingested food items and the environmental variables. Arrows represents the environmental parameters (Temp: water temperature; Sal: salinity; DO: dissolved oxygen; Rain: Rainfall) (*p < 0.05). Triangles (▲) represents food items (MP: microplastics; *Aclu: A. clupeoides*; *Cede: C. edentulus; Rbah: R. bahiensis; Cspi: C. spixii; Sste: S. stellifer*; Poly: Polychaeta; Shri: penaeid shrimp). Circles (○) represents the interactions among the factors habitats, seasons, species and ontogenetic phases. Dotted circles represent the groups.

DISCUSSION

Seasonal patterns of estuarine habitat use

Snooks are large predators (SOSA-LÓPEZ et al., 2005) that extensively use the whole diversity of estuarine habitats (STEVENS; BLEWETT; POULAKIS, 2007). For this reason, species have adaptations to avoid competition for resources and to seek optimal developmental conditions (DANTAS; BARLETTA, 2016; DUTKA-GIANELLI, 2014; PETERS; MATHESON JR.; TAYLOR, 1998). As other species their habitats use and feeding behaviours are highly variable and depend on fluctuations of the salinity gradient in the estuarine ecosystem (BARLETTA et al., 2005) and the ecological requirements of each ontogenetic phase (CONTENTE; STEFANONI; GADIG, 2009; MCMICHAEL; PETERS; PARSONS, 1989).

Regarding habitat use, young juveniles of snooks were not captured in the main channel of the Goiana Estuary. It is likely due to the preference of earlier phases in using the protected mangrove creeks as developmental grounds (BARLETTA et al., 2003). Indeed, according to Peters et al., (1998) and Taylor et al., (2000) newly hatched larvae of *C. undecimalis* use shallow waters in the coastal zone and adjacent beaches. Later, larvae migrate to mangrove creeks seeking for food and shelter from predators (LIMA et al., 2016), a usual movement pattern observed in other species that use estuaries [*e.g*. the Acoupa weakfish, *Cynoscion acoupa* (FERREIRA et al., 2016) and the Flagfin mojarra, *Eucinostomus melanopterus* (RAMOS et al., 2016)].

It is probable that larvae of *C. undecimalis* rarely leave the creeks, as emphasized in a survey conducted in the Goiana Estuary, where only one larvae was captured in the estuarine main channel (LIMA; BARLETTA; COSTA, 2015). The tidal creeks are also important to the metamorphosis of this species (DANTAS; BARLETTA, 2016), where larvae might spend around 20 days before becoming a juvenile (MCMICHAEL; PETERS; PARSONS, 1989). According to the nursery concept (BECK et al., 2001; DANTAS et al., 2012), this habitat is used as nursery ground for *C. undecimalis* (BARBOUR; ADAMS, 2012), since no young juvenile (b90mm)was captured in the main channel. It suggests that they remain in the tidal creeks of the lower estuary, until 90 mm (STEVENS; BLEWETT; POULAKIS, 2007).

Similarly to *C. undecimalis*, larvae (LIMA; BARLETTA; COSTA, 2015) and young juvenile of *C. mexicanus* do not occur in the estuary main channel. The early development of these two species might be similar, relying on mangrove tidal creeks as feeding and nursery grounds, such as observed for snooks in the sub-tropical Western Atlantic (ALVAREZ-

LAJONCHÈRE; TSUZUKI, 2008). The use of mangroves by earlier phases of snooks was corroborated by Sazima (2002) and Ramos et al., (2011).

Later, during the late rainy season, larger juveniles of *C. undecimalis* (> 90 mm) migrate from the mangrove creeks to the upper estuary in the early dry season, a pattern compatible with other Central American tropical estuaries (ALIAUME et al., 2000). Once in the upper estuary, juveniles of *C. undecimalis* use the favourable rive-like conditions with great availability of shelter, food, and lack of marine predators, to develop (FERREIRA et al., 2016). The highest density and lowest biomass of juveniles imply that many individuals with small body sizes are using the upper estuary during the early dry season as secondary nursery ground (Dantas et al., 2012). Juveniles might also inhabit the river (STEVENS; BLEWETT; POULAKIS, 2007).

Juveniles of *C. mexicanus* remain in the mangrove tidal creeks until ~150 mm (RAMOS et al., 2011). Then, they migrate to their secondary nursery, also in the upper estuary, but during the early rainy season.

Alongside snooks, other species also rely on the upper reaches of the estuary as nursery grounds (DANTAS et al., 2012; DANTAS; BARLETTA; COSTA, 2015; FERREIRA et al., 2016; RAMOS et al., 2016). Thereby, the conservation of these habitats, especially the upper estuary, must be of high priority to ensure the health of fish populations, and consequently fishery yields and income generation, at least for the tropical Western Atlantic (BARLETTA; CYSNEIROS; LIMA, 2016).

Sub-adults of *C. undecimalis* inhabit all estuarine and coastal habitats studied, but their distribution is strongly associated with areas of moderate salinities. This phase occurs in the middle estuary, where transitional features provide results in intermediate salinities. When river discharge increases, during the early and late rainy seasons, they move downstream to the lower estuary. Sub-adults of *C. mexicanus* have similar distribution patterns to their juveniles, inhabiting the main channel during the early and late rainy seasons. During the rainy period, larger sub-adults (> 300 mm) also inhabit the coastal zone. However, during the early and late dry seasons, they migrate to the river.

When snooks become adults, they also use the entire estuarine main channel, but in lower densities, suggesting that they use these habitats as a migratory corridor to the river (BOUCEK; REHAGE, 2013). In addition, adults snooks also use the coastal zone as feeding and spawning grounds (LOWERRE-BARBIERI et al., 2014).

<u>Feeding ecology</u>

Centropomidae occupy high trophic levels, even in earlier developmental phases, when compared to other fishes that use estuaries (DANTAS et al., 2013; RAMOS et al., 2014; SILVA et al., 2018). They rarely prey on zooplankton and have large invertebrates and fishes as the most important resources (BLEWETT; HENSLEY; STEVENS, 2006; DUTKA-GIANELLI, 2014). Juveniles of *C. undecimalis* feed mainly on Polychaeta while in the upper estuary, during the early dry season (DANTAS; BARLETTA; COSTA, 2015; FERREIRA et al., 2018). At the coastal zone, juveniles change their diet to small fishes (MCMICHAEL; PETERS; PARSONS, 1989), including planktivore pelagic fishes *A. clupeoides* and *R. bahiensis*, and the zoobenthivore demersal fish as *S. stellifer*.

On the other hand, juveniles of *C. mexicanus* prey mainly on *R. bahiensis* and Penaeid shrimp, and occasionally on Polychaeta in the upper estuary. When salinity decreases in the late rainy season, juveniles spread across the middle and lower estuaries feeding on a variety of prey, including *A. clupeoides, R. bahiensis*, Penaeid shrimp, Polychaeta and *S. stellifer* (SAZIMA, 2002).

Sub-adults and juveniles of both species of snooks have similar diets, shifting their feeding behaviour according to habitat use. In the uppermost reaches of the estuary, they feed mainly on Penaeid shrimps and Polychaeta. In the lower estuary and coastal zone, sub-adults of *C. undecimalis* raise their trophic level, feeding on *A. clupeoides*, *R. bahiensis*, and Penaeid shrimp, while sub-adults of *C. mexicanus* feed on *A. clupeoides, R. bahiensis* and Polychaeta in the middle and lower estuaries.

Adults of *C. undecimalis* have a diverse diet, which shifts according to the availability of prey in estuarine habitats. In the upper estuary, they feed on young Penaeid shrimp and *A. clupeoides*, highly available in habitats of low salinity (Lima et al., 2015). In the middle and lower estuary, they feed mainly on zoobenthivore demersal fishes, as *C. spixii* and *S. stellifer* but planktivore pelagic fishes are also part of their diet while there. This coincided with the highest availability of juveniles of *C. spixii* (DANTAS et al., 2012) and *S. stellifer* (DANTAS; BARLETTA; COSTA, 2015) in these same habitats. In the coastal zone their diet is based almost exclusively on fishes, principally planktivore pelagic species as A. clupeoides, *C. edentulus* and *R. bahiensis* and zoobenthivore demersal fishes *C. spixii* and *S. stellifer* in a smaller proportion.

Otherwise, the diet of adults of *C. mexicanus* is based on planktivore pelagic fishes (*A. clupeoides, C. edentulus and R. bahiensis*) and Penaeid shrimp, such as observed in snooks of Florida (BLEWETT; HENSLEY; STEVENS, 2006). In addition, adult *C. mexicanus* ingest a lower quantity and diversity of prey at the coastal zone, when compared to *C. undecimalis*.

These patterns of feeding ecology show that each ontogenetic phase of the three species have similar prey preferences. However, prey have different importance within a given habitat and season, indicating that these patterns avoid dietary overlaps at inter- and intra-specific levels as observed for species of Ariidae, Sciaenidae, Gerreidae and Haemulidae (DANTAS et al., 2013; RAMOS et al., 2014; SILVA et al., 2018).

C. undecimalis is the most abundant Centropomidae species in the Goiana Estuary. This is the largest species of Centropomidae, and their predominance might be a size-related competitive advantage. As a result they reach higher trophic levels, when compared to any other snook (EMMERSON; RAFFAELLI, 2004). The fact that this species is a top predator is emphasized in the Everglades mangroves, where *C. undecimalis* compete with alligators and bull sharks for estuarine resources (MATICH et al., 2017). However, in the Goiana Estuary, the major species to compete with latter stages of Centropomidae is the Acoupa weakfish (*Cynoscion acoupa*, Sciaenidae). Different from adult snooks, *C. acoupa* is a marine estuarine dependent species, feeding heavily on zoobenthivore demersal fishes (FERREIRA et al., 2016), which in turn are in a higher trophic level than the planktivore pelagic fishes consumed by the Centropomidae.

This might be related to the ambush foraging strategy used by snooks to capture pelagic prey (WAINWRIGHT et al., 2006). Somehow, this strategy avoids dietary overlaps in an inter-species level, supporting the co-existence of both estuarine top predators. However, at the intra-specific level, the use of different habitats by the different ontogenetic phases is the essential strategy to optimize the use of resources and to minimize competition (DANTAS et al., 2013; FERREIRA et al., 2016; RAMOS et al., 2014; WAINWRIGHT; RICHARD, 1995), which ranges from dietary overlaps to cannibalism (ADAMS; WOLFE, 2006; SAZIMA, 2002).

Microplastics contamination

The seasonal fluctuations of the estuarine ecocline are responsible not only for the distribution of food resources for the fish assemblages (BARLETTA et al., 2005, 2017a), but also determine the availability of microplastics in the estuarine ecosystem (BARLETTA; LIMA; COSTA, 2019; CHEUNG; CHEUNG; FOK, 2016; LIMA; BARLETTA; COSTA, 2015). Therefore, the presence of predator, prey and microplastics within the same environment allow for interactions among them that result in contamination according to foraging patterns (habitat, feeding ecology and ontogenetic shifts).

All ontogenetic phases of both snooks ingested microplastics, independent of habitat or season. Indeed, more than a half of the individuals of each species are contaminated, and filaments are the predominant type of microplastic ingested, as usually observed for other species and elsewhere (GÜVEN et al., 2017; LUSHER; MCHUGH; THOMPSON, 2013; PAZOS et al., 2017; POSSATTO et al., 2011).

The introduction of microplastics particles in the Goiana Estuary have multiple potential sources. Goiana City (80,000 habitats), 5 km upstream of the head of the estuary, is likely the major contributor of pollutants to the river and estuary section (BARLETTA; COSTA, 2009). Indeed, the introduction of microplastics to the environment from clothes washing is well known (HARTLINE et al., 2016; NAPPER; THOMPSON, 2016). Along the estuary, other activities might also input microplastics (*e.g.* sand mining, small fisher's settlements, and sugarcane plantations). However, the most concerning activity is artisanal fishery. It releases microplastics to the environment mostly during the use and maintenance of fishing gears (BARLETTA; LIMA; COSTA, 2019).

The dynamics of microplastic ingestion by snooks is associated with the fluctuation of salinity gradient in the estuary, which in turn affects the availability of all pollutants (LIMA; COSTA; BARLETTA, 2014). The ingestion of microplastics was much higher seawards (lower estuary and coastal zone), especially in the rainy season, when river runoff increases. Those habitats have a higher availability of microplastics in the water column (LIMA; COSTA; BARLETTA, 2014) due to intense coastal fishing activities (POSSATTO et al., 2011),. It has been reported to be an important source of microplastics for estuarine systems (LI; TSE; FOK, 2016; LIMA; COSTA; BARLETTA, 2014). Additionally, river runoff is a major source of microplastics to the estuarine environment (CHEUNG; CHEUNG; FOK, 2016; LEBRETON et al., 2017; LIMA et al., 2016), and during the late rainy season microplastics peak in density (water column) in the lower portion of the Goiana Estuary (Lima et al., 2014). Therefore, higher ingestion of microplastics by snooks have a direct relationship with the intensification of river runoff and of fishing activities in coastal habitats when these two major inputs of microplastics overlap.

Nevertheless, contamination by microplastics is also linked to the feeding behaviour of snooks. Overall, fishes that ingested a greater biomass and prey on higher trophic levels were the most contaminated. Juvenile snooks registered the lowest ingestion of microplastics, whereas adult the highest. It emphasizes that a feeding intensity (due to physiology) associated with the ingestion of prey from different trophic levels, and level of habitat contamination, condition the ingestion of microplastics. When sub-adult of snooks onset the

dietary shift towards piscivory, the number of microplastics ingested reach intermediate levels, since this phase rely on both invertebrates and fishes. Adults are at the highest trophic level, feeding mainly on fish. Therefore, it explains the highest contamination by microplastics in this phase. In addition, peaks of fish ingestion by adults coincides with peaks of microplastics ingestion and contamination.

Centropomus undecimalis is the Centropomidae species most contaminated by microplastics. The highest ingestion of microplastic occurred in adults at the lower estuary during the early dry season, at the same time when they rely on zoobenthivore demersal fishes. These are the prey with the highest trophic level within the diet of *C. undecimalis*. *C. mexicanus* is the second most contaminated species and the sub-adults are most at risk, followed by adults, because the proportion of invertebrates and fishes is almost the same for sub-adults and adults of *C. mexicanus* and, thus, their ingestion of microplastics might be similar. Moreover, the highest contamination rates occurs in the middle and lower estuaries, and coastal zone, during the rainy periods.

Microplastic ingestion has been registered on multiple levels of the marine trophic webs (FOSSI et al., 2012; LUSHER; MCHUGH; THOMPSON, 2013; SUN et al., 2017). Organisms can ingest microplastics while grazing or foraging on prey where these particles are available and, thus, the nearby microplastics are accidentally ingested (THOMPSON, 2004). It may also occur intentionally, when those particles are mistaken by food (WRIGHT; THOMPSON; GALLOWAY, 2013).

Additionally, a third pathway of microplastic contamination may occur when a predator feeds on a prey that is already contaminated. If a particle of microplastic that were within the digestive tract of the prey is transferred to the predator during the digestive process, it would characterize trophic transfer of microplastic along the food web (AU et al., 2017; FARRELL; NELSON, 2013; FERREIRA et al., 2016; NELMS et al., 2018). This type of contamination is more intense in the upper trophic levels, because top predators ingest larger prey and have a more intense predatory activity. It would also result in a momentary build-up in the gut contents of fishes because, in addition to microplastics accidentally ingested, the microplastics that were inside of the prey will remain in the gastrointestinal tract of the predator after the digestion of the prey, since they take longer to be excreted than ordinary food items (NELMS et al., 2018). Moreover, top predators rely on a greater biomass of food, increasing the probability of consuming a contaminated prey, being therefore more susceptible to microplastic trophic transfer.

The relationship observed between microplastics contamination and the feeding behaviour of snooks, might be an evidence of trophic transfer of microplastics. Snooks have a high ingestion rate of microplastic when compared to fishes of lower trophic levels (JOVANOVIĆ, 2017; POSSATTO et al., 2011; VENDEL et al., 2017). In addition, many fishes preyed by snooks are proven to be contaminated with microplastics (DANTAS; BARLETTA; COSTA, 2015; POSSATTO et al., 2011; RAMOS; BARLETTA; COSTA, 2012; SILVA et al., 2018), suggesting the likelihood of trophic transfer. Indeed, during stomach contents analyses, several undigested prey were retrieved from the digestive tract of snooks. The gut contents of these prey items (exclusively fishes) were also analysed in search of microplastics. Among the 41 food items in these conditions retrieved, 24 were contaminated, evidencing the likelihood of trophic transfer between prey and predator (Fig. 7).

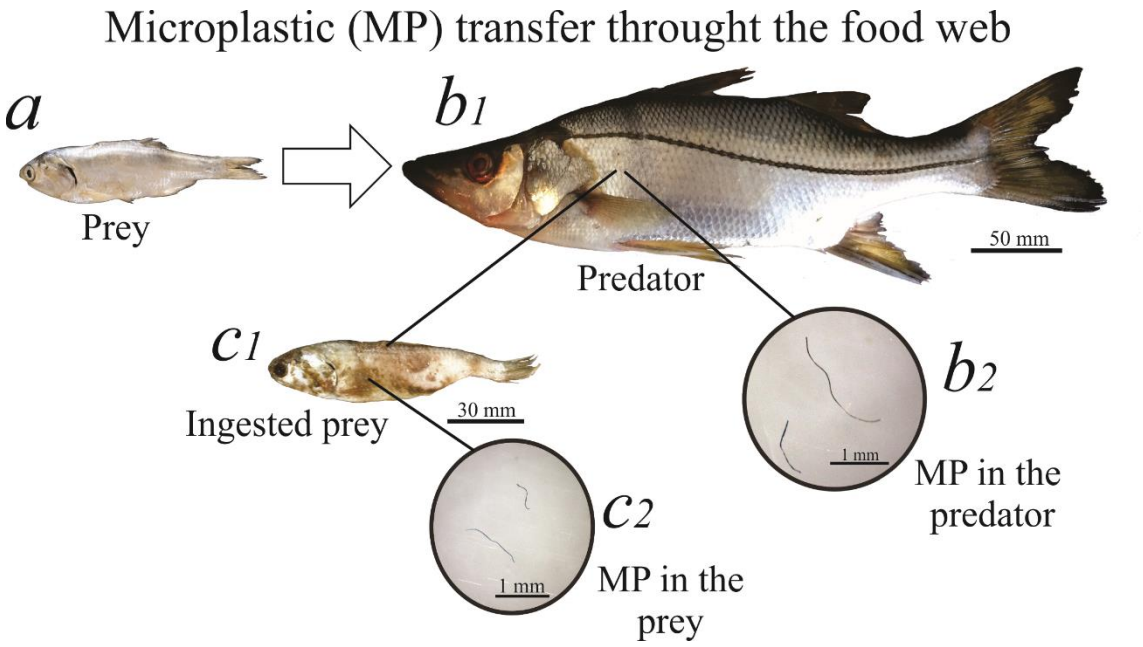

Figure 7 - Evidence of trophic transfer of microplastics from a planktivorous prey (a). The gut contents analysis of a C. mexicanus (b1) revealed that the predator ingested microplastics (b2) and A. clupeoides (c1). Further analysis in the gut contents of A. clupeoides (c1) showed that it had also ingested microplastics (c2).

Studies on the consequences of microplastic contamination in fishes are from laboratory experiments performed under unrealistic environmental conditions, when fishes are usually submitted to a restrict diet and great densities of microplastics in the water (BATEL et al.,

2018; CHOI et al., 2018; DE SÁ; LUÍS; GUILHERMINO, 2015). Therefore, the relationship between the effects of microplastic ingestion and fish behaviour are discordant.

Surveys have reported that ingestion of microplastics lead to behavioural changes, such as decreased predatory performance (DE SÁ; LUÍS; GUILHERMINO, 2015). On the other hand, Tosetto et al., (2017) did not observed a relationship between microplastic contamination and changes in fish behaviour.

Laboratory or field studies that relate fish (of every ontogenetic phase) and microplastics ingestion are still in their infancy. However, these studies are urgently needed, especially for commercially exploited species as snook, which reach the local and international market. These studies might help establishing health regulations and therefore re-shaping the market, as well as species and environmental connectivity conservation.

CONCLUSIONS

This study highlights that accurate sampling design along spatial and temporal factors, provide evidences on how environmental variability affects the ecological behaviour and microplastic contamination of snooks in a tropical estuarine ecosystem (Fig. 8). In the Goiana Estuary, the seasonal fluctuation of the estuarine ecocline have a major role in the distribution and feeding patterns of snooks. Several ecological and biological activities, as well as dietary preferences differ among ontogenetic phases, but are similar between the two most important species of snooks in this estuary. These species use the same habitats in different seasonal periods, probably to avoid competition for estuarine resources.

The estuarine ecocline is also an important environmental variable, which explain the distribution of microplastics, and determine their availability to the food web. This availability is likely to overlap with the distribution of snooks throughout the seasonal cycle, increasing the chances of interaction, and therefore contamination. Indeed, microplastic ingestion was ubiquitous in snooks, and highly associated with the food spectrum and use of specific habitats. The rates of microplastic ingestion increased from juvenile to adult phase, when they shifted from a diet mostly based on invertebrates to a diet based on pelagic and demersal fishes and shrimp.

Further studies are still necessary to evaluate in more detail the ingestion of microplastics (type, size and/or colour) and its association with habitats and feeding ecology, as well as its consequences at multiple trophic levels. Microplastics trophic transfer is indeed a relevant pathway for food web contamination. The status of snooks stocks must be assessed along the Central and South American coast, where fishery management is usually inefficient

or non-existent. However, some actions can be immediately enforced; including the protection of nursery grounds from the different anthropogenic disturbances.

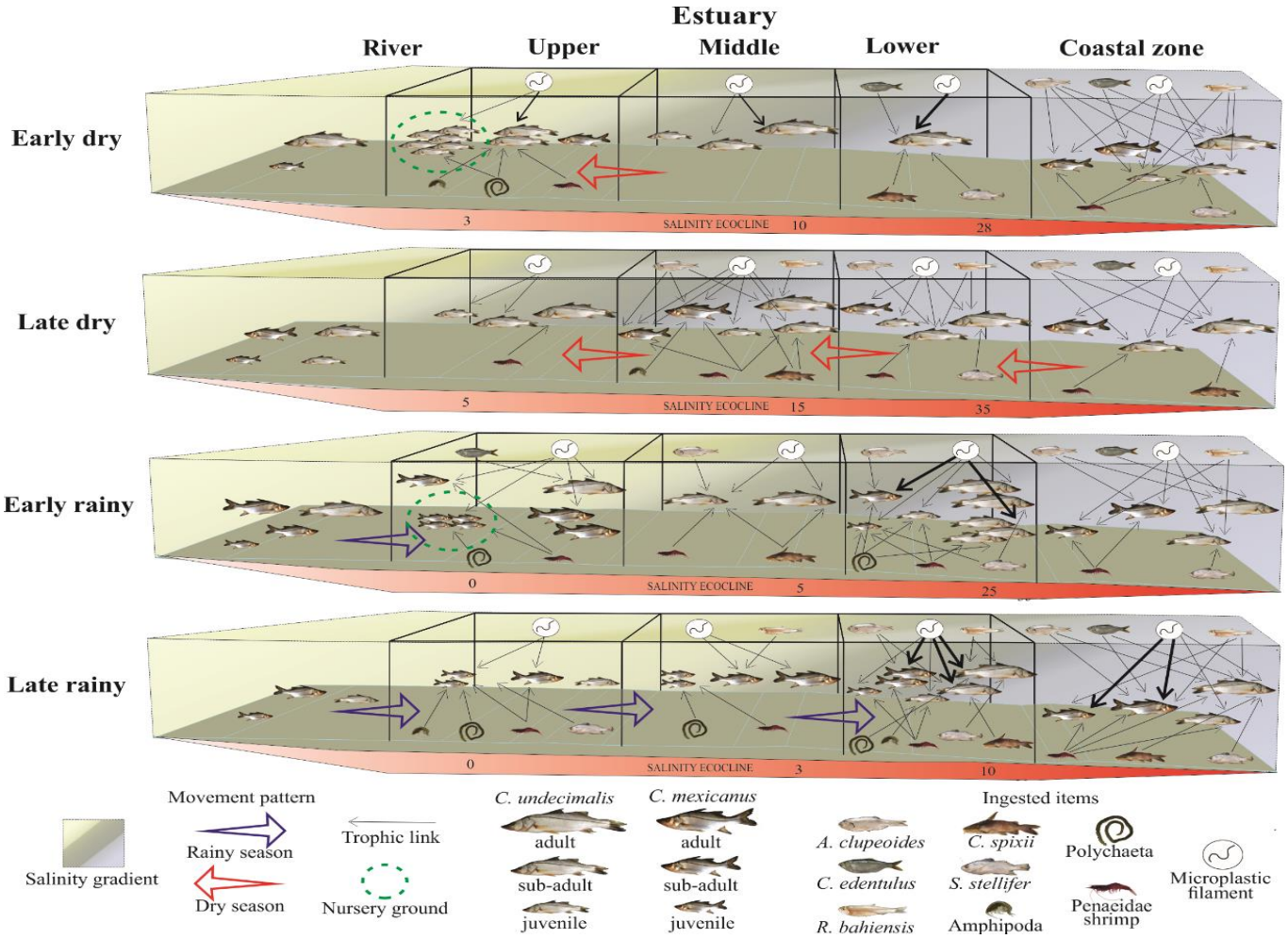

Figure 8 - Conceptual model of the habitat use (density of fishes), feeding ecology (main food items) and microplastic contamination (thickness of the arrows represent the contamination levels) in snooks along the estuarine ecocline.

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APÊNDICE 1. Material suplementar referente ao capítulo 1.

SUPPLEMENTARY MATERIAL

USE OF ESTUARINE RESOURCES BY TOP PREDATOR FISHES - HOW DO ECOLOGICAL PATTERNS AFFECT RATES OF CONTAMINATION BY MICROPLASTICS?

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Sampling design

Samplings were conducted during neap tides to minimize tidal influence. The net was 8.72m long, with 35mm mesh-size in the body, 22mm in the codend and fitted with a 5mm mesh codend cover, to ensure the capture of different ontogenetic phases (length classes)

(DANTAS et al., 2012; BARLETTA et al., 2005). All individuals were frozen immediately after sampling.

For the feeding ecology study, each fish represented a replicate for a specific area, season and ontogenetic phase (Fig. S1 and S2). All fishes captured for the movement patterns study were used in the feeding ecology and microplastic contamination analyses. However, to increase accuracy, additional samplings were performed from December 2006 to August 2008 in the main channel, during the late dry and late rainy seasons of each annual cycle. Additionally, twelve intertidal creeks from the lower estuary were sampled using a fyke net (10mm mesh-size), between April and May 2008. Moreover, coastal zone samples were obtained monthly between 2013 and 2015 from the artisanal fishery fleet. All fishes collected during these additional samplings were used exclusively for the feeding and microplastics contamination studies (Fig. S2).

Statistical analyses

Prior to statistical analyses, data were Box-Cox transformed to increase normality (BOX; COX, 1964). The assumptions for the analysis of variance (ANOVA) were tested using the Levene and Kolmogorov-Smirnov tests (LEVENE, 1960; UNDERWOOD, 1997). A three-way ANOVA, with a 5% level of significance, was performed to assess whether density and biomass of the Centropomidae species, weight of each food item and number of microplastics ingested varied among the areas, seasons and ontogenetic phases (Statistica12). For the feeding ecology study, only the most frequent items were considered. Whenever significant differences were observed, the *post-hoc* Bonferroni test ($\alpha < 0.05$) was used to determine the sources of variance (QUINN; KEOUGH, 2002).

A Canonical Correspondence Analysis (CCA) (CANOCO 5) was performed to assess correlations among environmental variables, food items and ingested microplastics for each ontogenetic phase of the Centropomidae species (TER BRAAK; SMILAUER, 2002). The most common items (top seven in Frequency of occurrence) preyed by snooks and microplastics were included in the analysis as number of items ingested. Data were log-transformed (rare species were not down-weighted, because only the most frequent were included in the analyses). To perform the analysis, a multiple least-squares regression was computed with the site scores (derived from weighted averages of microplastics and food contents) as dependent variables, and the environmental data (salinity, precipitation, water temperature and dissolved oxygen) as independent variables. The dependent variables were analysed through a direct gradient to extract variability patterns in relation to the independent variables (TER BRAAK, 1986; Palmer, 1993).

The CCA focused on a symmetric and bi-plot scaling, where independent variables were represented by eigenvectors radiating from the ordination origin. To determine which environmental variables were significant in the variability of microplastics and food items ingestion, a reduced model of Monte Carlo Permutation test (1,000 permutations) was used (TER BRAAK; SMILAUER, 2002). Environmental variables were tested through a stepwise routine.

Swept area and habitat use data

Trawls lasted 15min and were made by a small fishing boat (8m) of 40 horsepower. Before and after samplings, the position was recorded by a GPS. The swept area (*A*) was calculated using the following equation:

$$A = D*h*X_2$$

Where *D* represents the length of the path swept and $h*X_2$ the wingspread of the net. Where, *h* is the length of the read-hope and X_2 is the fraction of the head-hope equal to the width of the path swept. Barletta et al. (2005) proposed that the ideal sweep speed for the optimal performance of the otter trawl used in this study is between 3.7 and 6 km h^{-1}, with X_2 ranging from 0.478 to 0.535. Trawls were performed within this speed range and we assumed an X_2 of 0.5. Estimations of catch per unit area (CPUA) were made using the number and weight of captured fish, divided by the swept area. Mean density (individuals per hectare) and biomass (grams per hectare) values were calculated for the interactions of factors, taking into account all six replicates per area per month, totalizing 216 samples (Barletta, 1999; Barletta et al., 2005, 2008; Dantas et al., 2013).

$$\text{Density} = (C_N \ A^{-1}) \ 10^{-4}$$

$$\text{Biomass} = (C_W \ A^{-1}) \ 10^{-4}$$

Where C_N represent the capture in number, C_W the capture in weight and *A* the swept area.

Ontogenetic phases

The ontogeny of Centropomidae species was divided into three phases (juveniles, sub-adults and adults) (Table 1). To differ between juveniles and sub-adults, each interval of data representing an ontogenetic phase had its growth coefficient calculated through the power function of the total length using the following model:

$$Wg = \beta_0 \, TL^{\beta 1} + \varepsilon$$

Where *Wg* (weight) is the dependent variable, *TL* is the independent variable, β_0 is the intercept and β_1 is the growth coefficient (HUXLEY, 1924). When β_1 is below 3 (negatively allometric), it means that those group of individuals grow faster in length than gain weight,

characterizing the juvenile phase (Fig. S3). When β_1 is above 3 (positively allometric), it means that those group of individuals gain weight faster than grow in length, characterizing the sub-adult phase (Fig. S3).

To distinguish sub-adults from adults, it was used the average size at first maturation (L_{50}) (Fig. S3) (LEWIS; FONTOURA, 2005). This procedure establish the relative frequency of maturated individuals through the following equation:

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

The *F* represents the frequency of maturated individuals of each size class interval, *L* the pivotal point of each size class interval and *a* and *b* are the parameters estimated by the least squares of the linearized form of the equation:

$$-\ln[(1/F)-1]-a+b*L$$

Therefore, the size at first maturation (L_{50}) is estimated by:

$$(L_{50})= -a/b$$

Index of relative importance (I_{RI})

The index of relative importance (I_{RI}) was applied to assess the relevance of the food items and microplastics ingested for the Centropomidae species (Tables S3 and S7). The index was calculated using the following equation:

$$I_{RI} = \%F_i * (\%N_i + \%M_i)$$

The $\%F_i$ represents the frequency of occurrence of a given item (*i*), it is expressed as the percentage of individuals that ingested the item *i*. The $\%N_i$ represents the composition in number of a given item (*i*), it is expressed as the number of item *i* ingested, in percentage, with respect of the total number of all items ingested by individuals (HYSLOP, 1980). The $\%M_i$, represents the composition in mass of a given item (*i*), it is expressed as the mass of item *i* ingested, in percentage, with respect to the total weight of items ingested by individuals (HYSLOP, 1980). Each item has an I_{RI} value for the combination of factors (habitat, season and ontogeny) (Tables S3 and S7).

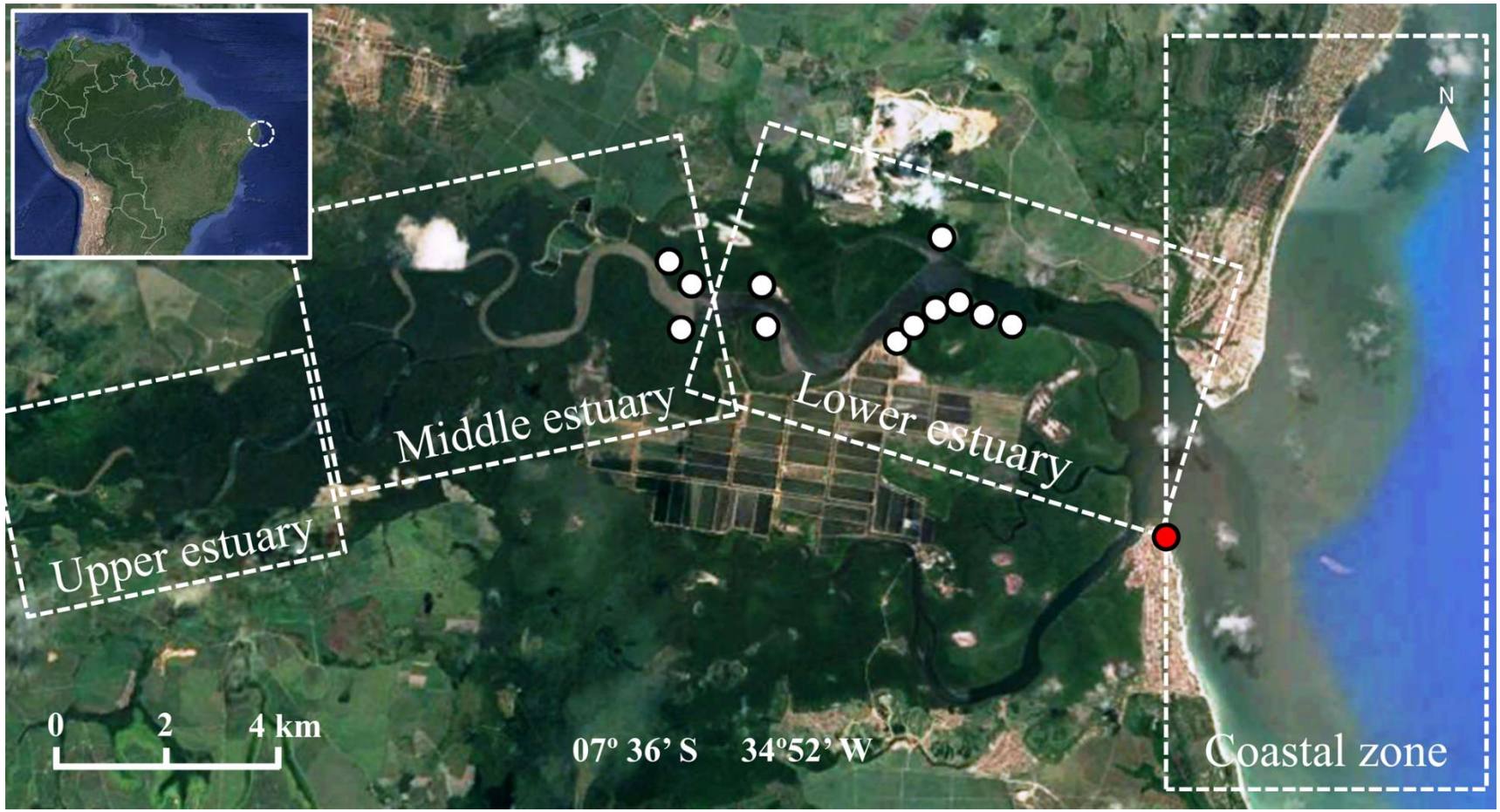

Figure S1 - Goiana Estuary in the Western Atlantic coast [upper estuary, middle estuary, lower estuary, coastal zone and mangrove creeks (○)]. Weather station (●).

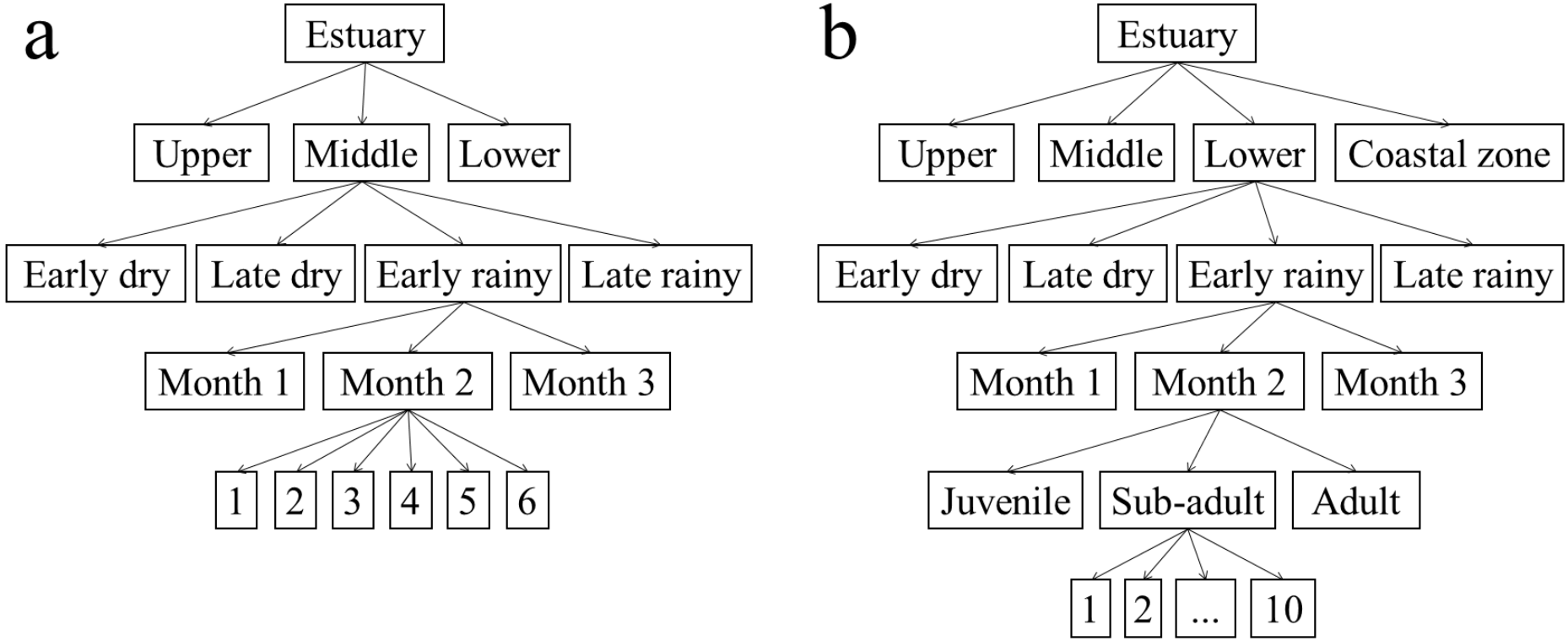

Figure S2 - Sampling design applied to the (a) habitat use and (b) feeding ecology studies. Figure adapted from Barletta et al. (2019).

