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Distribuição espaço-temporal e ecologia alimentar das diferentes fases ontogenéticas de *Achirus lineatus* (Pleuronectiformes: Achiridae) no estuário do Rio Goiana (PE/PB) RESEX ACAÚ/GOIANA

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Dissertação apresentada ao Programa de Pós-Graduação em Oceanografia do Departamento de Oceanografia da Universidade Federal de Pernambuco, como requisito para obtenção do grau de Mestre em Oceanografia.
Área de concentração: Oceanografia biológica

Orientador: Prof. Dr. Mário Barletta
Coorientador: Dr. André Ricardo de Araújo Lima

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Aprovada em 06 de fevereiro de 2018.

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RESUMO

Este estudo teve por objetivo descrever o padrão de distribuição, ecologia alimentar e contaminação por microplásticos na população de *Achirus lineatus* do estuário do Rio Goiana em PE/PB em relação à variação espaço-temporal e ontogenética. O canal principal do estuário foi dividido em três áreas (superior, intermediária e inferior) levando em consideração o gradiente de salinidade e a morfologia do estuário. Foram realizadas 6 amostragens mensais em cada área durante um ciclo anual completo, que foi dividido de acordo com a sazonalidade local em início da chuva (março a maio), final da chuva (junho a agosto), início da seca (setembro a novembro) e final da seca (dezembro a fevereiro), totalizando 216 arrastos. No total, 2421 espécimes de *A. lineatus* foram coletados no canal principal do estuário do rio Goiana, com uma densidade média total de 122.2 ind. ha-1 e biomassa média 1143.3 g. ha-1. As fases ontogenéticas foram classificadas como juvenis (< 39 mm), subadultos (40-49 mm) e adultos (> 50 mm). A espécie se distribui ao longo de todo o canal principal, durante todo o ciclo sazonal. As maiores densidades e biomassas médias foram observadas no estuário superior. Juvenis, subadultos e adultos apresentaram maiores densidades e biomassas no estuário superior durante o início da estação chuvosa, caracterizando este habitat e período sazonal com área de berçário, reprodução e alimentação (p <0,01). A dieta foi composta por anelídeos (poliquetas), microcrustáceos, macrocrustáceos, peixes teleósteos, nemátoda e microplásticos. Todas as fases ontogenéticas se alimentam preferencialmente de poliquetas (%I_{RI} > 13,06 e 100), caracterizando a espécie como zoobentívora durante todo o seu ciclo de vida. Porém, os juvenis e subadultos se alimentaram de copépodas e anfípodas como itens secundários, mostrando uma tendência zooplânctivora. Adultos se alimentaram secundariamente de peixes e macrocrustáceos mostrando uma tendência piscívora. Microplásticos contaminaram todas as fases ontogenéticas. Os subadultos apresentaram frequência de ocorrência de (FO = 11,7%), seguidos dos adultos (FO = 8,08%) e juvenis (FO = 7,93%). A maior ingestão de microplásticos principalmente na cor azul ocorreu nos adultos no final da estação chuvosa, independente das áreas do estuário (p <0,01). A contaminação por microplásticos na população de *A. lineatus*, está provavelmente associada ao forrageamento bentônico. Também, o fato de a contaminação ocorrer principalmente durante a estação chuvosa indica que ela está associada ao período de maior descarga do rio, quando os microplásticos provenientes do continente são carregados em direção ao mar. *A. lineatus*, tem uma grande importância ecológica nos ecossistemas estuarinos, devido a sua grande densidade e biomassa, funcionando como um elo entre níveis tróficos inferiores e superiores. Além de fornecer energia, eles também podem transferir essa classe de contaminante ao longo da teia trófica, produzindo efeitos como lesões no trato digestivo, diminuindo a capacidade predatória e até efeitos tóxicos nocivos (causados pela adsorção de poluentes orgânicos persistente), podendo atingir populações humanas que dependem destes recursos.

Palavras-chave: Estuário tropical. Dieta. Contaminação por microplástico. Linguado. Achiridae

ABSTRACT

This study aims to describe the patterns of distribution, feeding ecology and contamination by microplastics of the *Achirus lineatus* population inhabiting the Goiana estuary as a function of their spatial-temporal variation and ontogeny. The Goiana estuary was divided into three areas (upper, middle and lower) according to the salinity gradient and their geomorphology. Samples were taken during an annual cycle, that was divided following the local seasons into early rainy (March-May), late rainy (June-August), early dry (September-November) and late dry (December-February), totalling 216 samples. A total of 2,421 specimens of *A. lineatus* were collected within the main channel of the estuary, with an average density of 122.2 ind. ha^{-1} and an average biomass of 1,143.3 g. ha^{-1}. The ontogenetic phases of *A. lineatus* were classified as juveniles (<39 mm), subadults (40-49 mm) and adults (>50 mm). The species was distributed in the main channel, during the entire seasonal cycle. The highest density and biomass were observed at the upper estuary. Juvenile, subadults and adults presented the highest density and biomass in the upper estuary during the early rainy season, characterizing this habitat, during this seasonal period, as nursery, reproduction and feeding grounds (p <0.01). The diet was composed mainly of annelids (polychaetes), microcrustaceans, macrocrustaceans, teleostean fishes, parasites and microplastics. However, all ontogenetic phases fed mainly on polychaetes (%I_{RI} > 13.06 and 100), characterizing them as zoobenthivores during the whole life cycle. Juveniles and subadults also fed on copepods and amphipods as secondary prey, showing a zooplanktivorus behavior. On the other hand, adults also fed on fish and macrocrustaceans, as secondary prey, showing a piscivorous behavior. Microplastics contaminated all ontogenetic phases. Subadults showed the higher frequency of occurrence (FO = 11.7%), followed by adults (FO = 8.08%) and juveniles (FO = 7.93%). The highest ingestion of microplastic filaments, especially blue color occurred in adults during the late rainy season, regardless of area (p <0.01). Contamination of *A. lineatus* population by microplastics is probably associated with benthic foraging, due to the highest densities and sinking of these filaments toward the substrate. The fact that contamination occurred mainly during the rainy season means that it is associated to river runoff, when microplastics from the continent are flushed towards the ocean. *Achirus lineatus* has a huge ecological importance in estuarine ecosystems, due to their higher density and biomass, acting as a link between lower and higher trophic levels. Besides energy flow, they also might be a pathway for the transfer of microplastics along the food web, producing injuries in the digestive tract, decreasing predatory efficiency, and causing toxicological effects (*e.g.* caused by adsorbed persistent organic pollutants) in other fish of economic and subsistence importance. These impacts can be reflected in the human population that depends on these estuarine resources.

Keywords: Flatfish. Achiridae. Diet. Plastic debris. Tropical estuary

LISTA DE ILUSTRAÇÕES

SUMÁRIO

1 INTRODUÇÃO GERAL

Estuário é um corpo de água costeiro semifechado que tem uma conexão com o oceano, influenciado pela maré, no qual a água do mar é misturada com a água doce proveniente da drenagem continental (PRITCHARD, 1967). O gradiente ambiental de um estuário é constantemente alterado por variações nos fatores abióticos, proporcionando desafios constantes para a distribuição dos organismos ao longo desse ambiente (BARLETTA & DANTAS, 2016a, b). Esse ecossistema é utilizado como local de alimentação, proteção, reprodução e berçário para diversas espécies de peixes e invertebrados, residentes ou que apenas utilizam seus recursos durante parte do seu ciclo de vida (BARLETTA-BERGAN et al., 2002a, b; BARLETTA et al., 2005; BARLETTA & DANTAS, 2016b, LIMA et al., 2014, 2015, 2016). Um habitat berçário pode ser caracterizado como um local em que se concentram grandes densidades de juvenis de uma determinada espécie, contribuindo assim para um maior recrutamento da população de indivíduos adultos, quando comparado com outros habitats (BECK et al., 2001).

Nesse contexto está inserido o estuário do Rio Goiana, um ambiente tropical, localizado no extremo leste da América do Sul, com uma grande diversidade de habitats que abriga uma rica fauna de peixes e crustáceos (BARLETTA & COSTA, 2009). Diversos estudos foram realizados avaliando a distribuição e a ecologia alimentar da ictiofauna local, como por exemplos as famílias Sciaenidae (DANTAS et al., 2015, FERREIRA et al., 2016), Ariidae (DANTAS et al., 2013) e Gerreidae (RAMOS et al., 2016), importantes representantes de assembleias dos peixes demersais. Estes organismos vivem e se alimentam sobre ou próximo ao fundo (FROESE & PAULY, 2000), consumindo principalmente presas e a matéria orgânica disponível no substrato, auxiliando na ciclagem de nutrientes, fornecendo um link entre diferentes habitats.

As espécies da família Achiridae por isso desempenham um papel crucial no fluxo energético, consumindo diretamente detritos, ou predando organismos detritívoros, como poliquetas (CHAVES & SERENATO, 1998), promovendo um importante elo entre invertebrados bentônicos, a matéria orgânica e os níveis tróficos superiores (DUARTE & ANDREATA, 2003). O gênero *Achirus* Lacépède 1802 é composto por espécies carnívoras, alimentando-se preferencialmente de peixes e invertebrados bentônicos, os representantes desse grupo são classificados como

eurihalinos, como é o caso da espécie *Achirus lineatus* Linnaeus 1758, amplamente distribuída em ecossistemas estuarinos (CARPENTER, 2002). Trabalhos que descrevem a distribuição e ecologia alimentar de *A. lineatus* são escassos, portanto, são necessários estudos que visam compreender o uso do espaço e o ciclo de vida dessa espécie.

O estudo sobre o desenvolvimento ontogenético refere-se a mudanças morfológicas, que ocorrem desde a fertilização do embrião até a formação do indivíduo adulto. A maioria das pesquisas em ontogenia é restrita a fases iniciais do desenvolvimento, sendo este um período crucial onde ocorre as principais mudanças morfológicas e características que serão mantidas na fase adulta (FUKUHARA, 1988).

No entanto, o comportamento desses organismos se altera em cada fase da vida, variando desde os estágios iniciais, quando os jovens migram da área de desova para a área de berçário, e posteriormente quando os juvenis amadurecem, no habitat utilizado na fase adulta. Essas mudanças dependem de fatores ambientais e ecológicos (GIBSON, 1997).

A compreensão dos hábitos alimentares de organismos nas suas diferentes fases ontogenéticas (juvenil, subadultos e adultos) possibilita o entendimento de muitos processos biológicos, bem como as relações ecológicas entre as populações de um ecossistema. Essas informações são importantes para o monitoramento sustentável de um dos principais recursos alimentar, para espécies importantes economicamente e da comunidade de uma forma geral, além de fornecer dados sobre a saúde ambiental, possibilitando assim medidas mitigatórias e a conservação do mesmo. Levando em consideração as informações apresentadas, este projeto tem como hipótese que a distribuição espaço-temporal, ecologia alimentar e a contaminação por microplásticos nas diferentes fases ontogenéticas de *Achirus lineatus* ocorre em função da flutuação das variáveis ambientais.

2 OBJETIVOS

2.1 Objetivo Geral

O presente estudo tem por objetivo principal determinar a distribuição, a ecologia alimentar incluindo a contaminação por microplásticos na espécie *Achirus lineatus* em função da sua variação espaço-temporal e ontogenética.

2.2 Objetivos específicos

- Investigar se a distribuição espacial e sazonal de *A. lineatus* ao longo do estuário está atribuída ao seu ciclo de vida e/ou a flutuações das condições ambientais da área de estudo;
- Identificar áreas utilizadas como berçário no estuário do Rio Goiana pela espécie *A. lineatus*;
- Descrever a variação nos itens ingeridos por *A. lineatus* em função de seu desenvolvimento ontogenético, sazonal e espacial no estuário do Rio Goiana;
- Investigar a possível contaminação por microplásticos pela espécie *A. lineatus*, de acordo com as fases ontogenéticas e sua distribuição.

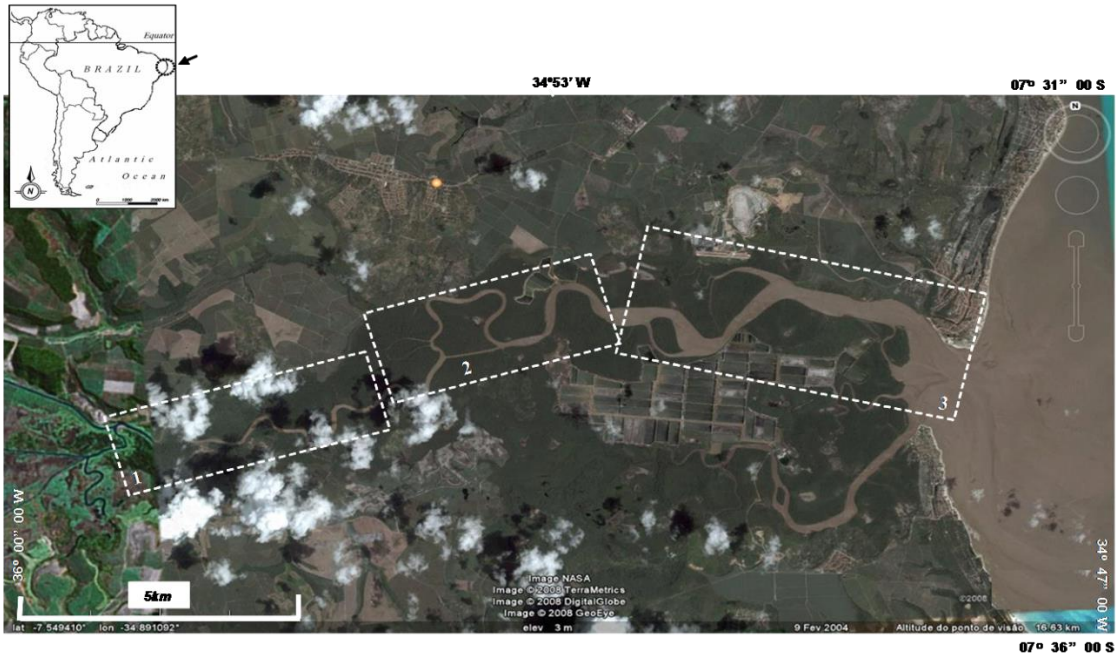
3 METODOLOGIA

3.1 Área de estudo

O estuário do Rio Goiana localiza-se na região Nordeste do Brasil na divisa dos estados de Pernambuco e Paraíba (7°32'-7°35' S e 34°50'-34°58' W) (Fig. 1), sendo formado pela confluência dos Rios Capibaribe Mirim e Tracunhaém (Barletta & Costa, 2009). O clima da região é tropical, com uma temperatura média do ar de 27 ºC (±2 ºC) ao longo do ano. A região apresenta quatro estações definidas de acordo com o nível de precipitação, que são divididas em: início do período chuvoso (março a maio), final da chuva (junho a agosto), início do período de seca (setembro a novembro) e o final da seca (dezembro a fevereiro) (Barletta & Costa, 2009).