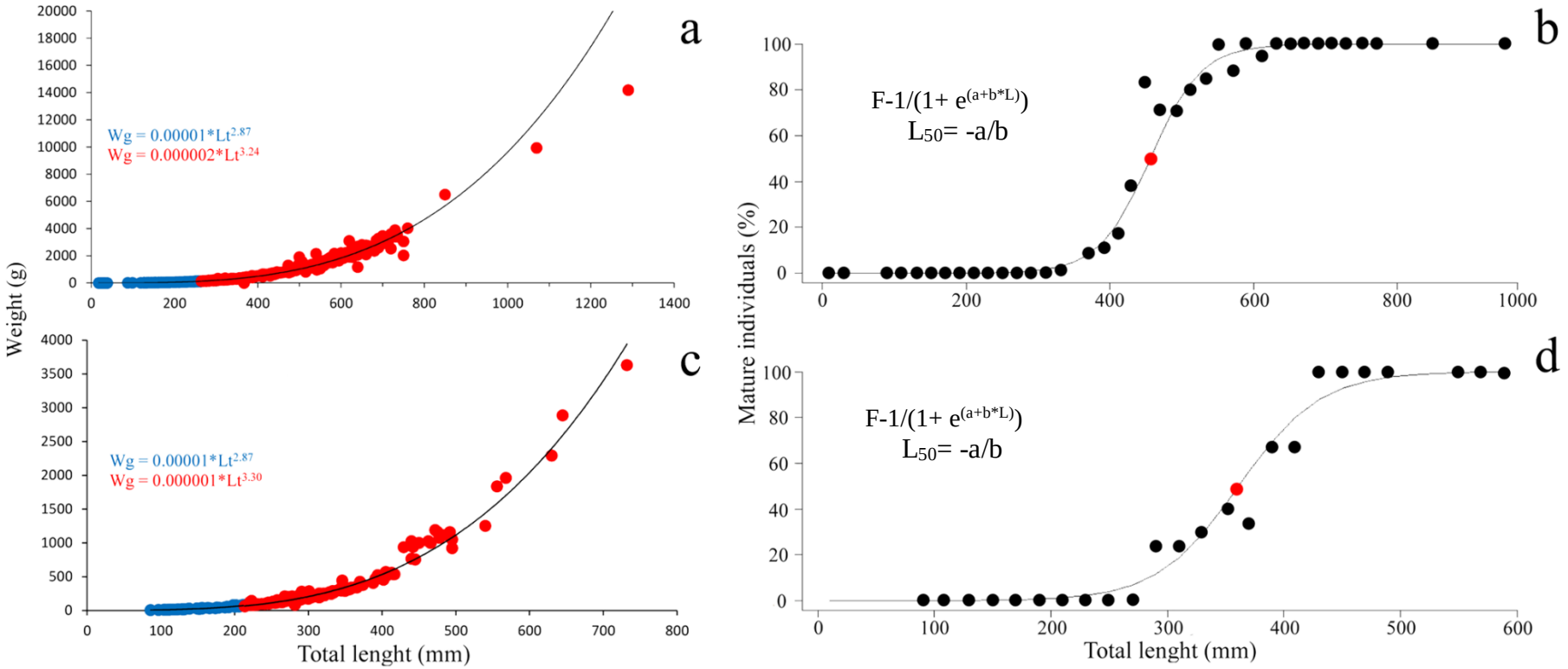

Figure S3 - The length *vs.* weight relationship and the equations (■ juveniles and ■ sub-adults/adults) for (a) *C. undecimalis* and (c) *C. mexicanus.* The coefficient of determination obtained from the linearization of Log-transformed data was 96% for *C. undecimalis* (n=360) and 98% for *C. mexicanus* (n=207). Estimations for the average length at first maturation (● L_{50}) of (b) *C. undecimalis* (L_{50} = 454 mm) and (d) *C. mexicanus* (L_{50} = 361 mm).

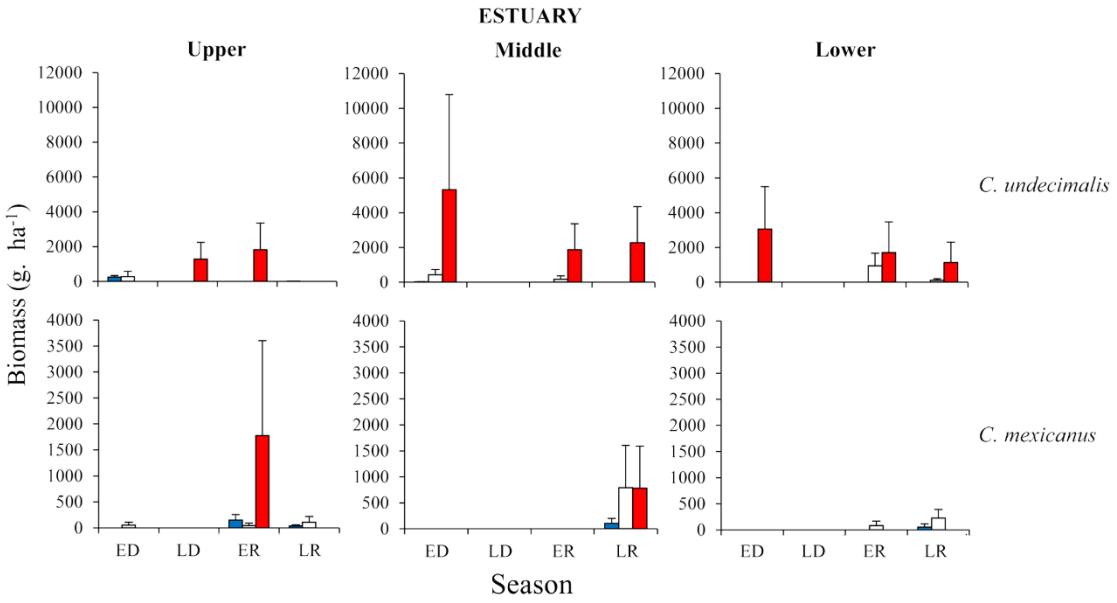

Figure S4 – Mean biomass (± SE) of Centropomidae species in the Goiana Estuary, according to habitats (upper, middle and lower estuaries), seasons (ED: early dry; LD: late dry; ER: early rainy; LR: late rainy) and ontogenetic phases (juveniles ■, sub-adults □ and adults ■).

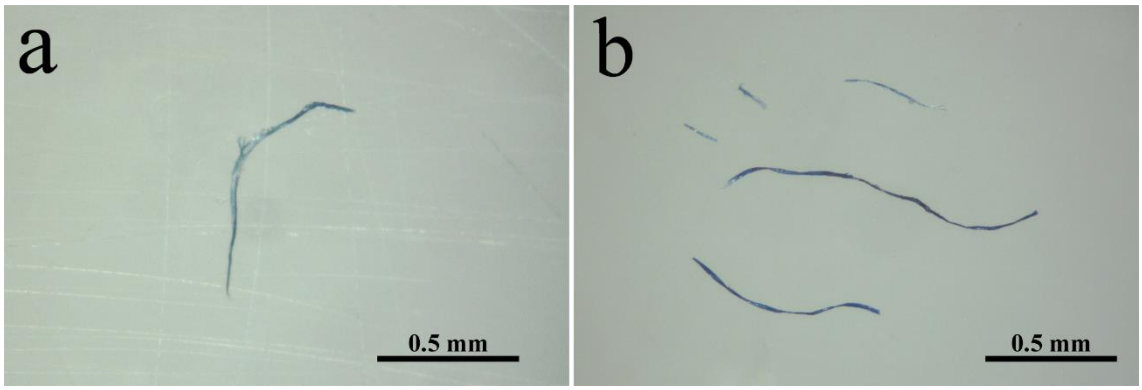

Figure S5 – Microplastics ingested by Centropomidae species: (a) filament with evidence of weathering and (b) multiple filaments with different sizes, ingested by the same individual.

Table S1 - Mean density and biomass (±SE), sample size and total mass of Centropomidae species used in the habitat use analysis [Juv (Juveniles); Sub (sub-adults); Ad (adults)] (216 samples collected between 2005 and 2006).

		Density (ind. ha^{-1})		**N**	**Biomass** (g ha^{-1})		**Mass (g)**
C. undecimalis	Juv	0.7	±0.35	24	23.8	±11.9	610.8
	Sub	0.59	±0.26	19	198.7	±84.7	4,036.5
	Ad	0.67	±0.23	20	1407.9	±575.4	28,906
	Total	**0.69**	**±0.17**	**63**	**573.69**	**±205.8**	**33553.3**
C. mexicanus	Juv	0.72	±0.28	27	28.9	±13.6	623
	Sub	0.56	±0.25	22	114.8	±72.1	2158
	Ad	0.2	±0.17	2	224.8	±170.2	2895
	Total	**0.52**	**±0.14**	**51**	**129.7**	**±65.2**	**5676**
Total		**1.15**	**±0.21**	**114**	**666.3**	**±204.1**	**39229.4**

Table S2 - Summary of the ANOVA for density and biomass of Centropomidae species in the Goiana estuary, according to habitat [U (upper); M (middle); L (lower)], season [ED (early dry); LD (late dry); ER (early rainy); LR (late rainy)] and ontogeny [Juv (juveniles); Sub (sub-adults); Adu (adults)]. Bonferroni's test was used to determinate the sources of variances [F (F-values); df (degree of freedom); *p*-value]. (ns: not significant) ($p < 0.05$).

	Density					**Biomass**				
	Factors	**F**	***df***	***p*-value**	***Post-hoc***	**Factors**	**F**	***df***	***p*-value**	***Post-hoc***
C. undecimalis	Season	4.61	3	0.01	ED	Season	4.39	3	0.01	ED
	Area	1.90	2	0.15	ns	Area	1.85	2	0.15	ns
	Phase	0.02	2	0.97	ns	Phase	0.02	2	0.97	ns
	Season *vs.* Area	2.27	6	0.06	ns	Season *vs.* Area	2.22	6	0.06	ns
	Season *vs.* Phase	3.85	6	0.01	ED Juv	Season *vs.* Phase	3.76	6	0.01	ED Juv
	Area *vs.* Phase	4.51	4	0.01	U Juv	Area *vs.* Phase	4.44	4	0.01	U Juv
	Season *vs.* Area *vs.* Phase	3.19	12	0.01	ED U Juv	Season *vs.* Area *vs.* Phase	3.11	12	0.01	ED U Juv
	Error		189					189		
C. mexicanus	Season	2.05	3	0.13	ns	Season	2.05	3	0.13	ns
	Area	3.31	2	0.03	U	Area	3.30	2	0.03	U
	Phase	1.59	2	0.20	ns	Phase	1.59	2	0.20	ns
	Season *vs.* Area	1.67	6	0.15	ns	Season *vs.* Area	1.67	6	0.15	ns
	Season *vs.* Phase	0.37	6	0.82	ns	Season *vs.* Phase	0.37	6	0.82	ns
	Area *vs.* Phase	1.10	4	0.35	ns	Area *vs.* Phase	1.10	4	0.35	ns
	Season *vs.* Area *vs.* Phase	0.69	12	0.69	ns	Season *vs.* Area *vs.* Phase	0.69	12	0.69	ns
	Error		189					189		

Table S3 - Food items ingested by *C. undecimalis* expressed as I_{RI}%, according to habitats [Upper; Middle; Lower; Coastal zone], seasons [Early dry; Late dry; Early rainy; Late rainy] and ontogetic phases [Juv (juveniles); Sub (sub-adults); Adu (adults)]. (-) no capture.

Items	Upper												Middle												Lower												Coastal zone											
	Early Dry			Late Dry			Early Rainy			Late Rainy			Early Dry			Late Dry			Early Rainy			Late Rainy			Early Dry			Late Dry			Early Rainy			Late Rainy			Early Dry			Late Dry			Early Rainy			Late Rainy		
	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu
Microplastic	9.7	1.8	-	26	19	0	-	-	55	0	-	-	-	100	100	25	50	38	100	72	55	-	-	100	0	-	53	8.3	53	16	49	75	44	11	41	47	55	67	46	-	70	65	-	7.1	55	53	7.1	57
Unidentified fish	0	14	-	0	70	0	-	-	0	0	-	-	-	0	0	0	0	12	0	8.3	34	-	-	0	0	-	0	14	22	3.2	3.6	3.4	0	6.1	8.1	12	0	0.4	0.3	-	0.3	6.3	-	44	4.1	11	15	0.5
Engraulidae	0	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	3.9	0	-	0	0.4	-	0	0	25	0	1.3
Anchovia clupeoides	0	0	-	0	0	0	-	-	46	0	-	-	-	0	0	0	0	6.6	0	0	3	-	-	0	0	-	0	0	12	0	0	0	0	0	22	0	6.2	4.6	17	-	1.9	0.6	-	0	21	0	0	8.2
Cetengraulis edentulus	0	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	0	0	0	0	0	-	-	0	0	-	19	0	0	0	0	0	0	0	0	0	0	0	5.5	-	0	11	-	0	5.8	0	0	4.1
Clupeidae	0	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	1.8	0	0	0	0	0	0	0	0	0.7	0	-	0.5	0	-	0	0	0	0	0.1
Rhinosardinia bahiensis	0	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	70	0	0	0	0	8.1	2.8	6.1	8.5	8.6	-	19	0	-	49	0	0	0	20
Harengula clupeola	0	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	19	0	0	-	4	0	-	0	0	0	0	0.6
Opisthonema oglinum	0	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	1.4	0	-	0	5.9	-	0	7.3	0	0	0.5
Odontognathus mucronatus	0	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	4.9	0	0	0	0	0	0	0	0	0	-	0.2	0	-	0	0	0	0	0
Gobidae	0	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	29	0	0	2.6	0	-	-	0	0	-	0	0	0	0	0.3	0	0	0	0	0	0	0	0	-	0	0	-	0	0	0	0	0
Bathygobius soporator	0	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	0	15	0	4.9	0	-	-	0	0	-	0	0	0	0	5.1	0	0	0	0	0	0	0	0	-	0	0	-	0	0	0	0	0
Eleotris pisonis	0	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	0	0	0	7.1	1.4	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	-	1.4	2.8	-	0	0	0	25	0
Cathorops spixii	0	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	1.7	26	0	0	3.4	-	-	0	0	-	12	0	0	0	0	0	0	0	4.9	0	0	0	0	-	0	1.3	-	0	0	0	0	1.5
Cynoscion acoupa	0	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	3.8	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	-	0	0	0	15	0.2
Stellifer stellifer	0	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	0	0	0	0	0	-	-	0	0	-	9.1	0	0	3.2	0	0	44	0	0	8.1	13	0	2.9	-	0	0	-	0	4.6	0	0	0.9
Stellifer rastrifer	0	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	3.1	-	0	0	0	0	0
Stellifer brasiliensis	0	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	8.1	0	0	0	-	0	0	-	0	0	0	0	0
Menticirrhus littoralis	0	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	1.5	-	0	0	0	0	0
Pomadasys corvinaeformis	0	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	1.4	-	0	0	0	0	0.8
Achirus lineatus	0	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	0	2.3	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	-	0	0	0	0	0
Centropomus undecimalis	0	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	7.4	0	0	3.3	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	-	0	0	0	0	0
Caranx latus	0	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	18	0	0	0	-	0	0	-	0	0	0	0	0
Diapterus rhombeus	0	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0.6	-	0	0	-	0	0	0	0	0
Eucinostomus melanopterus	0	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	15	-	0	0.8	-	0	0	0	0	0
Eugerres brasilianus	0	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	1.7	-	0	0	-	0	0	0	0	0
Trichiurus lepturus	0	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0.3	-	0	0	-	0	0	0	0	0
Myrophis punctatus	7.8	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	-	0	0	0	0	0
Hemiramphus brasiliensis	0	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	-	0	0	0	0	0.2
Unidentified invertebrate	0	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	0	0	0	0.6	0	-	-	0	100	-	0	0	0	0	0	0	0	46	0	0	0	0	0	-	0.2	0	-	0	0	0	0	0.1
Amphipoda	0	4.4	-	74	0	0	-	-	0	0	-	-	-	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	19	0	0	0	0	0	-	0	0	-	0	0	0	0	0
Paguridae	0	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0.2	1.4	0	0	0	0	0	0	0	-	0	0	-	0	0	0	7.7	0
Isopoda	0	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	5.5	0	0	0	0	0	0	-	0	0	-	0	0	0	0	0
Polychaeta	81	26	-	0	0	0	-	-	0	0	-	-	-	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	3.8	4.4	0	18	0	4.1	0	0	0	-	0	0	-	0	0	0	0	0
Anomalocardia flexuosa	0	0	-	0	5.2	0	-	-	0	0	-	-	-	0	0	0	0	0	0	0.7	0.5	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	-	0	0	0	0	0
Mytella falcata	0	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0.2	-	0	0	0	0	0
Gastropoda	0	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	2.1	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	-	0	0	0	0	0
Penaeid shrimp	1.4	54	-	0	0	100	-	-	0	0	-	-	-	0	0	0	1.5	0	0	0.6	0.8	-	-	0	0	-	0	0	0	2.6	30	8.6	0	0	16	0	0	11	0.4	-	1.6	0	-	0	0	11	31	0.1
Callinectes danae	0	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	1.5	0	0	0	0	-	-	0	0	-	0	77	0	0	2	1.3	0	0	0	0	0	1.6	0.6	-	0	0	-	0	0	0	0	3.8
Ucides cordatus	0	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	2.5	0	0	0	0	0	0	0	0	0	0	-	0	0.2	-	0	0	0	0	0
Mangrove fragments	0	0	-	0	5.9	0	-	-	0	0	-	-	-	0	0	75	2.7	0	0	0	1.3	-	-	0	0	-	7.5	0	7.4	0	5	5.5	5.6	0	0	0	0	0.4	0	-	0	0.2	-	0	1.9	0	0	0
Seaweed	0	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0.8	0	0	0	0	0	0	0	0	0	1.3	-	0.7	0	-	0	0.4	0	0	0
Sediment	0.5	0	-	0	0	0	-	-	0	0	-	-	-	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	1.4	0	0	0	0	0	0	0	0	-	0	0	-	0	0	0	0	0

Table S4 -Food items ingested by *C. undecimalis* expressed as FO% (frequency of occurrence), according to habitats [Upper; Middle; Lower; Coastal zone], seasons [Early dry; Late dry; Early rainy; Late rainy] and ontogetic phases [Juv (juveniles); Sub (sub-adults); Adu (adults)]. (-) no capture.

Items	Upper												Middle												Lower												Coastal zone											
	Early Dry			Late Dry			Early Rainy			Late Rainy			Early Dry			Late Dry			Early Rainy			Late Rainy			Early Dry			Late Dry			Early Rainy			Late Rainy			Early Dry			Late Dry			Early Rainy			Late Rainy		
	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu
Microplastic	57	33	-	100	100	0	-	-	100	0	-	-	0	50	100	100	57	38	100	78	70	-	-	100	0	-	100	100	57	29	76	78	67	20	50	60	75	64	47	0	67	50	-	40	58	75	33	50
Unidentified fish	0	67	-	0	100	0	-	-	0	0	-	-	0	0	0	0	0	25	0	22	40	-	-	0	0	-	0	100	43	14	20	11	0	10	25	40	0	7.1	6.7	0	6.7	17	-	60	17	25	33	7.1
Engraulidae	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	7.1	0	0	0	4.2	-	0	0	25	0	3.6
Anchovia clupeoides	0	0	-	0	0	0	-	-	50	0	-	-	0	0	0	0	0	13	0	0	10	-	-	0	0	-	0	0	14	0	0	0	0	0	25	0	25	7.1	27	0	6.7	4.2	-	0	25	0	0	14
Cetengraulis edentulus	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	33	0	0	0	0	0	0	0	0	0	0	0	13	0	0	13	-	0	17	0	0	11
Clupeidae	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	14	0	0	0	0	0	0	0	0	7.1	0	0	6.7	0	-	0	0	0	0	3.6
Rhinosardinia bahiensis	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	29	0	0	0	0	25	20	25	7.1	20	0	33	8.3	-	40	0	0	0	18
Harengula clupeola	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	25	0	0	0	6.7	0	-	0	0	0	0	3.6
Opisthonema oglinum	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	7.1	0	0	0	8.3	-	0	8.3	0	0	3.6
Odontognathus mucronatus	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	14	0	0	0	0	0	0	0	0	0	0	6.7	0	-	0	0	0	0	0
Gobidae	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	29	0	0	11	0	-	-	0	0	-	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0
Bathygobius soporator	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	13	0	11	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0
Eleotris pisonis	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	11	10	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.7	4.2	-	0	0	0	33	0
Cathorops spixii	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	14	13	0	0	10	-	-	0	0	-	33	0	0	0	0	0	0	0	25	0	0	0	0	0	0	4.2	-	0	0	0	0	7.1
Cynoscion acoupa	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	14	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	33	3.6
Stellifer stellifer	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	33	0	0	14	0	0	33	0	0	20	25	0	13	0	0	0	-	0	17	0	0	7.1
Stellifer rastrifer	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8.3	-	0	0	0	0	0
Stellifer brasiliensis	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	20	0	0	0	0	0	0	-	0	0	0	0	0
Menticirrhus littoralis	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4.2	-	0	0	0	0	0
Pomadasys corvinaeformis	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4.2	-	0	0	0	0	3.6
Achirus lineatus	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0.1	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0
Centropomus undecimalis	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	14	0	0	11	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0
Caranx latus	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	20	0	0	0	0	0	0	-	0	0	0	0	0
Diapterus rhombeus	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	6.7	0	0	0	-	0	0	0	0	0
Eucinostomus melanopterus	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	13	0	0	4.2	-	0	0	0	0	0
Eugerres brasilianus	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	6.7	0	0	0	-	0	0	0	0	0
Trichiurus lepturus	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	6.7	0	0	0	-	0	0	0	0	0
Myrophis punctatus	29	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0
Hemiramphus brasiliensis	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	3.6
Unidentified invertebrate	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	11	0	-	-	0	33	-	0	0	0	0	0	0	0	30	0	0	0	0	0	0	6.7	0	-	0	0	0	0	3.6
Amphipoda	0	33	-	100	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	20	0	0	0	0	0	0	0	0	-	0	0	0	0	0
Paguridae	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	4	11	0	0	0	0	0	0	0	0	0	0	-	0	0	0	33	0
Isopoda	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	33	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0
Polychaeta	71	33	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	24	11	0	20	0	20	0	0	0	0	0	0	-	0	0	0	0	0
Anomalocardia flexuosa	0	0	-	0	50	0	-	-	0	0	-	-	0	0	0	0	0	0	0	11	10	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0
Mytella falcata	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4.2	-	0	0	0	0	0
Gastropoda	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	14	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0
Penaeid shrimp	14	67	-	0	0	50	-	-	0	0	-	-	0	0	0	0	14	0	0	11	10	-	-	0	0	-	0	0	0	14	40	22	0	0	50	0	0	14	6.7	0	6.7	0	-	0	0	25	33	3.6
Callinectes danae	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	14	0	0	0	0	-	-	0	0	-	0	100	0	0	16	11	0	0	0	0	0	14	6.7	0	0	0	-	0	0	0	0	11
Ucides cordatus	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	14	0	0	0	0	0	0	0	0	0	0	0	0	4.2	-	0	0	0	0	0
Mangrove fragments	0	0	-	0	50	0	-	-	0	0	-	-	0	0	0	100	14	0	0	0	10	-	-	0	0	-	67	0	29	0	28	22	33	0	0	0	0	7.1	0	0	0	4.2	-	0	8.3	0	0	0
Seaweed	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	14	0	0	0	0	0	0	0	0	0	13	0	13	0	-	0	8.3	0	0	0
Sediment	14	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0

Table S5 - Food items ingested by *C. undecimalis* expressed as FN% (frequency in number), according to habitats [Upper; Middle; Lower; Coastal zone], seasons [Early dry; Late dry; Early rainy; Late rainy] and ontogetic phases [Juv (juveniles); Sub (sub-adults); Adu (adults)]. (-) no capture.

Items	Upper												Middle												Lower												Coastal zone											
	Early Dry			Late Dry			Early Rainy			Late Rainy			Early Dry			Late Dry			Early Rainy			Late Rainy			Early Dry			Late Dry			Early Rainy			Late Rainy			Early Dry			Late Dry			Early Rainy			Late Rainy		
	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu
Microplastic	20	5.6	-	50	33	0	-	-	75	0	-	-	0	100	100	50	52	38	100	60	59	-	-	100	0	-	58	17	64	27	44	76	57	37	57	50	58	44	44	0	66	38	-	17	50	55	14	45
Unidentified fish	0	11	-	0	33	0	-	-	0	0	-	-	0	0	0	0	0	8.3	0	13	17	-	-	0	0	-	0	17	12	9.1	5.9	2.7	0	7.1	7.1	13	0	2.3	2.1	0	1.5	10	-	50	7.5	9.1	29	2.2
Engraulidae	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	4.7	0	0	0	1.5	-	0	0	9.1	0	1.1
Anchovia clupeoides	0	0	-	0	0	0	-	-	25	0	-	-	0	0	0	0	0	8.3	0	0	6.9	-	-	0	0	-	0	0	4	0	0	0	0	0	7.1	0	8.3	2.3	10	0	1.5	1.5	-	0	15	0	0	7.9
Cetengraulis edentulus	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	21	0	0	0	0	0	0	0	0	0	0	0	6.3	0	0	5.9	-	0	5	0	0	3.4
Clupeidae	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	4	0	0	0	0	0	0	0	0	2.3	0	0	1.5	0	-	0	0	0	0	1.1
Rhinosardinia bahiensis	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	36	0	0	0	0	7.1	6.3	8.3	14	13	0	9	7	-	33	0	0	0	20
Harengula clupeola	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	8.3	0	0	0	7.5	0	-	0	0	0	0	1.1
Opisthonema oglinum	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	2.3	0	0	0	15	-	0	5	0	0	2.2
Odontognathus mucronatus	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	9.1	0	0	0	0	0	0	0	0	0	0	1.5	0	-	0	0	0	0	0
Gobidae	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	9.5	0	0	3.3	0	-	-	0	0	-	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0
Bathygobius soporator	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	8.3	0	3.3	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0
Eleotris pisonis	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	3.3	3.4	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.5	4.4	-	0	0	0	14	0
Cathorops spixii	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	4.8	33	0	0	3.4	-	-	0	0	-	5.3	0	0	0	0	0	0	0	7.1	0	0	0	0	0	0	2.9	-	0	0	0	0	2.2
Cynoscion acoupa	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	4.8	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	14	1.1
Stellifer stellifer	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	5.3	0	0	9.1	0	0	14	0	0	6.3	17	0	4.2	0	0	0	-	0	5	0	0	2.2
Stellifer rastrifer	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.9	-	0	0	0	0	0
Stellifer brasiliensis	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	6.3	0	0	0	0	0	0	-	0	0	0	0	0
Menticirrhus littoralis	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.5	-	0	0	0	0	0
Pomadasys corvinaeformis	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.5	-	0	0	0	0	1.1
Achirus lineatus	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	4.2	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0
Centropomus undecimalis	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	9.5	0	0	6.7	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0
Caranx latus	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	6.3	0	0	0	0	0	0	-	0	0	0	0	0
Diapterus rhombeus	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	2.1	0	0	0	-	0	0	0	0	0
Eucinostomus melanopterus	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	4.2	0	0	2.9	-	0	0	0	0	0
Eugerres brasilianus	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	4.2	0	0	0	-	0	0	0	0	0
Trichiurus lepturus	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	2.1	0	0	0	-	0	0	0	0	0
Myrophis punctatus	20	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0
Hemiramphus brasiliensis	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	2.2
Unidentified invertebrate	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	3.3	0	-	-	0	100	-	0	0	0	0	0	0	0	21	0	0	0	0	0	0	1.5	0	-	0	0	0	0	1.1
Amphipoda	0	11	-	50	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	20	0	0	0	0	0	0	0	0	-	0	0	0	0	0
Paguridae	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	1	2.7	0	0	0	0	0	0	0	0	0	-	0	0	0	14	0
Isopoda	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	14	0	0	0	0	0	0	0	0	-	0	0	0	0	0
Polychaeta	47	61	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	6.9	5.4	0	14	0	13	0	0	0	0	0	0	-	0	0	0	0	0
Anomalocardia flexuosa	0	0	-	0	17	0	-	-	0	0	-	-	0	0	0	0	0	0	0	3.3	3.4	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0
Mytella falcata	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.5	-	0	0	0	0	0
Gastropoda	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	4.8	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0
Penaeid shrimp	10	11	-	0	0	100	-	-	0	0	-	-	0	0	0	0	4.8	0	0	3.3	3.4	-	-	0	0	-	0	0	0	9.1	27	5.4	0	0	14	0	0	21	2.1	0	6	0	-	0	0	27	14	1.1
Callinectes danae	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	4.8	0	0	0	0	-	-	0	0	-	0	67	0	0	4	2.7	0	0	0	0	0	4.7	2.1	0	0	0	-	0	0	0	0	4.5
Ucides cordatus	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	1.5	-	0	0	0	0	0
Mangrove fragments	0	0	-	0	17	0	-	-	0	0	-	-	0	0	0	50	4.8	0	0	0	3.4	-	-	0	0	-	11	0	8	0	6.9	5.4	14	0	0	0	0	2.3	0	0	0	1.5	-	0	10	0	0	0
Seaweed	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	4	0	0	0	0	0	0	0	0	0	4.2	0	3	0	-	0	2.5	0	0	0
Sediment	3.3	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0

Table S6 - Food items ingested by *C. undecimalis* expressed as FW% (frequency in weight), according to habitats [Upper; Middle; Lower; Coastal zone], seasons [Early dry; Late dry; Early rainy; Late rainy] and ontogetic phases [Juv (juveniles); Sub (sub-adults); Adu (adults)]. (-) no capture.

Items	Upper												Middle												Lower												Coastal zone											
	Early Dry			Late Dry			Early Rainy			Late Rainy			Early Dry			Late Dry			Early Rainy			Late Rainy			Early Dry			Late Dry			Early Rainy			Late Rainy			Early Dry			Late Dry			Early Rainy			Late Rainy		
	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu
Microplastic	0.02	0.01	-	1.45	0.01	0	-	-	0.01	0	-	-	0	100	100	0.37	0	0	100	0	0.01	-	-	100	0	-	0.01	0.05	0.01	0.01	0.02	0.06	0.01	2.33	0.01	0.01	0.01	0.01	0.01	0	0.01	0.01	-	0.01	0.01	0.01	0.01	0.01
Unidentified fish	0	10.2	-	0	93.2	0	-	-	0	0	-	-	0	0	0	0	0	9.49	0	10.8	46.7	-	-	0	0	-	0	12.1	23.3	1.98	6.28	21.6	0	19.8	15.7	6.77	0	0.28	0.16	0	1.47	1.64	-	19.4	5.68	24.6	1.37	0.58
Engraulidae	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	18.9	0	0	0	1.46	-	0	0	68.4	0	13.4
Anchovia clupeoides	0	0	-	0	0	0	-	-	100	0	-	-	0	0	0	0	0	11	0	0	15.7	-	-	0	0	-	0	0	53.9	0	0	0	0	0	54.3	0	11.3	24.7	17.8	0	16.3	3.41	-	0	28.6	0	0	14.5
Cetengraulis edentulus	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	40.1	0	0	0	0	0	0	0	0	0	0	0	12.2	0	0	20.9	-	0	13.4	0	0	11.7
Clupeidae	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	4.73	0	0	0	0	0	0	0	1.72	0	0	3.27	0	-	0	0	0	0	0.15
Rhinosardinia bahiensis	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	87.3	0	0	0	0	15.5	2.78	11.1	36.5	6.67	0	27.5	9.06	-	80.6	0	0	0	22.8
Harengula clupeola	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	52.8	0	0	0	30.2	0	-	0	0	0	0	5.58
Opisthonema oglinum	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	5.72	0	0	0	8.08	-	0	41.1	0	0	3.39
Odontognathus mucronatus	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	8.45	0	0	0	0	0	0	0	0	0	0	0.18	0	-	0	0	0	0	0
Gobidae	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	51.4	0	0	11.8	0	-	-	0	0	-	0	0	0	0	3.86	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0
Bathygobius soporator	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	34.8	0	25.7	0	-	-	0	0	-	0	0	0	0	40.9	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0
Eleotris pisonis	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	37.9	6.93	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11.7	16.9	-	0	0	0	35	0
Cathorops spixii	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	2.35	42.1	0	0	21.8	-	-	0	0	-	33.5	0	0	0	0	0	0	0	6.66	0	0	0	0	0	0	7.02	-	0	0	0	0	6.06
Cynoscion acoupa	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	11.2	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	15.2	1.02
Stellifer stellifer	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	24.6	0	0	2.18	0	0	99.8	0	0	19.5	24.8	0	5.69	0	0	0	-	0	9.45	0	0	2.68
Stellifer rastrifer	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8.71	-	0	0	0	0	0
Stellifer brasiliensis	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	19.6	0	0	0	0	0	0	-	0	0	0	0	0
Menticirrhus littoralis	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9.67	-	0	0	0	0	0
Pomadasys corvinaeformis	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9.25	-	0	0	0	0	8.07
Achirus lineatus	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	2.62	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0
Centropomus undecimalis	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	21.5	0	0	12.6	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0
Caranx latus	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	50.7	0	0	0	0	0	0	-	0	0	0	0	0
Diapterus rhombeus	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	2.09	0	0	0	-	0	0	0	0	0
Eucinostomus melanopterus	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	45	0	0	3.3	-	0	0	0	0	0
Eugerres brasilianus	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	7.39	0	0	0	-	0	0	0	0	0
Trichiurus lepturus	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0.31	0	0	0	-	0	0	0	0	0
Myrophis punctatus	12.2	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0
Hemiramphus brasiliensis	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0.27
Unidentified invertebrate	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0.02	0	-	-	0	100	-	0	0	0	0	0	0	0	46.5	0	0	0	0	0	0	0.19	0	-	0	0	0	0	0.01
Amphipoda	0	2.33	-	98.6	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	6.98	0	0	0	0	0	0	0	0	-	0	0	0	0	0
Paguridae	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	2.33	7.07	0	0	0	0	0	0	0	0	0	0	-	0	0	0	1.15	0
Isopoda	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0.01	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0
Polychaeta	85.9	17.4	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	3.68	25.6	0	24.4	0	0.66	0	0	0	0	0	0	-	0	0	0	0	0
Anomalocardia flexuosa	0	0	-	0	1.96	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0.78	0.02	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0
Mytella falcata	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0
Gastropoda	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	4.12	0	0	0	0	-	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0
Penaeid shrimp	1.46	70.1	-	0	0	100	-	-	0	0	-	-	0	0	0	0	1.51	0	0	0.34	2.43	-	-	0	0	-	0	0	0	0.13	23.4	25	0	0	7.86	0	0	11.9	0.77	0	8.92	0	-	0	0	7.04	47.3	0.22
Callinectes danae	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	1.3	0	0	0	0	-	-	0	0	-	0	87.9	0	0	4.43	6.59	0	0	0	0	0	0.23	1.91	0	0	0	-	0	0	0	0	9.51
Ucides cordatus	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	8.17	0	0	0	0	0	0	0	0	0	0	0	0	0.12	-	0	0	0	0	0
Mangrove fragments	0	0	-	0	4.85	0	-	-	0	0	-	-	0	0	0	99.6	6.64	0	0	0	6.35	-	-	0	0	-	1.77	0	9.82	0	5.27	14.1	0.2	0	0	0	0	0.02	0	0	0	0.38	-	0	1.81	0	0	0
Seaweed	0	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	9.78	0	0	0	0	0	0	0	0	0	0.06	0	0.36	0	-	0	0.01	0	0	0
Sediment	0.48	0	-	0	0	0	-	-	0	0	-	-	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0	0	0	9.78	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0

Table S7 - Food items ingested by *C. mexicanus* expressed as I_{RI}%, according to habitats [Upper; Middle; Lower; Coastal zone], seasons [Early dry; Late dry; Early rainy; Late rainy] and ontogetic phases [Juv (juveniles); Sub (sub-adults); Adu (adults)]. (-) no capture.

Items	Upper												Middle												Lower												Coastal zone											
	Early Dry			Late Dry			Early Rainy			Late Rainy			Early Dry			Late Dry			Early Rainy			Late Rainy			Early Dry			Late Dry			Early Rainy			Late Rainy			Early Dry			Late Dry			Early Rainy			Late Rainy		
	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu
Microplastic	-	0	-	8.6	-	-	78	10	100	67	75	-	-	-	-	-	63	26	25	-	-	45	76	20	-	-	-	-	100	-	18	56	-	3.7	61	-	-	67	0	-	-	20	-	22	27	-	54	36
Unidentified fish	-	100	-	0	-	-	0	0	0	0	0	-	-	-	-	-	3.5	0	0	-	-	0	2.4	57	-	-	-	-	0	-	0.1	3.1	-	0	15	-	-	0	0	-	-	0	-	19	3.1	-	13	5.2
Engraulidae	-	0	-	0	-	-	0	0	0	0	12	-	-	-	-	-	2.8	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-	-	0	0	-	-	0	-	0.7	23	-	0	0.2
Anchovia clupeoides	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	19	63	0	-	-	0	0	0	-	-	-	-	0	-	2	0	-	0	5.2	-	-	9.3	0	-	-	0	-	1.8	42	-	0	0.2
Cetengraulis edentulus	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-	-	0	100	-	-	0	-	0	0	-	0	3.8
Clupeidae	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-	-	0	0	-	-	0	-	6.3	0	-	0.4	0
Rhinosardinia bahiensis	-	0	-	0	-	-	8.5	0	0	0	0	-	-	-	-	-	6.6	0	0	-	-	0	20	0	-	-	-	-	0	-	0	0	-	0	0	-	-	0	0	-	-	0	-	44	0	-	17	51
Harengula clupeola	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-	-	0	0	-	-	0	-	0	0	-	2.6	0
Opisthonema oglinum	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-	-	0	0	-	-	0	-	0.5	0	-	0	0.4
Odontognathus mucronatus	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-	-	0	0	-	-	80	-	0	0	-	0	0
Lycengraulis grossidens	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-	-	0	0	-	-	0	-	0.4	0	-	0	0
Gobidae	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	2.4	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-	-	15	0	-	-	0	-	0	0	-	0	0
Stellifer stellifer	-	0	-	0	-	-	0	0	0	10	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0.3	0	-	0	0	-	-	0	0	-	-	0	-	0	0	-	0	0
Pomadasys corvinaeformis	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-	-	0	0	-	-	0	-	0	0	-	0	0.9
Mugil liza	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-	-	0	0	-	-	0	-	0.2	0	-	0	0
Hemiramphus brasiliensis	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-	-	0	0	-	-	0	-	0	0	-	0	0.1
Unidentified invertebrate	-	0	-	0	-	-	0	0	0	0	1.3	-	-	-	-	-	1.8	0	0	-	-	0	1.3	13	-	-	-	-	0	-	0	0	-	0	0	-	-	0	0	-	-	0	-	0	0	-	0	0
Amphipoda	-	0	-	0	-	-	0	0	0	0.6	0	-	-	-	-	-	0.8	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-	-	0	0	-	-	0	-	0	0	-	0	0
Mysidacea	-	0	-	0	-	-	2.8	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	8.2	-	-	0	0	-	-	0	-	0	0	-	0	0
Paguridae	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	3.7	0	-	0	0	-	-	0	0	-	-	0	-	1.7	0	-	0	0
Isopoda	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0.1	0	-	0	0	-	-	0	0	-	-	0	-	0	0	-	0.5	0
Polychaeta	-	0	-	0	-	-	0.9	0	0	3.9	6.9	-	-	-	-	-	0	2	0	-	-	6.6	0	0	-	-	-	-	0	-	56	34	-	0	0	-	-	0	0	-	-	0	-	0	0	-	0	0
Penaeid shrimp	-	0	-	91	-	-	5.1	90	0	18	1.3	-	-	-	-	-	0.4	8.9	0	-	-	38	0	0	-	-	-	-	0	-	12	0.6	-	96	9	-	-	7	0	-	-	0	-	1.5	4.8	-	11	1.4
Callinectes danae	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	75	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	1.7	-	-	0	0	-	-	0	-	2	0	-	0.3	0.6
Aratus pisonii	-	0	-	0	-	-	4.4	0	0	0	3.8	-	-	-	-	-	0	0	0	-	-	11	0	0	-	-	-	-	0	-	1.8	5.2	-	0	0	-	-	0	0	-	-	0	-	0	0	-	0	0
Mangrove fragments	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	10	-	-	-	-	0	-	6.9	0.5	-	0	0	-	-	0	0	-	-	0	-	0	0	-	0.2	0
Seaweed	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-	-	1.8	0	-	-	0	-	0	0	-	0.2	0

Table S8 - Food items ingested by *C. mexicanus* expressed as FO% (frequency of occurrence), according to habitats [Upper; Middle; Lower; Coastal zone], seasons [Early dry; Late dry; Early rainy; Late rainy] and ontogetic phases [Juv (juveniles); Sub (sub-adults); Adu (adults)]. (-) no capture.

Items	Upper												Middle												Lower											
	Early Dry			Late Dry			Early Rainy			Late Rainy			Early Dry			Late Dry			Early Rainy			Late Rainy			Early Dry			Late Dry			Early Rainy			Late Rainy		
	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu
Microplastic	-	0	-	50	-	-	75	100	100	88	86	-	-	-	-	-	90	67	100	-	-	40	100	100	-	-	-	-	100	-	50	100	-	67	78	-
Unidentified fish	-	100	-	0	-	-	0	0	0	0	0	-	-	-	-	-	20	0	0	-	-	0	20	100	-	-	-	-	0	-	4.5	14	-	0	22	-
Engraulidae	-	0	-	0	-	-	0	0	0	0	14	-	-	-	-	-	10	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-
Anchovia clupeoides	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	20	67	0	-	-	0	0	0	-	-	-	-	0	-	4.5	0	-	0	11	-
Cetengraulis edentulus	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-
Clupeidae	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-
Rhinosardinia bahiensis	-	0	-	0	-	-	8.3	0	0	0	0	-	-	-	-	-	10	0	0	-	-	0	20	0	-	-	-	-	0	-	0	0	-	0	0	-
Harengula clupeola	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-
Opisthonema oglinum	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-
Odontognathus mucronatus	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-
Lycengraulis grossidens	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-
Gobidae	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	10	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-
Stellifer stellifer	-	0	-	0	-	-	0	0	0	13	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	4.5	0	-	0	0	-
Pomadasys corvinaeformis	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-
Mugil liza	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-
Hemiramphus brasiliensis	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-
Unidentified invertebrate	-	0	-	0	-	-	0	0	0	0	14	-	-	-	-	-	20	0	0	-	-	0	20	100	-	-	-	-	0	-	0	0	-	0	0	-
Amphipoda	-	0	-	0	-	-	0	0	0	13	0	-	-	-	-	-	10	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-
Mysidacea	-	0	-	0	-	-	8.3	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	22	-
Paguridae	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	18	0	-	0	0	-
Isopoda	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	4.5	0	-	0	0	-
Polychaeta	-	0	-	0	-	-	8.3	0	0	25	14	-	-	-	-	-	0	33	0	-	-	20	0	0	-	-	-	-	0	-	68	29	-	0	0	-
Penaeid shrimp	-	0	-	50	-	-	17	100	0	38	14	-	-	-	-	-	10	33	0	-	-	20	0	0	-	-	-	-	0	-	27	14	-	67	33	-
Callinectes danae	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	100	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	11	-
Aratus pisonii	-	0	-	0	-	-	8.3	0	0	0	14	-	-	-	-	-	0	0	0	-	-	20	0	0	-	-	-	-	0	-	14	29	-	0	0	-
Mangrove fragments	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0		-	-	-	-	0	-	36	14	-	0	0	-
Seaweed	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-

Items	Coastal zone											
	Early Dry			Late Dry			Early Rainy			Late Rainy		
	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu
Microplastic	-	33	0	-	-	50	-	56	75	-	73	59
Unidentified fish	-	0	0	-	-	0	-	26	25	-	20	18
Engraulidae	-	0	0	-	-	0	-	3.7	25	-	0	4.5
Anchovia clupeoides	-	8.3	0	-	-	0	-	7.4	25	-	0	4.5
Cetengraulis edentulus	-	0	50	-	-	0	-	0	0	-	0	14
Clupeidae	-	0	0	-	-	0	-	15	0	-	6.7	0
Rhinosardinia bahiensis	-	0	0	-	-	0	-	37	0	-	20	27
Harengula clupeola	-	0	0	-	-	0	-	0	0	-	6.7	0
Opisthonema oglinum	-	0	0	-	-	0	-	7.4	0	-	0	4.5
Odontognathus mucronatus	-	0	0	-	-	50	-	0	0	-	0	0
Lycengraulis grossidens	-	0	0	-	-	0	-	3.7	0	-	0	0
Gobidae	-	8.3	0	-	-	0	-	0	0	-	0	0
Stellifer stellifer	-	0	0	-	-	0	-	0	0	-	0	0
Pomadasys corvinaeformis	-	0	0	-	-	0	-	0	0	-	0	4.5
Mugil liza	-	0	0	-	-	0	-	3.7	0	-	0	0
Hemiramphus brasiliensis	-	0	0	-	-	0	-	0	0	-	0	4.5
Unidentified invertebrate	-	0	0	-	-	0	-	0	0	-	0	0
Amphipoda	-	0	0	-	-	0	-	0	0	-	0	0
Mysidacea	-	0	0	-	-	0	-	0	0	-	0	0
Paguridae	-	0	0	-	-	0	-	19	0	-	0	0
Isopoda	-	0	0	-	-	0	-	0	0	-	6.7	0
Polychaeta	-	0	0	-	-	0	-	0	0	-	0	0
Penaeid shrimp	-	8.3	0	-	-	0	-	15	25	-	27	14
Callinectes danae	-	0	0	-	-	0	-	19	0	-	6.7	9.1
Aratus pisonii	-	0	0	-	-	0	-	0	0	-	0	0
Mangrove fragments	-	0	0	-	-	0	-	0	0	-	6.7	0
Seaweed	-	8.3	0	-	-	0	-	0	0	-	6.7	0

Table S9 - Food items ingested by *C. mexicanus* expressed as FN% (frequency in number), according to habitats [Upper; Middle; Lower; Coastal zone], seasons [Early dry; Late dry; Early rainy; Late rainy] and ontogetic phases [Juv (juveniles); Sub (sub-adults); Adu (adults)]. (-) no capture.

Items	Upper												Middle												Lower												Coastal zone											
	Early Dry			Late Dry			Early Rainy			Late Rainy			Early Dry			Late Dry			Early Rainy			Late Rainy			Early Dry			Late Dry			Early Rainy			Late Rainy			Early Dry			Late Dry			Early Rainy			Late Rainy		
	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu
Microplastic	-	0	-	17	-	-	63	20	100	68	67	-	-	-	-	-	44	47	50	-	-	57	78	40	-	-	-	-	100	-	26	50	-	7.4	57	-	-	67	0	-	-	40	-	21	22	-	44	33
Unidentified fish	-	100	-	0	-	-	0	0	0	0	0	-	-	-	-	-	10	0	0	-	-	0	11	20	-	-	-	-	0	-	1.1	3.3	-	0	4.8	-	-	0	0	-	-	0	-	19	5.6	-	7.7	9.3
Engraulidae	-	0	-	0	-	-	0	0	0	0	6.7	-	-	-	-	-	5.1	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-	-	0	0	-	-	0	-	4.8	17	-	0	1.9
Anchovia clupeoides	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	15	20	0	-	-	0	0	0	-	-	-	-	0	-	1.1	0	-	0	9.5	-	-	6.7	0	-	-	0	-	2.4	50	-	0	0.9
Cetengraulis edentulus	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-	-	0	100	-	-	0	-	0	0	-	0	2.8
Clupeidae	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-	-	0	0	-	-	0	-	11	0	-	1.9	0
Rhinosardinia bahiensis	-	0	-	0	-	-	3.1	0	0	0	0	-	-	-	-	-	10	0	0	-	-	0	5.6	0	-	-	-	-	0	-	0	0	-	0	0	-	-	0	0	-	-	0	-	24	0	-	12	43
Harengula clupeola	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-	-	0	0	-	-	0	-	0	0	-	7.7	0
Opisthonema oglinum	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-	-	0	0	-	-	0	-	2.4	0	-	0	0.9
Odontognathus mucronatus	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-	-	0	0	-	-	60	-	0	0	-	0	0
Lycengraulis grossidens	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-	-	0	0	-	-	0	-	1.6	0	-	0	0
Gobidae	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	2.6	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-	-	6.7	0	-	-	0	-	0	0	-	0	0
Stellifer stellifer	-	0	-	0	-	-	0	0	0	4	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	2.2	0	-	0	0	-	-	0	0	-	-	0	-	0	0	-	0	0
Pomadasys corvinaeformis	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-	-	0	0	-	-	0	-	0	0	-	0	1.9
Mugil liza	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-	-	0	0	-	-	0	-	0.8	0	-	0	0
Hemiramphus brasiliensis	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-	-	0	0	-	-	0	-	0	0	-	0	0.9
Unidentified invertebrate	-	0	-	0	-	-	0	0	0	0	6.7	-	-	-	-	-	5.1	0	0	-	-	0	5.6	20	-	-	-	-	0	-	0	0	-	0	0	-	-	0	0	-	-	0	-	0	0	-	0	0
Amphipoda	-	0	-	0	-	-	0	0	0	4	0	-	-	-	-	-	5.1	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-	-	0	0	-	-	0	-	0	0	-	0	0
Mysidacea	-	0	-	0	-	-	19	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	12	-	-	0	0	-	-	0	-	0	0	-	0	0
Paguridae	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	4.3	0	-	0	0	-	-	0	0	-	-	0	-	19	0	-	0	0
Isopoda	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	1.1	0	-	0	0	-	-	0	0	-	-	0	-	0	0	-	3.8	0
Polychaeta	-	0	-	0	-	-	3.1	0	0	8	6.7	-	-	-	-	-	0	6.7	0	-	-	14	0	0	-	-	-	-	0	-	27	33	-	0	0	-	-	0	0	-	-	0	-	0	0	-	0	0
Penaeid shrimp	-	0	-	83	-	-	9.4	80	0	16	6.7	-	-	-	-	-	2.6	27	0	-	-	14	0	0	-	-	-	-	0	-	24	3.3	-	93	14	-	-	13	0	-	-	0	-	4	5.6	-	17	3.7
Callinectes danae	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	50	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	2.4	-	-	0	0	-	-	0	-	4.8	0	-	1.9	1.9
Aratus pisonii	-	0	-	0	-	-	3.1	0	0	0	6.7	-	-	-	-	-	0	0	0	-	-	14	0	0	-	-	-	-	0	-	4.3	6.7	-	0	0	-	-	0	0	-	-	0	-	0	0	-	0	0
Mangrove fragments	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0		-	-	-	-	0	-	8.7	3.3	-	0	0	-	-	0	0	-	-	0	-	0	0	-	1.9	0
Seaweed	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-	-	6.7	0	-	-	0	-	0	0	-	1.9	0

Table S10 - Food items ingested by *C. mexicanus* expressed as FW% (frequency in weight), according to habitats [Upper; Middle; Lower; Coastal zone], seasons [Early dry; Late dry; Early rainy; Late rainy] and ontogetic phases [Juv (juveniles); Sub (sub-adults); Adu (adults)]. (-) no capture.

Items	Upper												Middle												Lower											
	Early Dry			Late Dry			Early Rainy			Late Rainy			Early Dry			Late Dry			Early Rainy			Late Rainy			Early Dry			Late Dry			Early Rainy			Late Rainy		
	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu
Microplastic	-	0	-	0.46	-	-	0.16	0	100	0.37	0.06	-	-	-	-	-	0.01	0.01	0.1	-	-	0.03	0.02	0.01	-	-	-	-	100	-	0.02	0.06	-	0.02	0.11	-
Unidentified fish	-	100	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0.61	0	0	-	-	0	1.34	93.4	-	-	-	-	0	-	0.11	15.9	-	0	45.5	-
Engraulidae	-	0	-	0	-	-	0	0	0	0	55.3	-	-	-	-	-	12.4	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-
Anchovia clupeoides	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	42.9	94.1	0	-	-	0	0	0	-	-	-	-	0	-	32	0	-	0	24.7	-
Cetengraulis edentulus	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-
Clupeidae	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-
Rhinosardinia bahiensis	-	0	-	0	-	-	57.9	0	0	0	0	-	-	-	-	-	31.1	0	0	-	-	0	97.3	0	-	-	-	-	0	-	0	0	-	0	0	-
Harengula clupeola	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-
Opisthonema oglinum	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-
Odontognathus mucronatus	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-
Lycengraulis grossidens	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-
Gobidae	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	12.4	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-
Stellifer stellifer	-	0	-	0	-	-	0	0	0	66.8	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	2.7	0	-	0	0	-
Pomadasys corvinaeformis	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-
Mugil liza	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-
Hemiramphus brasiliensis	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-
Unidentified invertebrate	-	0	-	0	-	-	0	0	0	0	0.07	-	-	-	-	-	0.49	0	0	-	-	0	1.33	6.23	-	-	-	-	0	-	0	0	-	0	0	-
Amphipoda	-	0	-	0	-	-	0	0	0	0.11	0	-	-	-	-	-	0.01	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-
Mysidacea	-	0	-	0	-	-	1.62	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	15.1	-
Paguridae	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	10.7	0	-	0	0	-
Isopoda	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0.66	0	-	0	0	-
Polychaeta	-	0	-	0	-	-	3.18	0	0	6.09	30.6	-	-	-	-	-	0	0.49	0	-	-	2.72	0	0	-	-	-	-	0	-	33.7	73.9	-	0	0	-
Penaeid shrimp	-	0	-	99.5	-	-	8.84	100	0	26.6	0.39	-	-	-	-	-	0.16	5.45	0	-	-	82.9	0	0	-	-	-	-	0	-	9.05	0.52	-	100	5.46	-
Callinectes danae	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	99.9	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	9.13	-
Aratus pisonii	-	0	-	0	-	-	28.3	0	0	0	13.6	-	-	-	-	-	0	0	0	-	-	14.3	0	0	-	-	-	-	0	-	5.47	9.5	-	0	0	-
Mangrove fragments	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0.38	-	-	-	-	0	-	5.56	0.13	-	0	0	-
Seaweed	-	0	-	0	-	-	0	0	0	0	0	-	-	-	-	-	0	0	0	-	-	0	0	0	-	-	-	-	0	-	0	0	-	0	0	-

Items	Coastal zone											
	Early Dry			Late Dry			Early Rainy			Late Rainy		
	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu
Microplastic	-	0.01	0	-	-	0.01	-	0	0.01	-	0.02	0.01
Unidentified fish	-	0	0	-	-	0	-	20.8	2.06	-	31.9	6.37
Engraulidae	-	0	0	-	-	0	-	6.09	38.9	-	0	0.89
Anchovia clupeoides	-	30.6	0	-	-	0	-	10.4	52.8	-	0	1.38
Cetengraulis edentulus	-	0	100	-	-	0	-	0	0	-	0	12.8
Clupeidae	-	0	0	-	-	0	-	11.4	0	-	1.88	0
Rhinosardinia bahiensis	-	0	0	-	-	0	-	40.1	0	-	40.8	61.2
Harengula clupeola	-	0	0	-	-	0	-	0	0	-	16.1	0
Opisthonema oglinum	-	0	0	-	-	0	-	1.48	0	-	0	4.19
Odontognathus mucronatus	-	0	0	-	-	100	-	0	0	-	0	0
Lycengraulis grossidens	-	0	0	-	-	0	-	4.01	0	-	0	0
Gobidae	-	54.3	0	-	-	0	-	0	0	-	0	0
Stellifer stellifer	-	0	0	-	-	0	-	0	0	-	0	0
Pomadasys corvinaeformis	-	0	0	-	-	0	-	0	0	-	0	8.92
Mugil liza	-	0	0	-	-	0	-	2.59	0	-	0	0
Hemiramphus brasiliensis	-	0	0	-	-	0	-	0	0	-	0	0.38
Unidentified invertebrate	-	0	0	-	-	0	-	0	0	-	0	0
Amphipoda	-	0	0	-	-	0	-	0	0	-	0	0
Mysidacea	-	0	0	-	-	0	-	0	0	-	0	0
Paguridae	-	0	0	-	-	0	-	0.89	0	-	0	0
Isopoda	-	0	0	-	-	0	-	0	0	-	0.41	0
Polychaeta	-	0	0	-	-	0	-	0	0	-	0	0
Penaeid shrimp	-	14.5	0	-	-	0	-	1.28	6.16	-	8.1	1.86
Callinectes danae	-	0	0	-	-	0	-	0.94	0	-	0.56	2.07
Aratus pisonii	-	0	0	-	-	0	-	0	0	-	0	0
Mangrove fragments	-	0	0	-	-	0	-	0	0	-	0.15	0
Seaweed	-	0.53	0	-	-	0	-	0	0	-	0.01	0

Table S11 - Summary of the ANOVA for the weight of items ingested by *C. undecimalis* in the Goiana estuary, according to habitat [U (upper); M (middle); L (lower); C (coastline)], season [ED (early dry); LD (late dry); ER (early rainy); LR (late rainy)] and ontogeny [Juv (juveniles); Sub (sub-adults); Adu (adults)]. Bonferroni's test was used to determinate the sources of variances [F (F-values); df (degree of freedom); *p*-value]. (ns: not significant) ($p < 0.05$).

		Items in number			
	Factors	**F**	***df***	***p*-value**	***Post-hoc***
A. clupeoides	Season	0.07	3	0.97	ns
	Area	5.22	3	0.01	C
	Phase	4.68	2	0.01	Adu
	Season *vs.* Area	0.72	9	0.68	ns
	Season *vs.* Phase	0.80	6	0.56	ns
	Area *vs.* Phase	2.41	6	0.02	C Adu
	Season *vs.* Area *vs.* Phase	0.38	18	0.98	ns
	Error		192		
C. edentulus	Season	0.56	3	0.64	ns
	Area	13.47	3	0.01	C
	Phase	17.31	2	0.01	Adu
	Season *vs.* Area	0.29	9	0.97	ns
	Season *vs.* Phase	0.56	6	0.76	ns
	Area *vs.* Phase	13.47	6	0.01	C Adu
	Season *vs.* Area *vs.* Phase	0.29	18	0.99	ns
	Error		192		
R. bahiensis	Season	0.70	3	0.55	ns
	Area	14.59	3	0.01	C
	Phase	4.07	2	0.01	Juv
	Season *vs.* Area	1.10	9	0.36	ns
	Season *vs.* Phase	2.08	6	0.06	ns
	Area *vs.* Phase	2.23	6	0.04	C Adu
	Season *vs.* Area *vs.* Phase	2.83	18	0.01	ED C Adu - LD C Sub
	Error		192		

Table S11 Continued.

C. spixii	Season	0.30	3	0.82	ns
	Area	0.99	3	0.39	ns
	Phase	3.71	2	0.02	Adu
	Season *vs*. Area	0.85	9	0.56	ns
	Season *vs*. Phase	0.33	6	0.91	ns
	Area *vs.* Phase	1.02	6	0.40	ns
	Season *vs.* Area *vs*. Phase	0.71	18	0.79	ns
S. stellifer	Season	0.63	3	0.59	ns
	Area	4.42	3	0.01	L
	Phase	8.85	2	0.01	Adu
	Season *vs*. Area	0.57	9	0.81	ns
	Season *vs*. Phase	0.46	6	0.83	ns
	Area *vs.* Phase	3.05	6	0.01	L Adu
	Season *vs.* Area *vs*. Phase	0.42	18	0.98	ns
	Error		192		
Amphipoda	Season	1.93	3	0.12	ns
	Area	0.70	3	0.55	ns
	Phase	0.46	2	0.62	ns
	Season *vs*. Area	0.66	9	0.73	ns
	Season *vs*. Phase	0.53	6	0.78	ns
	Area *vs.* Phase	1.14	6	0.33	ns
	Season *vs.* Area *vs*. Phase	1.16	18	0.29	ns
	Error		192		
Polychaeta	Season	4.50	3	0.01	ED
	Area	2.98	3	0.03	U
	Phase	0.82	2	0.43	ns
	Season *vs*. Area	4.56	9	0.01	ED U
	Season *vs*. Phase	1.33	6	0.24	ns
	Area *vs.* Phase	2.09	6	0.06	ns
	Season *vs.* Area *vs*. Phase	2.30	18	0.01	ED U Juv
	Error		192		
Penaeid shrimp	Season	0.17	3	0.91	ns
	Area	1.23	3	0.29	ns
	Phase	2.57	2	0.07	ns
	Season *vs*. Area	2.04	9	0.03	ED C
	Season *vs*. Phase	0.69	6	0.65	ns
	Area *vs.* Phase	0.57	6	0.74	ns
	Season *vs.* Area *vs*. Phase	1.34	18	0.16	ns
	Error		192		

Table S12 - Summary of the ANOVA for the weight of items ingested by *C. mexicanus* in the Goiana estuary, according to habitat [U (upper); M (middle); L (lower); C (coastline)], season [ED (early dry); LD (late dry); ER (early rainy); LR (late rainy)] and ontogeny [Juv (juveniles); Sub (sub-adults); Adu (adults)]. Bonferroni's test was used to determinate the sources of variances [F (F-values); df (degree of freedom); *p*-value]. (ns: not significant) ($p < 0.05$).

	Items in number				
	Factors	**F**	***df***	***p*-value**	***Post-hoc***
A. clupeoides	Season	1.96	3	0.11	ns
	Area	1.95	3	0.12	ns
	Phase	1.38	2	0.25	ns
	Season *vs.* Area	3.66	9	0.01	LD M
	Season *vs.* Phase	0.49	6	0.81	ns
	Area *vs.* Phase	1.01	6	0.41	ns
	Season *vs.* Area *vs.* Phase	1.36	18	0.15	ns
	Error		192		
C. edentulus	Season	13.80	3	0.01	ED
	Area	26.71	3	0.01	C
	Phase	8.14	2	0.01	Adu
	Season *vs.* Area	13.80	9	0.01	ED C
	Season *vs.* Phase	11.38	6	0.01	ED Adu
	Area *vs.* Phase	8.14	6	0.01	C Adu
	Season *vs.* Area *vs.* Phase	11.38	18	0.01	ED C Adu
	Error		192		
R. bahiensis	Season	4.78	3	0.01	LD
	Area	43.44	3	0.01	C
	Phase	33.36	2	0.01	Sub
	Season *vs.* Area	6.08	9	0.01	LR C
	Season *vs.* Phase	4.49	6	0.01	LR Sub
	Area *vs.* Phase	24.26	6	0.01	C Sub
	Season *vs.* Area *vs.* Phase	7.69	18	0.01	LR C Adu
	Error		192		

Table S12 Continued.

S. stellifer	Season	0.80	3	0.49	ns
	Area	0.80	3	0.49	ns
	Phase	1.59	2	0.20	ns
	Season *vs*. Area	1.06	9	0.38	ns
	Season *vs*. Phase	0.80	6	0.57	ns
	Area *vs.* Phase	0.80	6	0.57	ns
	Season *vs.* Area *vs*. Phase	1.06	18	0.38	ns
	Error		192		
Amphipoda	Season	0.87	3	0.45	ns
	Area	0.87	3	0.45	ns
	Phase	0.81	2	0.44	ns
	Season *vs*. Area	1.04	9	0.40	ns
	Season *vs*. Phase	1.06	6	0.38	ns
	Area *vs.* Phase	1.06	6	0.38	ns
	Season *vs.* Area *vs*. Phase	0.97	18	0.48	ns
	Error		192		
Polychaeta	Season	7.43	3	0.01	ER
	Area	7.13	3	0.01	L
	Phase	2.34	2	0.09	ns
	Season *vs*. Area	9.14	9	0.01	ER L
	Season *vs*. Phase	2.51	6	0.02	ER Juv
	Area *vs.* Phase	2.40	6	0.02	L Juv
	Season *vs.* Area *vs*. Phase	2.39	18	0.01	ER L Juv
	Error		192		
Penaeid shrimp	Season	3.55	3	0.01	ED
	Area	0.68	3	0.56	ns
	Phase	0.12	2	0.88	ns
	Season *vs*. Area	1.00	9	0.43	ns
	Season *vs*. Phase	0.88	6	0.50	ns
	Area *vs.* Phase	2.04	6	0.06	ns
	Season *vs.* Area *vs*. Phase	1.02	18	0.43	ns
	Error		192		

Table S13 - Summary of the ANOVA for the number of microplastic ingested by Centropomidae species in the Goiana estuary, according to habitats [U (upper); M (middle); L (lower); C (coastal zone)], seasons [ED (early dry); LD (late dry); ER (early rainy); LR (late rainy)] and ontogenetic phases [Juv (juveniles); Sub (sub-adults); Adu (adults)]. Bonferroni's test was used to determinate the sources of variances [F (F-values); df (degree of freedom); *p*-value]. (ns: not significant) ($p < 0.05$).

		Microplastics in number			
	Factors	**F**	***df***	***p*-value**	***Post-hoc***
C. undecimalis	Season	2.20	3	0.08	ns
	Area	9.36	3	0.01	U
	Phase	5.10	2	0.01	Adu
	Season *vs*. Area	3.82	9	0.01	ER L
	Season *vs*. Phase	3.04	6	0.01	LD Sub
	Area *vs.* Phase	3.11	6	0.01	C Adu
	Season *vs.* Area *vs*. Phase	1.98	18	0.01	ED L Adu
	Error		192		
C. mexicanus	Season	50.32	3	0.01	LR
	Area	0.28	3	0.83	ns
	Phase	24.20	2	0.01	Sub
	Season *vs*. Area	7.32	9	0.01	LD M - ER C - ER L - LR L - LR M - LR C
	Season *vs*. Phase	8.11	6	0.01	LR Sub
	Area *vs.* Phase	13.55	6	0.01	L Sub
	Season *vs.* Area *vs*. Phase	5.12	18	0.01	LD M Sub - ER L Sub - LR M Sub - LR L Sub - LR C Sub - LR C Adu
	Error		192		

Table S14 - Summary of the Canonical Correspondence Analysis (CCA) using four environmental variables (water temperature, salinity, dissolved oxygen and rainfall) and the main food items ingested in number by Centropomidae species according to the factors (habitat, season and ontogenetic phase).

Statistic	Axis I	Axis II	Environmental variables	*p*-value
Eigenvalue	0.118	0.032	Dissolved oxygen (mg l^{-1})	0.01
Pseudo-canonical correlation %	58.8	35.8	Salinity	0.01
Explained fitted variation of species-environmental variables %	67	18.6	Rainfall (mm)	0.58
			Water temperature (ºC)	0.71

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5 ARTIGO 2 - DYNAMICS OF MARINE DEBRIS INGESTION BY PROFITABLE FISHES ALONG THE ESTUARINE ECOCLINE

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Dynamics of marine debris ingestion by profitable fishes along the estuarine ecocline

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ABSTRACT

The dynamics of microfilament (< 5mm) ingestion were evaluated in three species of snooks. The ingestion of different colours and sizes of microfilaments were strongly associated with the spatio-temporal estuarine use and ontogenetic shifts of snooks. Their feeding ecology was also analysed to assess dietary relationships with patterns of contamination. All species were highly contaminated with microfilaments. The highest ingestion of microfilaments occurred in the adults, when fishes became the main prey item and also during the peak of fishing activities, in the rainy season. This suggests that trophic transfer, in addition to periods of high availability of microfilaments are important pathways for contamination. The ingestion of microfilaments of different colours and sizes was likely influenced by input sources. Blue microfilaments were frequently ingested, and appear to have both riverine and estuarine inputs, since they were ingested in all seasons and habitats. Purple and red microfilaments were more frequently ingested in the lower estuarine habitats. The length of microfilaments was also associated with environmental variability. Longer microfilaments were ingested in habitats with greater riverine influence, the opposite was observed for shorter microfilaments. Therefore, microfilament contamination in snooks are a consequence of their ecological patterns of estuarine uses through different seasons and life history stages.

Keywords: Microplastics; plastic ingestion; microfilaments; top predators.

INTRODUCTION

Marine debris are among the greatest environmental concerns of the XXI century, especially because they are ubiquitous contaminants of a range of aquatic ecosystems (COSTA; BARLETTA, 2015; ERIKSEN et al., 2014; FISCHER et al., 2016). Moreover, the global production of plastics, one of the most common marine debris, is increasing, with annual productions exceeding 300 million tonnes (LUSHER; HOLLMAN; MENDOZA-HILL, 2017). Plastics are widely used in industry and domestically, with little prospect of their use decreasing as they are a versatile, cheap and durable material (ANDRADY; NEAL, 2009).

The introduction of debris into the aquatic environment occurs by accident or intentionally by improper disposal practices, such as illegal dumping of sewage and solid wastes into rivers and oceans (LEBRETON et al., 2017). Additionally, the fishing industry is recognized as one of the major sources of marine debris, responsible for the introduction of tonnes of items as the result of in situ maintenance, abrasion and environmental exposure of fishing gears (POSSATTO et al., 2011; THOMPSON, 2004).

Once in the aquatic environment, marine debris tend to breakdown, into smaller particles (< 5mm) due to the weathering processes caused by hydrodynamic forces and photodegradation (LUSHER; HOLLMAN; MENDOZA-HILL, 2017)(LUSHER; HOLLMAN; MENDOZA-HILL, 2017; THOMPSON, 2004). Because of their diminutive size, small particles of marine debris are more likely to be ingested by marine biota. Indeed, marine debris ingestion has been reported in a wide variety of taxa, from planktonic to nektonic species (STEER et al., 2017; SUN et al., 2017), and studies have reported high contamination rates, with more than 60% contamination of fishes caught in field surveys (NADAL; ALOMAR; DEUDERO, 2016; PAZOS et al., 2017). The high concentrations and wide distribution of marine debris means that they can interact with every trophic guild, being directly ingested and transferred across trophic levels, which can explain the resulting high contamination rates found in top predator fishes (FERREIRA et al., 2018; SETÄLÄ; FLEMING-LEHTINEN; LEHTINIEMI, 2014). One of the strongest links between marine wildlife and humans are through top predators, and so marine debris may indirectly affect human populations when these resources are consumed (SANTILLO; MILLER; JOHNSTON, 2017; WANG et al., 2019).

Marine debris can affect wildlife through chemical transfer of adsorbed organic and inorganic pollutants (TEUTEN et al., 2007). It is another pathway for organic pollutants and

heavy metal contamination through the food web (BATEL et al., 2018; LEÓN et al., 2018). However, when compared to other more relevant contamination sources (*e.g.* prey species) marine debris is not acknowledged as the main vector for organic pollutants (KOELMANS et al., 2016). Despite marine debris ingestion by fish and its persistence in the environment, in the present literature (BARLETTA; LIMA; COSTA, 2019) they are still not considered to be a pressing issue in regards to public health. However, the understanding of ecological and oceanographic features are essential tools to evaluate the dynamics of marine debris ingestion by fish, and will likely influence the characteristics of contaminants (*e.g.* size, shape and colour). Which in turn, might be indicative of the availability of marine debris, distance of input sources and help clarify if fish prefer a specific set of particles.

Snooks (Centropomidae) are one of the most important living resources exploited by American coastal fisheries, with annual landings of ≈ 13,000 tons on the east side of the continents (FAO, 2017). Adult snooks are usually found in the outermost portion of the estuary (FERREIRA; BARLETTA; LIMA, 2019) but also use habitats with greater structural complexity and migrate towards the inner habitats of the estuary in search of food and shelter (DANTAS; BARLETTA, 2016). Earlier stages are usually associated with nursery grounds in the mangrove creeks and upper estuary (FERREIRA; BARLETTA; LIMA, 2019; STEVENS; BLEWETT; POULAKIS, 2007). Snooks are one of the main estuarine top predators, occupying a demersal habitat, feeding mainly on fishes and macrocrustaceans (MATICH et al., 2017).

Considering the ecological importance of snooks as estuarine top predators and their economic relevance in the tropical western Atlantic, studies on the patterns of microfilament contamination in these species will serve as important indicators of potential risks to humans. The aim of this study is to investigate the spatio-temporal patterns of contamination with different sizes and colours of microfilaments (marine debris) in three important commercially exploited species in the Goiana Estuary (Brazil). The Centropomidae species *Centropomus undecimalis, C. mexicanus* and *C. pectinatus*, were sampled throughout their ontogeny (different life history stages) to correlate their patterns of contamination with ecological use of the estuary, and establish possible pathways of microfilaments ingestion.

RESULTS

Microfilaments were the major type of marine debris ingested by the snooks, representing more than 98% of the 773 particles ingested. Other types of marine debris such

as hard particles (< 1%), soft particles (< 1%) and paint chips (< 0.3%) were also found, but they not included in the analysis due to their low ingestion rates (Fig. S1).

From a total of 529 fishes analysed, 306 (~58%) were contaminated with microfilaments. In effect, 58% of *C. undecimalis* (1.51 ± 0.13 particles individual^{-1}; 149 individuals), 65% of *C. mexicanus* (1.43 ± 0.11 part. ind.$^{-1}$; 117 individuals) and 51% of *C. pectinatus* (1.21 ± 0.18 part. ind.$^{-1}$; 40 individuals) were contaminated. To evaluate the potential input sources of marine debris to the environment, the ingested microfilaments were measured and divided into six different colours (blue, purple, green, red, white and black).

Size and colours of ingested microfilaments

Individuals of *C. undecimalis* and *C. mexicanus* ingested longer microfilaments in the upper estuary (1.41 ± 0.20 mm and 1.52 ± 0.09 mm, respectively) and smaller sizes in the coastal zone (1.08 ± 0.05 mm and 1 ± 0.06 mm, respectively) (Table S1). *C. pectinatus* ingested longer microfilaments in the lower estuary (1.63 ± 0.44 mm), but in the coastal zone they followed the same trend as the other species (1.02 ± 0.08 mm) (Fig. 1).

Interactions among habitat *vs.* season *vs.* ontogenetic phases significantly affected the size of filaments ingested (Fig. 1; Table S2). For *C. undecimalis*, the longest microfilaments were ingested by juveniles in the upper estuary, during the early dry season (1.55 ± 0.38 mm).

For *C. mexicanus*, the longest microfilaments were ingested in the upper estuary, during the early rainy season (adults) (1.96 ± 0.28 mm) and late rainy season (juveniles) (1.77 ± 0.20 mm). For *C. pectinatus*, the longest microfilaments were recorded in sub-adults in the lower estuary, during the early rainy season (1.99 ± 0.53 mm). Smaller microfilaments were commonly ingested in the upper reaches of the estuary, however, no significant differences were detected.

Fishes were more prone to be contaminated in the lower estuary and during the rainy season (Table S1). Regardless of colour, adult snooks registered the highest rates of contamination. The majority of filaments ingested by snooks were blue (75.9%), followed by red (6.9%), green (6%), purple (5.8%), white (4.9%) and black (0.1%).

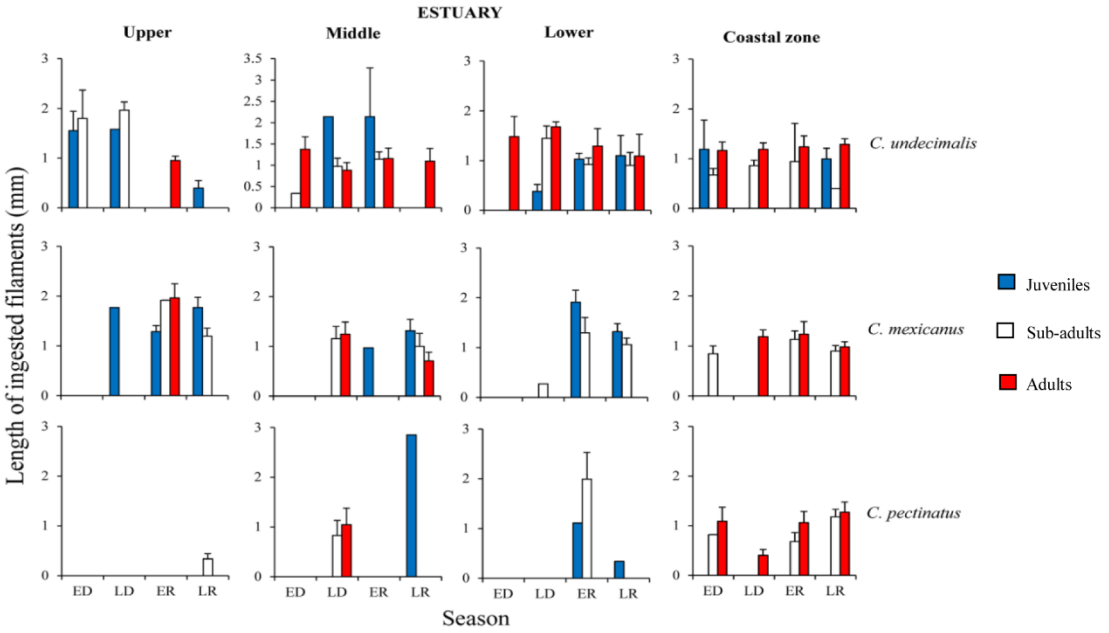

Figure 1. Mean (SE±) length of microfilaments ingested by the snooks, regarding different habitats (upper, middle, lower estuary and coastal zone), seasons [ED (early dry), LD (late dry), ER (early rainy) and LR (late rainy)] and ontogenetic phases.

For snooks of all ontogenetic phases the highest contamination rates of blue microfilaments occurred during the rainy season in the lower estuary and coastal zone (Fig. 2, 3 and S2). However, juveniles (1.4 ± 0.23 part. ind.$^{-1}$) and sub-adults (2.44 ± 0.66 part. ind.$^{-1}$) of *C. undecimalis* had the highest contaminations in the lower estuary, during the early rainy season ($p < 0.01$) (Fig. 2; Table S3).

Sub-adults of *C. mexicanus* were mostly contaminated during the late rainy season in the middle (2 ± 0.77 part. ind.$^{-1}$) and lower (2.11 ± 0.67 part. ind.$^{-1}$) estuaries ($p < 0.01$) (Fig. 3; Table S4). Sub-adults and adults of *C. pectinatus* were more contaminated in the coastal zone, during the late rainy season (0.94 ± 0.29 part. ind.$^{-1}$ and 1.28 ± 0.45 part. ind.$^{-1}$, respectively) ($p < 0.01$) (Fig. S2; Table S5).

A similar pattern was detected for purple microfilaments. Higher contamination rates were observed in all species inhabiting the outermost habitats (Fig. 2, 3 and S2). Sub-adult of *C. mexicanus* had the highest ingestion rates in the lower estuary, during the early rainy season (0.37 ± 0.19 part. ind.$^{-1}$; $p < 0.05$) (Fig. 3; Table S4). Meanwhile, adults of *C. undecimalis* (Fig. 2; Table S3) and *C. pectinatus* (Fig. S2; Table S5) were most contaminated in the coastal zone ($p < 0.01$), during the late rainy season (0.21 ± 0.14 part. ind.$^{-1}$ and 0.42 ± 0.21 part. ind.$^{-1}$, respectively).