Esse estuário possui uma grande diversidade de habitats, como o canal principal, canais de maré, e praias arenosas dispostas na foz do estuário (Dantas *et al.*, 2015, Lima *et al.*, 2014, Ramos *et al.*, 2014, Lacerda *et al.*, 2014) (Fig. 1). Estes habitats apresentam uma grande importância ecológica e econômica, além de servirem como fonte de subsistência para as comunidades ribeirinhas (Barletta & Costa, 2009).

Figura 1 - Estuário do Rio Goiana, as áreas delimitadas por [- - -] indicam o estuário superior (1), o estuário intermediário (2), o estuário inferior (3).

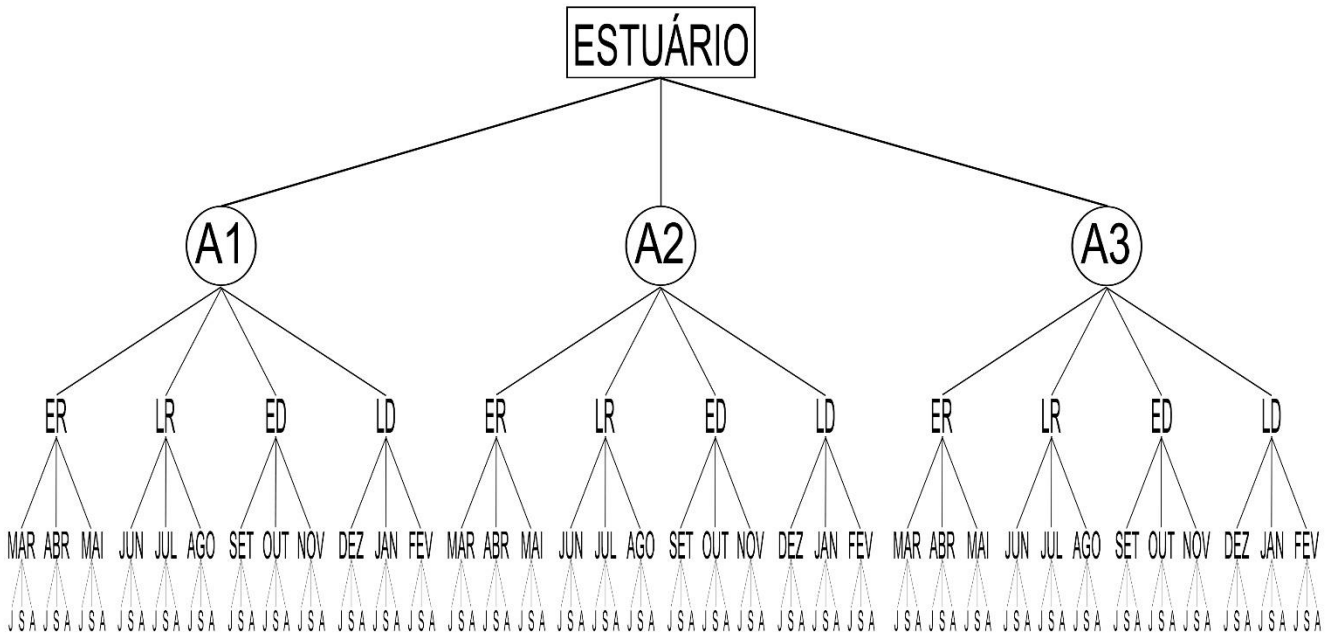


Fonte: Google Earth.

3.2 Coleta de dados

Foi planejado um desenho amostral (Fig. 2) para a realização das coletas no canal principal do Estuário do Rio Goiana (Fig. 1). As diversas fases ontogenéticas de *A. lineatus* (juvenis, subadultos e adultos) foram coletadas em cada área do estuário e em cada estação, conservadas em formol ou congeladas em freezer para as análises. A coleta da ictiofauna e das variáveis abióticas deste projeto têm sido realizadas desde 2005, no âmbito de 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), autorizados por licença ambiental para atividades com fins científicos SISBIO: 11050-1.

Figura 2- Esquema ilustrativo do desenho amostral proposto para as amostragens no canal principal do Rio Goiana. (A1: estuário superior; A2: estuário intermediário; A3: estuário inferior) (ER: início da chuva; LR: final da chuva; ED: início da seca; LD: final da seca) (J: juvenis; S: subadultos; A: adultos).



3.3 Parâmetros Abióticos

Juntamente com a coleta dos dados bióticos, foram aferidos os parâmetros abióticos da água, como temperatura Cº, salinidade (Salinometer WTW LF 197), oxigênio dissolvido (mg/L) (Oximeter WTW Oxi 340) e transparência (Disco de Secchi - cm). Os dados de precipitação total mensal (mm) foram compilados da estação meteorológica mais próxima (INMET, 2014).

3.4 Amostragem da ictiofauna no canal principal

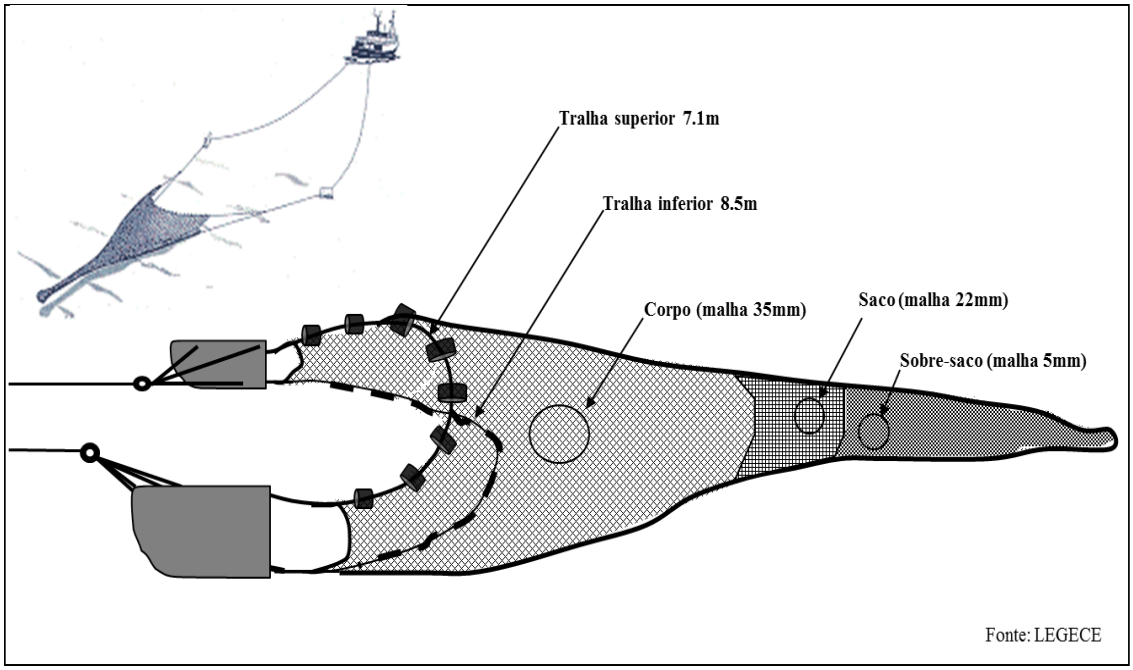
A coleta dos exemplares de maior tamanho corporal foi realizada com auxílio de uma rede de arrasto com portas, utilizando malhas de 35 mm na asa e no corpo da rede, 22 mm no saco e 5 mm no sobre saco, os diversos tamanhos de malha foram utilizados para obtenção de uma melhor representatividade das diferentes classes de tamanho da espécie. A metodologia de coleta está descrita detalhadamente em Dantas et al., (2010).

No momento em que foram realizadas as coletas no canal principal, também foram tomadas medidas de profundidade e a distância que a rede de arrasto percorreu durante cada amostragem, com a finalidade de calcular a área arrastada. Utilizando as informações obtidas referentes à área arrastada de cada amostra realizada, foi possível determinar a captura por unidade de área, com o intuito de estimar a densidade e a biomassa dos indivíduos, através da relação do número e peso dos espécimes com a área de coleta (SPARRE & VENEMA, 1997), expressos da seguinte forma:

$$Densidade = n/A\ (ind.m^{-2})$$
$$Biomassa = peso/A(g.m^{-2})$$

Onde, *n* é o número de indivíduos, *A* é a área arrastada pela rede durante 10 minutos de arrasto.

Figura 3 -Rede de arrasto com portas utilizada para amostragem no canal principal.



3.5 Processamento e classificação das amostras

Após cada coleta os exemplares foram etiquetados, os indivíduos de menor tamanho corporal foram preservados em formol e os de maior tamanho congelados, em seguida ambos foram armazenados em um banco de amostras. Em laboratório os indivíduos foram triados e identificados (MENEZES & FIGUEREDO, 2000), e tiveram suas medidas biométricas tomadas em comprimento total (mm) e peso (g).

3.6 Classificação ontogenética

As fases ontogenéticas foram classificadas para um melhor entendimento do ciclo de vida de *A. lineatus*. Os espécimes foram divididos em diferentes classes de tamanho, iniciando a fase juvenil pelo período de transformação da larva < 5 mm (RICHARDS, 2004) obtido por literatura especializada. Para distinguir os indivíduos juvenis dos subadultos foi utilizado o ponto de inflexão da curva do Peso *vs.* Comprimento (RAMOS et al., 2014), no qual se deu entre 5 a 39 mm (Fig. 5). Para estimar o comprimento da fase adulta, foi utilizado o comprimento da primeira maturação sexual (L_{50}) obtido através da função logística realizada utilizando os dados oriundos da análise macroscópica das gônadas (VAZZOLER, 1996) (Fig.6). Diferindo assim os indivíduos subadultos (40-49 mm) dos adultos em > 50 mm (Fig.7).

Figura 4 - Curva entre o peso vs. comprimento para *Achirus lineatus* do Estuário do Rio Goiana. Seta indica o ponto de inflexão da curva em 39 mm. ● juvenil (<39 mm), ● subadultos (40-49 mm) e ● adultos (>50 mm).

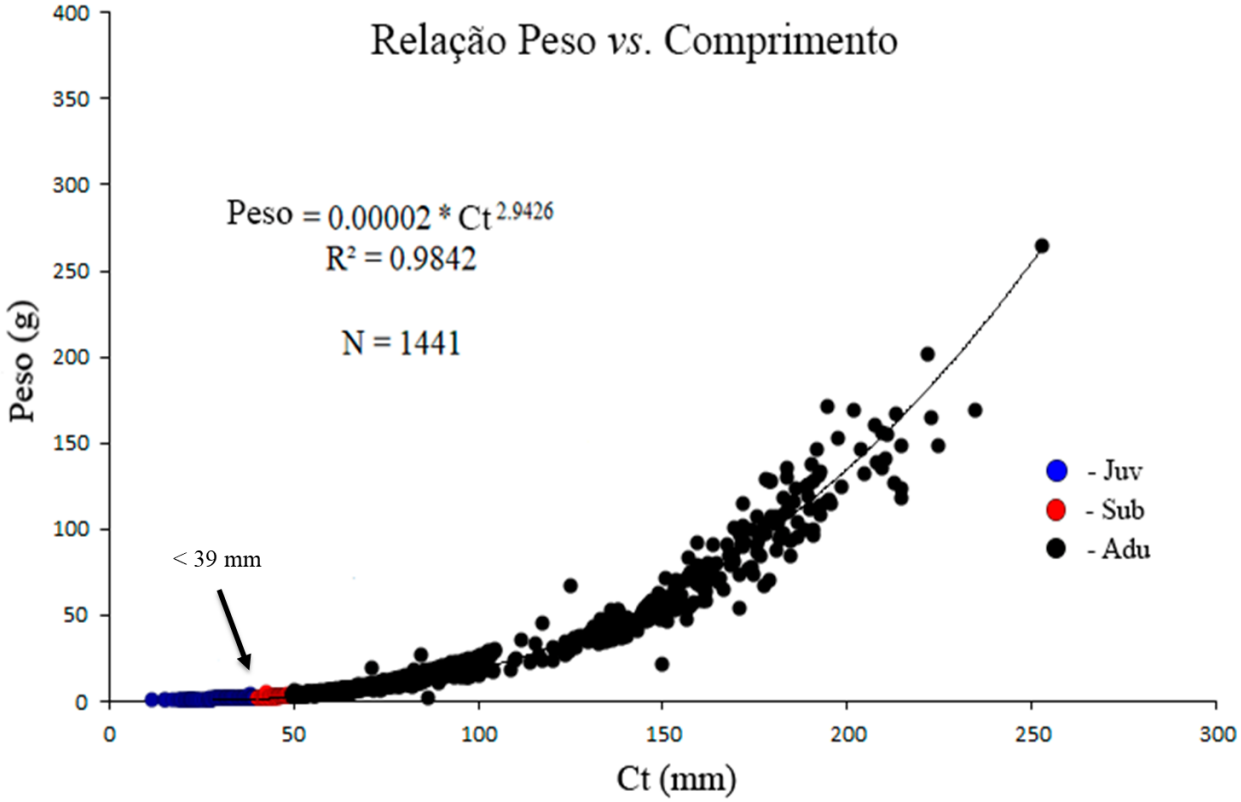


Figura 5 - Curva logística que definiu a primeira maturação de *Achirus lineatus* em 50 mm.