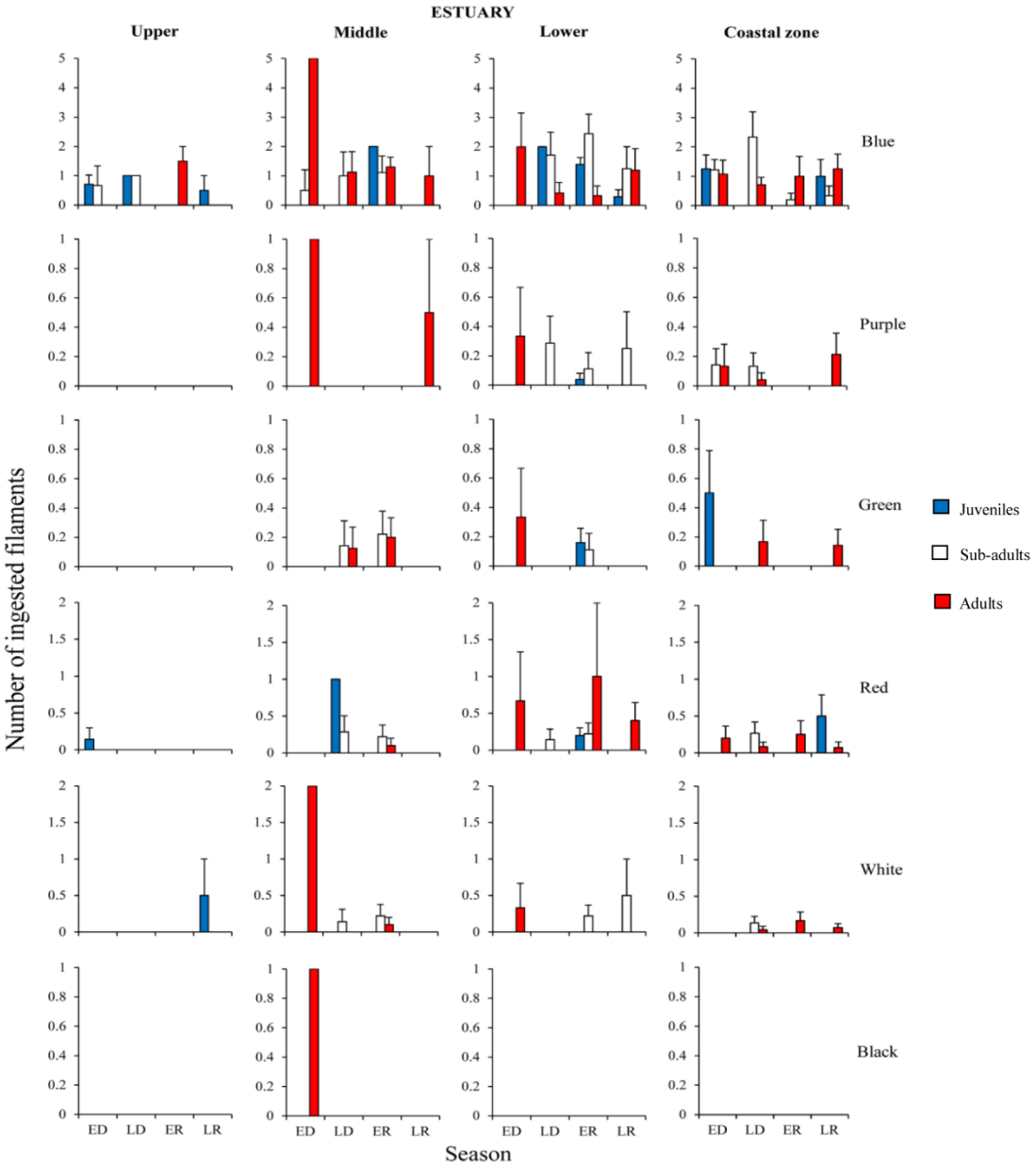

Figure 2. Mean (SE±) number of different colours of microfilaments ingested by the *C. undecimalis*, regarding different habitats (upper, middle, lower estuary and coastal zone), seasons [ED (early dry), LD (late dry), ER (early rainy) and LR (late rainy)] and ontogenetic phases.

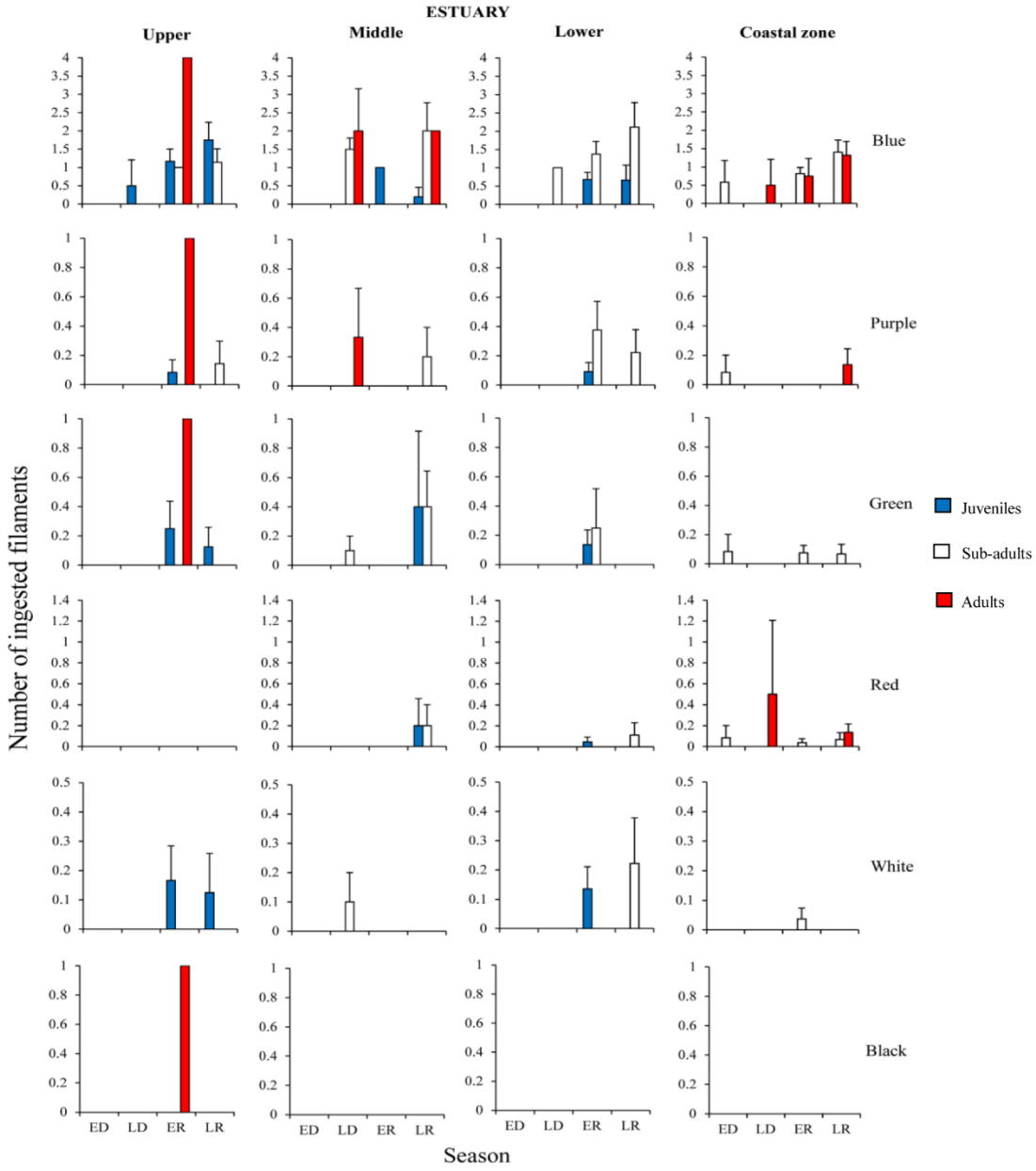

Figure 3. Mean (SE±) number of different colours of microfilaments ingested by the *C. mexicanus*, regarding different habitats upper, middle, lower estuary and coastal zone), seasons [ED (early dry), LD (late dry), ER (early rainy) and LR (late rainy)] and ontogenetic phases.

Green microfilaments were ingested throughout the habitats used by *C. undecimalis* and *C. mexicanus* (Fig.2 and 3). The highest ingestion rates for *C. undecimalis* occurred in sub-adults (0.22 ± 0.15 part. ind.$^{-1}$) and adults (0.2 ± 0.13 part. ind.$^{-1}$) in the middle estuary during the early rainy season ($p < 0.05$). Additionally, juveniles had the highest concentrations (0.16 ± 0.09 part. ind.$^{-1}$) in the lower estuary during the early rainy season ($p < 0.05$) (Fig. 2; Table S3). In the coastal zone, the highest contamination of juveniles was recorded during the early

dry season (0.5 ± 0.28 part. ind.-1), and in adults during late dry and late rainy seasons (0.16 ± 0.14 part. ind.$^{-1}$ and 0.14 ± 0.11 part. ind.$^{-1}$, respectively) ($p < 0.05$).

Red microfilaments ingestion peaked in adult *C. undecimalis* in the lower estuary, during the early rainy season (1 ± 1 part. ind.$^{-1}$) (Fig. 2, 3 and S2). Adults of *C. mexicanus* ingested more red microfilaments in the coastal zone, during the late dry season (0.5 ± 0.7 part. ind.$^{-1}$) and sub-adults of *C. pectinatus* ingested more in the middle estuary, during the late dry season (0.5 ± 0.5 part. ind.$^{-1}$).

The highest ingestion rates of white microfilaments were detected in sub-adults of *C. mexicanus* in the lower estuary, during the late rainy season (0.22 ± 0.15 part. ind.$^{-1}$; *p < 0.01*) (Fig. 3; Table S4). Peaks of ingestion were registered for juvenile *C. undecimalis* in the upper estuary, during the late rainy season (0.5 ± 0.5 part. ind.$^{-1}$), in the lower estuary for both, sub-adults of *C. undecimalis* during the late rainy season (0.22 ± 0.14 part. ind.$^{-1}$) and for sub-adults of *C. pectinatus* during the early rainy season (0.5 ± 0.5 part. ind.$^{-1}$) (Fig. 2 and S2; Table S3 and S5).

Feeding behaviour

The diet of snooks included a wide range of prey, which were grouped into six major ecological/taxonomic groups (pelagic fishes, demersal fishes, macrocrustaceans, microcrustaceans, bristle worms and organic matter) (Table S6).

Juveniles consumed the most variable diet, with macrocrustaceans and bristle worms being the most important (Fig. S3, S4 and S5). The highest ingestion of bristle worms and organic matter was recorded for juveniles of *C. mexicanus* in the lower estuary, during the early rainy season (140.8 ± 62.1 mg ind^{-1} and 23.2 ± 14.8 mg ind^{-1}, respectively; *p < 0.01*) (Fig. S4; Table S7). In contrast to the other species, juveniles of *C. undecimalis* had higher intakes of both pelagic and demersal fishes in the lower estuary and coastal zone.

Sub-adults snooks exhibited a transitional feeding behaviour, preying mostly on macrocrustaceans, microcrustaceans and bristle worms in the inner sections of the estuary. In the outer sections they fed mostly on pelagic fishes, demersal fishes and macrocrustaceans. For sub-adults of *C. undecimalis*, the highest ingestion of pelagic fishes occurred in the coastal zone, during the late dry season (3,166.7 ± 1,395.5 mg ind^{-1}; $p < 0.05$) (Fig. S3; Table S8). Pelagic fishes were also the main prey of sub-adult *C. mexicanus*, which registered the highest ingestion rates of this resource in the coastal zone, during the early rainy season (3,205.7 ± 579.2 mg ind^{-1}; *p < 0.01*) (Fig. S4; Table S7). Sub-adults of *C. pectinatus* had the highest ingestion rates of both macrocrustaceans and organic matter in the coastal zone,

during the late rainy season (282.7 ± 104.8 mg ind^{-1} and 17.1 ± 9.8 mg ind^{-1}, respectively; $p < 0.01$) (Fig. S5; Table S9).

Adult snooks fed mostly on pelagic fishes, demersal fishes and macrocrustaceans, with pelagic fishes being the main food resource. Adults of *C. undecimalis* had the highest ingestion of pelagic fishes in the coastal zone throughout the seasonal cycle ($p < 0.05$) [early dry (8,328.9 ± 2,643.7 mg ind^{-1}), late dry (6,654.4 ± 2,407 mg ind^{-1}), early rainy (7,509.2 ± 4,018.9 mg ind^{-1}) and late rainy (3,936.4 ± 1,306.8 mg ind^{-1}) seasons] (Fig. S3; Table S8). Similarly, adult *C. mexicanus* had the highest ingestion of pelagic fishes in the coastal zone, but only during the late rainy season (6,971.4 ± 3,272.3 mg ind^{-1}; $p < 0.01$) (Fig. S4; Table S7). Meanwhile, adults of *C. pectinatus* registered the highest intake of demersal fishes in the coastal zone, during the early rainy season (3,584.2 ± 3,584.2 mg ind^{-1}; $p < 0.01$) (Fig. S5; Table S9).

Influences of environmental variability in the patterns of microfilament contamination

The CCA was used to evaluate the relationship among the different colours of microfilaments, main food resources ingested and the environmental parameters of the ecosystem (Fig. 4). Axis I of the analysis explained 61.8% of the data variability, being negatively correlated with salinity ($p < 0.01$), dissolved oxygen ($p < 0.01$) and Secchi depth ($p < 0.01$) (Fig. 4). Axis I represented the salinity ecocline of the ecosystem. The positive section of this axis represented the innermost habitats (upper and middle estuaries) and the negative section the outermost habitats (lower estuary and coastal zone). The axis II explained 19.5% of the variability, being positively correlated with water temperature, salinity, dissolved oxygen and Secchi depth and negatively correlated with rainfall. Axis II described the seasonality. Its negative section represented the increased influence of river discharge in the ecosystem, which occurs during the rainy seasons. The positive section represented the increased oceanic influence that is more intense during the dry seasons.

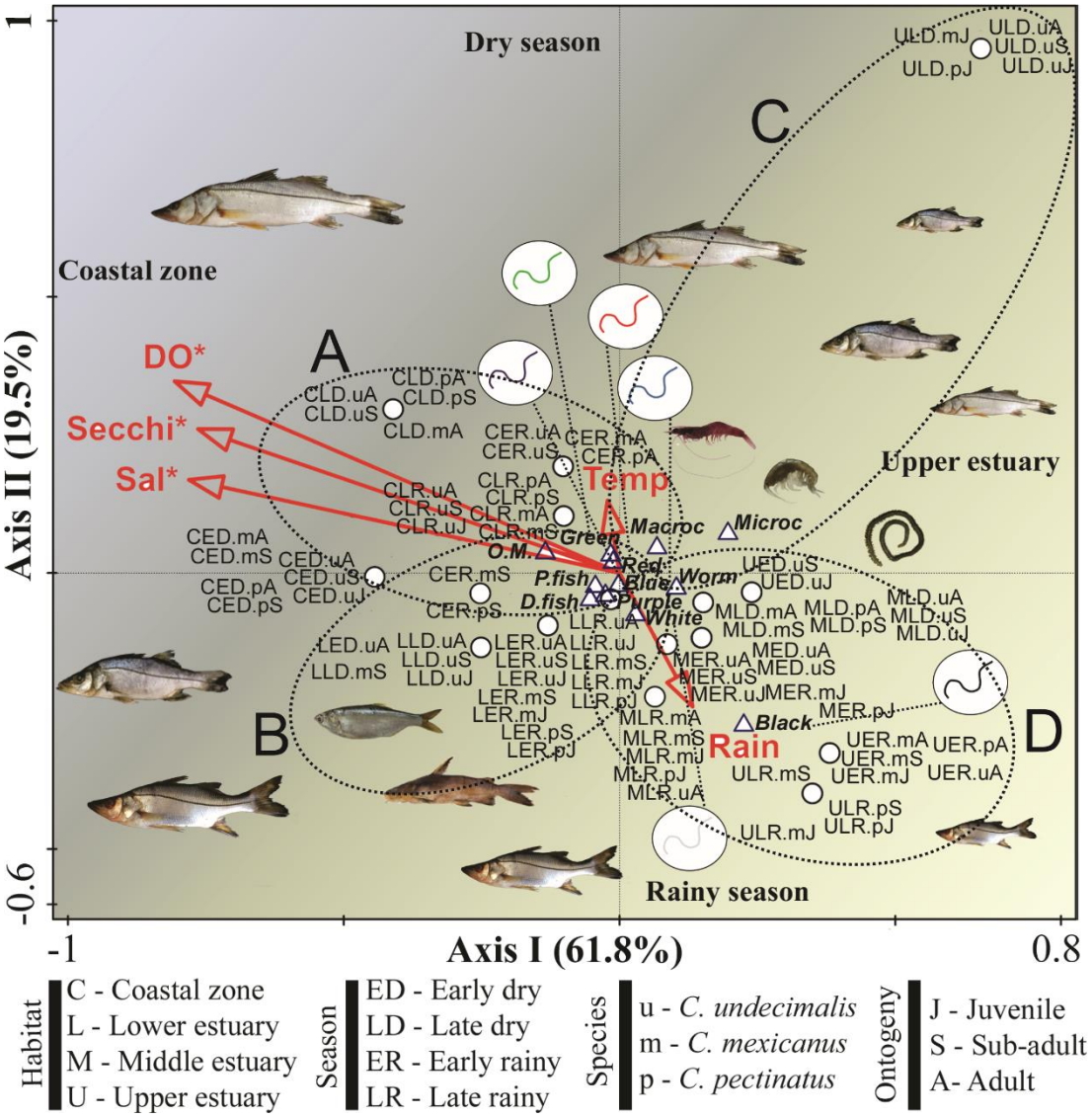

Figure 4. Canonical Correspondence Analysis (CCA) for the correlations among the different colours of microfilaments ingested, main food groups and environmental variables. Vectors represent the environmental variables [Sal (salinity), Secchi (Secchi depth), DO (dissolved oxygen), Temp (water temperature) and Rain (rainfall). Circles represent the interactions among the factors habitats, seasons, and ontogenetic phases of species. Triangles represent the colours of microfilaments ingested [Blue (blue microfilaments), Purple (purple microfilaments), Green (green microfilaments), Red (red microfilaments), White (white microfilaments) and Black (black microfilaments)] and the main food groups [Pfish (pelagic fishes), Dfish (demersal fishes), Macroc (macrocrustacens); Microcr (microcrustaceans); Worm (bristle worms) and O.M. (organic matter)].

Blue, purple, green and red microfilaments were placed near to the intersection of both axes. These were the filament colours which caused the highest contamination rates among the snooks. Additionally, they were placed slightly towards the negative section of axis I because of higher ingestions rates in the outermost habitats (Fig. 4 and Table S10). Group A represented snooks contaminated in the coastal zone and in the lower estuary, during the dry season (Fig. 4). Whereas group B included the most contaminated fishes, mainly from the lower estuary and coastal zone, during the rainy seasons. Group C and D represented the individuals that ingested microfilaments in the habitats with prevailing riverine influence, during the dry and rainy seasons, respectively.

The food groups, pelagic and demersal fishes were positively correlated with salinity, dissolved oxygen, Secchi depth and rainfall. Despite being consumed in all habitats and seasons, most items were consumed in the lower estuary and coastal zone, especially in the rainy seasons. Moreover, the CCA plotted the pelagic fishes and demersal fishes close to blue and purple microfilaments, suggesting that snooks have similar patterns of consumption/contamination for these items.

Macrocrustaceans and organic matter were positively correlated with temperature and negatively correlated with rainfall. However, macrocrustaceans were associated with the innermost habitats and organic matter with the outermost habitats (Fig. 4 and Table S10). White microfilaments, bristle worms and microcrustaceans were also associated with the innermost habitats, being mostly ingested in the upper and middle estuaries but also in the lower estuary, during the rainiest seasons (Fig. 4 and Table S10).

DISCUSSION

Microfilaments are widely distributed in aquatic ecosystems, with reports of many contaminated (GÜVEN et al., 2017; JOVANOVIĆ, 2017) and few non-contaminated taxa (VENDEL et al., 2017). Hydrodynamic forces and distance from significant sources are the major factors influencing microfilament availability in the environment (BARLETTA; LIMA; COSTA, 2019; CHEUNG; CHEUNG; FOK, 2016). As a result, microfilament concentrations vary greatly among different ecosystems and even among habitats (LIMA; COSTA; BARLETTA, 2014). Habitats along environmental gradients are susceptible to seasonal variations that alter microfilament availability (GÜNDOĞDU et al., 2018). Microfilament availability in the environment and contamination levels are directly linked to the patterns of habitat use by fishes (FERREIRA et al., 2018; PETERS; BRATTON, 2016; SANCHEZ; BENDER; PORCHER, 2014). This increases the likelihood of microfilament transfer

throughout the trophic chain and may ultimately lead to human contamination (FARRELL; NELSON, 2013). Therefore, to understand patterns of microfilament contamination it is important to understand the ecological behaviour of fishes for both environmental conservation and future food safety.

Ontogenetic changes through the life cycles of snook species play an important role in their ecological behaviour, leading to shifts in habitat use and feeding ecology (BLEWETT; HENSLEY; STEVENS, 2006), which could also affect the dynamics of microfilament ingestion. The feeding behaviour of snooks strongly reflects the availability of microfilaments ingested, regardless of colour and size. The highest contamination rates were recorded, when the feeding behaviour of snooks switched to concentrating on prey of higher trophic levels (*e.g.* pelagic and demersal fishes).

Juveniles of *C. undecimalis* and *C. mexicanus* were classified as opportunist predators and juveniles of *C. pectinatus* as zoobenthivorous (ELLIOTT et al., 2007). Sub-adult snooks were classified as opportunistic predators and adults as piscivorous. The highest ingestion rates of microfilaments were registered in adults of *C. undecimalis*, followed by sub-adults of *C. undecimalis* and adults of *C. mexicanus* and *C. pectinatus*.

Contamination with microfilaments is, in this case, a result of the trophic transfer and, as a result, species of higher trophic levels were more contaminated (FERREIRA et al., 2018; NELMS et al., 2018). Trophic transfer of microfilaments occurs when a contaminated prey is ingested and during the digestive process the microfilaments that were within the digestive tract of the prey are transferred to the predator (TOSETTO; WILLIAMSON; BROWN, 2017). Evidence of this process has been observed in other estuarine fishes, such as Sciaenidae, Acoupa weakfish (*Cynoscion acoupa*) and little croaker (*Stellifer stellifer*). In both cases, the adult phase fed mostly on fishes and had the highest levels of contamination with microfilaments (DANTAS; BARLETTA; COSTA, 2015; FERREIRA et al., 2016). Trophic transfer was also reported for the Brazilian mojarra (*Eugerres brasilianus*) and the flagfin mojarra (*Eucinostomus melanopterus*) (RAMOS; BARLETTA; COSTA, 2012).

Further evidence that indicates the contribution of trophic transfer to the contamination rates of microfilaments was evinced through the prey ingested by snooks. Some food items, at an early stage of digestion, were retrieved from the guts of snooks. Those items had their digestive tract inspected in search of microfilaments. From the 41 ingested items analysed in these conditions, 58% were contaminated with microfilaments (Fig. 5).

Moreover, proximity to the input source appears to be a major aspect influencing the different colours of microfilaments ingested. However, the predominance of contamination

with blue microfilaments, is so vast that their ingestion is usually greater than the total of the other colours of microfilaments (FERREIRA et al., 2018; SILVA et al., 2018). The high ingestion rate of microfilaments, in comparison to low concentrations in the water column (LIMA; COSTA; BARLETTA, 2014) and sediment (COSTA et al., 2011) implies that there is an active selection and/or pre-concentration process operating, either through selective consumption by younger snooks or through bioaccumulation through the food web.

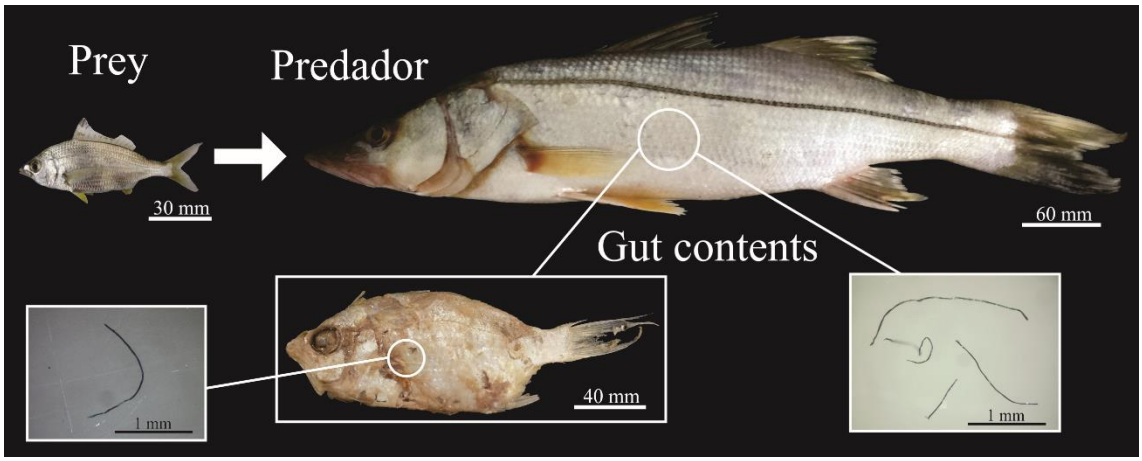

Figure 5. Evidence of trophic transfer observed between the predator (*C. undecimalis*) and prey (*Eucinostomus melanopterus*). Both individuals were contaminated by microfilaments.

Goiana City is a major upstream source of contaminants, and the artisanal fishery fleet is close to the mouth of the estuary (BARLETTA; COSTA, 2009). Urban effluents (DE FALCO et al., 2018; SUTTON et al., 2016) and the fishing activity (mainly blue microfilaments) (BROWNE et al., 2011; IVAR DO SUL; COSTA, 2014) are then indicated as the main sources of microfilaments in the estuary. Due to their widespread intake by snooks, blue microfilaments are likely to have both riverine and estuarine origins.

Blue microfilaments were ingested throughout the length of the estuary, during the entire seasonal cycle, by all Centropomidae species. The highest ingestion rates occurred during the rainy seasons in the lower estuary, and its surroundings habitats (middle estuary and coastal zone). This is likely a consequence of higher microfilaments availability, coinciding with the peak in the fishing activity in the estuary (POSSATTO et al., 2011). Indeed, fishes are regularly reported with microfilament contamination that originates from fishing gear (ANDRADY, 2011; CARDOZO et al., 2018; SILVA et al., 2018).

The highest contamination rates of purple microfilaments occurred in the lower estuary and coastal zone by older snooks. It is likely a result of increased weathering of marine debris into microfilaments. In turn, microfilaments may alter their colour, size and/or physical characteristics (*i.e.* weathered purple microfilaments may resemble blue microfilaments). The

reason for the highest ingestion rates of this colour being recorded in the sub-adult and adults of all species is their ecological behaviour. These ontogenetic phases had higher densities in these habitats and fed mostly on prey of higher trophic levels, thus increasing the chances of trophic transfer.

The ingestion of green microfilaments occurred differently among the species. *C. undecimalis* ingested green microfilaments throughout the seasonal cycle and its ontogeny, except in the upper estuary. On the other hand, *C. mexicanus* was mostly contaminated in the upper estuary, specifically during the early rainy season. Meanwhile, *C. pectinatus* ingested very few green microfilaments.

Red microfilaments were mostly ingested in the lower estuary, especially in the coastal zone. Higher contamination rates were detected in the lower and coastal habitats, it is, therefore, likely that the main input source for this colour of filament is from coastal waters, which may be carried into the estuary by waves and tides. Snooks were also contaminated with red microfilaments in the upper estuary, but to a lesser degree. Indeed, the highest ingestion rates were recorded in the middle and lower portions of the estuary and occurred when the saline intrusion was dislocated to these habitats, during the dry and rainy seasons, respectively (FERREIRA et al., 2016). The saline intrusion works as a barrier, preventing the passage of contaminants carried by the oceanic waters towards the upper reaches of the estuary (LIMA; BARLETTA; COSTA, 2015). This results in reduced availability and contamination rates with microfilaments of oceanic origin, such as red microfilaments.

Microfilaments carried by the river flow tend to become trapped within the estuarine/oceanic boundary, due to the barrier effect caused by the confluence of riverine and oceanic waters (GÜNDOĞDU et al., 2018). The ingestion of white microfilaments was strongly associated with the rainy season in all habitats. This is indicative of an origin related in river discharge, with sewage being the likely main input source of white microfilaments.

A number of studies have reported the possibility that macro marine debris (> 5mm) and even micro marine debris are intentionally ingested by marine biota, due to the resemblance of debris to natural prey (PROVENCHER et al., 2010; WRIGHT; THOMPSON; GALLOWAY, 2013). Taking into account that white microfilaments are more similar in colour and size to microcrustaceans (a group formed mostly of zooplankton), white microfilaments would be expected to be preferentially ingested by juveniles and sub-adults in the upper and middle estuaries, where microcrustaceans form a large proportion of their diet. However, no associations were observed between microcrustaceans and microfilaments,

suggesting that microfilament ingestion associated with microcrustacean prey is not a relevant pathway for contamination of snooks.

The largest difference in the average size of microfilaments ingested by snooks was correlated with the habitat in which they were ingested (availability). In habitats with greater riverine influence, fishes ingested longer filaments. Whereas, in the outermost habitats with greater oceanic influence, fishes ingested shorter filaments. These patterns are likely the result of the proximity of the contaminant and their input source (FERREIRA et al., 2018). Rivers receive great amounts of debris mostly from cities located along their margins, which are important pathways for the transportation of debris from land-based sources into the ocean (ZHANG, 2017). Hydrodynamics are an important erosive agent to marine debris, which breaks down into smaller particles (THOMPSON, 2004). Thereby, the lower estuary and the coastal zone are the habitats most likely to have smaller particles due to intense turbulence caused by the convergence of riverine discharge and tidal flow, and the consequent breakdown of larger particles. This is reflected in the smaller sizes of microfilaments ingested by fishes in the lower phases of the estuary.

Additionally, another trend for the ingestion of longer filaments was observed. Juveniles of *C. mexicanus* and sub-adults of *C. pectinatus* also ingested longer filaments in the lower estuary, but only during the early rainy season. This occurred concomitantly with the peak of the fishery activity in the Goiana Estuary, which is responsible for an intense input of microfilaments into the lower estuary (POSSATTO et al., 2011).

Supposedly, the larger the fish, the greater would be the chances to ingest bigger microfilaments(RAMOS; BARLETTA; COSTA, 2012). Additionally, macrofilaments are more readily detected by fishes (due to their greater dimensions), but this category was rarely ingested. No evidence of selective ingestion of microfilaments, either in size or colour, were observed among the ontogenetic phases of snooks, suggesting that direct ingestion of marine debris from the water column by predatory fishes such as snooks is not relevant .

Evidence suggests that trophic transfer is the most important influence on the total quantity of microfilaments in Centropomidae species, with different contamination rates recorded through ontogeny. Moreover, the peaks of ingestion of microfilaments of different colours, seems to be associated with the proximity to their input sources, being closely correlated with the seasonal variability of the salinity structure in the estuary. Multiple factors were noteworthy contributors of the contamination patterns, including ecological behaviour of each species, seasonality, river discharge, hydrodynamics of the estuarine boundary and local fishery fleet (Fig. 6). The size of filaments ingested by the fishes is clearly associated with the

salinity ecocline. The ingestion of different colours of microfilaments is likely a result of their availability in the environment to prey. No evidence indicated fish preferences for specific colours or sizes of microfilaments.

Studies on the consequences of marine debris ingestion for biota are still incomplete, especially regarding multilevel trophic processes. Effects on animals from chemical additives that are potential endocrine disruptors (GALLOWAY; COLE; LEWIS, 2017); their relevance as vectors for organic pollutants adsorbed from the environment (KOELMANS et al., 2016; ROCHMAN et al., 2013) and influence on the behaviour patterns of fish (DE SÁ; LUÍS; GUILHERMINO, 2015; TOSETTO; WILLIAMSON; BROWN, 2017) are becoming more common in the literature. However, the trophic transfer of marine debris through the food web (FARRELL; NELSON, 2013; FERREIRA et al., 2018; NELMS et al., 2018) still poses a great concern that extends to human health, due to the consumption of contaminated seafood (WANG et al., 2019). Little information is available on plastic effects on human health (WANG et al., 2019), but a recent survey conducted on mice, reported accumulation of particles in vital organs, impairing molecular functions (DENG et al., 2017). Human contamination with marine debris (including the microfilaments dealt with here), is probably related to length and intensity of exposure to contaminated food, including fish. That is, daily, weekly, yearly and life-long patterns of fish consumption. As for other pollutants, such as mercury, if risk arises, a choice might have to be made about which fish to consume based on its trophic position and age. How much and how often fish is consumed can be managed in order to control pollutants intake. In the case of marine debris, it is still uncertain if such strategies will be sufficient to reduce human exposure, and more trophic web-based information is needed to clarify that point.

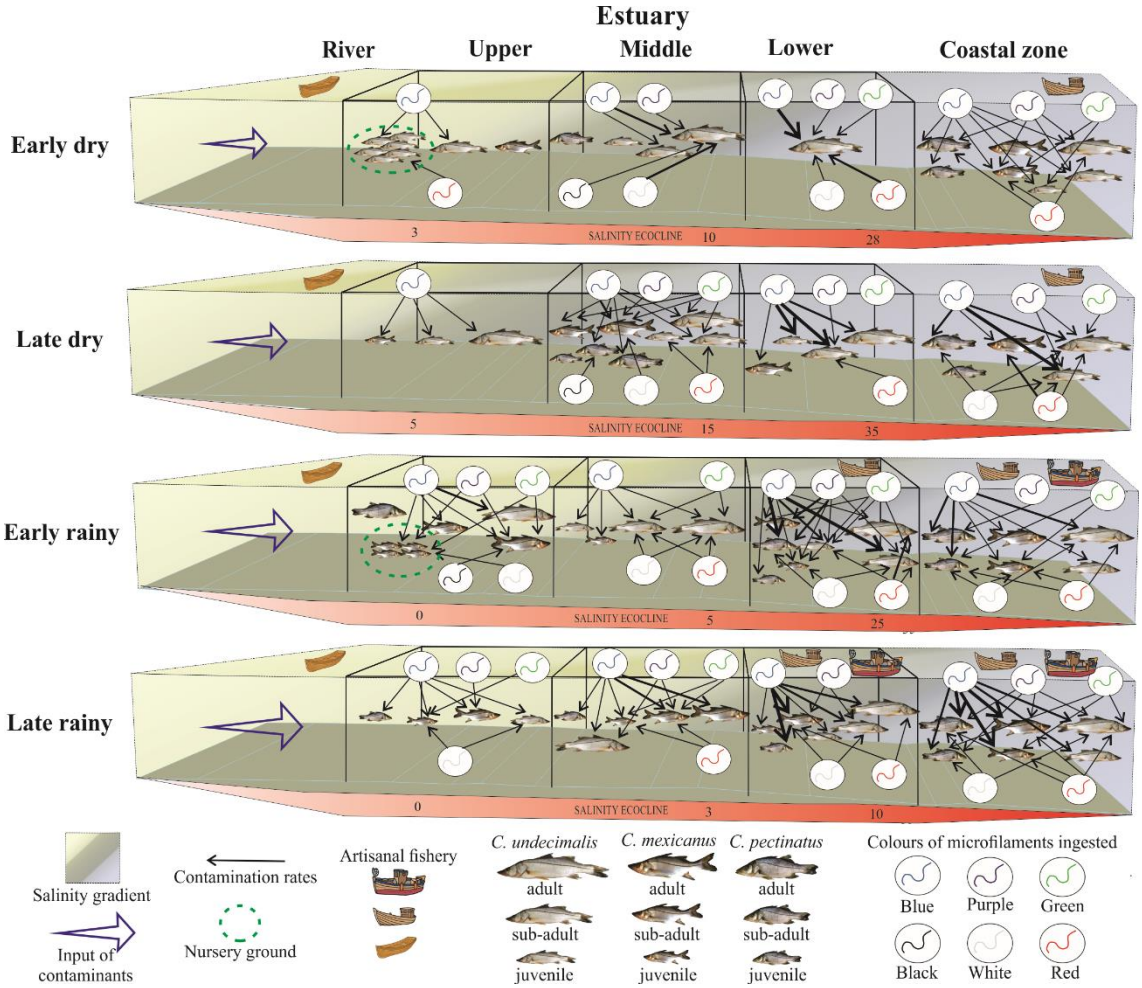

Figure 6. Conceptual model for the ingestion rates of different colours of microfilaments by snooks, regarding different habitats, seasons and ontogenetic phases.

Considering the ongoing magnitude of the marine debris problem, its potential hazards for wildlife and human beings we recommend assessments of marine debris ingestion for key species within regional food webs, as well as aquaculture products. Many species have been found to be contaminated, and the inventory only grows on a daily basis. The current study suggests that marine debris can enter food webs at different levels, through different routes, through different habitats and life phases. Juvenile snooks ingested microfilaments in their diets (other fish and invertebrates), starting at their nursery grounds, and likely have to deal with these loads, and further contamination, through their entire life history, eventually passing it on to their predators.

Future studies should, therefore, focus on methods that allow comparisons among studies of different ecosystems and taxa, developing sampling and laboratory protocols to improve identification of marine debris and avoid contamination biases. These include, among others, sample designs encompassing trophic, spatial and temporal variability,

including an appropriate number of replicates. Despite of procedural blanks being an important step for controlled experiments in laboratorial environments (LUSHER; HOLLMAN; MENDOZA-HILL, 2017), the effects of airborne contamination might be reduced on field surveys that implement robust sample designs in alignment with a substantial sample size, such as evinced by the patterns of ingestion observed among the different areas, seasons and ontogenetic phases of snooks species. The development of statistical approaches, such as aquatic community modelling, will advance our understanding of how contamination by microfilaments and other pollutants (*e.g*. heavy metals) (BARLETTA et al., 2012) are correlated with trophic level, life history and the salinity ecocline, as well as other environmental gradients.

METHODS

The Goiana Estuary is located in the western tropical Atlantic Ocean. Fishes were captured from 2005 to 2015, from different habitats within the estuary (upper, middle, lower estuary, and coastal zone) and seasons (early dry, late dry, early rainy and late rainy seasons) (BARLETTA; COSTA, 2009; FERREIRA et al., 2018) (Fig. S6 and S7). Fishes samples were taken following all ethical requirements and licenced by the Environment Ministry of Brazil (SISBIO permit number: 11050).

Prior to commencing fish sampling, environmental parameters (salinity, temperature, dissolved oxygen and Secchi depth) of bottom waters were recorded and rainfall data were compiled from a local weather station. After capture, all specimens were immediately frozen and transported to the laboratory. Three species of Centropomidae were used in this study: *C. undecimalis, C. mexicanus* and *C. pectinatus*. Individuals were divided into three ontogenetic phases (juveniles, sub-adults and adults) to evaluate the contamination patterns throughout their life cycle (Table S11).

In the laboratory, precautionary measures were taken to avoid airborne and inter-sampling contamination. To avoid airborne and inter-sampling contamination the working station and all equipment used in the evisceration were cleaned with distilled water and absolute ethanol, prior to the procedures for the identification of digestive tracts contents (DI BENEDITTO; OLIVEIRA, 2019; RIOS-FUSTER et al., 2019). Then, tweezers, scissors, scalpels and Petri dishes were also oven dried and double checked for contamination before the next use (FERREIRA et al., 2018; SILVA et al., 2018). Procedural blanks were not made. However, a robust sample design were applied in the study (Fig. S7) encompassing different estuarine areas, seasons and ontogenetic phases, which included a great number of individuals

of three species of snooks (n= 529). Additionally, 100% cotton lab coats and latex disposable gloves were used during all procedures (DI BENEDITTO; OLIVEIRA, 2019; RIOS-FUSTER et al., 2019).

Fishes were then eviscerated and their digestive tracts (stomach and intestine) were removed. Their gut contents were analysed in glass covered Petri dishes (to allow identification through the lid and avoid airborne contamination) using a stereomicroscope with a digital camera attached. Items suspected of being marine debris were visually identified, separated into covered Petri dishes and oven dried in 70 ºC for 48 h (DANTAS et al., 2019; LUSHER et al., 2017). Petri dishes were kept closed during the entire identification process, with the exception of when the items were transferred to another Petri dish to be oven dried and after the confirmation for storage in the database.

Withered items were considered non-synthetic organic matter and were discarded (LUSHER et al., 2017). Those items that did not changed their shape (not shrivel due to water loss and had a homogeneous thickness), physical consistency (being not easily broken or fragmented) and visual features (colour or brightness), were identified as marine debris and classified according to length, type (hard debris, soft debris, rubber crumbs, paint chips or microfilaments) and colours (blue, purple, red, green, black and white) (LUSHER et al., 2017). Despite this method being a good procedure for the identification and further exclusion of non-synthetic materials from the sample, it is not a useful tool to identify the polymer the plastic debris (DI BENEDITTO; OLIVEIRA, 2019; FERREIRA et al., 2018; LIMA; COSTA; BARLETTA, 2014). Then, marine debris were counted, weighed (± 0.0001g), photographed and measured using the image analysis package AxioVision LE. Contaminants larger than 5 mm were not included in the study. Food items ingested by each species were counted, weighted, and categorised into six food groups (pelagic fishes, demersal fishes, macrocrustaceans, microcrustaceans, bristle worms and organic matter), according to ecological and taxonomic criteria (Table S6).

Three-way analysis of variance was used to identify significant differences in the lengths of microfilaments ingested, the number of each different coloured microfilament and the weight of each category of food item, according to the factors: habitat, season, ontogenetic phase, and their interactions. All data were Box-Cox transformed (BOX; COX, 1964) and the ANOVA assumptions were tested. In addition, a Canonical Correspondence Analysis (CCA) was performed to investigate ecological correlations between environmental data and both the colour of microfilaments and food groups ingested by snooks [dependent variables as values

of I_{RI} (Index of relative importance) (HYSLOP, 1980)] (TER BRAAK; SMILAUER, 2002). Significant differences were accepted when $\alpha < 0.05$. For details, see supplementary material.

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APÊNDICE 1. Material suplementar referente ao capítulo 2.

SUPPLEMENTARY MATERIAL

Dynamics of marine debris ingestion by profitable fishes along the estuarine ecocline

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METHODS

Study Area

The Goiana Estuary is located in the Western Tropical Atlantic Ocean, the annual water temperature is above 26 ºC, with minor fluctuations (max. 31 ºC). The estuary was spatially divided into four habitats: upper, middle, lower estuary, and coastal zone (Supplementary Fig. S6). The ecosystem seasonality is mainly influenced by the variability in rainfall, characterizing four seasons: early dry (Sep-Nov), late dry (Dec-Feb), early rainy (Mar-May)

and late rainy (Jun-Aug). Rainfall is also responsible for the seasonal pulses in river flow and water quality shifts (BARLETTA; COSTA, 2009).

Along the estuary, there are several activities that produce solid wastes that are inadequately disposed, and are potential sources of marine debris pollution across a wide range of size fractions that also include microplastics (< 5mm). Aproximatey five kilometres upstream of the estuary, in the Goiana River, there is a city of almost 80,000 habitants, where untreated sewage effluents and urban runoff go straight into the river that drains into the estuary. Sugarcane plantations extend along the margins of the entire Goiana River towards the lower estuary, and are responsible for the input of fertilizers and pesticides into the estuary. Moreover, in the upper estuary, there are also activities such as subsistence fishery and dredging of the main channel for sand mining. In the middle estuary, the main pollution sources are the sugarcane plantations, artisanal fishery, untreated sewage from a small fishing community and deforestation of the mangrove forest (BARLETTA; COSTA, 2009).

At the lower estuary and coastal zone, the main pollution sources are the fishery fleet and unplanned urbanization, where no basic sanitation is in place. Municipal services are precarious and wastes are inefficiently collected. These two habitats are under greater anthropogenic pressure. One of the main threats are the contaminants carried by the river, which are introduced along its flow, and the contaminants transported from the oceans by waves and the tidal action. Other important sources of pollutants are the disposal of effluents from a shrimp farm and a cement industry, both located in the lower estuary, and the sewage disposal from the fishing villages, located in both margins of the river mouth. However, the most relevant potential source of pollutants is likely the fishing activity, which is focused on these habitats, due to the important landing of commercial species (BARLETTA et al., 2017).

Sampling design

Fishes were captured in the main channel of the Goiana Estuary from 2005 to 2015 encompassing different habitats and seasons (early dry, late dry, early rainy and late rainy). The upper, middle and lower portions of the estuary were sampled monthly (six replicates), using an otter trawl (forest green multifilament nylon net) between December 2005 and November 2006 (Supplementary Fig. S6) (FERREIRA et al., 2016). Additionally, monthly samplings (six replicates) were conducted in these same habitats, during the late dry and late rainy seasons, between December 2006 and August 2009. The mangrove creeks, located in the lower estuary, were sampled using a fyke net (forest green multifilament nylon net) (three replicates) between April and May 2008 (Supplementary Fig. S6). The net was fixed at the

entrance of the creeks during high tide and then fishes were collected during the subsequent low tide. Additionally, specimens from the coastal zone adjacent to the estuary were captured monthly by the artisanal fishing fleet from 2013 to 2015 (Supplementary Fig. S6). After capture, all specimens were immediately frozen and transported to the laboratory.

Prior to fish samplings, environmental parameters were registered from bottom and surface waters [salinity (Salinometer WTW LF 197); temperature and dissolved oxygen (Oximeter WTW oxi 340); and Secchi depth]. Rainfall data were compiled from the local weather station (Supplementary Fig. S6).

Laboratory procedures

Three species of Centropomidae were used in this study: *C. undecimalis* (Common snook), *C. mexicanus* (Largescale fat snook) and *C. pectinatus* (Tarpon snook). Individuals were divided into three ontogenetic phases (juveniles, sub-adults and adults) to evaluate the diet shifts and possible contamination with microdebris through their life cycle (Supplementary Table **S**11) [4].

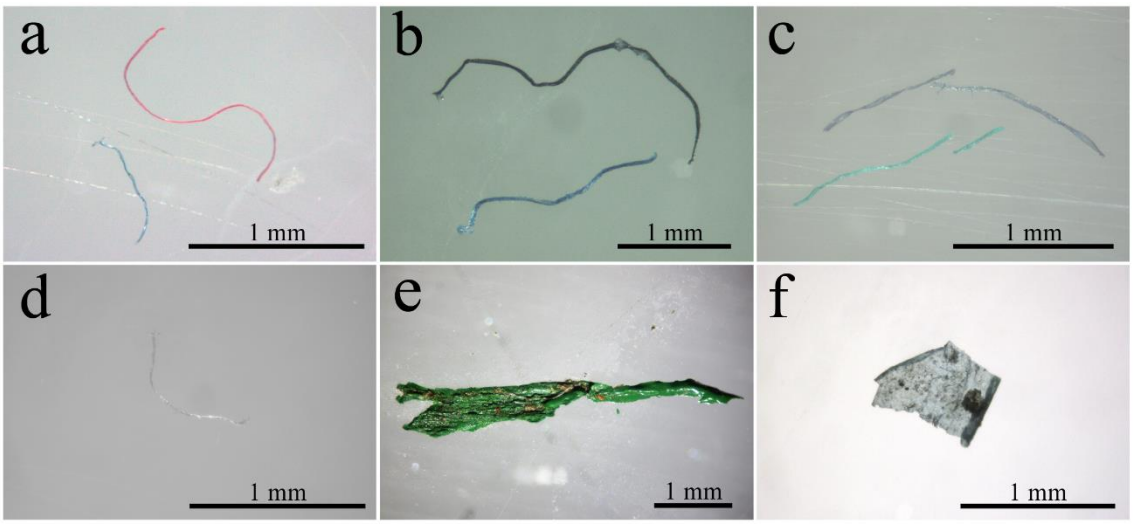
In the laboratory, fishes were eviscerated and the digestive tracts (stomach and intestine) were removed, their contents were analysed in a covered Petri dish using a stereomicroscope (Zeiss, Stemi 200) with a digital camera attached (Canon Powershot G10). Food items (actual prey) were visually sorted and identified to the lowest possible taxonomic level (MENEZES; FIGUEIREDO, 1980; RUPPERT; FOX; BARNES, 2004). Then, food items were counted, weighted and categorised into six groups (pelagic fishes, demersal fishes, macrocrustaceans, microcrustaceans, bristle worms and organic matter) (Supplementary Table S6). These groups take into account ecological and taxonomic criteria. Thus, different prey within the same group has a similar foraging behaviour and each group represents a specific role within the community.

Statistical analysis

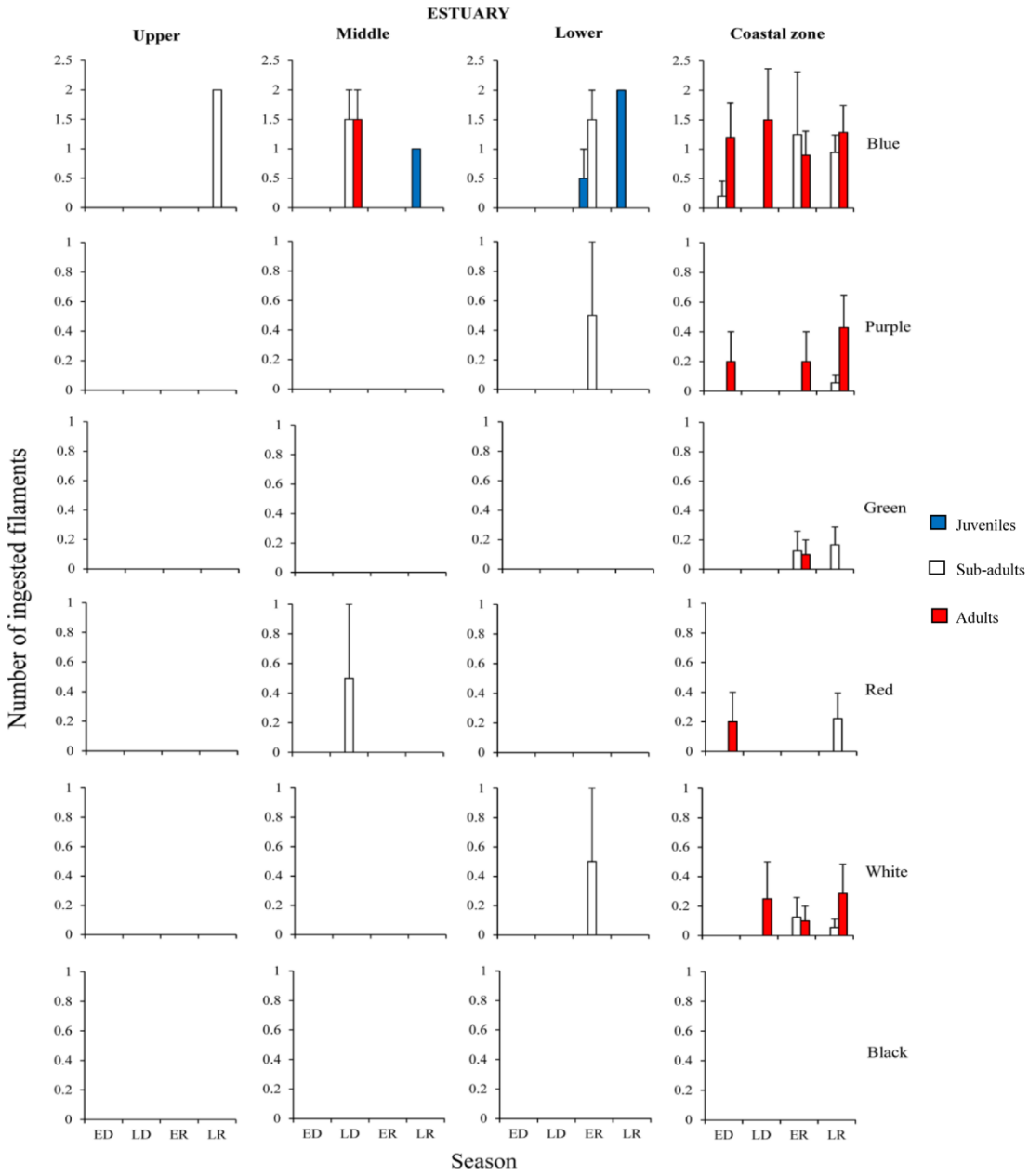
To identify significant differences in length of microfilaments ingested, number of colours, and weight of grouped food items, a three-way analysis of variance was applied for the factors: habitats, seasons and ontogenetic phases, and their interactions. Previously, to meet the ANOVA assumptions, all data were Box-Cox transformed (BOX; COX, 1964) and tested for homogeneity of variances, using the Levene test, and the goodness of fit to a normal distribution, was tested using the Kolmogorov-Smirnov test(UNDERWOOD, 1997).

Whenever significant differences were detected in the ANOVA, the sources of variance were identified using the Bonferroni *post hoc* test (QUINN; KEOUGH, 2002).

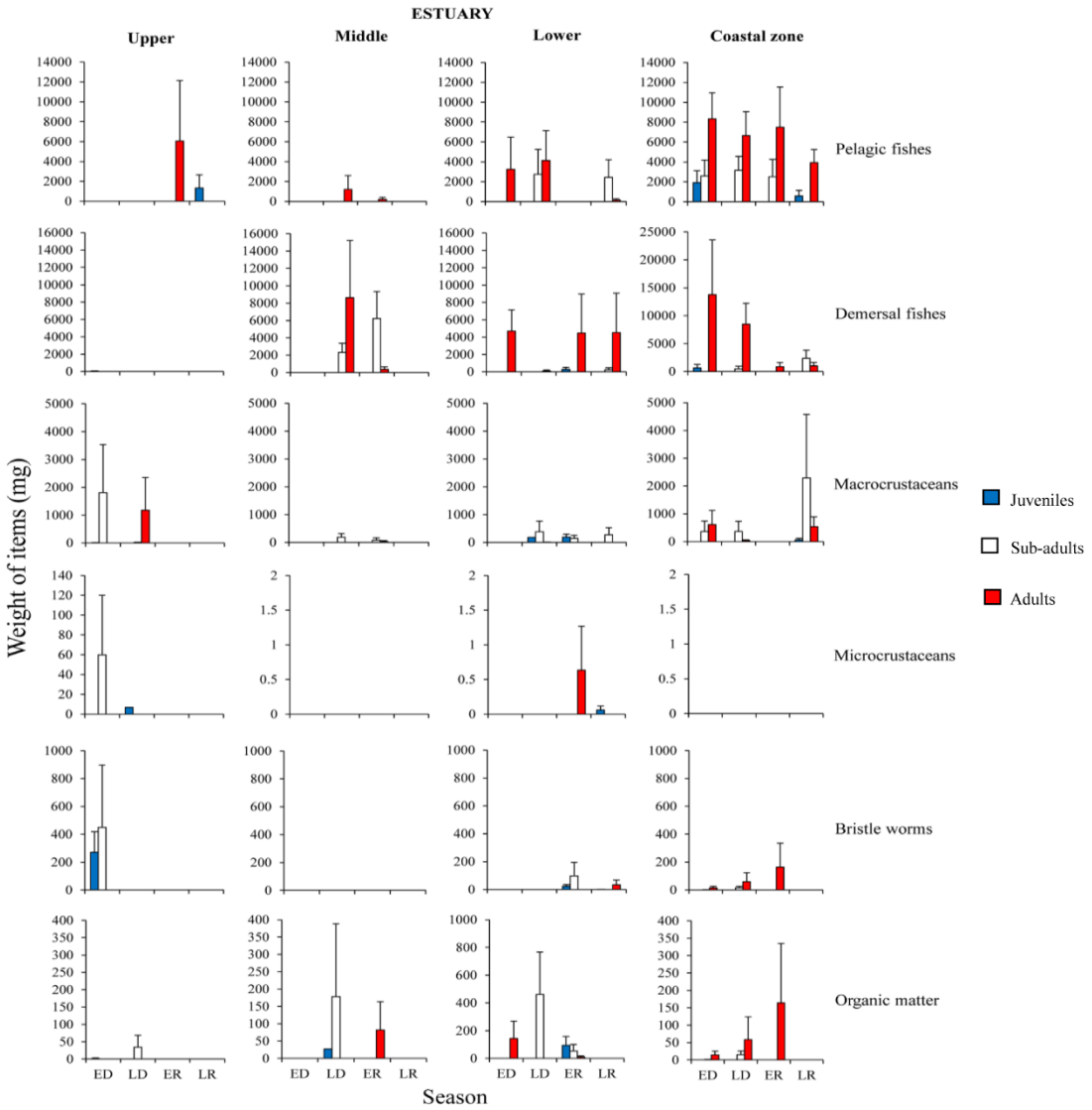
To investigate the ecological correlations, a Canonical Correspondence Analysis (CCA) was performed using CANOCO 5 software. This analysis investigates the influence of environmental data (independent variables) on the different colours of microfilaments and food groups ingested by Centropomidae species (dependent variables) (TER BRAAK; SMILAUER, 2002). The dependent variables were evaluated as values of I_{RI} (Index of relative importance) (HYSLOP, 1980). A triplot was produced displaying the dependent variables as geometric shapes and the independent variables as vectors. A Monte-Carlo permutation (100 permutes) was used to determinate which environmental variables significantly affected the dependent data (TER BRAAK; SMILAUER, 2002). All statistical analysis were conducted using a 0.05 level of significance.



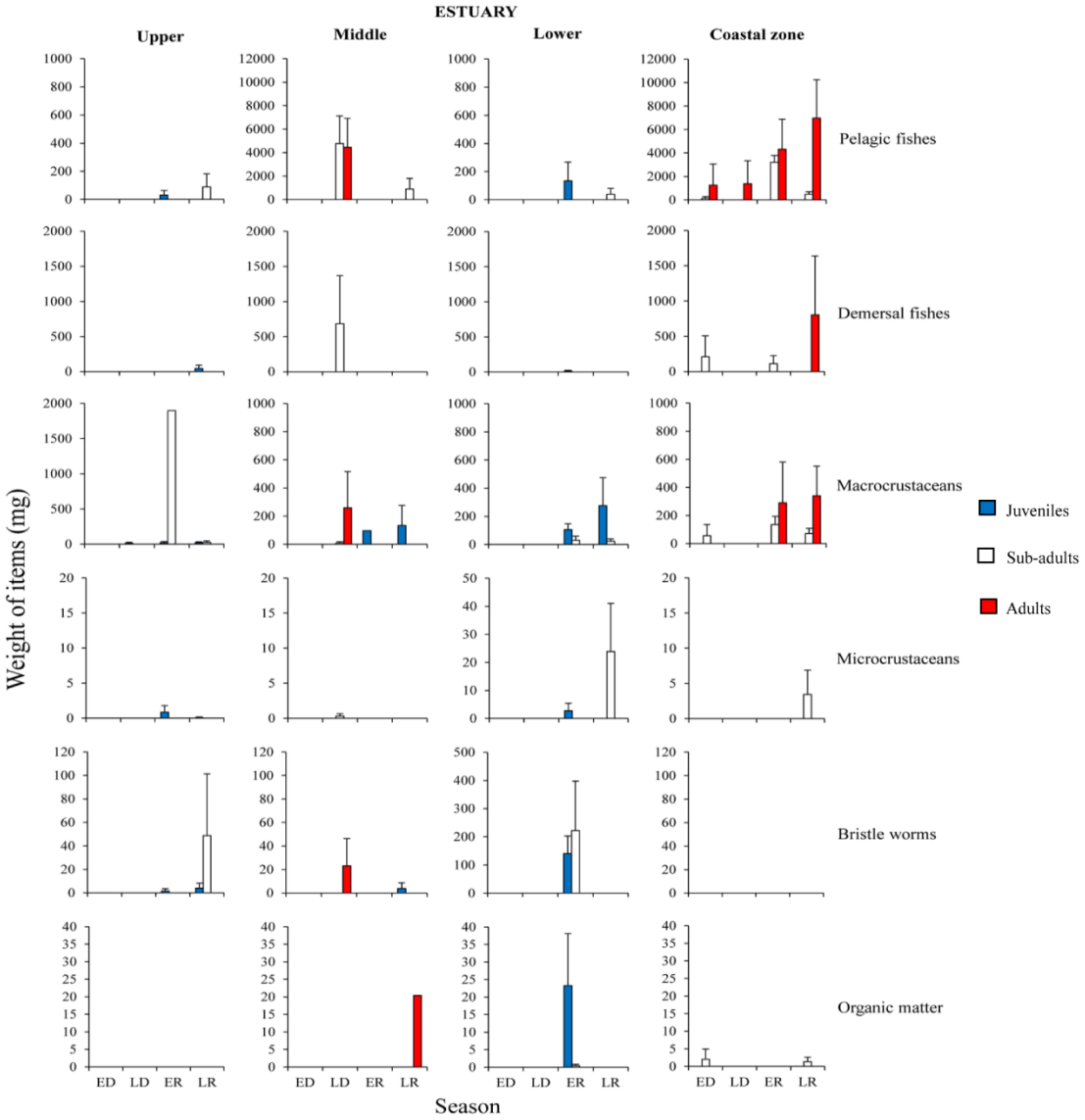
Supplementary Figure 1. Examples of microfilaments observed in the digestive tract of snooks. (a) Blue and red filaments, (b) black and blue filaments, (c) green and blue filaments, (d) white filaments, (e) green paint chips and (f) a grey soft particle.



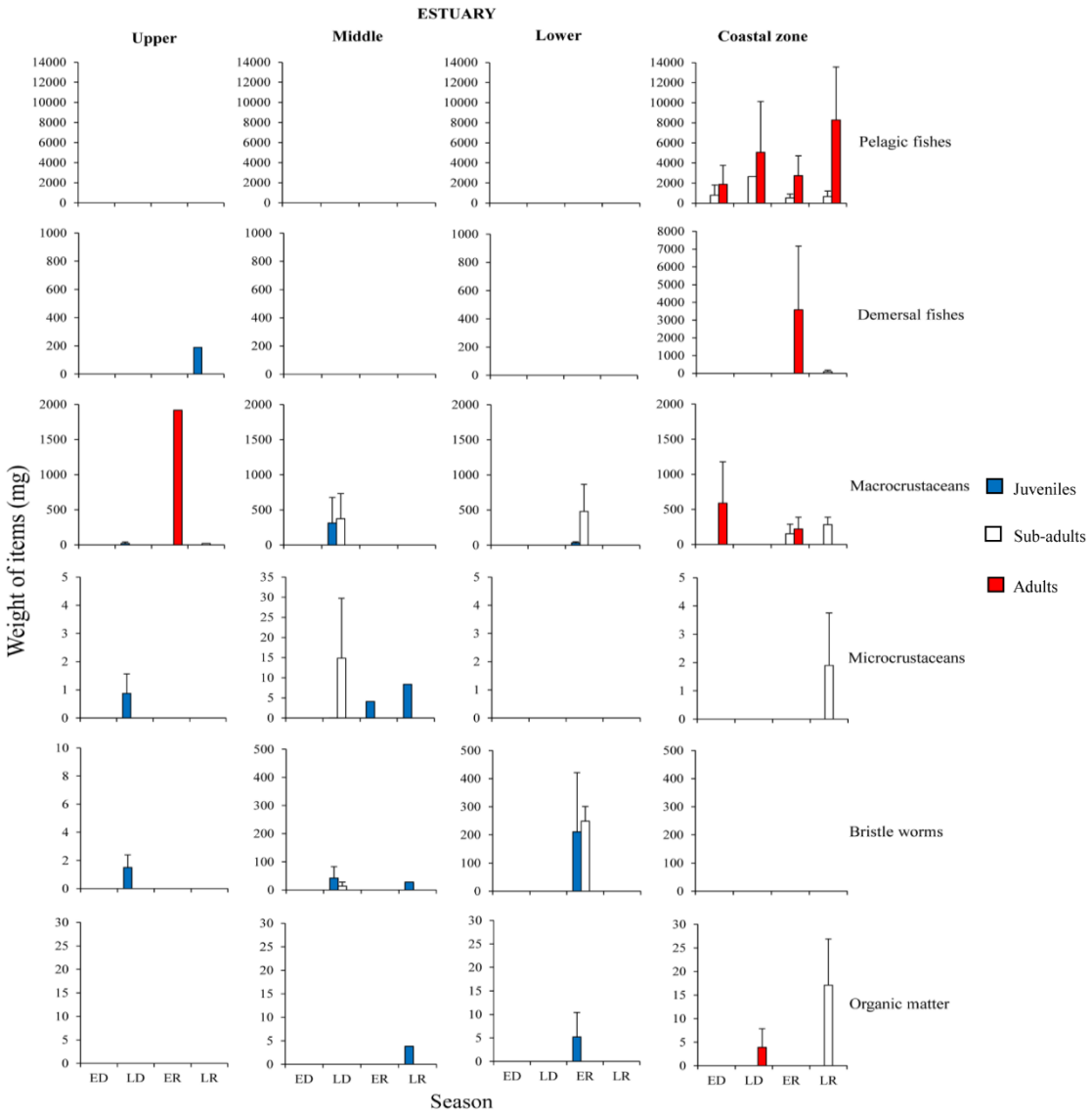
Supplementary Figure 2. Mean (SE±) number of different colours of filaments ingested by the *C. pectinatus*, regarding different habitats (upper, middle, lower estuary and coastal zone), seasons [ED (early dry), LD (late dry), ER (early rainy) and LR (late rainy)] and ontogenetic phases.



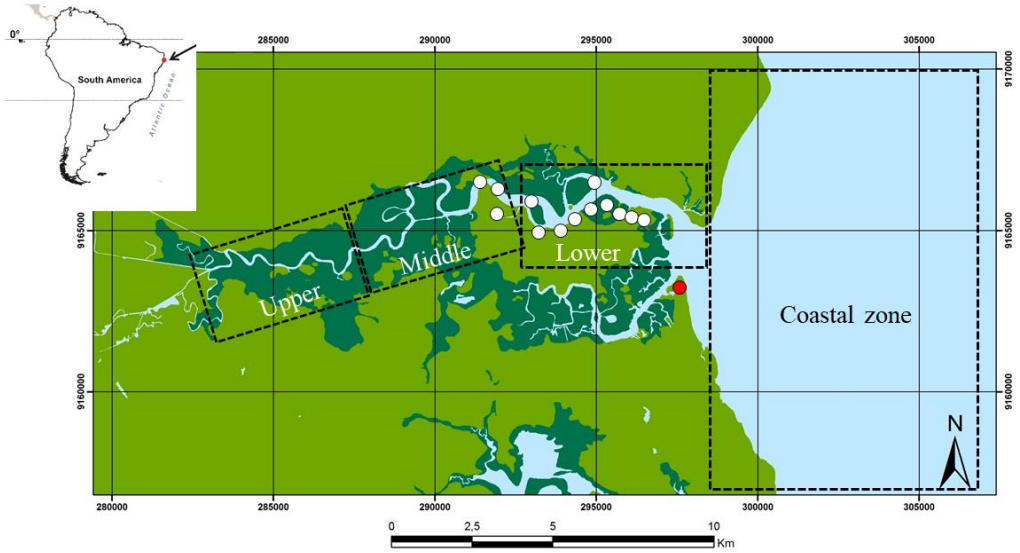
Supplementary Figure 3. Mean (SE±) weight of food groups of *C. undecimalis*, from different habitats (upper, middle, lower estuary and coastal zone), seasons [ED (early dry), LD (late dry), ER (early rainy) and LR (late rainy)] and ontogenetic phases.



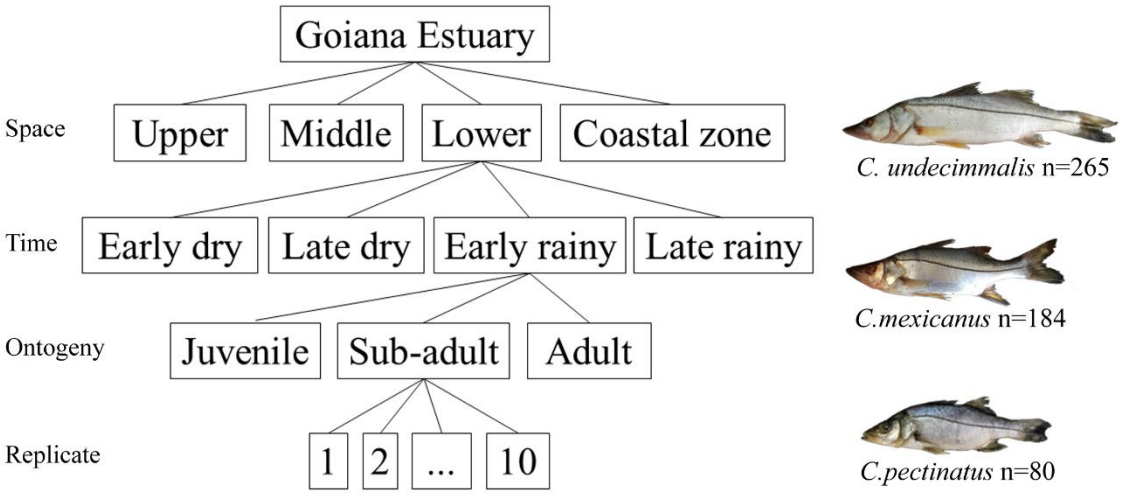
Supplementary Figure 4. Mean (SE±) weight of food groups of *C. mexicanus*, from different habitats (upper, middle, lower estuary and coastal zone), seasons [ED (early dry), LD (late dry), ER (early rainy) and LR (late rainy)] and ontogenetic phases.



Supplementary Figure 5. Mean (SE±) weight of food groups of *C. pectinatus*, from different habitats (upper, middle, lower estuary and coastal zone), seasons [ED (early dry), LD (late dry), ER (early rainy) and LR (late rainy)] and ontogenetic phases.



Supplementary Figure 6. Studied habitats in the Goiana Estuary: upper, middle, lower portions of the estuary, coastal zone, and (white circles) mangrove creeks. Local weather station (red circle).



Supplementary Figure 7. Sampling design applied on the study of marine debris ingestion by Centropomidae species, according to habitat, season and ontogenetic variability.

Supplementary Table 1. Mean (SE±) number, percentage and size of microfilaments ingested by snooks, according to the factors (habitat, season, and ontogenetic phase), regardless of interactions (N= number of fish in the sample).

Species	Blue filament		Purple filament		Green filament		Red filament		White filament		Black filament		Total filament	Size (mm)	
C. undecimalis	Mean ±SE	(%)	Mean ±SE	(%)	Mean ±SE	(%)	Mean ±SE	(%)	Mean ±SE	(%)	Mean ±SE	(%)	Mean ±SE	Mean ±SE	N
Habitat															
Upper	0.73 ±0.16	88		0		0	0.05 ±0.05	6	0.05 ±0.05	6		0	0.84 ±0.17	1.41 ±0.20	15
Middle	1.16 ±0.23	70	0.05±0.03	3	0.14 ±0.05	9	0.14 ±0.54	9	0.14 ±0.06	9	0.02 ±0.02	1	1.64 ±0.33	1.15 ±0.09	67
Lower	1.44 ±0.18	75	0.09 ±0.03	5	0.09 ±0.04	5	0.23 ±0.07	12	0.07 ±0.04	4		0	1.92 ±0.23	1.10 ±0.07	126
Coastal	1.14 ±0.18	75	0.10 ±0.04	7	0.08 ±0.03	6	0.12 ±0.03	8	0.07 ±0.02	5		0	1.49 ±0.21	1.08 ±0.05	190
Season															
Early Dry	1.14 ±0.19	75	0.12 ±0.05	8	0.06 ±0.03	4	0.12 ±0.06	8	0.06 ±.04	4	0.02 ±0.02	1	1.5 ±0.26	1.14 ±0.10	73
Late Dry	1.16 ±0.22	78	0.06 ±0.03	4	0.08 ±0.04	5	0.13 ±0.04	9	0.05 ±0.02	4		0	1.48 ±0.27	1.07 ±0.06	111
Early Rainy	1.30 ±0.17	73	0.02 ±0.02	1	0.13 ±0.4	7	0.19 ±0.06	11	0.12 ±0.04	7		0	1.72 ±0.22	1.09 ±0.07	136
Late Rainy	1.14 ±0.28	71	0.16 ±0.08	10	0.08 ±0.05	5	0.12 ±0.05	8	0.10 ±0.05	6		0	1.59 ±0.34	1.16 ±0.08	78
Phase															
Juvenile	1.16 ±0.14	77	0.02 ±0.02	1	0.12 ±0.05	8	0.18 ±0.06	12	0.02 ±0.02	1		0	1.52 ±0.17	1.10 ±0.10	75
Sub-adult	1.44 ±0.22	78	0.09 ±0.03	5	0.06 ±0.02	3	0.13 ±0.04	7	0.11 ±0.03	6		0	1.81 ±0.28	0.97 ±0.06	149
Adult	1.05 ±0.15	71	0.09 ±0.04	7	0.10 ±0.03	7	0.14 ±0.04	10	0.07 ±0.02	5	0.01 ±0.01	1	1.96 ±0.19	1.22 ±0.06	175

Supplementary Table 1. Continued.

Species	Blue filament		Purple filament		Green filament		Red filament		White filament		Black filament		Total filament	Size (mm)	
C. mexicanus	Mean ±SE	(%)	Mean ±SE	(%)	Mean ±SE	(%)	Mean ±SE	(%)	Mean ±SE	(%)	Mean ±SE	(%)	Mean ±SE	Mean ±SE	N
Habitat															
Upper	1.37 ±0.21	79	0.09 ±0.05	5	0.15 ±0.08	9		0	0.09 ±0.05	5	0.03 ±0.03	2	1.65 ±0.30	1.52 ±0.09	56
Middle	1.4 ±0.25	78	0.08 ±0.05	4	0.2 ±0.1	11	0.08 ±0.05	4	0.04 ±0.02	2		0	1.8 ±0.31	1.11 ±0.12	44
Lower	1.12 ±0.19	71	0.16 ±0.06	11	0.12 ±0.06	8	0.05 ±0.03	3	0.12 ±0.05	8		0	1.59 ±0.27	1.42 ±0.12	65
Coastal	0.97 ±0.13	84	0.04 ±0.03	4	0.04 ±0.02	4	0.08 ±0.02	7	0.01 ±0.01	1		0	1.16 ±0.15	1.0 ±0.06	100
Season															
Early Dry	0.5 ±0.31	73	0.06 ±0.06	9	0.06 ±0.06	9	0.06 ±0.06	9		0		0	0.69 ±0.35	0.84 ±0.15	10
Late Dry	1.33 ±0.27	82	0.05 ±0.05	4	0.05 ±0.05	4	0.05 ±0.05	4	0.05 ±0.05	4		0	1.55 ±0.33	1.17 ±0.16	28
Early Rainy	0.92 ±0.11	71	0.09 ±0.03	7	0.15 ±0.05	11	0.02 ±0.01	2	0.08 ±0.03	6	0.01 ±0.01	1	1.28 ±0.17	1.47 ±0.10	84
Late Rainy	1.37 ±0.16	79	0.09 ±0.04	5	0.08 ±0.03	5	0.09 ±0.03	5	0.04 ±0.02	2	0	0	1.71 ±0.20	1.11 ±0.06	130
Phase															
Juvenile	0.85 ±0.13	65	0.05 ±0.03	4	0.17 ±0.07	13	0.11 ±0.04	9	0.11 ±0.04	9		0	1.24 ±0.20	1.63 ±0.10	68
Sub-adult	1.19 ±0.12	80	0.08 ±0.02	6	0.09 ±0.03	6	0.07 ±0.02	5	0.04 ±0.02	3		0	1.49 ±0.15	1.07 ±0.06	140
Adult	1.28 ±0.26	80	0.14 ±0.07	9	0.02 ±0.02	2	0.11 ±0.05	7		0	0.03 ±0.02	2	1.6 ±0.33	1.15 ±0.09	57

Supplementary Table 1. Continued.

Species	Blue filament		Purple filament		Green filament		Red filament		White filament		Black filament		Total filament	Size (mm)	
C. pectinatus	Mean ±SE	(%)	Mean ±SE	(%)	Mean ±SE	(%)	Mean ±SE	(%)	Mean ±SE	(%)	Mean ±SE	(%)	Mean ±SE	Mean ±SE	N
Habitat															
Upper	0.28 ±0.28	100		0		0		0		0		0	0.28 ±0.28	0.33 ±0.10	2
Middle	0.7 ±0.26	88		0		0	0.1 ±0.11	13		0		0	0.8 ±0.29	1.16 ±0.30	8
Lower	1.2 ±0.37	75	0.2 ±0.2	13		0		0	0.2 ±0.2	13		0	1.6 ±0.51	1.63 ±0.44	7
Coastal	1 ±0.19	72	0.12 ±0.04	9	0.08 ±0.05	6	0.08 ±0.05	6	0.11 ±0.04	7		0	1.36 ±0.23	1.02 ±0.08	79
Season															
Early Dry	0.7 ±0.33	78	0.1 ±0.1	11		0	0.1 ±0.07	11		0		0	0.9 ±0.38	1.06 ±0.25	9
Late Dry	0.71 ±0.26	86		0		0	0.06 ±0.09	7	0.06 ±0.05	7		0	0.82 ±0.31	0.66 ±0.13	14
Early Rainy	1 ±0.38	74	0.13 ±0.09	10	0.08 ±0.07	6		0	0.13 ±0.07	10		0	1.30 ±0.43	1.03 ±0.16	31
Late Rainy	1 ±0.21	67	0.14 ±0.06	9	0.10 ±0.07	7	0.14 ±0.07	9	0.10 ±0.05	7		0	1.45 ±0.28	1.18 ±0.12	43
Phase															
Juvenile	0.31 ±0.17	100		0		0		0		0		0	0.31 ±0.08	1.43 ±0.74	3
Sub-adult	0.97 ±0.26	72	0.05 ±0.03	4	0.11 ±0.03	8	0.13 ±0.04	10	0.08 ±0.04	6		0	1.32 ±0.21	1.07 ±0.12	50
Adult	1.18 ±0.23	73	0.21 ±0.09	13	0.03 ±0.07	2	0.03 ±0.07	2	0.14 ±0.06	9		0	1.57 ±0.29	1.02 ±0.12	40

Supplementary Table 2.

Summary of the ANOVA for the length of microfilaments ingested by snooks in the Goiana Estuary, according to factors area [U (upper); M (middle); L (lower); C (coastal zone)], season [ED (early dry); LD (late dry); ER (early rainy); LR (late rainy)] and ontogeny [Juv (juveniles); Sub (sub-adults); Adu (adults)]. Bonferroni's test was used to determinate the sources of variances [F (F-values); df (degrees of freedom); *p*-value]. (ns: not significant) (*$p < 0.05$).