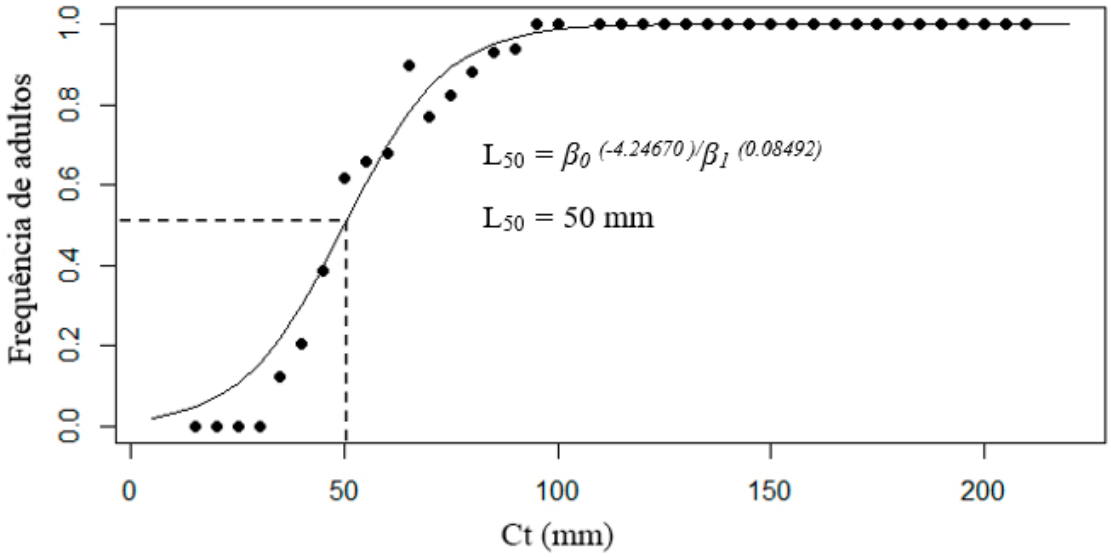
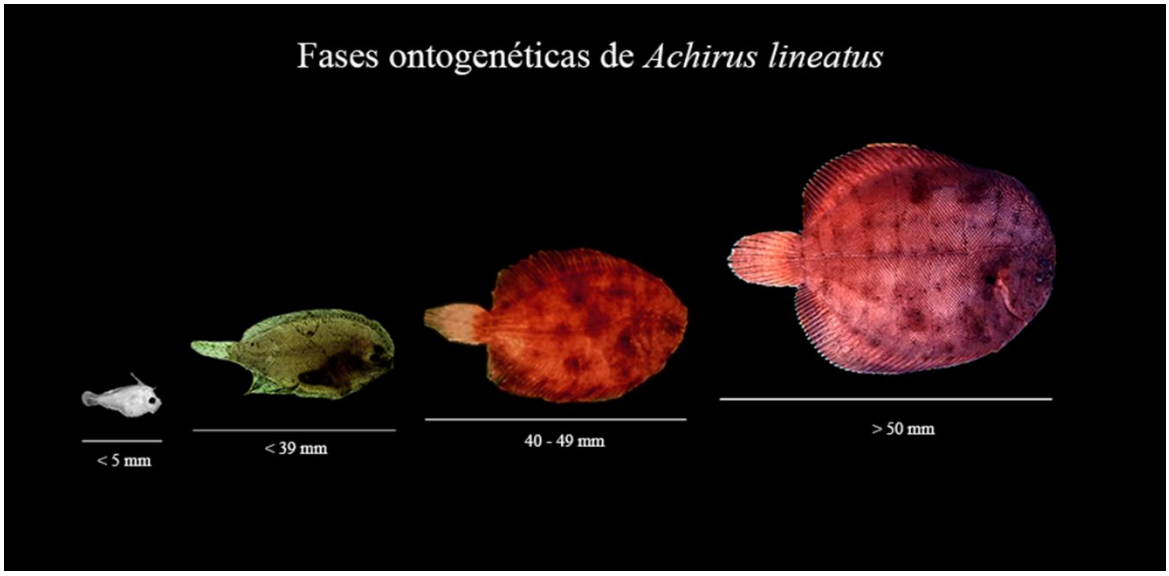


Figura 6 - Classificação ontogenética de *Achirus lineatus*. Larva (< 5 mm), juvenil (<39 mm), subadulto (40-49 mm) e adulto (>50 mm).



Fonte: Própria.

3.7 Análise do conteúdo estomacal

Depois de obter os dados morfométricos de cada exemplar capturado, foram realizadas as análises dos conteúdos estomacais, que foram removidos e triados. Os itens alimentares foram identificados até o menor nível taxonômico possível, com o auxílio de literatura especializada (BRUSCA & BRUSCA, 2002; RUPPERT et al., 2005; STACHOWITSCH, 1992). Em seguida, os itens foram lavados com água destilada, secos e pesados em balança analítica. Os itens considerados microplásticos (< 5 mm) foram submetidos a teste para confirmação de que não eram matéria orgânica natural, no qual foram colocados em uma estufa a 70 °C por 48 horas, e após a confirmação, no qual se dava a partir da observação de que esses microplásticos não se deterioravam e não tinham a sua coloração alterada, eles foram fotografados e classificados de acordo com sua possível origem e cor.

Para investigar os principais itens alimentares utilizados pela espécie e determinar seu grau de relevância, foi utilizado o Índice de Importância Relativa (PINKAS et al., 1971), que consiste da seguinte equação:

$$\mathrm{IIR} = \%F_i * (\%N_i + \%P_i)$$

No qual F_i é o valor referente à frequência de ocorrência dos itens alimentares, N_i representa a porcentagem numérica dos itens e P_i a porcentagem por peso de cada item alimentar (HYNES, 1950; HYSLOP, 1980).

3.8 Análise estatística

Com o intuito de alcançar a normalidade dos dados, eles foram submetidos ao processo de transformação, através do método Box-Cox (BOX & COX, 1964). Em seguida, foi aplicado o teste de Levene (UNDERWOOD, 1997) para testar a homocedasticidade dos tratamentos.

A análise de variância (ANOVA) foi utilizada para testar se a alimentação, a densidade e a biomassa total dos indivíduos apresentaram diferenças significativas em relação aos fatores temporais, espaciais e ontogenéticos. Testes a posteriori (Bonferroni com $\alpha = 0,05$) foram utilizados para definir as fontes de variância (QUINN & KEOUGH, 2002). A análise canônica de correspondência (CCA) foi realizada para constatar as interações ecológicas entre as variáveis ambientais (Precipitação, salinidade, temperatura, profundidade de Secchi e oxigênio dissolvido), a distribuição das diferentes fases ontogenéticas e sua alimentação (PALMER, 1993; TER BRAAK, 1986).

4 SPATIO-TEMPORAL DISTRIBUTION, FEEDING ECOLOGY AND MICROPLASTICS CONTAMINATION OF A BENTHIC FISH IN A TROPICAL ESTUARY

4.1 Introduction

Estuaries are important ecosystems that connect land and sea. It is influenced by tides, that seawater mixing with fresh water from the continental drainage (Pritchard, 1967). The environmental gradient of an estuary, especially salinity, is constantly shifted by the seasonal variation on abiotic factors, providing incessant challenges for organisms to be distributed across this environment (Barletta & Dantas, 2016a; b). This ecosystem is used by riverine, estuarine and marine fauna for feeding, protection, reproduction and nursery (Barletta-Bergan *et al*., 2002a, b; Barletta *et al*., 2005; Barletta & Dantas, 2016b; Lima *et al*., 2014, 2015, 2016). A nursery habitat can be characterized as a place where above average juvenile densities of a species are concentrated, thereby contributing to an increased recruitment of population of adult individuals when compared to other habitats (Beck *et al*., 2001).

Indeed, estuarine ecosystems play an important role in the development and survivor rates of estuarine and marine species. In this way, they are important for the local economy and subsistence of surrounding communities around tropical world (Barletta *et al*., 2017). Nevertheless, these ecosystems are vulnerable to anthropogenic impacts *e.g.* dredging spoils and plastics debris (Barletta *et al*., 2016; Costa & Barletta, 2016). Microplastics as contaminants are a well-known problem in aquatic habitats, as evidenced in many marine environments (Boerger *et al*., 2010; Bråte *et al*., 2016; Dantas *et al*., 2012; Possatto *et al*., 2011; Ramos *et al*., 2012; Ferreira *et al*., 2016). The ingestion of microplastics by fishes can be hazardous, causing internal injuries, decrease in predatory efficiency, and induce toxicity (de Sá *et al*., 2015; Moore, 2008; Teuten *et al*., 2007; Barboza *et al*., 2017).

Microplastics (<5 mm) are part of the large amount of plastics found in marine and freshwater environments (Arthur *et al*., 2009). Microplastics adsorb pollutants *e.g.* persistent organic pollutants (Oehlmann *et al*., 2009; Frias *et al*., 2010; Rochman *et a*l., 2013) and *e.g.* heavy metals (Ashton *et al*., 2010; Holmes *et al*., 2012), which might be bioaccumulated and biomagnified across the food web (Batel *et al*., 2016; Teuten *et al*.,

2009). However, few studies about spatio-temporal fluctuations of microplastic contamination in estuarine fishes diet have been carried out taking into consideration different ontogenetic stages.

Goiana Estuary has diverse habitats for shelter (*e.g*. mangroves forest, intertidal and main channels, sandy beaches, seagrass and beach rocks) used for spawning, nursery, feeding and protection by many species of molluscs, crustaceans and fishes (Barletta & Costa, 2009). Several studies have been carried out in this estuary, evaluating the ecology of fish species in relation to their distribution (Dantas *et al*., 2010, 2015; Lima et al. 2014), feeding ecology (Ramos *et al*., 2014, Ferreira et al, 2016), contamination by heavy metals (Costa *et al*., 2009, Barletta *et al.*, 2012) and microplastics (Ferreira *et al*., 2016; Lima *et al*., 2016). According to these studies, demersal fishes in this estuary feed mainly on molluscs, polychaetes, amphipods, copepods, fishes and detritus that are available in the substrate, assisting in the cycling of nutrients, providing a link between different habitats and trophic levels.

The estuarine demersal species *Achirus lineatus* play a role in the flow of energy directly consuming debris, or predating detritivore organisms, such as polychaetes (Chaves & Serenato, 1998). This species promotes an important link between secondary consumers and higher trophic levels (Duarte & Andreata, 2003). Being an euryhaline species, it is widely distributed across estuarine ecosystems (Carpenter, 2002). Interestingly, its behaviour changes at each stage of the life cycle. Juveniles migrate from the spawning area to the nursery area, and later subadults move to feeding grounds used by adults. These changes are triggered by seasonal fluctuations of the salinity ecocline (Barletta *et al*., 2005; 2008; 2016b).

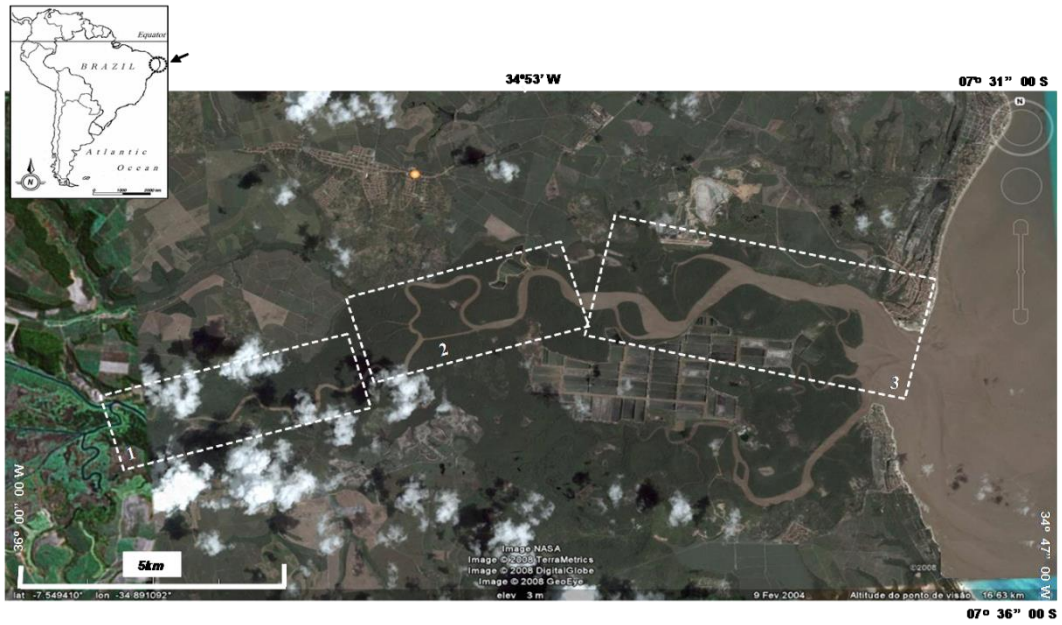
An understanding of the feeding ecology of different ontogenetic stages (juvenile, subadult and adult) makes possible the understanding of many biological and ecological processes (*e.g.* contamination) between the fish community and estuarine habitats. Based on this information, the present study aims to describe the distribution, feeding ecology and contamination by microplastics as a function of the spatio-temporal and ontogenetic variation of *A. lineatus*. The hypothesis tested in this study is that the distribution, diet and contamination of *Achirus lineatus*, occur as a function of their ontogeny and spatio-temporal distribution in the different estuarine habitats.

4.2 Materials and Methods

4.2.1 Study area

The estuary of the Goiana River is located in the Northeast region of Brazil (7°32'-7°35' S e 34°50'-34°58' W) (Fig.7). The Goiana River is formed by the confluence of the rivers Capibaribe Mirim and Tracunhaém, the predominant vegetation in the floodplain is the mangrove forest (Barletta & Costa, 2009). The climate of the region is tropical, with an average 27 ºC (±2 ºC) throughout the year. The region presents four seasons defined according to rainfall, which are divided into: early rainy (March to May), late rainy (June to August), early dry (September to November) and late dry (December to February) (Barletta & Costa, 2009). This estuary has an important diversity of habitats, such as the main channel, tidal creeks, and sandy beaches on both sides of the river mouth (Dantas *et al*., 2015, Lima *et al*., 2014, Ramos *et al*., 2014, Lacerda *et al*., 2014). Goiana estuary is a marine protected area (MPA) due to its variety of habitats which have ecological and economical (Barletta & Costa, 2009).

Figure 7 - Goiana Estuary divided in three areas (Upper; Middle and Lower estuary) represented by



4.2.2 Sampling

Samples were taken at the main channel of estuary of Goiana River, that was divided in three areas according to the salinity gradient. The upper estuary had salinities under 5; the middle estuary from 5 to 20 and the lower estuary salinities above 20 (Barletta & Costa, 2009). From December 2005 to November 2006, six replicate samples per month were taken from each area of the estuary (upper, middle and lower) (n = 216). Sampling used an otter trawl net with 8.72 m long, and mesh size 35 mm in the body and 22 mm at the cod end. To obtain a better representation of the different size classes of the species, an additional 5 mm mesh was used as cover at the cod end. The initial and final positions of the tow in the main channel were recorded by means of a GPS. Measurements of depth and distance trawled during each sampling were also taken (Dantas *et al*., 2010). Based on time and distance trawled and net parameters, it was possible to determine the catch per unit area (CPUA), with the purpose of estimating density and biomass of individuals caught, through the relation of the number and weight of the specimens (Sparre & Venema 1997).

Abiotic parameters such as water temperature C°, salinity (Salinometer WTW LF 197), dissolved oxygen (mg/L) (Oximeter WTW Oxi 340) and Secchi depth (cm) were concomitantly recorded. Rainfall data (monthly total in mm) were compiled from the nearest meteorological station (INMET, 2014).

4.2.3 Laboratory procedures

After each sampling, specimens were labelled, preserved in formalin (4%) and stored in a sample collection. Later, individuals were identified (Menezes & Figueiredo, 2000), measured for total length (mm) and weighted (g). Each individual had their organs removed, weighed and stored for analysis of gonad maturation and stomach contents.

To separate juvenile from subadults, the inflection point of the length (Lt) *vs.* weight (Wg) curves was used (Ramos *et al.,* 2014). To distinguish subadults from adults, the length of the first gonadal maturation L_{50} (Vazzoler, 1996) was used. Then, Lt *vs.* Wg relationship and L_{50} logistic curve determined the ontogenetic stages: juvenile (5-39 mm), sub adult (> 40-49 mm) and adult (> 50 mm) (Supplementary Material S1).

The items contained in each digestive tract were identified to the lowest possible taxonomic level with the aid of specialized literature (Brusca & Brusca, 2002; Ruppert *et al*., 2005; Stachowitsch, 1992). All laboratory material used were washed in distilled water and oven dried, lab coats were cotton and gloves latex. After the identification of items, they were washed with distilled water, dried and weighed in an analytical balance. The items suspected of being microplastics, were submitted to a test to guarantee that they were not organic matter (oven dried (70 °C) for 48 h). After that, microplastics were photographed and categorized by colour and type.