		Items in number				
	Factors	**F**	***df***	***p*-value**		***Post-hoc***
C. undecimalis	Season	2.33	3	0.07	ns	
	Area	19.78	3	0.01	*	U
	Phase	24.04	2	0.01	*	Adu
	Season *vs*. Area	8.37	9	0.01	*	ER.L
	Season *vs*. Phase	7.67	6	0.01	*	LD.Sub
	Area *vs.* Phase	18.44	6	0.01	*	C.Ad
	Season *vs.* Area *vs*. Phase	3.82	18	0.01	*	ED.U.Juv
C. mexicanus	Season	77.26	3	0.01	*	ED
	Area	3.73	3	0.01	*	U
	Phase	22.50	2	0.01	*	Sub
	Season *vs*. Area	18.62	9	0.01	*	ER.C - LR.M - LR.C - ER.L - ER.U
	Season *vs*. Phase	11.05	6	0.01	*	LR.Sub
	Area *vs.* Phase	27.74	6	0.01	*	U.Juv
	Season *vs.* Area *vs*. Phase	12.62	18	0.01	*	ER.U.Ad - LR.U.Juv - ER.L.Juv
C. pectinatus	Season	9.81	3	0.01	*	ER - LR
	Area	78.58	3	0.01	*	C
	Phase	44.25	2	0.01	*	Juv
	Season *vs*. Area	16.90	9	0.01	*	LR.C
	Season *vs*. Phase	7.60	6	0.01	*	*
	Area *vs.* Phase	35.73	6	0.01	*	C.Ad - C.Sub
	Season *vs.* Area *vs*. Phase	8.46	18	0.01	*	ER.L.Sub

Supplementary Table 3.

Summary of the ANOVA for the colour of microfilaments in number ingested by *C. undecimalis* in the Goiana Estuary, according to factors area [U (upper); M (middle); L (lower); C (coastline)], season [ED (early dry); LD (late dry); ER (early rainy); LR (late rainy)] and ontogeny [Juv (juveniles); Sub (sub-adults); Adu (adults)]. Bonferroni's test was used to determinate the sources of variances [F (F-values); df (degrees of freedom); *p*-value]. (ns: not significant) (*$p < 0.05$).

		Items in number				
	Factors	**F**	***df***	***p*-value**		***Post-hoc***
Blue filaments	Season	2.55	3	0.06	ns	
	Area	6.40	3	0.01	*	U
	Phase	2.90	2	0.06	ns	
	Season *vs.* Area	4.32	9	0.01	*	ER.L
	Season *vs.* Phase	2.37	6	0.03	*	LD.Juv
	Area *vs.* Phase	3.66	6	0.01	*	C.Ad
	Season *vs.* Area *vs.* Phase	2.22	18	0.01	*	ER.L.Juv - ER.L.Sub
Purple filaments	Season	0.77	3	0.50	ns	
	Area	4.02	3	0.01	*	C
	Phase	3.82	2	0.02	*	Juv
	Season *vs.* Area	0.74	9	0.67	ns	
	Season *vs.* Phase	1.80	6	0.09	ns	
	Area *vs.* Phase	2.00	6	0.06	ns	
	Season *vs.* Area *vs.* Phase	1.11	18	0.34	ns	
Green filaments	Season	2.42	3	0.06	ns	
	Area	3.11	3	0.02	*	U
	Phase	0.90	2	0.40	ns	
	Season *vs.* Area	1.74	9	0.08	ns	
	Season *vs.* Phase	1.68	6	0.12	ns	
	Area *vs.* Phase	1.45	6	0.19	ns	
	Season *vs.* Area *vs.* Phase	1.20	18	0.05	*	ER.M.Sub - ER.M.Ad - ED.C.Juv - LD.C.Ad - ER.C.Ad

Supplementary Table 3. Continued.

		Items in number				
	Factors	**F**	***df***	***p*-value**		***Post-hoc***
Red filaments	Season	2.30	3	0.07	ns	
	Area	3.88	3	0.01	*	U
	Phase	0.87	2	0.41	ns	
	Season *vs.* Area	1.43	9	0.17	ns	
	Season *vs.* Phase	1.80	6	0.09	ns	
	Area *vs.* Phase	1.11	6	0.35	ns	
	Season *vs.* Area *vs.* Phase	1.22	18	0.24	ns	
White filaments	Season	1.19	3	0.31	ns	
	Area	1.28	3	0.27	ns	
	Phase	3.12	2	0.04	*	Juv
	Season *vs.* Area	1.11	9	0.35	ns	
	Season *vs.* Phase	1.27	6	0.26	ns	
	Area *vs.* Phase	1.77	6	0.10	ns	
	Season *vs.* Area *vs.* Phase	1.32	18	0.17	ns	

Supplementary Table 4.

Summary of the ANOVA for the colour of microfilaments in number ingested by *C. mexicanus* in the Goiana Estuary, according to factors area [U (upper); M (middle); L (lower); C (coastal zone)], season [ED (early dry); LD (late dry); ER (early rainy); LR (late rainy)] and ontogeny [Juv (juveniles); Sub (sub-adults); Adu (adults)]. Bonferroni's test was used to determinate the sources of variances [F (F-values); df (degrees of freedom); *p*-value]. (ns: not significant) (*$p < 0.05$).

		Items in number				
	Factors	**F**	***df***	***p*-value**		***Post-hoc***
Blue filaments	Season	51.44	3	0.01	*	LR
	Area	0.24	3	0.86	ns	
	Phase	43.74	2	0.01	*	Sub
	Season *vs.* Area	10.04	9	0.01	*	ER.U - LR.M -LD.M - LR.C - ER.L - LR.U
	Season *vs.* Phase	12.34	6	0.01	*	LR.Sub
	Area *vs.* Phase	14.21	6	0.01	*	L.Sub
	Season *vs.* Area *vs.* Phase	6.37	18	0.01	*	LD.M.Sub - LR.C.Sub
Purple filaments	Season	2.55	3	0.06	ns	
	Area	0.82	3	0.48	ns	
	Phase	1.28	2	0.27	ns	
	Season *vs.* Area	2.24	9	0.02	*	ER.L
	Season *vs.* Phase	0.73	6	0.62	ns	
	Area *vs.* Phase	2.07	6	0.06	ns	
	Season *vs.* Area *vs.* Phase	1.7	18	0.04	*	ER.L.Sub
Green filaments	Season	3.25	3	0.02	*	ER
	Area	0.20	3	0.88	ns	
	Phase	2.52	2	0.08	ns	
	Season *vs.* Area	1.91	9	0.06	ns	
	Season *vs.* Phase	0.68	6	0.66	ns	
	Area *vs.* Phase	1.92	6	0.07	ns	
	Season *vs.* Area *vs.* Phase	0.85	18	0.63	ns	

Supplementary Table 4. Continued.

		Items in number				
	Factors	**F**	***df***	***p*-value**		***Post-hoc***
Red filaments	Season	1.36	3	0.25	ns	
	Area	5.17	3	0.01	*	U
	Phase	0.92	2	0.39	ns	
	Season *vs.* Area	0.53	9	0.84	ns	
	Season *vs.* Phase	1.05	6	0.38	ns	
	Area *vs.* Phase	1.71	6	0.11	ns	
	Season *vs.* Area *vs.* Phase	0.76	18	0.74	ns	
White filaments	Season	3.39	3	0.01	*	ED LD ER LR
	Area	2.64	3	0.06	ns	
	Phase	3.84	2	0.02	*	Adu
	Season *vs.* Area	1.92	9	0.06	ns	
	Season *vs.* Phase	3.47	6	0.01	*	ER.Juv
	Area *vs.* Phase	2.47	6	0.02	*	U.Juv - L.Sub
	Season *vs.* Area *vs.* Phase	3.22	18	0.01	*	LR.L.Sub

Supplementary Table 5.
Summary of the ANOVA for the colour of microfilaments in number ingested by *C. pectinatus* in the Goiana Estuary, according to factors area [U (upper); M (middle); L (lower); C (coastal zone)], season [ED (early dry); LD (late dry); ER (early rainy); LR (late rainy)] and ontogeny [Juv (juveniles); Sub (sub-adults); Adu (adults)]. Bonferroni's test was used to determinate the sources of variances [F (F-values); df (degrees of freedom); *p*-value]. (ns: not significant) (*$p < 0.05$).

		Items in number				
	Factors	**F**	***df***	***p*-value**		***Post-hoc***
Blue filaments	Season	4.16	3	0.01	*	LR
	Area	23.91	3	0.01	*	C
	Phase	8.18	2	0.01	*	Juv
	Season *vs.* Area	3.89	9	0.01	*	LR.C
	Season *vs.* Phase	0.40	6	0.87	ns	LR.Sub
	Area *vs.* Phase	10.97	6	0.01	*	C.Ad - C.Sub
	Season *vs.* Area *vs.* Phase	1.92	18	0.01	*	LR.C.Sub - LR.C.Ad
Purple filaments	Season	2.18	3	0.09	ns	
	Area	7.52	3	0.01	*	C
	Phase	5.00	2	0.01	*	Adu
	Season *vs.* Area	2.03	9	0.03	*	LR.C
	Season *vs.* Phase	0.99	6	0.42	ns	
	Area *vs.* Phase	6.06	6	0.01	*	C.Ad
	Season *vs.* Area *vs.* Phase	1.26	18	0.21	ns	
Green filaments	Season	1.46	3	0.22	ns	
	Area	4.34	3	0.01	*	C
	Phase	1.79	2	0.16	ns	
	Season *vs.* Area	1.46	9	0.16	ns	
	Season *vs.* Phase	1.07	6	0.37	ns	
	Area *vs.* Phase	1.79	6	0.10	ns	
	Season *vs.* Area *vs.* Phase	1.07	18	0.37	ns	

Supplementary Table 5. Continued.

		Items in number				
	Factors	**F**	***df***	***p*-value**		***Post-hoc***
Red filaments	Season	0.66	3	0.57	ns	
	Area	2.14	3	0.09	ns	
	Phase	1.85	2	0.15	ns	
	Season *vs.* Area	1.43	9	0.17	ns	
	Season *vs.* Phase	1.52	6	0.17	ns	
	Area *vs.* Phase	0.79	6	0.57	ns	
	Season *vs.* Area *vs.* Phase	1.08	18	0.35	ns	
White filaments	Season	1.74	3	0.15	ns	
	Area	6.04	3	0.01	*	C
	Phase	2.80	2	0.06	ns	
	Season *vs.* Area	1.13	9	0.33	ns	
	Season *vs.* Phase	0.77	6	0.58	ns	
	Area *vs.* Phase	3.16	6	0.01	*	C.Ad
	Season *vs.* Area *vs.* Phase	0.58	18	0.90	ns	

Supplementary Table 6.
Food items ingested by snooks grouped into ecologic/taxonomic categories.

Food items Groups			
Pelagic fishes	Engraulidae *Anchovia clupeoides* *Cetengraulis edentulus* Clupeidae *Rhinosardinia bahiensis* *Harengula clupeola* *Opisthonema oglinum* *Odontognathus mucronatus* *Lycengraulis grossidens* *Mugil liza* *Caranx latus* *Trichiurus lepturus* *Hemiramphus brasiliensis*	**Demersal fishes**	*Cathorops spixii* *Achirus lineatus* *Cynoscion acoupa* *Stellifer stellifer* *Stellifer rastrifer* *Stellifer brasiliensis* *Menticirrhus littoralis* *Centropomus undecimalis* *Pomadasys corvinaeformis* *Diapterus rhombeus* *Eucinostomus melanopterus* *Eugerres brasilianus* Gobidae *Bathygobius soporator* *Eleotris pisonis* *Myrophis punctatus*
Macrocrustaceans	Paguridae Penaeidae shrimp *Callinectes danae* *Aratus pisonii* *Ucides cordatus*	**Microcrustaceans**	Amphipoda Mysidacea Copepoda Isopoda
Bristle worms	Nereidae Syllidae	**Organic matter**	Mangrove fragments Seaweed

Supplementary Table 7.

Summary of the ANOVA for the weight of food groups ingested by *C. mexicanus* in the Goiana Estuary, according to factors area [U (upper); M (middle); L (lower); C (coastal zone)], season [ED (early dry); LD (late dry); ER (early rainy); LR (late rainy)] and ontogeny [Juv (juveniles); Sub (sub-adults); Adu (adults)]. Bonferroni's test was used to determinate the sources of variances [F (F-values); df (degrees of freedom); *p*-value]. (ns: not significant) (*$p < 0.05$).

		Items in number				
	Factors	**F**	***df***	***p*-value**		***Post-hoc***
Pelagic fishes	Season	4.79	3	0.01	*	ED
	Area	19.74	3	0.01	*	C
	Phase	14.15	2	0.01	*	Juv
	Season *vs.* Area	8.07	9	0.01	*	ER.C - LR.C
	Season *vs.* Phase	1.67	6	0.12	ns	
	Area *vs.* Phase	6.88	6	0.01	*	C.Sub - C.Ad
	Season *vs.* Area *vs.* Phase	4.29	18	0.01	*	ER.C.Sub - LR.C.Ad
Demersal fishes	Season	0.08	3	0.96	ns	
	Area	1.33	3	0.26	ns	
	Phase	0.93	2	0.39	ns	
	Season *vs.* Area	0.71	9	0.69	ns	
	Season *vs.* Phase	0.76	6	0.59	ns	
	Area *vs.* Phase	0.96	6	0.44	ns	
	Season *vs.* Area *vs.* Phase	1.09	18	0.35	ns	
Macrocrustaceans	Season	5.56	3	0.01	*	ER
	Area	1.66	3	0.17	ns	
	Phase	0.37	2	0.68	ns	
	Season *vs.* Area	1.14	9	0.33	ns	
	Season *vs.* Phase	0.76	6	0.59	ns	
	Area *vs.* Phase	2.96	6	0.01	*	C.Sub
	Season *vs.* Area *vs.* Phase	1.37	18	0.14	ns	
Microcrustaceans	Season	1.20	3	0.30	ns	
	Area	1.11	3	0.34	ns	
	Phase	1.16	2	0.31	ns	
	Season *vs.* Area	0.78	9	0.63	ns	
	Season *vs.* Phase	1.47	6	0.18	ns	
	Area *vs.* Phase	0.72	6	0.63	ns	
	Season *vs.* Area *vs.* Phase	0.91	18	0.55	ns	

Supplementary Table 7. Continued.

		Items in number				
	Factors	**F**	***df***	***p*-value**		***Post-hoc***
Bristle worms	Season	6.16	3	0.01	*	ER
	Area	5.96	3	0.01	*	L
	Phase	2.09	2	0.12	ns	
	Season *vs*. Area	7.87	9	0.01	*	ER.C
	Season *vs*. Phase	2.19	6	0.04	*	ER.Juv
	Area *vs.* Phase	2.11	6	0.06	ns	
	Season *vs.* Area *vs*. Phase	2.11	18	0.01	*	ER.L.Juv
Organic matter	Season	1.97	3	0.11	ns	
	Area	1.90	3	0.12	ns	
	Phase	1.43	2	0.24	ns	
	Season *vs*. Area	2.63	9	0.01	*	ER.L
	Season *vs*. Phase	2.49	6	0.02	*	ER.Juv
	Area *vs.* Phase	2.57	6	0.02	*	L.Juv
	Season *vs.* Area *vs*. Phase	2.18	18	0.01	*	ER.L.Juv

Supplementary Table 8.
Summary of the ANOVA for the weight of food groups ingested by *C. undecimalis* in the Goiana Estuary, according to factors area [U (upper); M (middle); L (lower); C (coastal zone)], season [ED (early dry); LD (late dry); ER (early rainy); LR (late rainy)] and ontogeny [Juv (juveniles); Sub (sub-adults); Adu (adults)]. Bonferroni's test was used to determinate the sources of variances [F (F-values); df (degrees of freedom); *p*-value]. (ns: not significant) (*$p < 0.05$).

		Items in number				
	Factors	**F**	***df***	***p*-value**		***Post-hoc***
Pelagic fishes	Season	1.75	3	0.15	ns	
	Area	34.07	3	0.01	*	C
	Phase	14.24	2	0.01	*	Adu
	Season *vs*. Area	2.06	9	0.03	*	ED.C - LD.C
	Season *vs*. Phase	1.88	6	0.08	ns	
	Area *vs*. Phase	4.80	6	0.01	*	C.Ad
	Season *vs*. Area *vs*. Phase	1.78	18	0.02	*	ED.C.Ad - LD.C.Ad - ER.C.Ad - LR.C.Ad - LD.C.Sub
Demersal fishes	Season	1.16	3	0.32	ns	
	Area	9.46	3	0.01	*	U
	Phase	12.91	2	0.01	*	Adu
	Season *vs*. Area	3.21	9	0.01	*	LD.M
	Season *vs*. Phase	1.66	6	0.13	ns	
	Area *vs*. Phase	6.87	6	0.01	*	C.Ad
	Season *vs*. Area *vs*. Phase	1.41	18	0.12	ns	
Macrocrustaceans	Season	0.21	3	0.88	ns	
	Area	2.57	3	0.06	ns	
	Phase	3.47	2	0.03	*	Sub
	Season *vs*. Area	3.01	9	0.01	*	LR.C
	Season *vs*. Phase	1.10	6	0.35	ns	
	Area *vs*. Phase	2.36	6	0.03	*	C.Ad
	Season *vs*. Area *vs*. Phase	1.34	18	0.16	ns	
Microcrustaceans	Season	0.94	3	0.41	ns	
	Area	1.10	3	0.34	ns	
	Phase	0.92	2	0.39	ns	
	Season *vs*. Area	0.96	9	0.47	ns	
	Season *vs*. Phase	1.02	6	0.40	ns	
	Area *vs*. Phase	0.94	6	0.46	ns	
	Season *vs*. Area *vs*. Phase	1.01	18	0.44	ns	

Supplementary Table 8. Continued.

		Items in number				
	Factors	**F**	***df***	***p*-value**		***Post-hoc***
Bristle worms	Season	1.11	3	0.34	ns	
	Area	1.93	3	0.12	ns	
	Phase	0.53	2	0.58	ns	
	Season *vs.* Area	2.40	9	0.01	*	ED.U
	Season *vs.* Phase	0.76	6	0.59	ns	
	Area *vs.* Phase	0.36	6	0.90	ns	
	Season *vs.* Area *vs.* Phase	0.82	18	0.67	ns	
Organic matter	Season	2.56	3	0.06	ns	
	Area	2.94	3	0.03	*	L
	Phase	1.06	2	0.34	ns	
	Season *vs.* Area	0.56	9	0.82	ns	
	Season *vs.* Phase	3.05	6	0.01	*	LD.Ad
	Area *vs.* Phase	0.59	6	0.73	ns	
	Season *vs.* Area *vs.* Phase	1.30	18	0.19	ns	

Supplementary Table 9.

Summary of the ANOVA for the weight of food groups ingested by *C. pectinatus* in the Goiana Estuary, according to factors area [U (upper); M (middle); L (lower); C (coastal zone)], season [ED (early dry); LD (late dry); ER (early rainy); LR (late rainy)] and ontogeny [Juv (juveniles); Sub (sub-adults); Adu (adults)]. Bonferroni's test was used to determinate the sources of variances [F (F-values); df (degrees of freedom); *p*-value]. (ns: not significant) (*$p < 0.05$).

	Items in number					
	Factors	**F**	***df***	***p*-value**		***Post-hoc***
Pelagic fishes	Season	1.57	3	0.19	ns	
	Area	25.15	3	0.01	*	C
	Phase	9.46	2	0.01	*	Adu
	Season *vs*. Area	1.57	9	0.12	ns	
	Season *vs*. Phase	1.45	6	0.19	ns	
	Area *vs.* Phase	9.46	6	0.01	*	C.Sub - C.Ad
	Season *vs.* Area *vs*. Phase	1.45	18	0.11	ns	
Demersal fishes	Season	1.60	3	0.18	ns	
	Area	2.87	3	0.03	*	C
	Phase	0.64	2	0.52	ns	
	Season *vs*. Area	1.47	9	0.15	ns	
	Season *vs*. Phase	2.13	6	0.06	ns	
	Area *vs.* Phase	1.49	6	0.18	ns	
	Season *vs.* Area *vs*. Phase	2.19	18	0.01	*	ER.C.Ad
Macrocrustaceans	Season	1.52	3	0.20	ns	
	Area	3.90	3	0.01	*	C
	Phase	2.73	2	0.06	ns	
	Season *vs*. Area	2.65	9	0.01	*	ER.C
	Season *vs*. Phase	1.73	6	0.11	ns	
	Area *vs.* Phase	2.31	6	0.03	*	C.Sub
	Season *vs.* Area *vs*. Phase	2.31	18	0.01	*	LR.C.Sub
Microcrustaceans	Season	0.85	3	0.46	ns	
	Area	1.46	3	0.22	ns	
	Phase	1.05	2	0.34	ns	
	Season *vs*. Area	0.70	9	0.70	ns	
	Season *vs*. Phase	0.74	6	0.61	ns	
	Area *vs.* Phase	0.59	6	0.73	ns	
	Season *vs.* Area *vs*. Phase	1.22	18	0.24	ns	

Supplementary Table 9. Continued.

		Items in number				
	Factors	**F**	***df***	***p*-value**		***Post-hoc***
Bristle worms	Season	2.39	3	0.06	ns	
	Area	2.51	3	0.06	ns	
	Phase	1.43	2	0.24	ns	
	Season *vs*. Area	3.41	9	0.01	*	ER.L
	Season *vs*. Phase	0.91	6	0.48	ns	
	Area *vs.* Phase	1.00	6	0.42	ns	
	Season *vs.* Area *vs*. Phase	1.02	18	0.43	ns	
Organic matter	Season	2.54	3	0.06	ns	
	Area	3.51	3	0.01	*	C
	Phase	1.92	2	0.14	ns	
	Season *vs*. Area	2.59	9	0.01	*	LR.C
	Season *vs*. Phase	3.06	6	0.01	*	LR.Sub
	Area *vs.* Phase	2.58	6	0.01	*	C.Sub
	Season *vs.* Area *vs*. Phase	3.06	18	0.01	*	LR.C.Sub

Supplementary Table 10.

Summary of the Canonical Correspondence Analysis (CCA) using six environmental variables (water temperature, Secchi depth, salinity, dissolved oxygen and rainfall) and the index of relative importance (I_{RI}) for the main food items ingested by snooks according to the factors habitats, seasons and ontogenetic phases.

Statistics	Axis I	Axis II	Environmental variables	*p*-value
Eigenvalue	0.095	0.031	Salinity	0.01
Pseudo-canonical correlation %	55	36.4	Dissolved oxygen (mg l^{-1})	0.01
Explained fitted variation of species-environmental variables %	61.8	19.5	Secchi depth (cm)	0.01
			Water temperature (ºC)	0.95
			Rainfall (mm)	0.95

Supplementary Table 11

Size classes of different ontogenetic phases of snooks.

	Juvenile	**Sub-adult**	**Adult**
C. undecimalis	< 263 mm	263 - 454 mm	> 454 mm
C. mexicanus	< 213 mm	213 - 361 mm	> 361 mm
C. pectinatus	< 146 mm	146 - 261 mm	> 261 mm

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6 ARTIGO 3 - HIGH INTAKE RATES OF MICROPLASTICS IN A WESTERN ATLANTIC PREDATORY FISH, AND INSIGHTS OF A DIRECT FISHERY EFFECT

Artigo publicado na revista Environmental Pollution.

High intake rates of microplastics in a Western Atlantic predatory fish, and insights of a direct fishery effect

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ABSTRACT

Microplastic contamination was investigated in the gut contents of an economically important estuarine top predator, *Cynoscion acoupa*, according to spatiotemporal and ontogenetic use of a tropical estuary. Microplastic contamination was found in more than half of the analysed fish. Ingested microplastics were classified by type, colour and length with most of the particles consisting of filaments (< 5 mm). Longer filaments were more frequently ingested in the upper estuary and smaller filaments in the lower estuary, as a result of differences in hydrodynamic forces and proximity to the probable input sources. The river is likely an important source of filaments to the estuary and filaments ingested in the upper estuary showed little sign of weathering, when compared with those from the lower estuary, which are subject to intense weathering and consequent break-up of particles to smaller sizes. Most filaments, of all colours, accumulated in adults of *C. acoupa*, which are more susceptible to contamination through both direct ingestion and trophic transference as they shift their feeding mode to piscivory. Moreover, the highest ingestion of filaments in adults occurred in the lower estuary, during the late rainy season, likely associated with the intense fishing activities in this habitat, which results in a greater input of filaments from fishing gear, which are mainly blue in colour. Overall, 44% of the ingested filaments were blue, 20% purple, 13%

black, 10% red and 12% white. The next most common colour, the purple filaments, are most likely blue filaments whose colour has weathered to purple. Red filaments were proportionally more ingested in the lower estuary, indicating a coastal/oceanic source. White and black filaments were more commonly ingested in the inner estuary, suggesting that they have a riverine origin and/or were actively ingested by juveniles and sub-adults, which inhabit the inner estuary and have zooplankton as an important food resource.

Keywords: Marine debris. Microplastic filaments. Seafood contamination. Fishery resource. Trophic transfer.

INTRODUCTION

The intensification of anthropogenic activities, especially in the mid-20th century, has resulted in many threats to marine wildlife. Impacts caused by dredging, overfishing, introduction of alien species, coastal habitat losses and illegal dumping of solid wastes in the marine environment are widely recorded (BARLETTA; CYSNEIROS; LIMA, 2016; BARNES, 2002; BARNES; MILNER, 2005; BLABER et al., 2000; COSTA; BARLETTA, 2015; JACKSON et al., 2001; LIMA et al., 2016; MYERS; WORM, 2003).

Plastic materials are one of the most frequent among the great variety of solid wastes illegally dumped in the marine environment (BARNES et al., 2009; GREGORY, 2009). The high and increasing loads of environmental pollutants recorded in the sedimentary cycle of terrestrial and marine environments suggest that plastics are a geological indicator of the Anthropocene (WILLIAMS et al., 2016). This results from the widespread use of plastic products since the 19th century; their flexibility and competitive prices making them suitable for a wide range of uses (THOMPSON et al., 2009; ZALASIEWICZ et al., 2016). Once in the aquatic environment, plastics undergo weathering processes, caused by waves, wind, tidal action and ultraviolet radiation, resulting in their mechanical breakdown into smaller particles (BROWNE; GALLOWAY; THOMPSON, 2007; WANG et al., 2016).

Microplastics are those particles smaller than 5 mm (Arthur et al., 2009; Lusher et al., 2017), which are ubiquitous in the marine environment (COLLIGNON et al., 2012; LIMA; COSTA; BARLETTA, 2014). The widespread occurrence of microplastic in the aquatic ecosystems results in an inevitable interaction with organisms (GREGORY, 2009), occurring mostly by ingestion (WRIGHT; THOMPSON; GALLOWAY, 2013). Indeed, many studies have reported microplastic ingestion by shellfish (DAVIDSON; DUDAS, 2016), marine mammals (FOSSI et al., 2012) and fishes in a variety of marine environments (BOERGER et

al., 2010; BRÅTE et al., 2016; DANTAS; BARLETTA; DA COSTA, 2012; FERREIRA et al., 2016; POSSATTO et al., 2011; RAMOS; BARLETTA; COSTA, 2012).

Although widely spread in the marine environment, including in remote oceanic islands (IVAR DO SUL et al., 2013; LIMA; BARLETTA; COSTA, 2016), microplastics are somewhat diluted in the open ocean but have recently been reported in higher concentration within semi-enclosed environments, such as estuaries (LIMA; COSTA; BARLETTA, 2014; ZHAO et al., 2014). Concentration of microplastics can be so high that their densities are comparable with those of eggs and larval fishes (LIMA; BARLETTA; COSTA, 2015). Microplastics are found in every habitat within the estuarine system, including intertidal mudflats (COSTA et al., 2011), mangrove forests (IVAR DO SUL et al., 2014), mangrove creeks (LIMA et al., 2016) and the main estuary channel (LIMA; COSTA; BARLETTA, 2014).

Microplastic pollution in estuaries has multiple and complex sources from urban settlements along their margins, nearby cities and fishing activities. However, the river basin has been identified as one of the main contributors of continental microplastics into estuaries (FOK; CHEUNG, 2015; LEBRETON et al., 2017; SILVA-CAVALCANTI et al., 2017). Since estuaries are pathways connecting rivers to the oceans, they function as a retainer of microplastics during drier months, as well as the main exporters of microplastics to coastal and high seas when runoff increases seaward (LEBRETON et al., 2017; LIMA; COSTA; BARLETTA, 2014).

This means that fishes and other organisms inhabiting any estuarine habitat and adjacent coastal environments are susceptible to ingest microplastics (DANTAS; BARLETTA; DA COSTA, 2012; FERREIRA et al., 2016; RAMOS; BARLETTA; COSTA, 2012). Once ingested, microplastics can be hazardous to the contaminated organisms, resulting in digestive injuries or a decrease in predatory efficiency that can induce starvation (DE SÁ; LUÍS; GUILHERMINO, 2015; MOORE, 2008; TEUTEN et al., 2007). In addition, they can be toxic through persistent organic pollutants that absorb onto microplastics and are bioaccumulated and biomagnified (OEHLMANN et al., 2009; ROCHMAN et al., 2013).

The contamination of important food species, is a pressing issue because of the potential implications for human health (SANTILLO; MILLER; JOHNSTON, 2017; TALSNESS et al., 2009). Thus, commercial target species should be a focus for studies on microplastic ingestion. Usually, fishes from higher trophic levels are primary targets of fishing activities because of the protein quality and higher sales prices (PINNEGAR et al., 2002).

Unfortunately, top predators are also more vulnerable to plastic debris contamination (AU et al., 2017; COLE et al., 2011). The build-up of plastic particles along the trophic chain through biotransference of microplastic from the contaminated prey may result in higher levels of contamination in top predator fishes (FERREIRA et al., 2016).

The 45 species of fish from the Sciaenidae family (CERVIGÓN et al., 1993), represent the most important taxonomic group for the South American coastal fisheries (CERVIGÓN et al., 1993), with landings of ~42,000 tons in Brazil (MPA, 2011). One of the most important commercial fish inhabiting Western Atlantic estuaries is the Acoupa weakfish *Cynoscion acoupa* (BARLETTA; BARLETTA-BERGAN; SAINT-PAUL, 1998; FERREIRA et al., 2016), which represents the most economically important species of the Sciaenidae family.

In the Goiana Estuary (northeast Brazil), juveniles of *C. acoupa* occur mainly, in the upper estuary, which is an extremely important habitat for the species since it is a nursery ground during the early rainy season (March to May). Sub-adults also inhabit the upper estuary, as it is an excellent feeding ground, with an absence of marine predators and reduced competition (FERREIRA et al 2016). Adults of *C. acoupa*, are one of the main predators inhabiting the coastline. They gather for foraging and reproduction in the estuary mouth where they are captured by the artisanal fishery (FERREIRA et al., 2016). Therefore, studying the effects of non-natural food items on *C. acoupa* is fundamental to understanding how microplastics might alter the ecology of the estuary.

Studies on microplastic distribution and interactions with marine biota are increasing in quantity and quality. Such studies must quantify the spatial and temporal variation in microplastics and the many factors that influence this (COSTA; BARLETTA, 2015; LUSHER et al., 2017; UNDERWOOD et al., 2017). Standard protocols for sampling, extraction and enumeration of microplastics ingested by fishes have also been developed to enable worldwide comparisons (LUSHER et al., 2017). Although several studies have focused on the contamination of fishes by plastic debris, few attempts have been made to understand spatiotemporal patterns of availability and ingestion of microplastics (BOERGER et al., 2010; BRÅTE et al., 2016; DANTAS; BARLETTA; COSTA, 2012; JOVANOVIĆ, 2017; LIMA; BARLETTA; COSTA, 2015; LUSHER; MCHUGH; THOMPSON, 2013; POSSATTO et al., 2011; SILVA-CAVALCANTI et al., 2017).

Both the distribution patterns of fishes and microplastic availability vary with the spatial and seasonal variability in environmental factors within tropical estuaries (BARLETTA et al., 2008; LIMA; BARLETTA; COSTA, 2015; LIMA; COSTA; BARLETTA, 2014). Any

investigation must include the role of the estuarine ecocline on fish ecological behaviour and on their encounter rate with microplastics (FERREIRA et al., 2016). This approach is important to detect which environmental variables are associated with patterns of microplastic ingestion through the life cycle of fish species, in addition to changes in their patterns of use within the estuary (FERREIRA et al., 2016).

A recent survey reported no relationship between ingested microplastic quantity and trophic level (GÜVEN et al., 2017). Although the evidence for trophic transfer was equivocal, there was no assessment of microplastics previously ingested by prey items, especially prey of piscivorous fishes by Güven et al (2017). Therefore, the present study assesses possible preferences for different types, colour and sizes of plastics ingested in relation to the main feeding mode and shows evidences of microplastic transference in the food web.

The aim of this study is to investigate whether the ecological patterns of this species (including categories of prey) are related with ingestion rates of the different categories of microplastic (colour and length) with respect to the seasonal and spatial shifts in the diet of different ontogenetic phases (juvenile, sub-adults and adults) in a tropical estuarine ecosystem.

MATERIAL AND METHODS

Study Area

The Goiana Estuary (~4,700 ha) is a tropical ecosystem, located in the easternmost portion of South America, which separates the humid coast from a semi-arid continent (Fig. 1). This ecosystem encompasses many habitats, such as the main channel (average depth of 6 m ±4m), tidal creeks surrounded by a dense mangrove forest and dissipative sandy beaches in the mouth of the estuary (BARLETTA; COSTA, 2009). The estuary is under influence of a semi-diurnal meso-tidal regime, with a tidal range of up to 2.5 m. The ecosystem is spatially divided into upper, middle and lower estuary, based on salinity gradient and geomorphology (BARLETTA; COSTA, 2009) (Fig. 1).

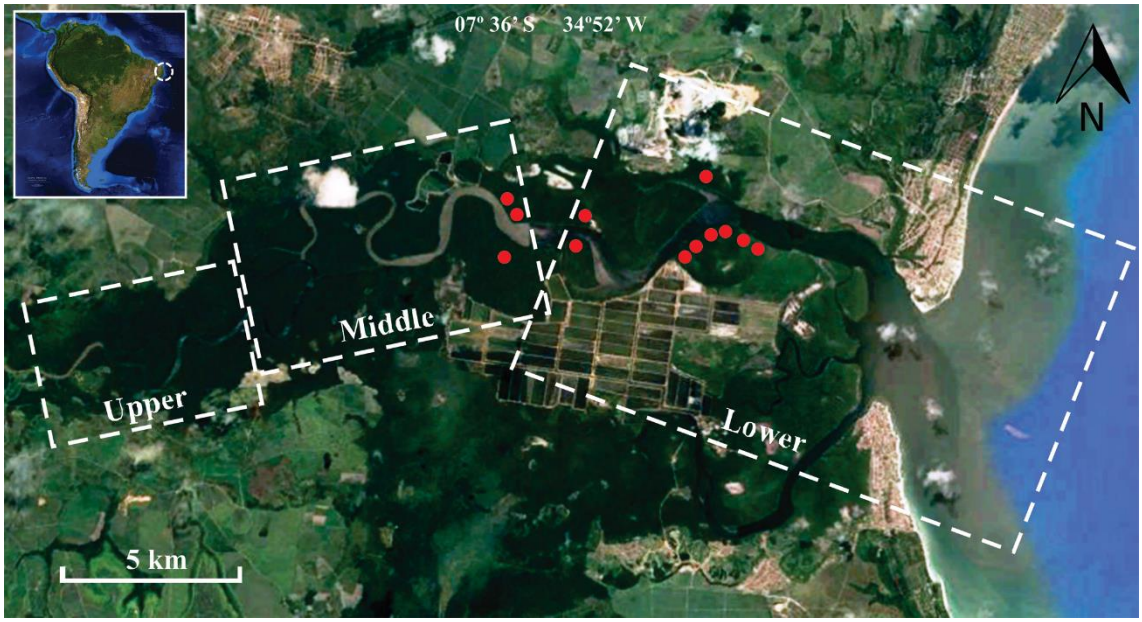

Figure 1 - Studied habitats in the Goiana Estuary, the mangrove creeks ● and the upper, middle and lower estuaries.

The climate is tropical with an average annual air temperature of 27 °C. Although temperature varies little around the year, well-defined dry and rainy periods are the main temporal influences. Variability in rainfall defines four seasons: early dry (September to November), late dry (December to February), early rainy (March to May) and late rainy (June to August) (BARLETTA; COSTA, 2009). During the dry season, freshwater input into the estuary is very low, but in the late rainy season, continental runoff increases. These hydrodynamic changes are responsible for much of the seasonal abiotic and biotic variability (FERREIRA et al., 2016).

Between 450 and 1,000 families rely on the natural resources of the Goiana Estuary for their traditional livelihoods (BARLETTA; COSTA, 2009). Villages and fishers' settlements have developed small-scale economic and subsistence activities throughout the estuary. In the upper estuary, common activities include sand mining and family farming. In the lower estuary, subsistence and commercial fishing of target fishes, crabs, prawns and oyster, and exploitation of shellfish (*Anomalocardia flexuosa* and *Tagelus plebeius*) are key economic activities (BARLETTA; COSTA, 2009). Moreover, the urban area of Goiana city is located about 5 km upstream from the upper estuary, and is responsible for the unregulated dumping of domestic and industrial effluents and solid wastes of ~79,000 inhabitants living along the river margins (BARLETTA; COSTA, 2009).

In the Goiana Estuary, contamination by plastic debris and microplastics (< 5 mm) is a known problem in the main channel and tidal creeks of the mangrove forest (IVAR DO SUL et al., 2014; LIMA et al., 2016; LIMA; BARLETTA; COSTA, 2015; LIMA; COSTA; BARLETTA, 2014). Demersal fishes inhabiting this system were reported to ingest microfibers, such as fishing lines and nylon nets derived from fishing activities (DANTAS; BARLETTA; COSTA, 2012; FERREIRA et al., 2016; POSSATTO et al., 2011; RAMOS; BARLETTA; COSTA, 2012).

The system is under the protection of Federal agencies. However, the Marine Protection Area of the type extractive reserve Acaú-Goiana (~6,700 ha), created in 2007, still does not have consolidated management plans to guarantee the sustainable use of fisheries resources, or to preserve the system in order to secure livelihoods (BARLETTA; COSTA, 2009; GUEBERT; BARLETTA; COSTA, 2013).

Fish sampling

To understand the spatio-temporal patterns of contamination throughout the life cycle of *C. acoupa*, different ontogenetic phases were sampled in the main estuarine habitats (main channel, mangrove creeks and coastal area) in different seasons. Environmental data from the surface and bottom waters were recorded before each sampling, including salinity, temperature, dissolved oxygen and water clarity (Secchi depth). Rainfall data were compiled from the Brazilian National Institute of Meteorology (INMET, 2014).

Six replicates from each of the three areas within the estuarine main channel (upper, middle, lower) were collected monthly between December of 2005 and November of 2006, using an otter trawl (Supplementary Material). Furthermore, the same sampling protocol was used during the late dry and late rainy seasons (3 months each), between December of 2006 and August of 2008 (DANTAS et al., 2010).

Fishes were also sampled in mangrove creeks of the lower estuary (three replicates) between April and May of 2008, using a fyke net (RAMOS et al., 2011) (Supplementary Material). Fishes caught from the mangrove creeks provided complementary data for analyses of microplastics in the lower estuary.

Additionally, monthly samples were obtained between 2013 and 2015 from the artisanal Goiana fishery fleet, located on the coastline adjacent to the estuarine mouth (lower estuary), from where all adults of *C. acoupa* were captured.

Sample processing

For the digestive tract analysis, some precautions were taken to avoid contamination of samples with microplastics from other sources, such as the use of 100% cotton lab coats and disposable latex gloves. Moreover, laboratory instruments (Petri dishes, scalpels, scissors and tweezers) were washed in distilled water, oven dried and checked for contamination before use.

Individuals were divided into three ontogenetic phases: juveniles (1.4–22 cm), sub-adults (22–34 cm) and adults (> 34 cm) (FERREIRA et al., 2016). Specimens of *C. acoupa* were eviscerated and contents were removed from the stomach and intestine. Subsequently, the empty stomachs and intestines were washed in distilled water to confirm that all contents were extracted.

Visual identification was made in a Petri dish. The contents were sorted using a stereomicroscope, and microplastics were separated from natural food items. Food items were counted, weighed and grouped into ecologic/taxonomic categories (demersal fishes, pelagic fishes, macrocrustaceans, microcrustaceans, worms and organic matter) (Table S1).

Items suspected of being microplastics were oven dried (70 °C) for 48 h, and filaments that shrivelled through water loss were categorised as organic matter. Some characteristics were taken into account to confirm that particles were actually microplastics, such as shape and physical consistency (not easily cut or broken). After confirmation, microplastics were photographed, measured, counted, weighted (analytical balance), and classified according to shape, size and colour. The classification by type and colour of microplastics was used to detect possible different input sources in the estuary. Then, the index of relative importance (I_{RI}) was applied to both food items and microplastics (Pinkas, 1971) (Supplementary Material).

Not all ontogenetic phases of *C. acoupa* were present in all estuarine habitats across all seasons. Juveniles were absent during the entire dry season and in the early rainy season in the lower estuary. Sub-adults were absent during the early dry and late rainy seasons in the upper estuary and during the late dry and early rainy season in the middle and lower estuaries. Adults were not caught in the upper and middle estuary throughout the year.

Data analysis

The Box-Cox transformation was applied in all data (BOX; COX, 1964). To test for the homogeneity of variances and the goodness of fit to a Gaussian distribution, the Levene and Kolmogorov-Smirnov tests were applied respectively (LEVENE, 1960; UNDERWOOD, 1997). A three-way analysis of variance was used to determinate whether the number and

mass of ingested items (microplastics and food items) differed seasonally, spatially or through ontogeny ($\alpha \leq 0.05$). Whenever significant differences were observed, the post hoc test of Bonferroni was applied to detect the sources of variance (QUINN; KEOUGH, 2002).

The Canonical Correspondence Analysis (CCA) was performed to investigate ecological interactions between the environmental data, as well as microplastic distribution in the water column (independent variables), and the I_{RI} values of ingested microplastics as well as type of prey (dependent variables) (PALMER, 1993; TER BRAAK; SMILAUER, 2002).

RESULTS

General patterns of plastic ingestion

In this study, 552 individuals of *C. acoupa* were analysed. Among them, 51% of guts were contaminated by plastic particles. The average number of particles ingested (3.03 ± 4.06 particles per digestive tract) varied greatly, ranging from 0 to 63 particles per individual (Table 1).

Table 1. Number (min-max) of filaments ingested by *C. acoupa* per area, season and ontogenetic phase. (-) no capture.

	Upper				**Middle**				**Lower**			
	ED	**LD**	**ER**	**LR**	**ED**	**LD**	**ER**	**LR**	**ED**	**LD**	**ER**	**LR**
Juvenile	0-6	0-5	0-6	0-7	2-3	0-2	0-7	0-5		-	-	0-15
Sub-adult	0	0-14	1-7	-	2	-	-	0-4	5-11	-	-	0-5
Adult	-	-	-	-	-	-	-	-	0-20	0-30	0-63	0-5

In total, 1,073 plastic particles were recorded. The vast majority, 99.9%, were plastic filaments, with only a very small proportion, < 0.01 percentage, of hard microplastics (2 particles). Of these filaments, 15 were categorised as mesoplastics (> 5 mm) and 1056 as microplastics (< 5 mm), which were divided into five categories according to colour: blue (44.6%), purple (19.8%), black (13.4%), red (10.0%) and white (12.2%) (Fig. 2).

Due to the low number of mesoplastics filaments and hard microplastics, their ingestion rates were not included in the following analyses. Filaments (microplastics) were ingested by all ontogenetic phases in all estuarine habitats and seasons, independent of the colour.

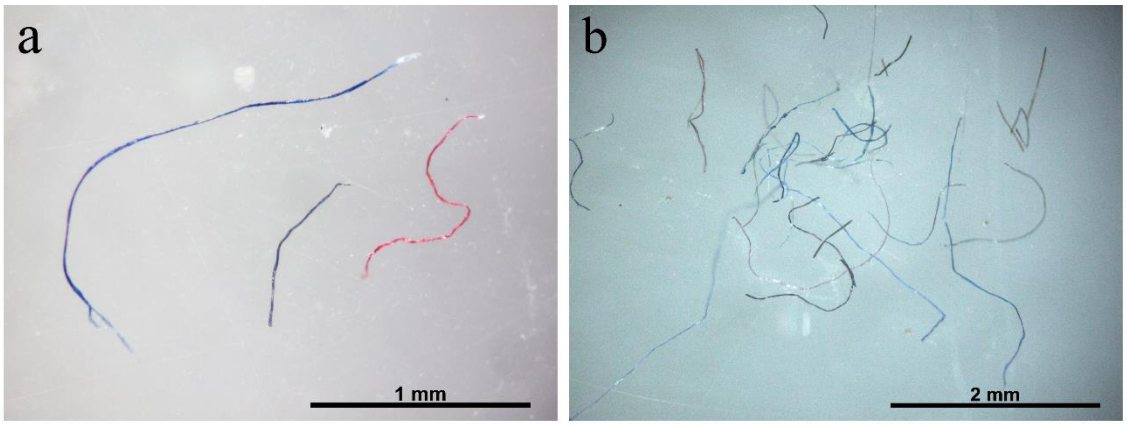

Figure 2 - Contamination level and examples of different colours of filaments found in the gut contents of *C. acoupa*. In (a) it is possible to identify different colours of microplastics found in the gut content of an adult *C. acoupa* (zoom: 50x), and in (b) it is shown a high contamination level registered in a single specimen (zoom: 20x).

Spatio-temporal patterns of filament ingestion by different ontogenetic pha**ses**

There was a significant three-way interaction between the factors season, area, and Ontogeny, highlighting complex patterns of microplastic ingestion (ANOVA; Fig. 3 and 4: Table S2 and S3).

Independent of colour, the average length of ingested filaments was longer in the innermost portions of the estuary; the middle (1.70 mm ± 0.76) and upper estuary (1.75 mm ± 0.87) ($p < 0.05$) (F=13.99) (Table 2 and S2). Juveniles (1.70 mm ± 0.83) and sub-adults (1.95 mm ± 0.35) also ingested longer filaments ($p < 0.05$) (F=68.75) (Table 2 and S2). Shorter filaments were ingested in the late rainy season (1.50 mm ± 0.75) (Table 2).

Juveniles ingested the longest filaments in all estuarine habitats and seasons (≥ 1.50 mm), with the exception of the middle estuary, during the late dry season (1.12 mm ± 0.51). A peak was observed for juveniles in the upper estuary, during the late dry season (2.29 mm ± 0.36) ($p < 0.05$) (F=8.34) (Fig. 3 and Table S2). The length of filaments ingested by sub-adults differed greatly throughout the estuary, ranging from 1.09 mm (± 0.77) in the middle estuary, during the early dry season to 1.94 mm (± 0.62) in the upper estuary, during the late dry season (Fig. 3). Adults ingested the shortest filaments (< 1.50 mm), except for the lower estuary, during the late dry season (1.59 mm ± 0.21) (Fig. 3).

According to length of the different colours of filaments, blue and purple filaments were similar lengths 1.53 mm (± 0.04) and 1.43 mm (± 0.07), respectively (Table S4). White (2.28 mm ±0.13) and black (1.86 mm ± 0.12) were the longest filaments ingested, and red were the shortest (1 mm ± 0.09) (Table S4).

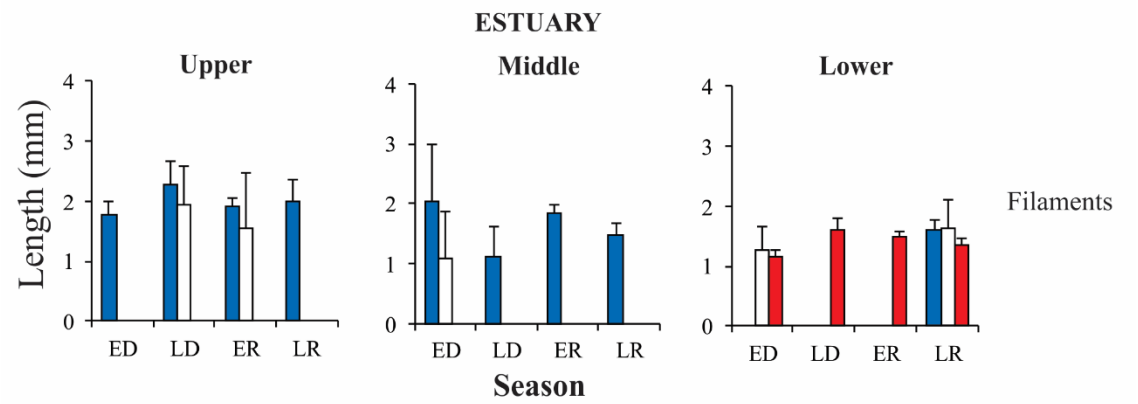

Figure 3 - Mean (± SE) length of filaments ingested by *C. acoupa* in the Goiana Estuary, according to factors area (upper, middle and lower), season [ED (early dry); LD (late dry); ER (early rainy); LR (late rainy)] and ontogeny (juveniles ■, sub-adults □ and adults ■).

Blue filaments were proportionally the most ingested particles by all ontogenetic phases during the entire annual cycle in all estuarine reaches (> 40%), followed by purple filaments (> 17%) (Table 2 and S4). In the upper and middle estuarine habitats, black and white filaments were proportionally the third most ingested particle (> 14%) (Table 2 and S4). Whereas in the lower estuary, the third most ingested filaments were red (13.7%) (Table 2 and S4). The third most ingested filaments by juveniles were in equal proportions black and white (15%), for sub-adults, white (15.7%), and for adults, red (15%). During the early and late dry seasons, red filaments were also the third most ingested (14.9% and 13.2%, respectively) (Table 2).

The highest ingestion of filaments in all colours was by adults in the lower estuary, during the early rainy season ($p < 0.05$) [blue filaments (F=4.72); purple filaments (F=2.72); black filaments (F=2.11); red filaments (F=4.27); white filaments (F=3.82)] (Fig. 4, Table S3 and S4). Interactions were also significant for sub-adults in the lower estuary, during the early dry season, but only for the black filaments ($p < 0.05$) [black filaments (F=2.11)] (Fig. 4 and Table S3).

Table 2
Average (±se) number of the different colours and lengths (independent of colour) of filaments ingested by *C. acoupa* per area, season and ontogenetic phase.

	Number of filaments													
	Blue filament		Purple filament		Black filament		Red filament		White filament		Total filaments	Length (mm)
Upper	0.42 (±0.03)	41.2%	0.24 (±0.02)	23.5%	0.15 (±0.01)	14.7%	0.07 (±0.01)	6.9%	0.14 (±0.01)	13.7%	1.01 (±0.07)	1.75 (±0.87)
Middle	0.46 (±0.02)	42.2%	0.24 (±0.01)	22.0%	0.16 (±0.01)	14.7%	0.06 (±0.01)	5.5%	0.17 (±0.01)	15.6%	1.09 (±0.07)	1.70 (±0.76)
Lower	2.63 (±0.25)	48.3%	1.01 (±0.09)	18.5%	0.59 (±0.06)	10.8%	0.75 (±0.07)	13.8%	0.47 (±0.04)	8.6%	5.41 (±0.52)	1.53 (±0.76)
Juvenile	0.48 (±0.04)	42.5%	0.24 (±0.03)	21.2%	0.17 (±0.02)	15.0%	0.07 (±0.01)	6.2%	0.17 (±0.02)	15.0%	1.14 (±0.08)	1.70 (±0.83)
Sub-adult	0.96 (±0.22)	47.1%	0.36 (±0.11)	17.6%	0.16 (±0.09)	7.8%	0.24 (±0.10)	11.8%	0.32 (±0.12)	15.7%	2 (±0.45)	1.95 (±0.35)
Adult	5.31 (±1.23)	49.4%	2.09 (±0.51)	19.5%	1.09 (±0.32)	10.1%	1.61 (±0.39)	15.0%	0.64 (±0.13)	6.0%	10.76 (±2.45)	1.66 (±0.51)
Early Dry	0.93 (±0.20)	44.9%	0.45 (±0.16)	21.7%	0.24 (±0.11)	11.6%	0.31 (±0.12)	15.0%	0.14 (±0.06)	6.8%	2.07 (±0.40)	1.70 (±0.74)
Late Dry	1.01 (±0.30)	43.2%	0.42 (±0.12)	17.9%	0.36 (±0.11)	15.4%	0.31 (±0.14)	13.2%	0.24 (±0.07)	10.3%	2.37 (±0.68)	1.95 (±0.94)
Early Rainy	1.03 (±0.20)	47.9%	0.45 (±0.08)	20.9%	0.25 (±0.05)	11.6%	0.21 (±0.06)	9.8%	0.21 (±0.03)	9.8%	2.16 (±0.39)	1.72 (±0.79)
Late Rainy	0.56 (±0.08)	42.4%	0.27 (±0.05)	20.5%	0.15 (±0.04)	11.4%	0.12 (±0.03)	9.1%	0.22 (±0.05)	16.7%	1.34 (±0.17)	1.50 (±0.75)

Blue filaments were by far the most frequently ingested microplastic (Table S4). This colour was ingested equally by juveniles in all estuarine habitats and seasons (< 0.5 filament per digestive tract), with the exception of the lower estuary, during the late rainy season, when juveniles ingested more blue filaments (0.92 filament ± 0.02) (Fig. 4). The sub-adults displayed higher ingestion rates of blue filaments than the juveniles, and had two peaks of ingestion: in the upper estuary, during the early rainy season (2 filaments ± 0.24) and in the lower estuary, during the early dry season (2 filaments ± 0) (Fig. 4). The adults were the ontogenetic phase with the highest ingestions rates of blue filaments, with a peak in the late dry (3.5 filaments ± 0.34) and early rainy (7.6 filaments ± 0.34) ($p < 0.05$) seasons in the lower estuary (Fig. 4 and Table S3).

Purple filaments were equally (< 0.35 filaments) ingested by juveniles in all estuarine habitats (Fig. 4). Sub-adults ingested, on average, more purple filaments in the middle estuary, during the early dry season (1 filament ± 0) and in the lower estuary, during the early dry season (1 filament ± 0) (Fig. 4). Adults had higher ingestion rates of purple filaments than other ontogenetic stages and showed a peak of ingestion in the lower estuary, during the early rainy season (2.7 filaments ± 0.14) ($p < 0.05$) (Fig. 4 and Table S3).

Black filaments were ingested by juveniles, during all seasons, in low numbers (< 0.4 filament), except in the middle estuary, during the early dry season, when peak numbers were recorded (1 filament ± 0.33) (Fig. 4). For the sub-adults, black filaments, were only ingested in the lower estuary, with a peak during the early dry season (2 filaments ± 0) which was greater than the ingestion rates of adults ($p < 0.05$) (Fig. 4 and Table S3). Adults showed low ingestion rates in the lower estuary, during the early dry season (0.12 filament ± 0.03), but two peaks, one in the late dry (1.08 filaments ± 0.11) and the other in the early rainy season (1.22 ± 0.07) were observed in the lower estuary (Fig. 4 and Table S3).

Few red filaments were ingested by juveniles in all estuarine habitats and seasons (< 0.15 filament), except for the upper estuary, during the early dry season (0.28 filament ± 0.04) (Fig. 4 and Table S4). Sub-adults ingested red filaments in much lower rates in the upper estuary (< 0.3 filament) than in the lower estuary (> 0.5 filament) (Fig. 4). The adults in the lower estuary, had the greatest variation in ingestion of red filaments, ranging from none in the late rainy season to 2.3 filaments (± 0.15), during the early rainy season ($p < 0.05$) (Fig. 4, Table S3 and S4).

White filaments were more ingested by juveniles in the lower (> 0.3 filament) and middle (> 0.25 filament) estuarine habitats than in the upper estuary (< 0.15 filament) (Fig. 4).

A peak in the middle estuary, during the early rainy season, was recorded for juveniles (0.66 filament ± 0.19) (Fig. 4). Ingestion rates of sub-adults varied greatly from 0.2 filaments (± 0.11) in the upper estuary, during the late dry season to 1.0 filament (± 0.20) in the upper estuary, during the early rainy season, and 1.0 filament (± 0.35) in the lower estuary during the early dry season) (Fig. 4). The ingestion of white filaments by adults of the lower estuary varied greatly, ranging from none recorded in the late rainy season to a peak of 0.94 filament (± 0.04) in the early rainy season ($p < 0.05$) (Fig. 4 and Table S3).

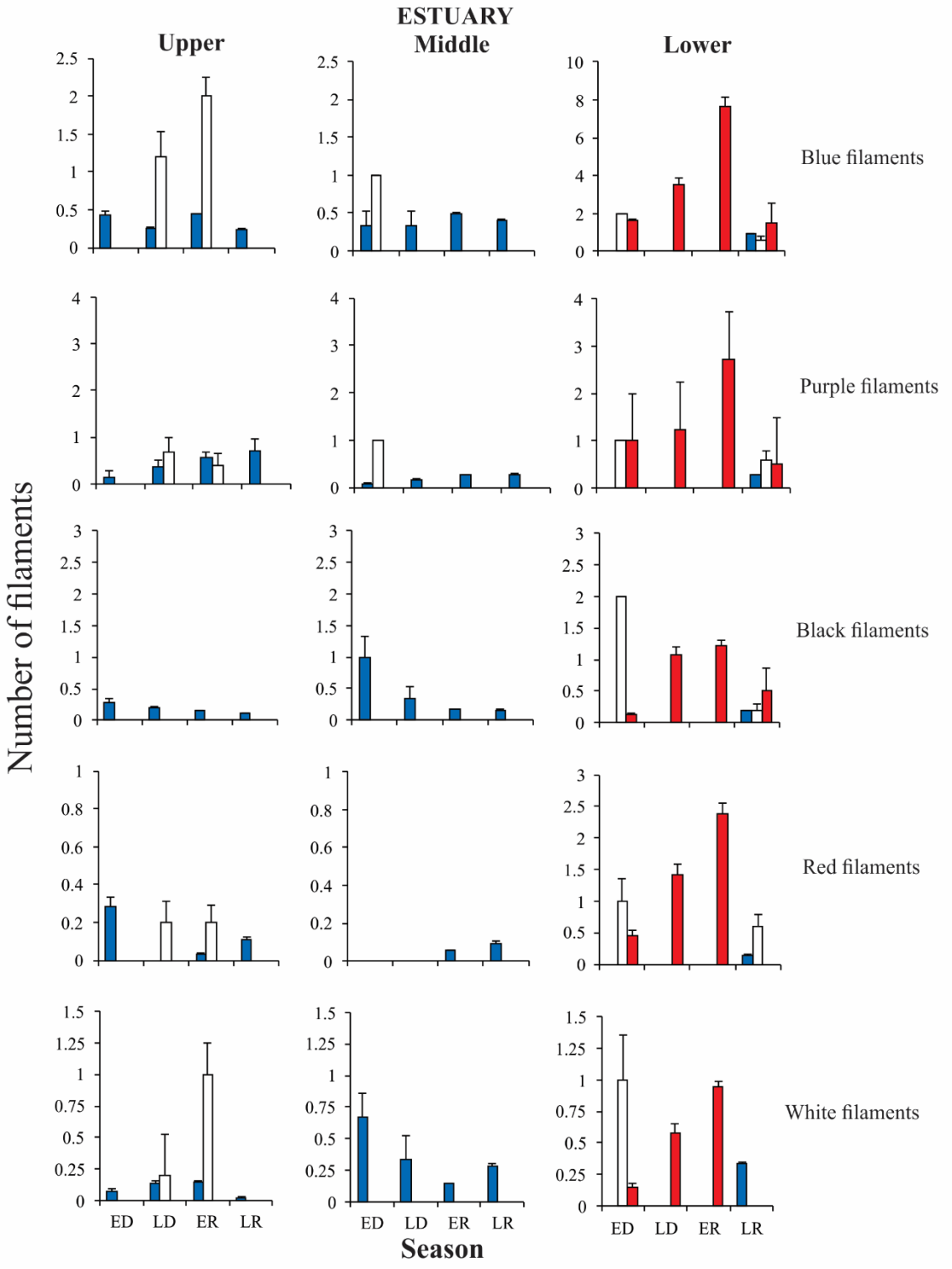

Figure 4 - Mean (± SE) number of different colours ingested by *C. acoupa* in the Goiana Estuary, according to factors area (upper, middle and lower), season [ED (early dry); LD (late dry); ER (early rainy); LR (late rainy)] and ontogeny (juveniles ■, sub-adults □ and adults ■).

Spatio-temporal patterns of food items ingestion by different ontogenetic phases

Food items were grouped into six ecological/taxonomic categories (Table S2) and analysed by weight; thus, accounting for the fact that heavier prey items provide more energy. The feeding habit of juveniles consisted mainly of prey from lower trophic levels, such as microcrustaceans from the zooplankton (Fig. 5 and Table S5), the utilisation of which varied through interacting factors (Fig. 5). The highest ingestion of microcrustaceans by juveniles occurred during the early rainy season in the upper (3.52 mg ± 0.12) and middle (3.02 mg ± 0.14) estuarine habitats ($F=4.58$; $p < 0.05$) (Fig. 5 and Table S5).

Sub-adults had an ontogenetic dietary shift, from opportunistic to piscivorous, preying mainly on higher trophic levels (Fig. 5). This life-history stage ingested mainly worms in the upper estuary, and demersal and pelagic fishes in the middle and lower estuarine habitats. However, the most important item for sub-adults, was demersal fishes (17,489.7 mg ± 11,330.0) in the lower estuary, during the early dry season ($F=4.58$; $p < 0.05$) (Fig. 5 and Table S5).

Adults are mainly piscivorous, preying on both demersal and pelagic fishes but also macrocrustaceans. The highest ingestion of demersal fish was in the lower estuary, during the late dry (11,650.74 mg ± 1,094.6) and early rainy (11,824.5 mg ± 748.0) seasons ($F=4.58$; $p < 0.05$) (Fig. 5 and Table S5). In addition, the highest ingestion of macrocrustaceans was in lower estuary, during the early dry (569.4 mg ± 125.3) and early rainy (336.92 mg ± 31.9) seasons ($F=8.06$; $p < 0.05$) (Fig. 5 and Table S5).

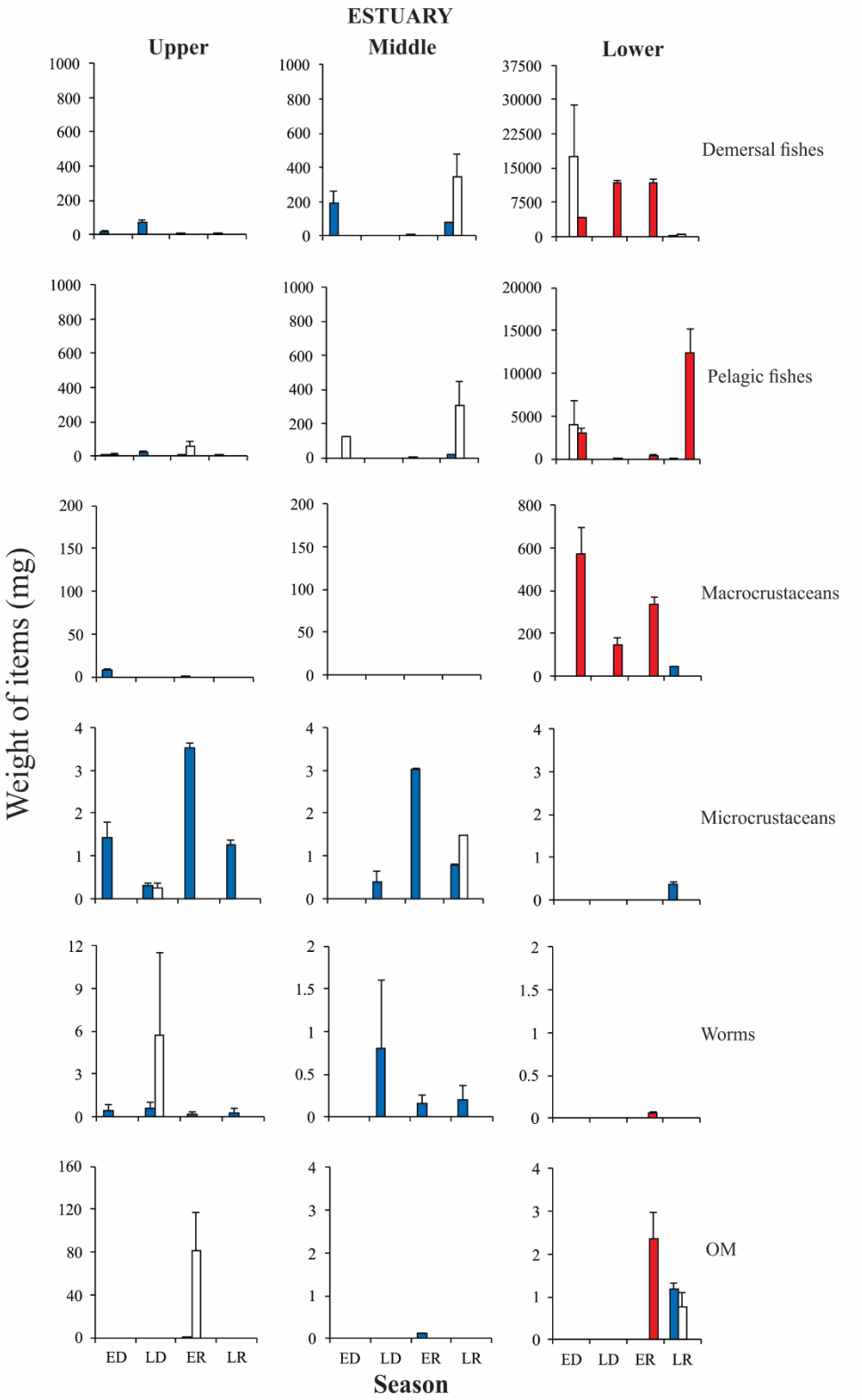

Figure 5 - Mean (± SE) weight of food groups ingested by *C. acoupa* in the Goiana Estuary, according to factors area (upper, middle and lower), season [ED (early dry); LD (late dry); ER (early rainy); LR (late rainy)] and ontogeny (juveniles ■, sub-adults □ and adults ■).

Influence of environmental parameters on patterns of filaments and food items ingestion

The CCA indicates correlations between the relative importance of different colours of filaments ingested and food items groups with the environmental variables (Fig. 6 and Table S6). The first axis represented the salinity gradient across the estuary and explained 55.2% of the variance; this axis was negatively correlated with dissolved oxygen ($p < 0.05$), salinity and Secchi depth ($p > 0.05$) (Fig. 6 and Table S6). The second axis represented the seasonality of the estuary, although not significantly, and explained 24.8% of the variance; this axis was positively correlated with temperature and negatively correlated with rainfall ($p > 0.05$) (Fig. 6 and Table S6).

All colours of plastic filaments (except white), demersal fishes and macrocrustaceans were positively correlated with the occurrence of *C. acoupa* adults in the lower estuary, corroborating the results of ANOVA (Table S3 and S4). Moreover, all colours of plastic filaments were negatively correlated with rainfall and positively with higher temperatures, emphasising the higher ingestion rates during the early and late dry seasons (Fig. 6 and Table 2).

Blue filaments were placed near to origin of the first axis (salinity gradient), as a result of the ubiquitous ingestion of blue filaments in all estuarine habitats (Fig. 6, Table 2 and S4). White filaments were the only colour of filaments that were negatively correlated with salinity. Thus, this colour were positively correlated with the innermost portions of the estuary, in the upper and, mostly, the middle estuary. Microcrustaceans displayed a similar trend to the white filaments. However, this food item showed stronger correlations with salinity (negative correlation) and were positively correlated with the upper estuary (Fig. 6).

Red filaments were strongly positively correlated with the most ocean-influenced areas (Fig. 6) of the lower estuary, during the early dry and late dry seasons (Table 2 and S4). Moreover, red filaments showed a similar trend to macrocrustaceans and demersal fishes, being associated with the adults and sub-adults of the lower estuary, during dry seasons (Fig. 6).

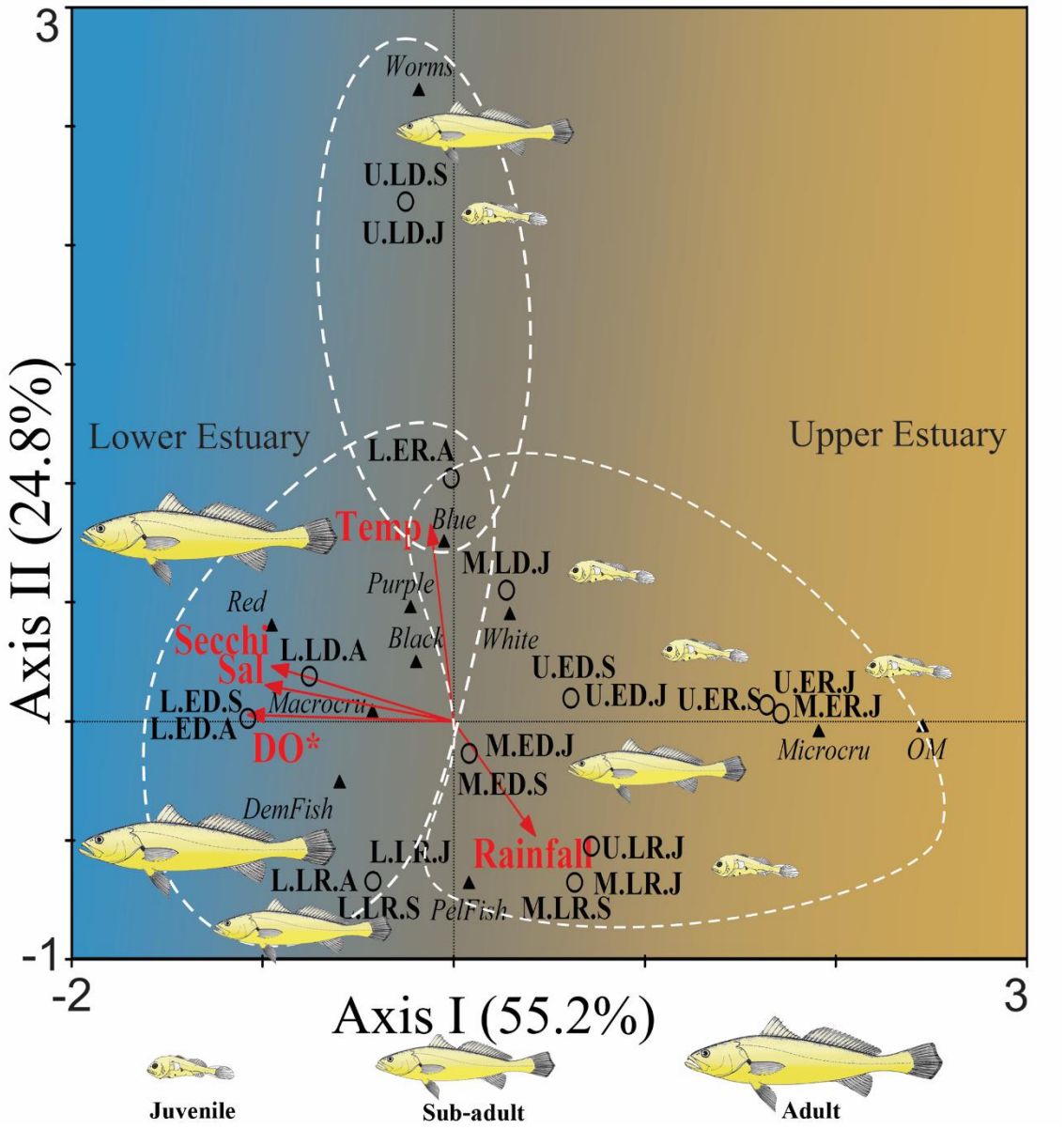

Figure 6 - Canonical Correspondence Analysis (CCA) for correlations between the different colours of filaments and food items groups with the environmental variables. Arrows represents the environmental parameters [Temp (water temperature); Secchi (Secchi depth); Sal (salinity); DO (dissolved oxygen); Rainfall] (*p < 0.05). Triangles (▲) represents colours of filaments and food items [Blue (blue filaments); Purple (purple filaments); Black (black filaments); Red (red filaments); White (white filaments); DemFish (demersal fishes); PelFish (pelagic fishes); Macrocu (macrocrustacens); Microcru (microcrustaceans); Worms (polychaeta worms); OM (organic matter)]. Circles (○) represents the interaction of factors, area [U (upper); M (middle); L (lower)], season [ED (early dry); LD (late dry); ER (early rainy); LR (late rainy)] and ontogeny [J (juveniles); S (sub-adults); A (adults)].

DISCUSSION

The ubiquitous occurrence of microplastics in aquatic ecosystems is of great concern, especially because the extensive use in fisheries, exponential increase in overall production and, consequently, in the improper disposal practices over the last century gives little prospect for improvement (BLABER, 2012; COSTA; BARLETTA, 2015). Another concern is that studies have reported that the amount of microplastics are comparable and sometimes surpass the abundance of zooplanktonic organisms in regional coastal zones (LIMA; BARLETTA; COSTA, 2015) or even in open seas areas (COLLIGNON et al., 2012; LIMA; BARLETTA; COSTA, 2016). It means that the chances of interactions between this class of pollutants and a variety of species are occurring, mostly through ingestion of these particles (BOERGER et al., 2010; FERREIRA et al., 2016; LUSHER et al., 2017; RAMOS; BARLETTA; COSTA, 2012). Fish are among the most studied group due to their importance to humans as both subsistence and economic food resources (SANTILLO; MILLER; JOHNSTON, 2017). However, knowledge of how the ingestion of microplastics is influencing the life cycle and ecological behaviour of fish species is still poorly understood. The Acoupa weakfish is a keystone species for the study of microplastic contamination because, as for many other species, it is a top predator of economic importance with a complex pattern of coastal and estuarine use throughout its life cycle (FERREIRA et al., 2016).

At least four types of microplastics are found in the Goiana Estuary, and at least two of them are directly linked to the fishing activity (LIMA; COSTA; BARLETTA, 2014). Although the most common and frequent types in the water column are soft and hard microplastics (LIMA; COSTA; BARLETTA, 2014), little or no ingestion of these types were recorded in comparison to filaments. Filaments were the most widespread type of microplastic ingested by *C. acoupa* (> 99%), the typical pattern described for many other demersal fish in other locations (BOERGER et al., 2010; DANTAS; BARLETTA; COSTA, 2012; LUSHER et al., 2016). The high intake of filaments by demersal fish most likely occurs due to the rapid sinking of this type of microplastic (LIMA; COSTA; BARLETTA, 2014), making it easily available to be ingested by accident during benthic foraging (RAMOS; BARLETTA; COSTA, 2012; VENDEL et al., 2017). Additionally, filaments may resemble natural food items, such as zooplankton (amphipods and copepods) and polychaetes (THOMPSON et al., 2004), resulting in them being preyed upon by mistake. This would mostly occur in juveniles and sub-adults of *C. acoupa* that prey on these food items.

Insights into the length of filaments ingested and contamination sources

Studies into the source of microplastic contamination have focussed on the chemical composition of particles, but few studies have been able to draw conclusions because of the ubiquitous occurrence of plastics in every aquatic habitat (IVAR DO SUL; COSTA, 2014). The use of fishes as bioindicators of microplastic contamination and sources can advance our knowledge through detailed sampling across spatial and seasonal scales (COSTA; BARLETTA, 2015; LUSHER et al., 2017). For the Goiana Estuary, there is a clear pattern of longer filament ingestion in the innermost estuarine habitats, where only juveniles and sub-adults of *C. acupa* are found. In addition, a significant peak of ingestion of longer filaments was detected in the upper estuary during the late rainy season. According to Lima et al. (2014), the salt wedge of the estuary function as a barrier accumulating microplastics upstream near the riverine area during the driest season, such as that observed in the Río de La Plata Estuary (ACHA et al., 2003). Filaments closer to the source of contamination, have had less time for weathering and, thus, ingestion of longer filaments near the river basin suggests this is closer to the sources of contamination (BROWNE; GALLOWAY; THOMPSON, 2007).

The upstream contamination by longer filaments might be related to riverine inputs; which in turn are likely derived from untreated dumping of sewage and unregulated solid wastes disposal from upstream cites, such as the Goiana city located five kilometres from the head of the estuary (LIMA; COSTA; BARLETTA, 2014). Moreover, smaller filaments in adults inhabiting exclusively the lower estuary might indicate contamination by weathered filaments from the upper estuary; again indicating an upstream source. Indeed, rivers discharges have been recognised as an important source of microplastics to the marine environment (LEBRETON; GREER; BORRERO, 2012; ZHAO et al., 2014).

Filaments ingested in the lower estuary might also have a coastal/oceanic origin, from fishing activity. The coastal environment has stronger hydrodynamic forces, and filaments are exposed to wind, waves and tidal action, causing the breakdown of filaments into smaller particles, due to the stronger weathering process (BROWNE; GALLOWAY; THOMPSON, 2007).

Interactions between filaments ingestion and ecological patterns

Microplastic ingestion of adult *C. acoupa* showed interactions between area of estuary and season and were the most contaminated ontogenetic phase in the lower estuary during the early rainy season, regardless of filament colour. This might be related to the shift in trophic guild of adults to feed almost exclusively on demersal, pelagic fishes, and macrocrustaceans, whose guts could be already contaminated with microplastics (BOERGER et al., 2010; DANTAS; BARLETTA; COSTA, 2012; TAYLOR et al., 2016; VENDEL et al., 2017). Therefore, the chances of trophic transfer (WRIGHT; THOMPSON; GALLOWAY, 2013) of filaments and direct intake due to increased foraging activity make this coastal top predator a biomonitor of environmental quality (FERREIRA et al., 2016).

Adults of *C. acoupa* need to ingest a large amount of prey, both in number and weight, to fulfil their energetic requirements. Hence, higher trophic levels are more susceptible to microplastic contamination due to the trophic transference (ERIKSSON; BURTON, 2003).

Ingestion of filaments by sub-adults was also higher than that reported for juveniles. This might also be attributed to them feeding at a higher trophic level than juveniles. Sub-adults feed, not only on demersal and pelagic fishes, but also on microcrustaceans and polychaete worms, suggesting a shift from an opportunistic juvenile to a piscivorous feeding mode (ELLIOTT et al., 2007) was responsible for sub-adults ingesting the next highest number of filaments.