The digestive tract contents were used to build a data matrix for the index of relative importance (%I_{RI}) (Pinkas *et al.,* 1971) which consists on the following equation:

$$\mathrm{IRI} = \%F_i * (\%N_i + \%P_i)$$

where F_i is the value of the frequency of occurrence per items, N_i represents the percentage in number per items, and P_i the percentage for weight items (Hynes, 1950; Hyslop, 1980). For transforming IRI in %I_{RI}, it was used the sum of all IRI values divided by the IRI value per each item.

4.2.4 Statistical analysis

In order to achieve normality, data were submitted to a transformation using the Box-Cox method (Box & Cox, 1964). Then the Levene test (Underwood, 1997) was applied to test for homoscedasticity of the treatments.

The Three-Way ANOVA, was used to test whether feeding, density and total biomass of the individuals presented significant differences in relation to temporal, spatial and ontogenetic factors. *A posteriori* Bonferroni test ($\alpha = 0.05$) was performed to define the sources of variance (Quinn & Keough, 2002).

The Canonical Correspondence Analysis (CCA - Canoco 5), was performed to verify possible ecological interactions between environmental variables (rainfall, salinity, temperature, Secchi depth and dissolved oxygen), the distribution of the different ontogenetic stage and their feeding preference and microplastic contamination (based on the Index of Relative Importance – IRI%) (Palmer, 1993; ter Braak, 1986; ter Braak and Šmilauer, 2012).

4.3 Results

4.3.1 Environmental variables

The upper estuary showed the lowest mean value of salinity (5 ± 1), DO (5 ± 1 mg L^{-1}) and Secchi depth (55 ± 5 cm) (Fig.8). Nevertheless, the highest mean records of water temperature (29 ± 1°C) during the dry season, when the lowest rainfall occurs (46 mm), creating a more effective intrusion of coastal waters into the estuary (Fig.8). During the rainy season, decreased salinity was observed towards the lower estuary (9 ± 1). Dissolved Oxygen (DO) showed the same trends (upper: 4.2 ± 1 mg L^{-1}; middle: 4.5 ± 1 mg L^{-1}; lower: 6.0 ±1 mg L^{-1}). Water temperature remained stable in all estuary (26.5 ± 1°C) during the rainy season (Fig.8).

4.3.2 Seasonal and spatial distribution of ontogenetic stages of A. lineatus

During a full annual cycle, a total of 2421 specimens of *Achirus lineatus* were sampled in the main channel of Goiana Estuary (69.2% adults, 16.8% sub adults, 14% juveniles) (Table S1). A total mean density of 122.2 ind. ha^{-1} and biomass 1143.3 g. ha^{-1} was recorded for *A. lineatus* (Table S1). The highest values of density (528 ind. ha^{-1}; $F = 5.44$; $p < 0.01$) and biomass (4996.7 g. ha^{-1}; $F = 5.39$; $p < 0.01$), were observed in the upper estuary during the early rainy season for adults (Table S2; Supplementary Material S2). The highest values of juveniles (density) and subadult (density and biomass) were detected during the rainy season in the upper and middle estuary. However, the highest biomass of juvenile was detected in the upper estuary during the early rainy season (Table S2; S2).

4.3.3 Feeding ecology

A total of 543 stomachs were analysed for their contents segregated into ontogenetic stages: 63 specimens were juveniles, 68 specimens were subadults and 410 specimens were adults. According to the Index of Relative Importance (IRI) and frequency of occurrence, the most important prey was Polychaeta regardless of area, season or ontogenetic stage (Table S3, Fig.9). Microplastic filaments (Fig.10) were detected mainly in adults during the late rainy season in the lower estuary (IRI = 6.15%)

(Table S3; Fig.9). Juveniles had microplastic filaments in gut contents during the early dry season at the upper estuary. Subadults were contaminated with microplastics in their stomachs contents in the middle estuary during the late rainy season (Table S3; Fig. 9).

The ANOVA (number and weight of ingested items) showed significant differences between feeding habitats, which are associated with a combination of factors area, season and ontogenetic stage (S3; Table S4; Fig. 11). Polychaetes were the main prey for adults during the rainy season (in number) in the upper estuary (Table S4; Fig. 11), and during the early dry season (in weight) in the middle estuary (Table S5 and S4). In addition, significant interactions (area *vs.* season *vs.* ontogenetic stage) were detected for polychaetes ingestions (number and weight). It suggests that the ingestion (in number and/or weight) of this prey varies in time and space during the development of this species (Fig. 11; S3). Ingestion of copepods in the middle estuary, and amphipods in the upper estuary, showed significant differences ($p < 0.01$), mainly for juveniles and subadults during the rainy season (Table S4, Fig. 11).

4.3.4 Microplastics contamination in stomach contents

Microplastic filaments in stomach contents showed significant differences for season ($F = 5.29$; $p < 0.01$) and ontogenetic stage ($F = 26.48$; $p < 0.01$) (Table S4; Fig. 11). Moreover, microplastics ingestion showed significant interactions among area *vs.* season *vs.* ontogenetic phase ($F = 2.62$; $p < 0.01$) (Table S4; Fig. 11). It suggests that the contamination by microplastics filaments occur as a function of the variables time, space and fish growth.

Microplastics recorded were filaments and were separated by colour (blue, white, red, black and green) (Fig. 10). Ingestion of blue filaments differed significantly among areas, season and ontogenetic phase (Table S6, Fig. 12). Blue filaments differed in the upper estuary ($F = 6.49$; $p < 0.01$), in the late rainy season ($F = 5.75$; $p < 0.01$), and the ontogenetic phase adults ($F = 24.23$; $p < 0.01$). Adults of *A. lineatus* ($F = 3.60$; $p < 0.05$) were contaminated by white filaments mostly in the upper estuary ($F = 7.76$; $p < 0.01$) (Table S6; Fig 12).

Figure 8 -Abiotic parameters data. a) Total monthly rainfall (mm) and mean ± SE of b) water temperature, c) water salinity, d) Secchi depth (cm) and e) dissolved oxygen in different seasons ED, Early dry (Sep-Oct-Nov); LD, Late dry (Dec-Jan-Feb); ER, Early rainy (Mar-Apr-May); LR, Late rainy (Jun-Jul-Aug) and areas (Upper: △ Middle: □ and Lower: ○) of the Goiana estuary.

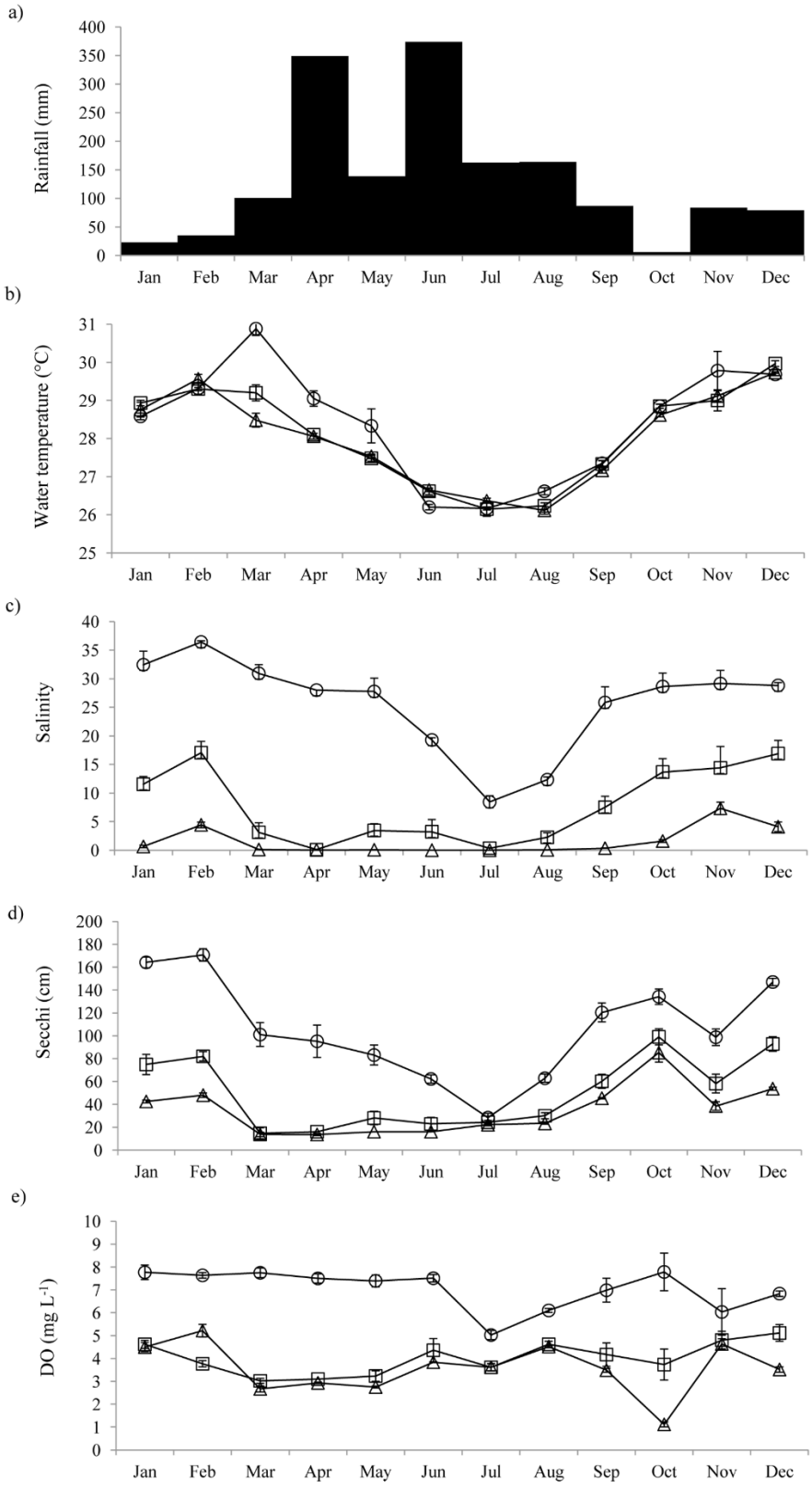


Figure 9 - Frequency of occurrence in percentage (FO%) of items ingested by *Achirus lineatus* in different ontogenetic phases (Juvenile: ; Subadults: ; Adults:), seasons (ED, early dry; LD, late dry; ER, early rainy; LR, late rainy) and areas (upper, middle and lower) of the Goiana estuary.

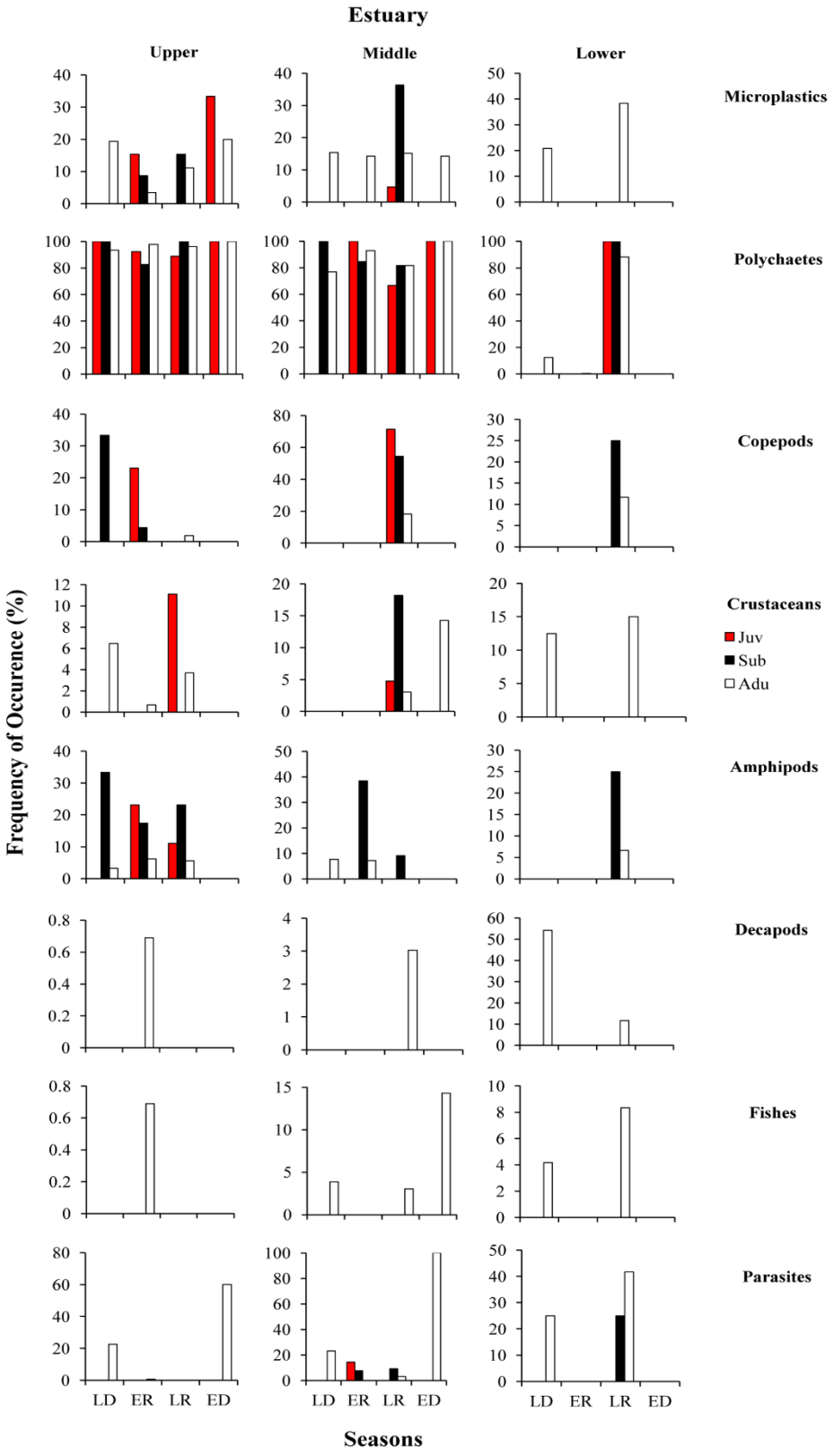


Figure 10 - Microplastics in the digestive tract of *Achirus lineatus*. a) Red filament; b) White filament; c) Blue filament in the digestive tract (zoom 10x) and d) zoom 20x (arrows indicate the zoom); e) Microplastic adhered in the stomach of *Achirus lineatus* with an mysidacea (zoom 10x) and f) zoom 40x (arrows indicate the zoom).

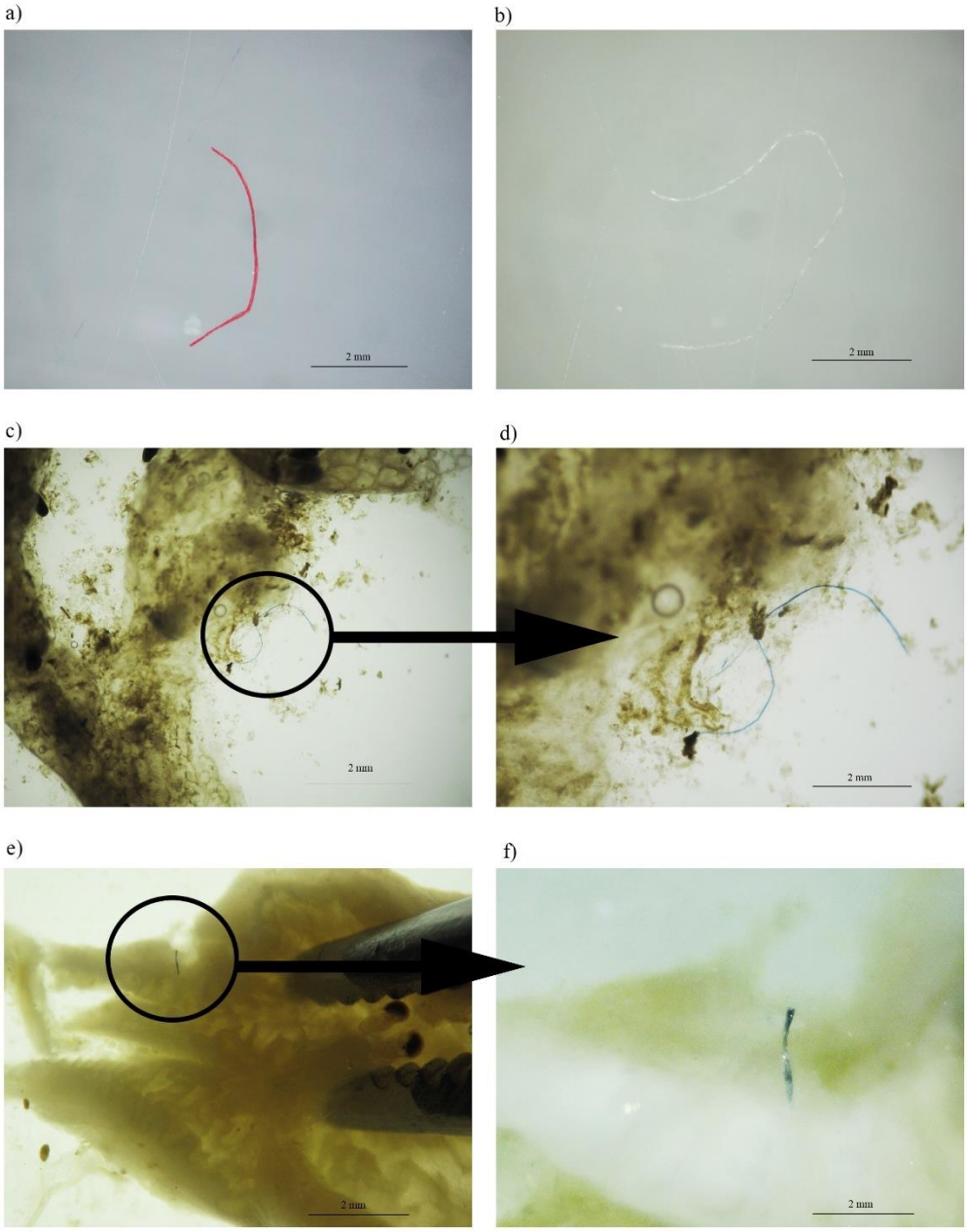


Figure 11 - Mean numbers of items ingested by *A. lineatus* in different ontogenetic phases (Juvenile: ■ Subadults: ■; Adults: □), seasons (ED, early dry; LD, late dry; ER, early rainy; LR, late rainy) and areas (upper, middle and lower) of the Goiana estuary.

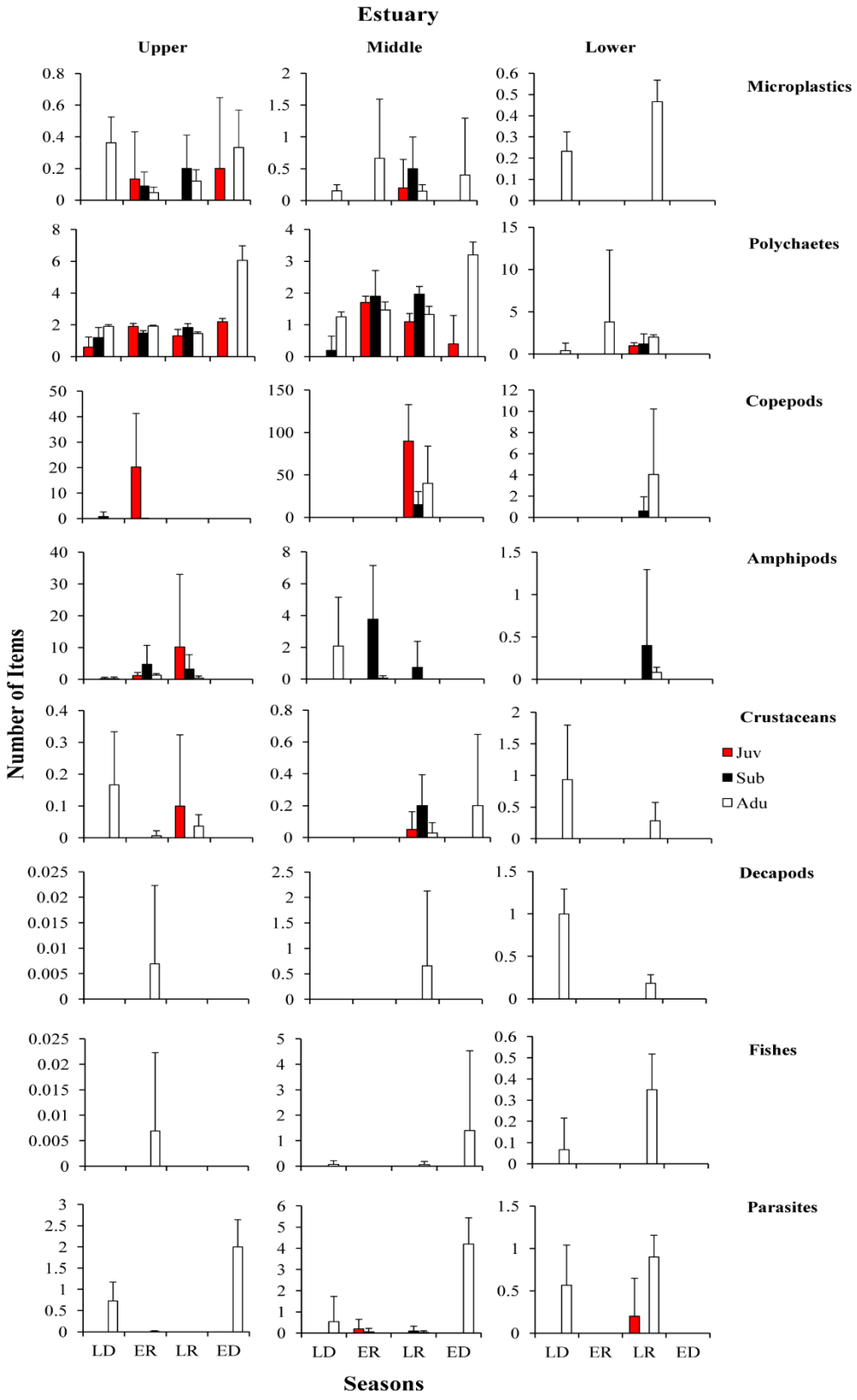


Figure 12 - Mean of number filaments ingested by *Achirus lineatus* in different ontogenetic phases (Juvenile: ■, Subadults:■; Adults:□), seasons (ED, early dry; LD, late dry; ER, early rainy; LR, late rainy) and areas (upper, middle and lower) of the Goiana estuary.

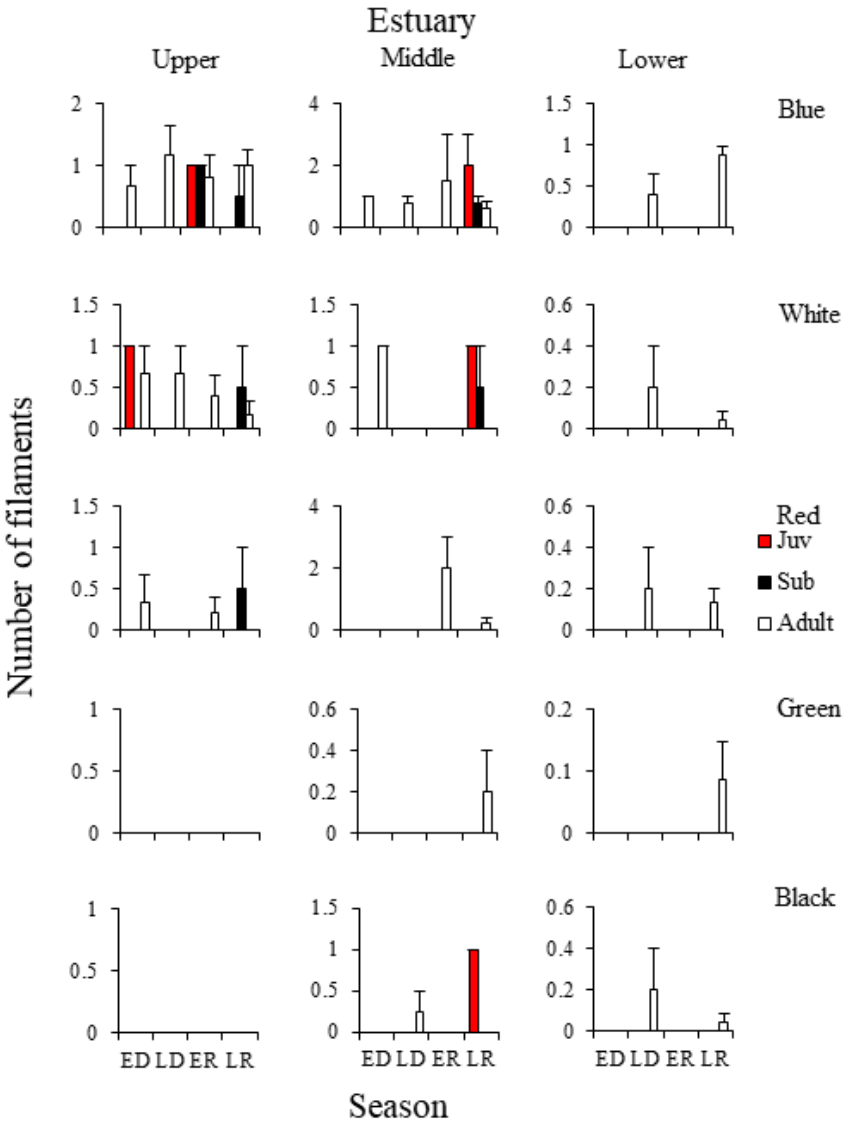
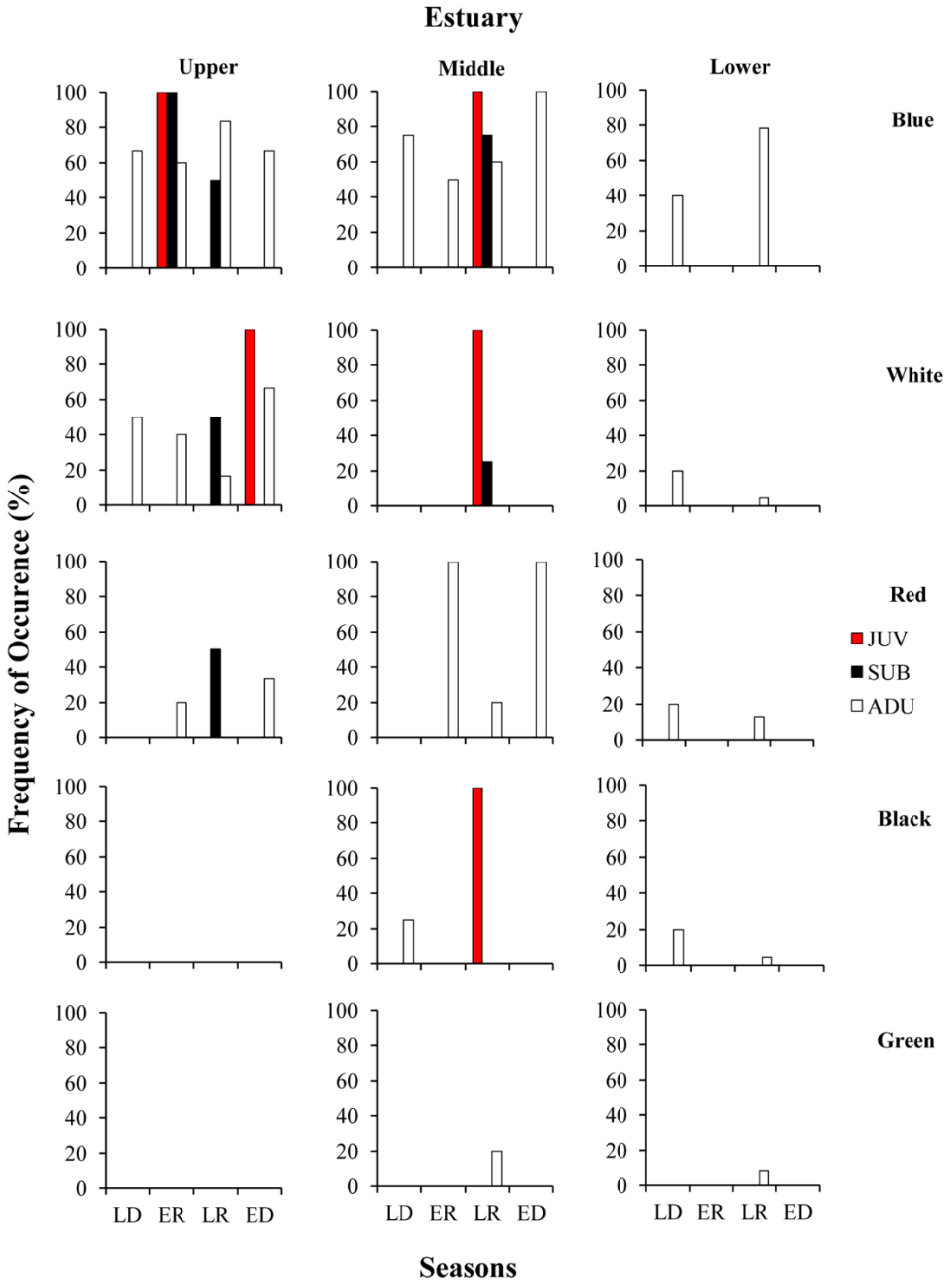


Figure 13 - Frequency of occurrence in percentage (FO%) different colours of filaments ingested by *Achirus lineatus* according to ontogenetic phases (Juvenile: ; Subadults: ; Adults:) seasons (ED, early dry; LD, late dry; ER, early rainy; LR, late rainy) and areas (upper, middle and lower) of the Goiana estuary.