Moreover, although the highest quantities of all colours of filaments were ingested by adults, blue filaments were by far the most frequent microplastic in the gut contents (Fig. 7). This is also a typical observation, reported worldwide, for several fish species (BOERGER et al., 2010; POSSATTO et al., 2011; LUSHER et al., 2016; VENDEL et al., 2017), further assessments are required to understand why this is a common colour ingested by fishes. However, the ingestion of various colours of filaments in different quantities might be a prime consequence of their availability in the environment. Purple filaments apparently have the same pattern of ingestion to blue filaments. This is possibly the result of this colour having a similar origin as that of the blue filaments, through fishing activities. For the Goiana Estuary it is likely that blue filaments are correlated with peak fishing activities in the rainy season, resulting in large numbers of filaments entering this habitat during the maintenance of ropes and fishing nets, which are commonly blue in colour (POSSATTO et al., 2011). Fishing effort is highest in the lower estuary because of the higher catches of commercially important fishes

and crustaceans (LIMA; COSTA; BARLETTA, 2014). In this estuarine habitat, higher ingestion rates were detected for all ontogenetic stages.

Filaments are ubiquitous in the water column of the Goiana Estuary, but their higher density is observed in the lower estuary during the rainy season as a result of the freshwater runoff to sea (LIMA; COSTA; BARLETTA, 2014). Thus, the higher intake of filaments in the rainy season might also be attributed to an increased availability of this contaminant when runoff increases (LIMA et al., 2014). Additionally, the lower estuary is under the influence of kilometres of mangrove forests and estuarine sandy beaches which have the ability to store and then release microplastics to surrounding habitats (COSTA et al., 2011; RYAN et al., 2009).

Red filaments were more frequently ingested in the lower estuary, during the entire dry season, when salinity was at its highest (FERREIRA et al., 2016) (Fig. 7). The positive correlation of red filaments with more oceanic conditions might indicate a marine source for this colour of contaminant, although the source of red filaments needs further investigation. Furthermore, the higher ingestion of red filaments correlated with the higher consumption of demersal fishes, pelagic fishes and macrocrustaceans by the sub-adult and adult phases in the lower estuary.

On the other hand, white and black filaments were proportionally more ingested in the innermost habitats of the estuary (upper and middle estuaries), by the juvenile and sub-adult phases of *C. acoupa*. In accordance with this pattern, juveniles and sub-adults exhibited opportunistic feeding on microcrustaceans of the zooplankton, especially in the upper and middle estuaries. It could be an indicator that this colour of microplastics are ingested as a result of their similarities in size and colour to zooplanktonic prey, such as amphipods and copepods (GÜVEN et al., 2017), as well as implying an upstream origin for this colours of filaments.

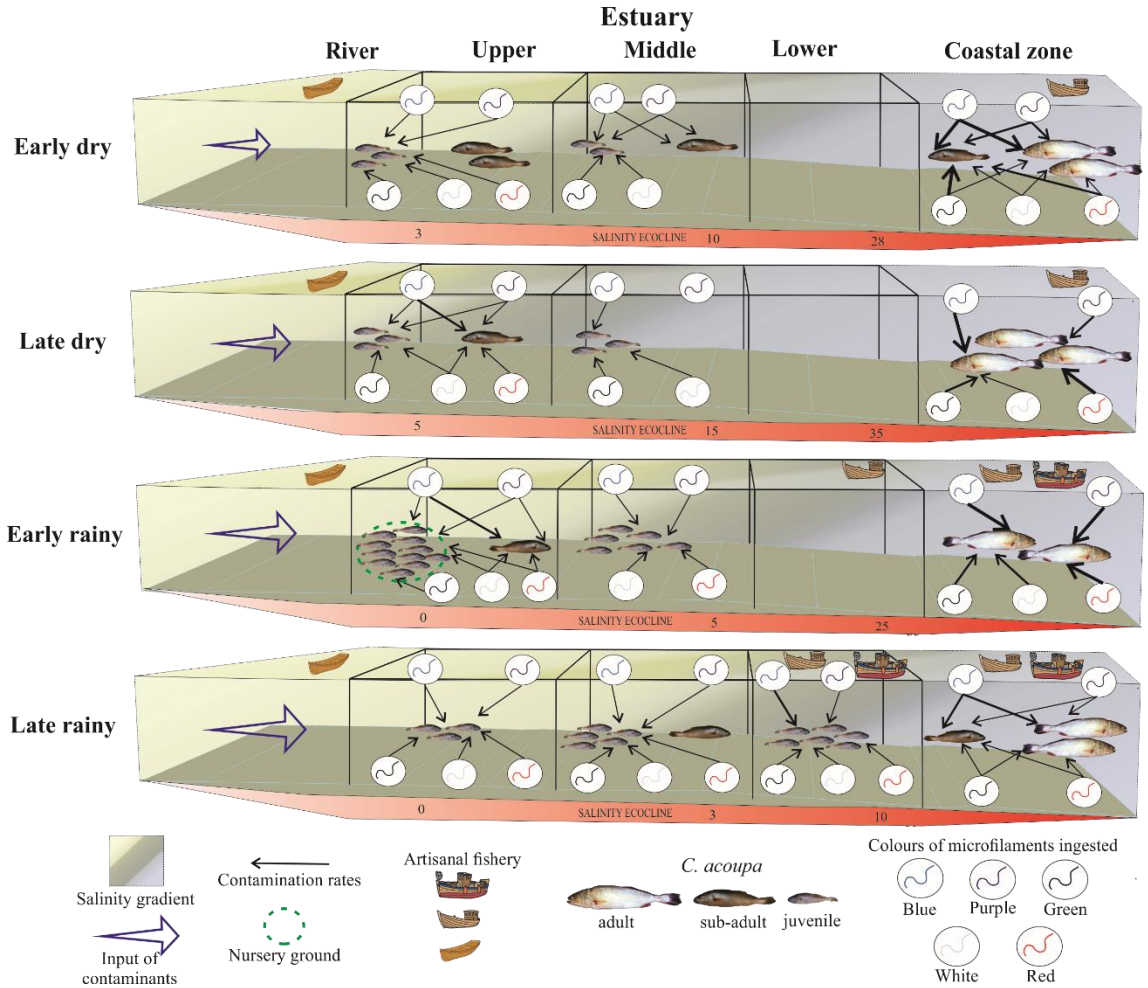

Figure 7 - Conceptual model for the ingestion rates of different colours of microfilaments by *C. acoupa*, regarding different habitats, seasons and ontogenetic phases.

Consequences of microplastic ingestion

Microplastics can be both a physical and chemical hazard to any fish that have ingested them. Impacts can vary from intestinal blockage, which can cause a false sensation of being well fed and induce starvation (MOORE, 2008) to digestive injuries that may decrease the predatory efficiency (TEUTEN et al., 2007). An analogous problem was evinced for planktofagous fish in Southeast Asia, which were highly contaminated by sawdust (BREWER et al., 2001). Microplastics and sawdust may have the same deleterious effects on fishes, reducing growth and reproduction rates, due to the decrease in the nutrient intakes.

Microplastics contain toxic additives and can also be a vector for organic pollutants (ROCHMAN et al., 2013; SANTILLO et al., 2017) due to their capacity to absorb and release pollutants (ROCHMAN et al., 2013; SANTILLO et al., 2017). Once in contact with the intestinal dermis of a contaminated fish, microplastics can potentially lead to bioaccumulation and biomagnification of organic contaminants (ROCHMAN et al., 2013).

The main concern of this study is that the acoupa weakfish is an important fishery resource in the east South American Coast. According to the Fishery and Aquaculture Ministry, *C. acoupa* represented one of the most important catches for both the industrial and artisanal fishery, and together with the whitemouth croaker (*Micropogonias furnieri*) and mullets (*Mugil* spp.) were responsible for ~83,000 tonnes of the marine fishery in 2011 in Brazil (MPA, 2011). The presence of up to 63 filaments in the digestive tract of a single adult *C. acoupa* raises food safety concerns for human populations. All adult *C. acoupa* were collected from the artisanal fishery, which local populations rely on for an important part of their diet. Further evaluations are therefore required to understand how microplastic contamination of fishes might affect human health through transference of toxins (SANTILLO et al., 2017).

CONCLUSIONS

This study found high ingestion rates of filaments by an economically important estuarine top predator, the Acoupa weakfish. The highest ingestion of filaments was recorded for adults inhabiting the lower estuary, independent of filament colour Interactions with spatial, temporal and biological factors and correlations with environmental variables, suggest that the increased intake in adult stages was due to trophic transference in addition to direct ingestion. Studies of spatial and temporal factors affecting the Acoupa weakfish, along the estuarine ecocline, have proven to be a good bioindicators of microplastic sources in the

coastal system; suggesting that longer filaments have a riverine origin and smaller filaments are associated to oceanic inputs.

Ontogenetic shifts in food preferences were consistent with the ingestion of filaments over spatial and temporal scales. From these results, it is suggested that the trophic transfer of microplastics are pronounced when fish shift to a higher trophic level during the ontogenetic development and start to accumulate microplastics. This is particularly true for piscivorous fish, such as the Acoupa weakfish, whose prey are highly mobile and can use several contaminated estuarine habitats.

Moreover, the high contamination levels of many species of finfish and shellfish need to be taken into account as a direct effect of the fishing activity, which is a major source of microplastic contamination to the environment. Thus, the estuarine fauna are especially vulnerable to microplastic contamination, due to the intense fishery exploitation of this ecosystem. Another issue is the microplastic bioaccumulation within human food resources, to improve knowledge further detailed studies are required to link year round levels of microplastic contamination, through the food web and across ontogenetic stages. It is only then that the risk to human health can be properly assessed.

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APÊNDICE 1. Material suplementar referente ao capítulo 3.

SUPPLEMENTARY MATERIAL

High intake rates of microplastics in a Western Atlantic predatory fish, and insights of a direct fishery effect

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Fishing methods

In the main channel of the estuary, fish were sampled using an otter trawl. The net was 8.7 m long, with ground rope of 8.5 m and head hope of 7.1m. The mesh size was 35 mm in the body, 22 mm in the cod end and 5 mm in the cod end cover, trawls lasted for 5 minutes. Sampling was conducted during neap tides in depths between 5 and 10 m.

In the mangrove creeks of the lower estuary, fish were sampled using a fyke net. The net was 35 m in length and 5 m in height. The mesh size was 10 mm, the net was fixed in the entrance of the creeks, during the high tide and retrieved during low tide to collect the fish.

Index of relative importance (I_{RI})

The index of relative importance (I_{RI}) was applied to the different colours of microplastics and food item groups. This index was calculated using the following equation (PINKAS; OLIPHANT; IVERSON, 1971):

$$I_{RI} = \%F_i * (\%N_i + \%M_i)$$

Where, $\%F_i$ represents the frequency of occurrence of a given item, and is the percentage of digestive tracts containing item *i*. The $\%N_i$, represents the composition in number of a given item, and is the number of item *i*, in percentage, of the total number of items in all digestive tracts analysed (HYSLOP, 1980). The $\%M_i$, represents the composition in mass of a given item, and is the mass of item *i*, in percentage, with respect to the total weight of items in all digestive tracts analysed (HYSLOP, 1980).

Table S1
Food items ingested by *C. acoupa* grouped into ecologic/taxonomic categories.

Food items Groups			
Demersal fishes	*Achirus lineatus*	**Pelagic fishes**	*Anchovia clupeoides*
	Bairdiella ronchus		*Cetengraulis edentulus*
	Cathorops spixii		*Opisthonema oglinum*
	Cathorops spixii egg		*Rhinosardinia bahiensis*
	Cynoscion acoupa		
	Diapterus rhombeus	**Macrocrustaceans**	Penaeidae shrimp
	Eleotris pisonis		*Callinectes danae*
	Pomadasys corvinaeformis		Paguridae
	Stellifer brasiliensis		*Anomalocardia flexuosa*
	Stellifer stellifer		*Mytilus* sp.
			Gastropoda
Microcrustaceans	Amphipoda	**Worms**	Syllidae
	Brachyura		Nereidae
	Copepoda		
	Isopoda	**Organic matter**	Seaweed
	Mysidacea		Plant fragments

Table S2

Summary of the ANOVA for the length of plastic filaments (mm), independent of colour ingested by *C. acoupa* in the Goiana Estuary, according to factors area [U (upper); M (middle); L (lower)], season [ED (early dry); LD (late dry); ER (early rainy); LR (late rainy)] and ontogeny [Juv (juveniles); Sub (sub-adults); Adu (adults)]. Bonferroni's test was used to determinate the sources of variances [F (F-values); df (degree of freedom); *p-value*]. (ns: not significant) (*$p < 0.05$).

	Itens in number				
	Factors	**F**	***df***	***p*-value**	***Post-hoc***
Length of filaments	Season	2.10	3	0.10	ns
	Area	13.99	2	0.01	* **U** M L
	Phase	68.75	2	0.01	* **Juv** Sub Adu
	Season *vs*. Area	8.57	6	0.01	ns
	Season *vs*. Phase	8.94	6	0.01	*
	Area *vs.* Phase	32.09	4	0.01	*
	Season *vs.* Area *vs*. Phase	8.34	12	0.01	*

Table S3
Summary of the ANOVA for number of plastic filaments ingested by *C. acoupa* in the Goiana Estuary, according to factors area [U (upper); M (middle); L (lower)], season [ED (early dry); LD (late dry); ER (early rainy); LR (late rainy)] and ontogeny [Juv (juveniles); Sub (sub-adults); Adu (adults)]. Bonferroni's test was used to determinate the sources of variances [F (F-values); df (degree of freedom); *p*-value]. (ns: not significant) (*$p < 0.05$).

	Itens in number				
	Factors	**F**	***df***	***p*-value**	***Post-hoc***
Blue filament	Season	1.73	3	0.16	ns
	Area	6.37	2	0.01	* U M **L**
	Phase	0.83	2	0.44	ns
	Season *vs*. Area	1.87	6	0.09	ns
	Season *vs*. Phase	2.44	6	0.03	*
	Area *vs*. Phase	21.85	4	0.01	*
	Season *vs*. Area *vs*. Phase	4.72	12	0.01	*
Purple filament	Season	1.22	3	0.30	ns
	Area	6.03	2	0.01	* U M **L**
	Phase	1.55	2	0.21	ns
	Season *vs*. Area	0.96	6	0.45	ns
	Season *vs*. Phase	2.84	6	0.01	*
	Area *vs*. Phase	16.71	4	0.01	*
	Season *vs*. Area *vs*. Phase	2.75	12	0.01	*
Black filament	Season	0.57	3	0.64	ns
	Area	2.70	2	0.07	ns
	Phase	5.96	2	0.01	* **Juv** Sub Adu
	Season *vs*. Area	1.45	6	0.20	*
	Season *vs*. Phase	2.42	6	0.02	*
	Area *vs*. Phase	12.03	4	0.01	*
	Season *vs*. Area *vs*. Phase	2.11	12	0.02	*

Table S3 Continued.

		Itens in number			
	Factors	**F**	***df***	***p*-value**	***Post-hoc***
Red filament	Season	0.46	3	0.71	ns
	Area	17.09	2	0.01	* U M **L**
	Phase	3.65	2	0.03	* Juv Sub **Adu**
	Season *vs.* Area	0.22	6	0.97	ns
	Season *vs*. Phase	4.36	6	0.01	*
	Area *vs*. Phase	13.43	4	0.01	*
	Season *vs.* Area *vs*. Phase	4.27	12	0.01	*
White filament	Season	3.15	3	0.03	* ED LD **ER** LR
	Area	1.96	2	0.14	ns
	Phase	3.09	2	0.05	* **Juv** Sub Adu
	Season *vs.* Area	0.89	6	0.50	ns
	Season *vs*. Phase	1.44	6	0.20	ns
	Area *vs*. Phase	10.29	4	0.01	*
	Season *vs.* Area *vs*. Phase	3.82	12	0.01	*

Table S4

Different colours of filaments ingested by *C. acoupa* expressed as percentage, according to factors area [U (upper); M (middle); L (lower)], season [ED (early dry); LD (late dry); ER (early rainy); LR (late rainy)] and ontogeny [Juv (juveniles); Sub (sub-adults); Adu (adults)]. (-) no capture.

Filaments	∑	Cum%	Upper %												Middle %												Lower %												Average Length (± se)
			Early Dry			Late Dry			Early Rainy			Late Rainy			Early Dry			Late Dry			Early Rainy			Late Rainy			Early Dry			Late Dry			Early Rainy			Late Rainy			
			Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	Juv	Sub	Adu	
Blue	471	44.6	37.5	0	-	33.3	60	-	42.4	55.6	-	32.1	-	-	14.3	50	-	25	0	-	45.3	-	-	32.3	0	-	-	40	50	-	-	44.7	-	-	51.3	49.5	30	60	1.53 ± 0.04
Purple	210	64.5	6.3	0	-	22.2	20	-	26.1	11.1	-	35.7	-	-	14.3	50	-	25	0	-	21.4	-	-	19.5	0	-	-	10	31.3	-	-	16.0	-	-	18.2	15.0	30	20	1.43 ± 0.07
Black	140	77.7	25	0	-	25.9	0	-	14.1	0	-	14.3	-	-	42.9	0	-	25	0	-	15.1	-	-	21.1	0	-	-	20	3.1	-	-	13.8	-	-	8.2	10.3	10	20	1.86 ± 0.12
Red	106	87.8	25	0	-	0	10	-	3.3	5.6	-	14.3	-	-	0.0	0	-	0	0	-	5.2	-	-	5.3	0	-	-	10	12.5	-	-	18.1	-	-	16.0	7.5	30	0	1.00 ± 0.09
White	129	100	6.3	0	-	18.5	10	-	14.1	27.8	-	3.6	-	-	28.6	0	-	25	0	-	13.0	-	-	21.8	0	-	-	20	3.1	-	-	7.4	-	-	6.3	17.8	0	0	2.28 ± 0.13
TOTAL	1056		16	0	-	27	10	-	92	18	-	28	-	-	7	2	-	4	0	-	192	-	-	133	0	-	-	10	32	-	-	94	-	-	269	107	10	5	1.57 ± 0.03

Table S5

Summary of the ANOVA for weight of food items groups ingested by *C. acoupa* in the Goiana Estuary, according to factors area [U (upper); M (middle); L (lower)], season [ED (early dry); LD (late dry); ER (early rainy); LR (late rainy)] and ontogeny [Juv (juveniles); Sub (sub-adults); Adu (adults)]. Bonferroni's test was used to determinate the sources of variances [F (F-values); df (degree of freedom); *p*-value]. (ns: not significant) (*$p < 0.05$).

		Itens in number			
	Factors	***F***	***df***	***p*-value**	***Post-hoc***
					*
Demersal Fish	Season	6.72	3	0.01	ED LD ER **LR**
					*
	Area	34.37	2	0.01	U M **L**
					*
	Phase	3.38	2	0.04	Juv **Sub** Adu
	Season *vs.* Area	2.58	6	0.01	*
	Season *vs.* Phase	7.13	6	0.01	*
	Area *vs.* Phase	18.13	4	0.01	*
	Season *vs.* Area *vs.* Phase	4.58	12	0.01	*
					*
Pelagic Fish	Season	3.90	3	0.01	ED LD ER **LR**
	Area	4.08	2	0.02	U M **L**
	Phase	1.83	2	0.16	ns
	Season *vs.* Area	0.73	6	0.63	ns
	Season *vs.* Phase	2.05	6	0.01	*
	Area *vs.* Phase	7.63	4	0.01	*
	Season *vs.* Area *vs.* Phase	1.52	12	0.12	ns
					*
Macrocrustacean	Season	5.31	3	0.01	ED **LD** ER LR
					*
	Area	9.22	2	0.01	U M **L**
					*
	Phase	7.43	2	0.01	Juv **Sub** Adu
	Season *vs.* Area	4.53	6	0.01	*
	Season *vs.* Phase	5.12	6	0.01	*
	Area *vs.* Phase	9.98	4	0.01	*
	Season *vs.* Area *vs.* Phase	8.06	12	0.01	*

Table S5 Continued.

	Itens in number				
	Factors	***F***	***df***	***p*-value**	***Post-hoc***
Microcrustacean	Season	6.47	3	0.01	* **ED** LD ER LR
	Area	15.20	2	0.01	* U M **L**
	Phase	65.26	2	0.01	* **Juv** Sub Adu
	Season *vs.* Area	2.62	6	0.01	*
	Season *vs.* Phase	7.95	6	0.01	*
	Area *vs.* Phase	12.42	4	0.01	*
	Season *vs.* Area *vs.* Phase	2.76	12	0.01	*
Worms	Season	0.35	3	0.78	ns
	Area	1.47	2	0.23	ns
	Phase	6.91	2	0.01	* **Juv** Sub Adu
	Season *vs.* Area	0.94	6	0.47	ns
	Season *vs.* Phase	0.67	6	0.67	ns
	Area *vs.* Phase	3.16	4	0.01	*
	Season *vs.* Area *vs.* Phase	0.53	12	0.89	ns
Organic Matter	Season	2.34	3	0.07	ns
	Area	0.62	2	0.54	ns
	Phase	0.40	2	0.67	ns
	Season *vs.* Area	0.96	6	0.45	ns
	Season *vs.* Phase	0.23	6	0.96	ns
	Area *vs.* Phase	0.25	4	0.90	ns
	Season *vs.* Area *vs.* Phase	1.07	12	0.38	ns

Table S6

Summary of Canonical Correspondence Analysis (CCA) using environmental variables (water temperature, Sechhi depth, salinity, dissolved oxygen and rainfall) and the index of relative importance (I_{RI}) for the colours of plastic filaments and food items groups ingested by *C. acoupa*, according to factors. (* $p < 0.05$).

C. acoupa	Axis 1	Axis 2	*p*-value	
Eigenvalue	0.409	0.184		
Species-environmental correlation	0.790	0.686		
Cumulative % variance of species data	16.9	24.5		
Cumulative % variance of species environmetal relation	55.2	80		
Correlation with environmental variables:				
Rainfall (mm)	0.294	-0.314	0.19	
Water temperature (ºC)	-0.074	0.542	0.35	ns
Salinity	-0.610	0.100	0.19	ns
Dissolved oxygen (mg l^{-1})	-0.666	0.018	0.01	*
Secchi depth (cm)	-0.588	0.151	0.98	ns

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7 CONCLUSÕES

O estuário do rio Goiana apresentou uma grande heterogeneidade entre seus hábitats, ocasionado por um forte gradiente de salinidade formado pelo balanço de massas entre a descarga fluvial e o fluxo de maré oceânico. Como consequência, diversos parâmetros abióticos foram influenciados, como a dissolução de oxigênio das águas estuarinas e a transparência da água, que por sua vez está diretamente associada com a penetração luz nas massas d'água do estuário, que é responsável por regular os processos oxido-redutivos.

Adicionalmente, o ecossistema mostrou uma resposta típica de estuários tropicais em relação a sazonalidade. A principal força responsável por governar o ciclo sazonal foi a pluviometria, que apresentou um marcante padrão distinguindo quatro períodos ao longo do ano. Entre os meses de setembro e novembro o ecossistema registrou índices pluviométricos muito baixos, que caracterizou o período do início da estiagem, nos meses seguintes, (dezembro a fevereiro) foram observados os menores índices pluviométricos da região, definindo o fim da estiagem.

Posteriormente, entre os meses de março e maio a pluviometria sofreu um forte incremento, caracterizando o período do início da chuva. Em seguida, os meses de junho e agosto registraram os maiores índices pluviométricos do ecossistema e caracterizaram o fim do período chuvoso.

A pluviometria é extremamente importante na dinâmica estuarina por influenciar na descarga do rio Goiana e alterar o balanço de massas entre o rio e o mar. O efeito da modificação dessa dinâmica é responsável por alterar o aporte de matéria orgânica no estuário, além da salinidade, que é o parâmetro chave na dinâmica estuarina e repercute diretamente nas demais variáveis ambientais.

A sucessão de espécies, a dinâmica alimentar e os padrões migratórios e reprodutivos da ictiofauna são diretamente associados com a variabilidade ambiental. Desta forma, entender os processos oceanográficos que ocorrem no estuário do rio Goiana é fundamental para descrever a dinâmica ecológica das espécies estuarinas. As espécies avaliadas no estudo (*C. undecimalis, C. mexicanus, C. pectinatus* e *C. acoupa*) são de grande relevância econômica por serem uma das principais espécies alvo da pesca costeira e estuarina no Atlântico Sul ocidental. Além disso, elas têm uma grande importância ecológica por serem caracterizadas como um dos principais predadores de topo dos estuários tropicais do Atlântico ocidental.

Os padrões de movimentação espacial das espécies estão intrinsecamente associados à dinâmica salina do ecossistema. Usualmente os indivíduos adultos de Centropomidae (*C.*

undecimalis, C. mexicanus e *C. pectinatus*) forrageiam nos hábitats externos do estuário do rio Goiana, no estuário inferior e principalmente na zona costeira, independente do período sazonal. Os espécimes adultos das três espécies de Centropomidae são classificadas como piscívoros, se alimentando principalmente de peixes e camarões.

C. undecimalis apresentou o maior nível trófico entre os Centropomidae, predando principalmente peixes demersais (*C. spixii* e *S. stellifer*) e pelágicos (*A. clupeoides, C. edentulus* e *R. bahiensis*). *C. mexicanus* apresentou uma maior amplitude de nincho e um menor nível trófico, quando comparado com *C. undecimalis,* e se alimentou principalmente de peixes pelágicos (*A. clupeoides, C. edentulus* e *R. bahiensis*) e camarões (*Litopaeneus* sp.). Dentre os indivíduos adultos, *C. pectinatus* apresentou o menor nível trófico, predando peixes pelágicos (*A. clupeoides* e *R. bahiensis*) e camarões.

Em relação a guilda funcional, os Centropomidae são classificados como espécies anfídromas e ocasionalmente migram estuário acima para forragear em diferentes hábitats. Entretanto, todo o processo reprodutivo é realizado na região costeira em diferentes períodos sazonais para evitar competição interespecífica. Após a eclosão as larvas utilizam o fluxo de maré para migrar até os canais de maré, localizados no estuário inferior, em busca de proteção dos predadores marinhos e maior disponibilidade de alimento. Os canais de maré são o primeiro hábitat berçário das espécies de Centropomidae, onde as larvas se desenvolvem até o estágio juvenil permanecendo até atingirem maiores dimensões corporais (*C. undecimalis* ≈ 90mm e *C. mexicanus* ≈ 150mm).

Posteriormente, os juvenis migram para o segundo hábitat berçário de forma intervalar para evitar competição interespecífica. *C. undecimalis* (6,49 ±2,89 ind. ha^{-1}) e *C. mexicanus* (4,13 ±1,98 ind. ha^{-1}) no estuário superior, durante o início da estiagem e início da chuva, respectivamente, e *C. pectinatus* possivelmente para rio. Os indivíduos juvenis ocupam principalmente o estuário superior por apresentar uma maior complexidade estrutural, fornecendo áreas de abrigo contra predadores e uma ampla disponibilidade de alimento.

A classificação da guilda trófica dos juvenis das espécies avaliadas é designada como oportunista. Eles apresentam um amplo leque alimentar, ingerindo invertebrados bentônicos, demersais e até peixes de pequenas dimensões. Os juvenis de *C. undecimalis* predam principalmente Polychaeta e camarões (*Litopaeneus* sp.), os juvenis de *C. mexicanus,* camarões, *A. clupeoides* e Polychaeta e os juvenis de *C. pectinatus* têm como matriz alimentar principalmente camarões, Amphipoda e Polychaeta.

Durante os períodos chuvosos, com a consequente amortização dos parâmetros ambientais, os juvenis também ocorrem nos hábitats externos do estuário. *C. undecimalis* e *C. mexicanus* migram para o estuário inferior, onde incluem uma maior parcela de peixes demersais e principalmente pelágicos (*A. clupeoides*) na sua dieta.

Os indivíduos subadultos de Centropomidae possuem um padrão de distribuição similar aos juvenis, ocupando principalmente os habitats internos do estuário (estuário superior e intermediário) com um hábito oportunista, predando Polychaeta, camarões, Amphipoda e peixes pelágicos. Entretanto, nos períodos chuvosos eles apresentam uma maior tendência a migrar para o estuário inferior e para a zona costeira. Quando os subadultos de *C. undecimalis, C. mexicanus* e *C. pectinatus* migram para os hábitats externos do estuário é observado uma mudança na sua matriz alimentar. Eles passam a restringir seu leque alimentar em presas de maiores níveis tróficos, como *A. clupeoides* e *R. bahiensis.* Apesar de permanecerem sendo classificados como oportunistas, essa mudança no seu hábito alimentar é um indicativo de transição da sua matriz alimentar para o hábito piscívoro.

Todas as espécies de Centropomidae ingeriram microplásticos e apresentaram elevados níveis de contaminação (*C. undecimalis* 56%, *C. mexicanus* 63% e *C. pectinatus* 50%), quando comparados com outras espécies avaliadas previamente no estuário do rio Goiana e em outros estuários tropicais. Os Centropomidae ingeriram microplásticos ao longo de todo o seu ciclo de vida, em todos os hábitats ocupados, independente do período sazonal avaliado. Os padrões de contaminação demonstraram uma nítida associação com os parâmetros ambientais, que determinam a disponibilidade dos contaminantes. Além de estarem associados com o hábito alimentar das espécies, que influencia na forma de entrada dos contaminantes no organismo.

De forma geral, os espécimes que ocuparam os hábitats externos do estuário apresentaram maiores taxas de contaminação por microplástico. Principalmente os que forrageiam no estuário inferior, por este hábitat apresentar uma maior disponibilidade de microplásticos na coluna d'água em decorrência do efeito de trapeamento gerado pelas massas d'água fluvial e costeira, que resulta em uma concentração de microplásticos e maiores densidades do contaminante na coluna d'água. Além disso, o estuário inferior é uma das áreas prioritárias de exploração da atividade pesqueira que é uma grande fonte de microplásticos para o ambiente aquático.

Os indivíduos adultos de *C. undecimalis* apresentaram altas taxas de contaminação, independente do habitat e estação sazonal avaliada, como uma consequência da sua matriz

alimentar, baseada principalmente em presas de maiores níveis tróficos. Esse tipo de comportamento alimentar potencializa o processo de transferência trófica de microplásticos, que ocorre quando um organismo se alimenta de uma presa que já estava previamente contaminada, de forma que durante o processo de digestão, os microplásticos que estavam no conteúdo estomacal da presa são transferidos para o predador, originando uma fonte adicional de ingestão de microplásticos, além da ingestão direta.

As maiores taxas de contaminação observadas nos adultos de *C. undecimalis* foram registradas no estuário inferior durante o início da estiagem (3,66 ±1,20 part. ind^{-1}). Nesse hábitat e período sazonal específico os adultos de *C. undecimalis* apresentaram uma maior dependência alimentar de *C. spixii* e *S. stellifer*, espécies demersais com um maior nível trófico do que as espécies pelágicas usualmente predadas por *C. undecimalis*, favorecendo o processo de transferência trófica.

Os indivíduos adultos de *C. mexicanus* e *C. pectinatus* também apresentaram altas taxas de ingestão de microplásticos. Porém, eles apresentaram menores taxas de contaminação quando comparados com os adultos de *C. undecimalis* por possuírem um menor nível trófico. Os maiores níveis de contaminação em *C. mexicanus* e *C. pectinatus* foram observados nos adultos e subadultos que ocuparam o estuário inferior e a zona costeira, principalmente durante o início e o fim do período chuvoso, como consequência da matriz alimentar que foi muito semelhante entre os adultos e subadultos. Adicionalmente, a atividade pesqueira foi uma importante fonte de microplásticos por registrar a ápice das suas operações justamente no estuário inferior e zona costeira nesse período do ano.

Os juvenis de *C. undecimalis, C. mexicanus* e *C. pectinatus* apresentaram as menores taxas de contaminação entre as fases ontogenéticas de Centropomidae por ocuparem principalmente os hábitats com menor disponibilidade de microplástico no ecossistema e por possuírem os menores níveis tróficos, sendo menos vulneráveis à transferência trófica. Entretanto, essa fase ontogenética também apresentou níveis consideráveis de contaminação, principalmente quando se analisa a proporção de juvenis contaminados (*C. undecimalis* 61%, *C. mexicanus* 58% e *C. pectinatus* 21%).

Os microplásticos ingeridos pelos Centropomidae e Sciaenidae apresentam uma grande heterogeneidade em relação ao formato, cor e dimensões, que estão associados principalmente à dinâmica ambiental de onde os peixes foram contaminados. Quanto ao formato, as partículas ingeridas são predominantemente filamentos (99%), possivelmente por

ser o tipo de microplástico mais facilmente assimilado pelos organismos marinhos durante a alimentação.

A principal fonte de entrada de microplásticos no ambiente estuarino é a descarga fluvial, o que pode ser constatado pela maior dimensão dos filamentos ingeridos no estuário superior (*C. undecimalis* 1,41 ±0,2 mm ind^{-1}, *C. mexicanus* 1,52 ±0,09 mm ind^{-1} e *C. acoupa* 1,75 ±0,87 mm ind^{-1}) e intermediário (*C. undecimalis* 1,14 ±0,09 mm ind^{-1}, *C. mexicanus* 1,1 ±0,12 mm ind^{-1}, *C. pectinatus* 1,16 ±0,3 mm ind^{-1} e *C. acoupa* 1,75 ±0,87 mm ind^{-1}). Esses hábitats apresentam uma menor hidrodinâmica, desta forma as partículas sofrem menor pressão intempérica e são menos suscetíveis a fragmentação. Os hábitats externos do ecossistema possuem uma forte hidrodinâmica e os microplásticos são expostos de forma mais intensa a ação das ondas e da maré. As massas d'água desses ambientes também possuem um maior tempo de residência, e permanecem por maiores períodos de tempo na interface rio-oceano, ocasionando uma maior fragmentação das partículas, como pode ser constatado nas menores dimensões de microplásticos ingeridos pelas espécies avaliadas no estuário inferior (*C. undecimalis* 1,09 ±0,07 mm ind^{-1}, *C. mexicanus* 1,42 ±0,13 mm ind^{-1}, *C. pectinatus* 1,63 ±0,44 mm ind^{-1} e *C. acoupa* 1,02 ±0,11 mm ind^{-1}) e na zona costeira (*C. undecimalis* 1,07 ±0,05 mm ind^{-1}, *C. mexicanus* 1 ±0,06 mm ind^{-1} e *C. pectinatus* 1,01 ±0,08 mm ind^{-1}).

Um comportamento anômalo pode ser observado em relação ao comprimento dos microplásticos ingeridos no estuário inferior durante o início da estação chuvosa, quando as partículas apresentaram dimensões muito superiores. Esse comportamento pode estar associado com o pico da atividade pesqueira, em razão desses fragmentos serem muito recentes, eles sofreram uma menor carga intempérica, resultando em maiores dimensões.

Dentre as diferentes cores de microplásticos ingeridos pelos Centropomidae e Sciaenidae, a cor azul foi a predominante (*C. undecimalis* 74%, *C. mexicanus* 78%, *C. pectinatus* 73% e *C. acoupa* 44%). Estudos que avaliam a contaminação em peixes usualmente constatam os filamentos azuis como os mais ingeridos, o que provavelmente é resultado do amplo uso dessa coloração nos mais diversos produtos manufaturados a partir de plásticos, além dela ser a mais utilizada em apetrechos de pesca. Os microplásticos azuis foram ingeridos ao longo de todos os hábitats e estações do ano, registrando maiores taxas de ingestão no estuário inferior e na zona costeira.

Os microplásticos de cor roxa apresentaram maiores taxas de contaminação nos espécimes que ocuparam o estuário inferior e a zona costeira. Possivelmente, isto está

associado com a grande hidrodinâmica desses hábitats, que resulta em uma maior carga intempérica nos microplásticos de cor azul, modificando sua coloração para roxo, como pode ser observado em diversos fragmentos que demonstram uma transição de coloração entre azul e roxo.

Em relação as fases ontogenéticas, os indivíduos subadultos e adultos apresentaram níveis consideravelmente maiores de ingestão de partículas roxas, que está relacionado com seu comportamento preferencial de distribuição ao longo do ecossistema, ocupando principalmente o estuário inferior e a zona costeira, onde os microplástico dessa cor são mais abundantes.

Os microplásticos vermelhos foram amplamente ingeridos no estuário inferior e zona costeira e registraram taxas de contaminação quase insignificantes no estuário interno. O padrão de contaminação pela cor vermelha nas porções internas do estuário sempre está restringido pela posição da cunha salina (estuário intermediário na estiagem e estuário inferior no período de chuva), que atua como uma barreira física, evidenciando uma origem oceânica para essas partículas que são trazidas por correntes litorâneas.

Os espécimes avaliados apresentaram maiores taxas de ingestão de microplásticos de coloração branca nas estações chuvosas, sugerindo a descarga fluvial como uma das principais fontes de entrada para o estuário. Os microplásticos de cor preta foram muito pouco ingeridos, o que pode estar associado com uma menor disponibilidade no ecossistema.

Apesar de estudos indicarem que a ictiofauna pode confundir os microplásticos com suas presas e ingeri-los de forma ativa, e os microplásticos brancos e pretos possuírem grande semelhança no formato e na cor com presas amplamente ingeridas pelas espécies avaliadas (filamentos brancos semelhantes a Amphipoda e Copepoda; filamentos pretos semelhantes a cerdas de Polychaeta). Esta hipótese não foi corroborada pelos dados levantados nesse estudo.

O hábito alimentar dos Centropomidae e Sciaenidae não está relacionado com a ingestão de nenhuma cor específica ou comprimento de fragmento, indicando que não existe uma ingestão ativa de microplásticos de forma que os peixes capturem especificamente as partículas as confundido com suas presas. Entretanto, a ecologia alimentar das espécies está diretamente associada com a quantidade de partículas ingeridas, independente da cor, comprimento e formato.

O ecossistema estuarino sofre grande pressão das ações antropogênicas, sendo historicamente um dos principais ambientes aquáticos explorados em razão do seu grande potencial econômico. Ações de monitoramento necessitam ser mais eficazes no controle da

perda e/ou degradação dos hábitats, principalmente nos ambientes berçários, que são essenciais para que as espécies completem seu ciclo de vida. No caso dos predadores do topo estuarinos, as ações devem ser redobradas nos canais de maré e no estuário superior, principalmente no início dos períodos de estiagem e chuvoso.

Apesar da atividade pesqueira ser de extrema importância nos aspectos econômico, social e cultural, ela é a principal responsável pelo declínio das populações de predadores de topo da ictiofauna. A implementação de uma dimensão mínima de captura relacionada ao tamanho de primeira maturação (tamanho inicial da fase adulta) dos espécimes deve ser imposta e fiscalizada pelos órgãos regulamentadores da pesca. Assim como a proibição total da captura das espécies durante as agregações reprodutivas, com o intuito de viabilizar a saúde do estoque pesqueiro.

Algumas das consequências da atividade antropogênica não são tão perceptíveis, porém são igualmente preocupantes. As espécies estuarinas apresentam altas taxas de ingestão de microplásticos e os predadores de topo são especialmente vulneráveis a esse tipo de contaminação. Os estudos relacionados as consequências da contaminação por microplásticos ainda estão em uma fase inicial. Porém, podem ser observadas alterações comportamentais e fisiológicas nos espécimes contaminados, além das partículas de microplástico agirem como vetores para entrada de metais pesados nos organismos.

Em razão dos predadores de topo estarem altamente contaminados, essas espécies deveriam ser incluídas em programas de biomonitoramento de ingestão de microplásticos (prioritariamente *C. acoupa* e *C. undecimalis*), para um levantamento a nível de grande escala dessa problemática. Essas espécies são as mais cobiçadas para consumo humano, desta forma estudos que avaliam o impacto da contaminação nos peixes devem ser intensificados para que medidas preventivas possam ser implementadas, como a estipulação de um limite máximo de consumo do pescado nas regiões mais afetadas.

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