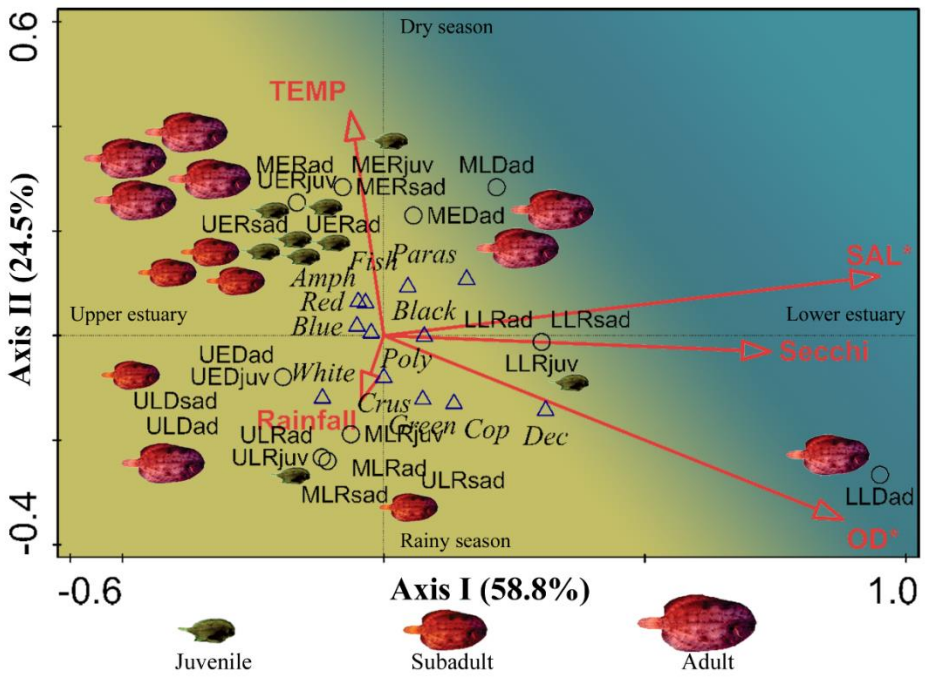
4.3.5 Environmental variables acting in the patterns of trophic niche shifts

The Canonical Correspondence Analysis (CCA) showed that the environmental variables salinity and dissolved oxygen ($p < 0.05$) were responsible for the formation of

Axis I. This axis represented the gradient of salinity in the Goiana Estuary and was responsible for 58.8% of the total variability in the data (Table S6; Fig. 8). Axis II was formed by temperature (positively) and rainfall (negatively), which represented the seasonality and was responsible for 24.5% of the total data variability (Table S7; Fig. 14).

The items ingested by *A. lineatus*, polychaetes and blue microplastic filaments, are plotted in the centre of data ordination. It suggests that these items have great importance as food items for all ontogenetic phases regardless of area and season. On the other hand, copepods and decapods were correlated with green filaments ingestion in the middle estuary, particularly during the late rainy season for juveniles and adults, respectively (Fig. 14). In addition, juveniles and subadults correlated with ingestion of crustaceans (*e.g*. mysidacea, ostracod, *Daphnia* sp) and white filaments in the upper estuary during the dry season.

Figure 14 - Canonical correspondence analysis (CCA) triplot for correlations between environmental variables and indices of relative importance (IRI) for items ingested by *Achirus lineatus*. The environmental parameters (Temp: Temperature; Sal: Salinity; DO: Dissolved oxygen; Secchi: Secchi depth; and Rainfall) are represented by arrows (*p< 0.05). The prey items (Blue; White; Red; Black; Green; Poly: Polychaetes; Amph: Amphipoda; Cop: Copepods; Crus: Crustaceans; Dec: Decapods; Fishes: Fishes; Paras: Parasites) are represented by△. The combinations between areas (U: Upper; M: Middle; L: Lower) seasons (ED: early dry; LD: late dry; ER: early rainy; LR: late rainy) and ontogenetic phases (juv: juveniles; sad: subadults; adu: adults) are represented by○.



4.4 Discussion

Seasonal patterns of salinity gradient in the Goiana Estuary were the most important element in structuring *A. lineatus* distribution in this ecosystem. This was also described for fish assemblage distributions in the tropical Caeté Estuary, eastern Amazon (Barletta *et al*., 2005); in Paranaguá Estuary, a tropical-subtropical estuary in southern Brazil (Barletta *et al*., 2008, 2016) and in the Río the la Plata Estuary, a temperate estuary in Uruguay-Argentina (Jaureguizar *et al*., 2004). Regardless of climate, seasonal fluctuations of salinity are the main influence on faunal distribution in estuaries (Barletta & Dantas, 2016a; b).

At Goiana Estuary, rainfall characterizes seasons over the year. These patterns create a complex environmental gradient, expressed in the water parameters (*e.g*. salinity ecocline, Secchi depth, dissolved oxygen), which depend on the Goiana River runoff. Differences in salinity values at the main channel create a mixing of waters (continental and marine) in the estuary, characterizing the early and late dry seasons which are most influenced by marine inputs. This forces the movement of organisms among estuarine habitats, changing with seasons (Barletta *et al*., 2005). Rainfall forces the decrease in salinity at the main channel of the estuary, due to the increase of the river runoff. Suspended organic matter then increases, decreasing Secchi depth values (~50 cm) for all areas. Early and late rainy seasons are characterized by increases of river runoff, causing water parameters in the estuary to be more riverine in character (Blaber *et al*., 1989; Barletta *et al*., 2005, 2008).

During the rainy season, the upper portion of the estuary showed greater importance for the distribution of *A. lineatus*, and for *Cathorops* spp (Ariidae) and *Stellifer* spp (Sciaenidae) (Dantas *et al.*, 2013; Dantas *et al.*, 2015). Due to their common habits as benthic/demersal fishes, all preferred areas with higher turbidity and abundant organic matter, ideal places for foraging benthic prey and avoiding predators (Dantas *et al*., 2010, 2012a, b). In this portion of the estuary, adults (> 50 mm) of *A. lineatus* showed the highest densities and biomass during the rainy season. This information corroborated the hypothesis that patterns of distribution change as a function of the spatio-temporal variation and ontogenetic phases of estuarine fishes, including *A. lineatus*. It suggests that adults use this area during the rainy season as spawning grounds. Lima *et al*. (2015) reported that eggs of *A. lineatus* were more abundant during the early rainy season, in the middle and lower estuary. Larvae of *A.*

lineatus showed highest densities in the lower estuary during the same season. Juveniles of *A lineatus* showed higher density in the upper estuary during the early rainy season and in the middle estuary during the late rainy season. It suggests that these areas are nursery grounds for this species during the rainy season. The upper portion of Goiana Estuary was also classified as a nursery area for estuarine Ariidae and Sciaenidae (Dantas *et al*., 2012a, b), as well as to other marine estuary-dependent fishes (Ferreira *et al*., 2016).

The ability of *A. lineatus* for tolerating wide salinity ranges provides it with a large home range distribution within the system. It facilitates avoiding predation, foraging on benthic prey and increases abundance in the whole ecosystem (Carpenter, 2002). Polychaetes (*Nereis* sp. Syllidae and Terebellidae) were the preferred prey for *A. lineatus*, indicating that they are zoobenthivores (Fig. 9). Nevertheless, each ontogenetic stage showed different behaviours, according to seasonal environmental variation as reported in many studies for other demersal estuarine species (Dantas *et al*., 2012; 2014; Ramos *et al*., 2014; 2016; Ferreira *et al*, 2016).

During the dry season, when the estuary has more characteristics of marine environment, adults and subadults, showed a more diverse diet (Fig. 9). Subadults fed on amphipods (Corophiidae) and copepods in the upper estuary, zooplanktivorous habits. Adults also prey on crustaceans (Mysidacea, Isopods and Ostracods) in the upper and lower estuary during late dry and late rainy season and in the middle estuary during late rainy and early dry season. Decapods (Peneidae, Paguridae and Astacidae) were prey mainly in the lower estuary during late rainy and late dry seasons. Fishes (*Myrophis punctatus* and *Gobionellus oceanicus*) were preyed in the middle (early dry) and lower estuary, especially during the late rainy season.

During the rainy season, juveniles also fed on amphipods in the upper estuary, and copepods (Cyclopoid and Calanoid) in the upper and middle estuary, where the copepods showed the highest densities in the Goiana Estuary (Lima *et al*., 2014). This habit showed the plasticity of *A. lineatus* to feed on a wide range of prey and may reveal a transition in the feeding habits of larvae and juveniles, showing a trend for zooplanktivory.

Nowadays, a great concern in fish ecology is the ubiquitous contamination, by microplastics. Being widespread in every ecosystem, microplastics easily interact with organisms (Lima *et al.,* 2015). Microplastic filaments were found in the digestive tract of adults of *A. lineatus* in the upper estuary, regardless season. In the lower estuary,

during the late rainy season, filaments of microplastic represented up to 38% of frequency in occurrence. This suggests direct association with benthic foraging (Dantas *et al*., 2012a, b; Ramos *et al*., 2012; Possatto *et al*., 2011; Lusher *et al*., 2013). Subadults presented 36% of microplastics occurrence in the middle estuary during the late rainy season. Microplastics were also found in the digestive tract of juveniles (up to 33%) in the early dry season at the upper estuary. This indicates that the nursery area was also contaminated by microplastics, as Ferreira *et al*., (2016) reported for juveniles of Acoupa weakfish, thereby compromising the health of juveniles.

The increase in microplastic occurrence in stomach contents according to ontogenetic stage, suggest a high rate of ingestion of these filaments during benthic foraging. This was also reported by Ferreira *et al*., (2016). In addition, it is possible that fish accumulate microplastics for their whole life. The great availability of these filaments in sediments is probably because of their rapid sinking (Lima *et al*., 2014). Most microplastics found in the digestive tract of *A. lineatus* were classified as blue filaments. Indeed, blue filaments are the most common coloured plastics found in stomach contents of other fishes of the Goiana Estuary (Possatto *et al*., 2011, Dantas *et al.*, 2012, Ramos *et al.*, 2012; Ferreira *et al*., 2016) and worldwide (Boerger *et al*., 2010; Lusher *et al*., 2015). This is probably associated with the use and discarding/loss of fishery gear as well as maintenance of fishing nets (Lima *et al*., 2014, 2015).

Previous studies across the world have identified the same problems of interactions between microplastics and feeding ecology of fishes (Boerger *et al*., 2010; Lusher *et al*., 2015; Bråte *et al*., 2016). In the Goiana Estuary, previous studies confirm contamination of the environment and several species with microplastics (Ferreira *et al*., 2016; Lima *et al*., 2014; Dantas *et al*., 2012; Ramos *et al*., 2012). Regardless of the type of microplastic, this contaminant is ingested worldwide by fish and is hazardous for biota, especially when microplastics are biotransferred along the food chain (Eriksson and Burton, 2003).

Ferreira *et al*., (2016) found that both juveniles and adults of *A. lineatus* were one of the favourite prey of *Cynoscion acoupa* (Scianidae). The authors suggested that high microplastics contamination in adults of *C. acoupa* could be a consequence of biotransference of microplastics along the food chain. In a laboratory experiment, Barboza *et al*., (2017) reported that contamination by microplastics can be dangerous, causing toxic effects, *e.g*. neurological effects, and showed that microplastics increase availability of other pollutants, which are hazardous for human health (WHO, 1990).

This may suggest that consumption of contaminated prey leads to accumulation of microplastics along their life cycle and may have impacts directly in the food web.

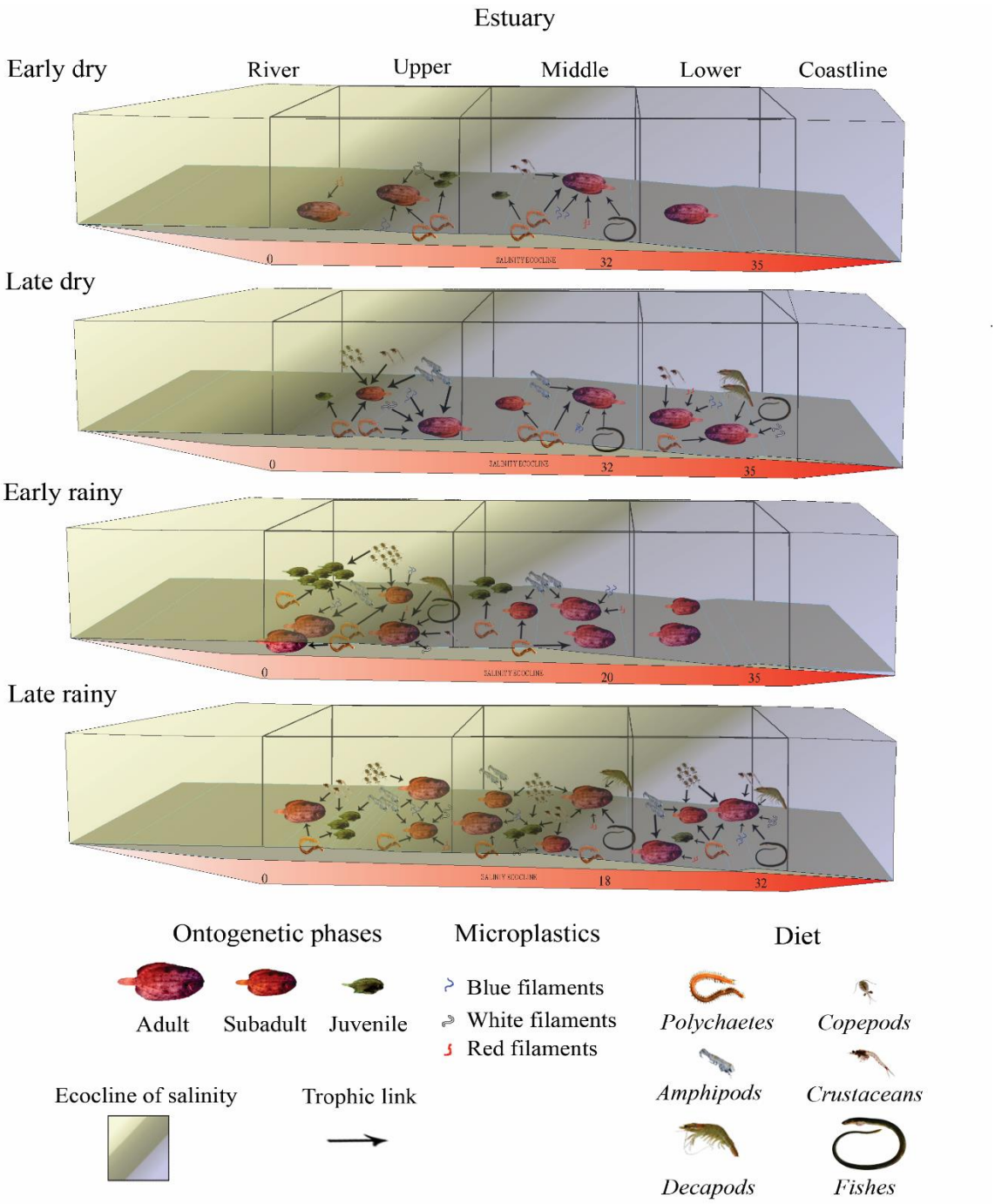
4.5 Conclusions

This study confirmed the hypothesis that distribution, feeding ecology and contamination by microplastics change according to areas of the estuary, seasonality, and ontogenetic stages of *Achirus lineatus*. The salinity ecocline was the most important factor to rule this environment. Contamination by microplastic filaments in the *A. lineatus* population at Goiana estuary occurred regardless ontogenetic phase and area, and had the highest values (number of filament per digestive tract) recorded during the late rainy season for adults, suggesting that they may accumulate microplastics in their digestive tract over their life span (Fig. 9).

The lined sole *Achirus lineatus* has an important role in the maintenance of the ecological process in estuaries where it occurs, due to the higher density and biomass of this species. They are also a preferential prey of top predators. The impacts generated in important areas, as the nursery ground (Fig. 9) and feeding areas may be a serious concern for the future of the species, pursuant to other organisms, which depend direct or indirectly of them. Besides that, this study demonstrated that *A. lineatus* may has the ability to transfer contaminants such as microplastic to higher levels of food web.

To better understand microplastics biotransference, and whether they could transfer adsorbed pollutants such as persistent organic pollutants (POPs) and mercury to the food web, more studies are necessary. If microplastics have the ability to be transferred along the trophic chain, the chances to accumulate these pollutants in the food web increase. This problem has direct consequences to human health.

Figura 15 - Conceptual model of temporal and spatial patterns of ontogenetic phase of *A. lineatus* diet and microplastic contamination in the Goiana Estuary.



5 CONCLUSÃO GERAL

Este estudo comprovou a hipótese testada de que a distribuição, a ecologia alimentar e a contaminação por microplásticos mudaram de acordo com as áreas do estuário, a sazonalidade e as fases ontogênicas de *Achirus lineatus*. Entretanto, a ecoclina de salinidade é o fator preponderante na estruturação desse ecossistema. Os indivíduos de *A. lineatus* têm por preferência habitar as áreas mais internas do estuário, provavelmente devido às melhores condições ambientais, que ajudam a evitar a predação, no entanto eles usam todo o canal principal durante todas as estações. Na estação chuvosa, os adultos do estuário superior, alimentam-se de grandes quantidades de poliquetas, aumentam em peso e em comprimento para iniciar sua atividade reprodutiva, que pode ocorrer nesta área. Após a desova, durante o início da estação chuvosa, os adultos migram para o estuário médio e inferior, onde se alimentam de crustáceos, decápodes e peixes, e onde também apresentam maiores taxas de contaminação por microplásticos. Os juvenis ocupam o estuário superior e intermediário durante a estação chuvosa, que são caracterizadas como áreas de berçário para a espécie. No estuário intermediário, se alimentam de poliquetas e copepodas, além de obterem proteção, através da turbidez intensa nessa área durante a estação chuvosa, evitando assim, predadores. Os subadultos têm o mesmo padrão de distribuição e ecologia alimentar dos adultos, porém, além de se alimentarem de poliquetas, eles também se alimentam de copepodas e anfípodas. Durante a estação seca, os subadultos de *A. lineatus* no estuário inferior, migram retornando ao estuário médio e superior. Nesta estação, eles predam principalmente poliquetas e crustáceos. A contaminação por filamentos microplásticos ocorreu independentemente das áreas, fases ontogenéticas e estação do ano. Os maiores valores deste contaminante foram registrados durante o final da estação chuvosa para os adultos, sugerindo que eles podem acumular esses contaminantes microplásticos no seu trato digestivo ao longo de todo seu ciclo de vida.

O linguado *Achirus lineatus* tem um papel fundamental na manutenção dos processos ecológicos no ecossistema estuarino, observado pela sua alta densidade e biomassa, e também são presas preferenciais para os predadores de topo nesse ecossistema. Os impactos gerados em áreas importantes, como o berçário e áreas utilizadas para alimentação, podem ser danosos para o futuro da espécie, e de outros organismos que dependem direta ou indiretamente deles. Além disso, este estudo sugere que organismos que promovem um elo entre o substrato e os maiores níveis trófico,

podem ter a capacidade de transferir contaminantes, bem como microplásticos e outros poluentes através da cadeia trófica.

Para melhorar o entendimento sobre como ocorre a biotransferência e se os contaminantes microplásticos, podem também transferir poluentes adsorvidos, como poluentes orgânicos persistentes (POP) e poluentes derivados de mercúrio para a teia trófica, são necessários mais estudos acerca deste assunto. Entretanto, se os microplásticos têm a capacidade de serem transferidos na cadeia trófica, as chances de acumulação desses contaminantes e dos poluentes que podem estar adsorvidos, aumentam. Por isto, este problema está diretamente associado à saúde humana.

REFERÊNCIAS

ARTHUR, C.; BAKER, J.; BAMFORD, H. Proceedings of the International Research Workshop on the Occurrence, Effects, and Fate of Microplastic Marine Debris, September 9-11, 2008, **University of Washington Tacoma**, Tacoma, WA, USA, 2009.

ASHTON, K.; HOLMES, L.; TURNER, A. Association of metals with plastic production pellets in the marine environment. **Marine pollution bulletin**, v. 60, n. 11, p. 2050-2055, 2010.

BARBOZA, L. G. A.; VIEIRA, L. R.; BRANCO, V.; FIGUEIREDO, N.; CARVALHO, F.; CARVALHO, C.; & GUILHERMINO, L. Microplastics cause neurotoxicity, oxidative damage and energy-related changes and interact with the bioaccumulation of mercury in the European seabass, *Dicentrarchus labrax* (Linnaeus, 1758). **Aquatic Toxicology**, 2017.

BARLETTA, M.; AMARAL, C. S.; CORRÊA, M. F. M.; GUEBERT, F.; DANTAS, D. V.; LORENZI, L.; & SAINT-PAUL, U. Factors affecting seasonal variations in demersal fish assemblages at an ecocline in a tropical–subtropical estuary. **Journal of Fish Biology**, v. 73, n. 6, p. 1314-1336, 2008.

BARLETTA, M.; & COSTA, M. F. Living and non-living resources exploitation in a tropical semi-arid estuary. **Journal of Coastal Research**, p. 371-375, 2009.

BARLETTA, M.; JAUREGUIZAR, A. J.; BAIGUN, C.; FONTOURA, N. F.; AGOSTINHO, A. A.; ALMEIDA-VAL, V. M. F.; VAL. A. L.; TORRES, R. A.; JIMENES-SEGURA, L. F.; GIARRIZZO, T.; FABRÉ, N. N.; BATISTA, V. S.; LASSO, C.; TAPHORN, D. G.; COSTA, M. F.; CHAVES, P. T.; VIEIRA, J. P.; CORRÊA, M. F. M. Fish and aquatic habitat conservation in South America: a continental overview with emphasis on neotropical systems. **Journal of fish biology**, v. 76, n. 9, p. 2118-2176, 2010.

BARLETTA, M.; BARLETTA-BERGAN, A.; SAINT-PAUL, U. S. G. H.; & HUBOLD, G. The role of salinity in structuring the fish assemblages in a tropical estuary. **Journal of fish biology**, v. 66, n. 1, p. 45-72, 2005.

BARLETTA-BERGAN, A.; BARLETTA, M.; SAINT-PAUL, U. Structure and seasonal dynamics of larval fish in the Caeté River Estuary in North Brazil. **Estuarine, Coastal and Shelf Science**, v. 54, n. 2, p. 193-206, 2002a.

BARLETTA-BERGAN, A.; BARLETTA, M.; SAINT-PAUL, U. Community structure and temporal variability of ichthyoplankton in North Brazilian mangrove creeks. **Journal of Fish Biology**, v. 61, n. sA, p. 33-51, 2002b.

BARLETTA, M.; LUCENA, L. R. R.; COSTA, M. F.; BARBOSA-CINTRA, S. C. T.; & CYSNEIROS, F. J. A. The interaction rainfall vs. weight as determinant of total mercury concentration in fish from a tropical estuary. **Environmental pollution**, v. 167, p. 1-6, 2012.

BARLETTA, M.; & DANTAS, D. V. Environmental Gradient. In: Kennish, M. J. **Encyclopedia of Estuaries**. Springer Netherlands, 237-242, 2016a.

BARLETTA, M.; CYSNEIROS, F. J. A.; LIMA, A. R. A. Effects of dredging operations on the demersal fish fauna of a South American tropical–subtropical transition estuary. **Journal of fish biology**, v. 89, n. 1, p. 890-920, 2016.

BARLETTA, M.; LIMA, A. R.; DANTAS. D. V.; OLIVEIRA, I. M.; NETO, J. R.; FERNANDES, C. A.; FARIAS, E. G. G.; FILHO, J. L. R.; COSTA, M. F. How Can Accurate Landing Stats Help in Designing Better Fisheries and Environmental Management for Western

Atlantic Estuaries?. In: **Coastal Wetlands: Alteration and Remediation**. Springer, Cham. p. 631-703, 2017.

BLABER, S. J. M.; BREWER, D. T.; SALINI, J. P. Species composition and biomasses of fishes in different habitats of a tropical northern Australian estuary: their occurrence in the adjoining sea and estuarine dependence. **Estuarine, Coastal and Shelf Science**, v. 29, n. 6, p. 509-531, 1989.

BATEL, A.; LINTI, F.; SCHERER, M.; ERDINGER, L.; & BRAUNBECK, T. Transfer of benzo [a] pyrene from microplastics to Artemia nauplii and further to zebrafish via a trophic food web experiment: CYP1A induction and visual tracking of persistent organic pollutants. **Environmental toxicology and chemistry**, v. 35, n. 7, p. 1656-1666, 2016.

TER BRAAK, C. JF. Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. **Ecology**, v. 67, n. 5, p. 1167-1179, 1986.

BECK, M. W.; HECK, KL Jr.; ABLE, K.W.; CHILDERS, D.L.; EGGLESTON, D.B.; GILLANDERS, B.M.; HALPERN, B.; HAYS, C.G.; HOSHINO, K.; MINELLO, T.J.; ORTH, R.J.; SHERIDAN, P.F.; WEINSTEIN, M.P. The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates: a better understanding of the habitats that serve as nurseries for marine species and the factors that create site-specific variability in nursery quality will improve conservation and management of these areas. **Bioscience**, v. 51, n. 8, p. 633-641, 2001.

BOX, G. E.P.; & COX, D. R. An analysis of transformations. **Journal of the Royal Statistical Society. Series B (Methodological)**, p. 211-252, 1964.

BOERGER, C. M.; LATTIN, G. L.; MOORE, S. L.; & Moore, C. J. Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre. **Marine pollution bulletin**, v. 60, n. 12, p. 2275-2278, 2010.

BRÅTE, I. L. N.; EIDSVOLL, D. P.; STEINDAL, C. C.; & THOMAS, K. V. Plastic ingestion by Atlantic cod (*Gadus morhua*) from the Norwegian coast. **Marine pollution bulletin**, v. 112, n. 1-2, p. 105-110, 2016.

BRUSCA, R.C.; & G.J. Brusca. Invertebrates, 2nd ed. **Sunderland: Sinauer Associates**. 966p, 2002.

CARPENTER, K.E.; DE ANGELIS, N. (Ed.). **The living marine resources of the Western Central Atlantic**. Food and agriculture organization of the United Nations, 2002.

CLARKE, K. R.; GORLEY, R. N. PRIMER v6: user manual/tutorial (Plymouth routines in multivariate ecological research). **Plymouth: Primer-E Ltd**, 2006.

CHAVES, P. T.C.; & SERENATO, A. Diversidade de dietas na assembléia de linguados (Teleostei, Pleuronectiformes) do manguezal da Baía de Guaratuba, Paraná, Brasil. **Revista Brasileira de Oceanografia**, v. 46, n. 1, p. 61-68, 1998.

COSTA, M. F.; BARBOSA, S. C; BARLETTA, M; DANTAS, D. V; KEHRIG, H. A; SEIXAS, T. G. & MALM, O. Seasonal differences in mercury accumulation in *Trichiurus lepturus* (Cutlassfish) in relation to length and weight in a Northeast Brazilian estuary. **Environmental Science and Pollution Research**, v. 16, n. 4, p. 423, 2009.

COSTA, M. F.; BARLETTA, M. Special challenges in the conservation of fishes and aquatic environments of South America. **Journal of fish biology**, v. 89, n. 1, p. 4-11, 2016.

DANTAS, D. V.; BARLETTA, M.; COSTA, M. F.; BARBOSA-CINTRA, S. C. T.; POSSATTO, F. E.; RAMOS, J. A. A.; LIMA, A. R. A.; & SAINT-PAUL, U. Movement patterns of catfishes (Ariidae) in a tropical semi-arid estuary. **Journal of Fish Biology**, v. 76, n. 10, p. 2540-2557, 2010.

DANTAS, D. V.; BARLETTA, M.; COSTA, M. F. The seasonal and spatial patterns of ingestion of polyfilament nylon fragments by estuarine drums (Sciaenidae). **Environmental Science and Pollution Research**, v. 19, n. 2, p. 600-606, 2012.

DANTAS, D. V.; BARLETTA, M.; RAMOS, J. D. A. A.; LIMA, A. R. A.; & COSTA, M. F. Seasonal diet shifts and overlap between two sympatric catfishes in an estuarine nursery. **Estuaries and Coasts**, v. 36, n. 2, p. 237-256, 2013.

DANTAS, D. V.; BARLETTA, M.; COSTA, M. F. Feeding ecology and seasonal diet overlap between *Stellifer brasiliensis* and *Stellifer stellifer* in a tropical estuarine ecocline. **Journal of fish biology**, v. 86, n. 2, p. 707-733, 2015.

DUARTE, G. A.S.; ANDREATA, J. V. Hábito alimentar das espécies de Achiridae e Cynoglossidae que ocorrem na Baía da Ribeira, Angra dos Reis, Rio de Janeiro, Brasil. **Títulos não-correntes**, v. 17, n. 1/2, 2012.

ERIKSSON, C.; BURTON, H. Origins and biological accumulation of small plastic particles in fur seals from Macquarie Island. **AMBIO: A Journal of the Human Environment**, v. 32, n. 6, p. 380-384, 2003.

FERREIRA, G. V.B.; BARLETTA, M.; LIMA, A. R.; DANTAS, D. V.; JUSTINO, A. K.; & COSTA, M. F. Plastic debris contamination in the life cycle of Acoupa weakfish (*Cynoscion acoupa*) in a tropical estuary. **ICES Journal of Marine Science**, v. 73, n. 10, p. 2695-2707, 2016.

FUKUHARA, O. Morphological and functional development of larval and juvenile Limanda yokohamae (Pisces: Pleuronectidae) reared in the laboratory. **Marine Biology**, v. 99, n. 2, p. 271-281, 1988.

FROESE, R.; PAULY, D (Ed.). **FishBase 2000: concepts designs and data sources**. WorldFish, 2000.

FRIAS, J. P. G. L.; SOBRAL, P.; FERREIRA, A. M. Organic pollutants in microplastics from two beaches of the Portuguese coast. **Marine Pollution Bulletin**, v. 60, n. 11, p. 1988-1992, 2010.

GIBSON, R. N. Behaviour and the distribution of flatfishes. **Journal of Sea Research**, v. 37, n. 3-4, p. 241-256, 1997.

HOLMES, L. A.; TURNER, A.; THOMPSON, R. C. Adsorption of trace metals to plastic resin pellets in the marine environment. **Environmental Pollution**, v. 160, p. 42-48, 2012.

HYNES, H. B. N. The food of fresh-water sticklebacks (Gasterosteus aculeatus and Pygosteus pungitius), with a review of methods used in studies of the food of fishes. **The Journal of Animal Ecology**, p. 36-58, 1950.

HYSLOP, E. J. Stomach contents analysis-a review of methods and their application. **Journal of Fish Biology**, 17:411-429, 1980

INMET. Instituto Nacional de Meteorologia. Ministério da Agricultura, Pecuária e Abastecimento. Disponível em: www.inmet.gov.br. 2014.

JAUREGUIZAR, A. J.; MENNI, R.; GUERRERO, R.; & LASTA, C. Environmental factors structuring fish communities of the Rıo de la Plata estuary. **Fisheries Research**, v. 66, n. 2-3, p. 195-211, 2004.

LACERDA, C. H. F.; BARLETTA, M.; DANTAS, D. V. Temporal patterns in the intertidal faunal community at the mouth of a tropical estuary. **Journal of fish biology**, v. 85, n. 5, p. 1571-1602, 2014.

LIMA, A. R. A.; COSTA, M. F.; BARLETTA, M. Distribution patterns of microplastics within the plankton of a tropical estuary. **Environmental Research**, v. 132, p. 146-155, 2014.

LIMA, A. R. A.; BARLETTA, M.; COSTA, M. F. Seasonal distribution and interactions between plankton and microplastics in a tropical estuary. **Estuarine, Coastal and Shelf Science**, v. 165, p. 213-225, 2015.

LIMA, A. R. A.; BARLETTA, M.; COSTA, M.F.; RAMOS, J.A.A.; DANTAS, D.V.; Justino, A.K.S.; & FERREIRA, G.V.B. Changes in the composition of ichthyoplankton assemblage and plastic debris in mangrove creeks relative to moon phases. **Journal of fish biology**, v. 89, n. 1, p. 619-640, 2016.

LUSHER, A. L.; MCHUGH, M.; THOMPSON, R. C. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. **Marine pollution bulletin**, v. 67, n. 1-2, p. 94-99, 2013.

LUSHER, A. L.; O'DONNELL, C.; OFFICER, R.; & O'CONNOR, I. Microplastic interactions with North Atlantic mesopelagic fish. **ICES Journal of Marine Science**, v. 73, n. 4, p. 1214-1225, 2015.

MENEZES, N A.; FIGUEIREDO, J. L. **Manual de peixes marinhos do sudeste do Brasil: Teleostei; 5**. Museu de Zoologia, Univ. de São Paulo, 2000.

MOORE, C. J. Synthetic polymers in the marine environment: a rapidly increasing, long-term threat. **Environmental research**, v. 108, n. 2, p. 131-139, 2008.

OEHLMANN, J.; SCHULTE-OEHLMANN, U.; KLOAS, W.; JAGNYTSCH, O.; LUTZ, I.; KUSK, K. O.; & Tyler, C. R. A critical analysis of the biological impacts of plasticizers on wildlife. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 364, n. 1526, p. 2047-2062, 2009.

PALMER, M. W. Putting things in even better order: the advantages of canonical correspondence analysis. **Ecology**, v. 74, n. 8, p. 2215-2230, 1993.

POSSATTO, F. E.; BARLETTA, M.; COSTA, M. F.; DO SUL, J. A. I.; & DANTAS, D. V. Plastic debris ingestion by marine catfish: an unexpected fisheries impact. **Marine pollution bulletin**, v. 62, n. 5, p. 1098-1102, 2011.

PRITCHARD, D. W. What is an Estuary: Physical View Point? In: LAUFF, G. H. (ed). Estuaries. Washington, **American Association for Advance of Science**, p. 3-5. 1967.

PINKAS.; OLIPHANT, S.; IVERSON, I. L. K. Food habits of albacore, bluefin tuna and bonito in Californian waters. **California Department of Fish and Game: Fish Bulletin**, v. 152, p. 1-105, 1971.

QUINN, G. P.; KEOUGH, M. J. **Experimental design and data analysis for biologists**. Cambridge University Press, 2002.

RAMOS, J. A.A.; BARLETTA, M.; COSTA, M. F. Ingestion of nylon threads by Gerreidae while using a tropical estuary as foraging grounds. **Aquatic Biology**, v. 17, n. 1, p. 29-34, 2012.

RAMOS, J. A. A.; BARLETTA, M., DANTAS, D. V.; LIMA, A. R. A.; & COSTA, M. F. Trophic niche and habitat shifts of sympatric Gerreidae. **Journal of Fish Biology**, v. 85, n. 5, p. 1446-1469, 2014.

RAMOS, J. A. A.; BARLETTA, M.; DANTAS, D. V.; COSTA, M. F. Seasonal and spatial ontogenetic movements of Gerreidae in a Brazilian tropical estuarine ecocline and its application for nursery habitat conservation. **Journal of fish biology**, v. 89, n. 1, p. 696-712, 2016.

ROCHMAN, C. M.; HOH, E.; KUROBE, T.; & TEH, S. J. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. **Scientific reports**, v. 3, p. 3263, 2013.

RICHARDS, W. J. (Ed.). **Early stages of Atlantic fishes: an identification guide for the western central north Atlantic, Two Volume Set**. CRC Press, 2005.

RUPPERT, E. E.; FOX, R. S.; BARNES, R. D. **Zoologia dos Invertebrados**. 7ª ed. 1168p. 2005.

DE SÁ, L. C.; LUÍS, L. G.; GUILHERMINO, L. Effects of microplastics on juveniles of the common goby (Pomatoschistus microps): confusion with prey, reduction of the predatory performance and efficiency, and possible influence of developmental conditions. **Environmental pollution**, v. 196, p. 359-362, 2015.

SPARRE, P.; & Venema, S. C. **Introducao a avaliação de mananciais de peixes tropicais**. Pt. 1: Manual. - Pt. 2: Exercicios. 1998.

STACHOWITSCH, M. **The invertebrates: an illustrated glossary**. Wiley-Liss, Inc., 1992.

TEUTEN, E. L.; ROWLAND, S. J.; GALLOWAY, T. S.; & THOMPSON, R. C. Potential for plastics to transport hydrophobic contaminants. **Environmental science & technology**, v. 41, n. 22, p. 7759-7764, 2007.

TEUTEN, E. L.; SAQUING, J. M.; KNAPPE, D. R.; BARLAZ, M. A.; JONSSON, S.; BJÖRN, A.; ROWLAND, S. J.; THOMPSON, R. C.; GALLOWAY, T.S.; YAMASHITA, R.; OCHI, D.; WATANUKI, Y.; MOORE, C.; VIET, P. H.; TANA, S. T.; PRUDENTE, M.; BOONYATUMANOND, R.; ZAKARIA, M. P.; AKKHAVONG, K.; OGATA, Y.; HIRAI, H.; IWASA, S.; MIZUKAWA, .K.; HAGINO, Y.; IMAMURA, A.; SAHA, M.; TAKADA, H. Transport and release of chemicals from plastics to the environment and to wildlife. **Philosophical Transactions of the Royal Society of London B: Biological Sciences**, v. 364, n. 1526, p. 2027-2045, 2009.

UNDERWOOD, A. J. **Experiments in ecology: their logical design and interpretation using analysis of variance**. Cambridge University Press, 1997.

VAZZOLER, A. E. A. M. Biologia da reprodução de peixes teleósteos: teoria e prática. **Maringá: Eduem**, v. 169, 1996.

W.H.O. METHYLMERCURY, environmental health criteria 101. **Geneva: World Health Organization**, p. 1-144, 1990.

APÊNDICE

SUPPLEMENTARY MATERIAL

S1. Characterization of the ontogenetic stages of *Achirus lineatus*, using the Lt *vs.* Wg equation (Juvenil ; Subadults ; Adults) and the estimation of average length of first maturation (L_{50}).

S2. Mean of density (ind. ha^{-1}) and biomass (g. ha^{-1}) of the *Achirus lineatus* in different ontogenetic stages (Juvenile Subadults Adults), seasons (ED, early dry; LD, late dry; ER, early rainy; LR, late rainy) and areas (upper, middle and lower) of the Goiana estuary.

S3. Mean weight (g) of items ingested by *Achirus lineatus* in different ontogenetic stages (Juvenile: Subadults: Adults:), seasons (ED, early dry; LD, late dry; ER, early rainy; LR, late rainy) and areas (upper, middle and lower) of the Goiana estuary.

Table S1. Mean of density (ind. ha^{-1}) and biomass (g. ha^{-1}) of *Achirus lineatus* according to ontogenetic phases (Juv: Juveniles; Sub: sub-adults; Adu: Adults), seasons (ED, early dry; LD, late dry; ER, early rainy; LR, late rainy) and areas (upper, middle and lower) of the Goiana estuary.

Table S2. Summary of the ANOVA results for total density (ind. ha^{-1}) and biomass (g. ha^{-1}) of *Achirus lineatus*. Differences among areas, seasons and ontogenetic phases were determined by Bonferroni's test *post hoc* comparisons. (*ns*: not significant) (F: F-values; df:degree of freedom; *p*-value) (Areas U: upper; M: middle; L: lower) (Seasons ED: early dry; LD: late dry; ER: early rainy; LR: late rainy) (Phases Juv: Juveniles; Sub: sub-adults; Adu: Adults) (**$p < 0.01$).

Table S3. Diet composition of *Achirus lineatus* expressed as percentage of the Index of Relative Importance (%I_{RI}), according to ontogenetic phases (Juv: Juvenile; Sub: subadult; Adu: Adult), Seasons (Early Dry, Late Dry, Early Rainy, Late Rainy) and Areas (Upper, Middle and Lower) of the Goiana Estuary. (-) no capture.

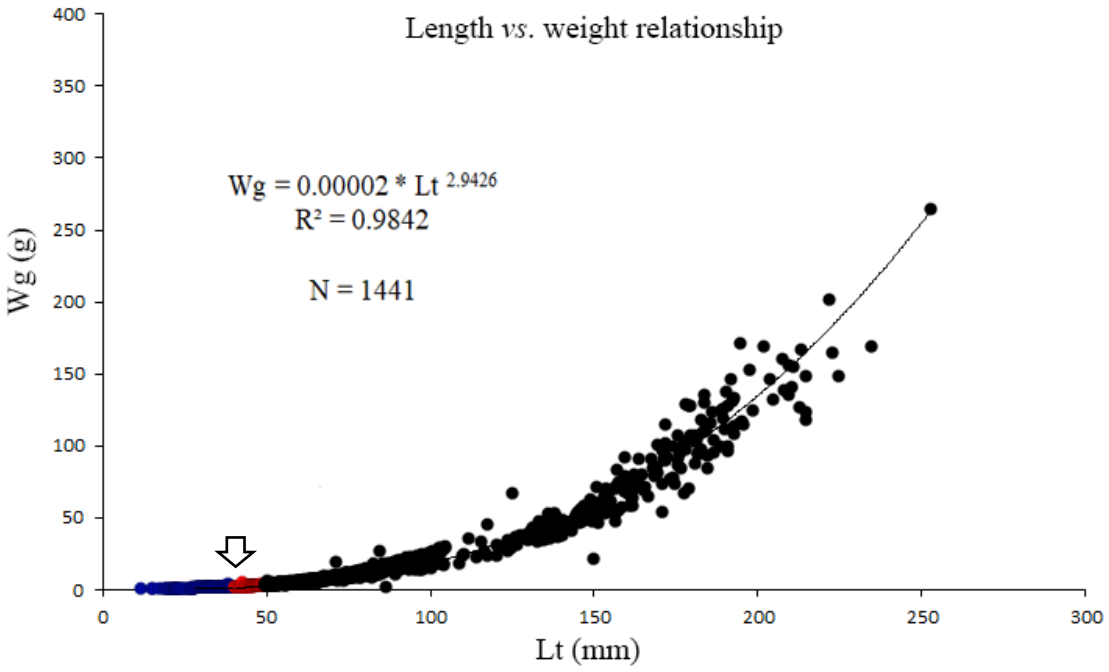
Table S4. Summary of the ANOVA results for mean number of items ingested by *Achirus lineatus*. Differences among areas, seasons and ontogenetic phases were determined by Bonferroni's test *post hoc* comparisons. (*ns*: not significant) (F: F-values; df:degree of freedom; *p*-value) (Areas U: upper; M: middle; L: lower) (Seasons ED: early dry; LD: late dry; ER: early rainy; LR: late rainy) (Phases Juv: Juveniles; Sub: sub-adults; Adu: Adults) (**$p < 0.01$).

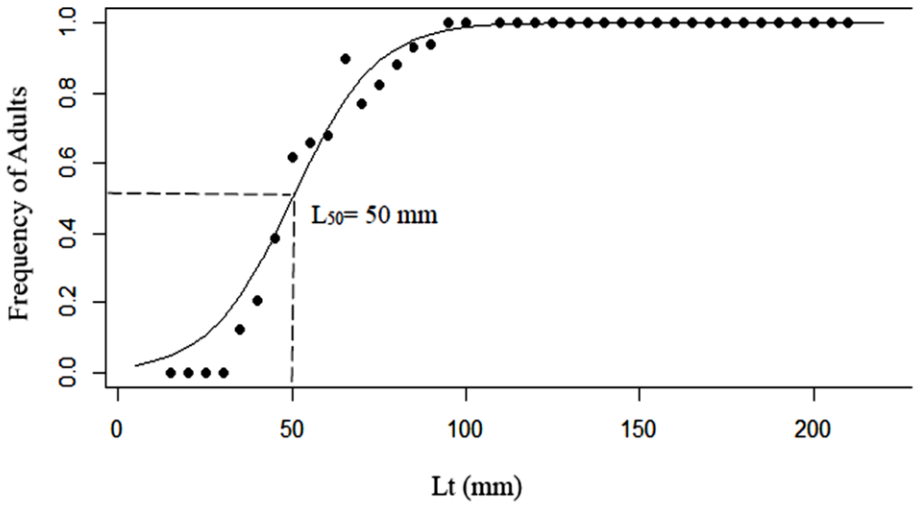
Table S5. Summary of the ANOVA results for mean weight (g) of items ingested by *Achirus lineatus*. Differences among areas, seasons and ontogenetic phases were determined by Bonferroni's test *post hoc* comparisons. (*ns*: not significant) (F: F-values; df:degree of freedom; *p*-value) (Areas U: upper; M: middle; L: lower) (Seasons ED: early dry; LD: late dry; ER: early rainy; LR: late rainy) (Phases Juv: Juveniles; Sub: sub-adults; Adu: Adults) (**$p < 0.01$).

Table S6. Summary of the ANOVA results results for the number of the plastics filaments ingested by *Achirus lineatus*. Differences among areas, seasons and ontogenetic phases were determined by Bonferroni's test *post hoc* comparisons. (*ns*: not significant) (F: F-values; df:degree of freedom; *p*-value) (Areas U: upper; M: middle; L: lower) (Seasons ED: early dry; LD: late dry; ER: early rainy; LR: late rainy) (Phases Juv: Juveniles; Sub: sub-adults; Adu: Adults) (*$p < 0.05$) (**$p < 0.01$).

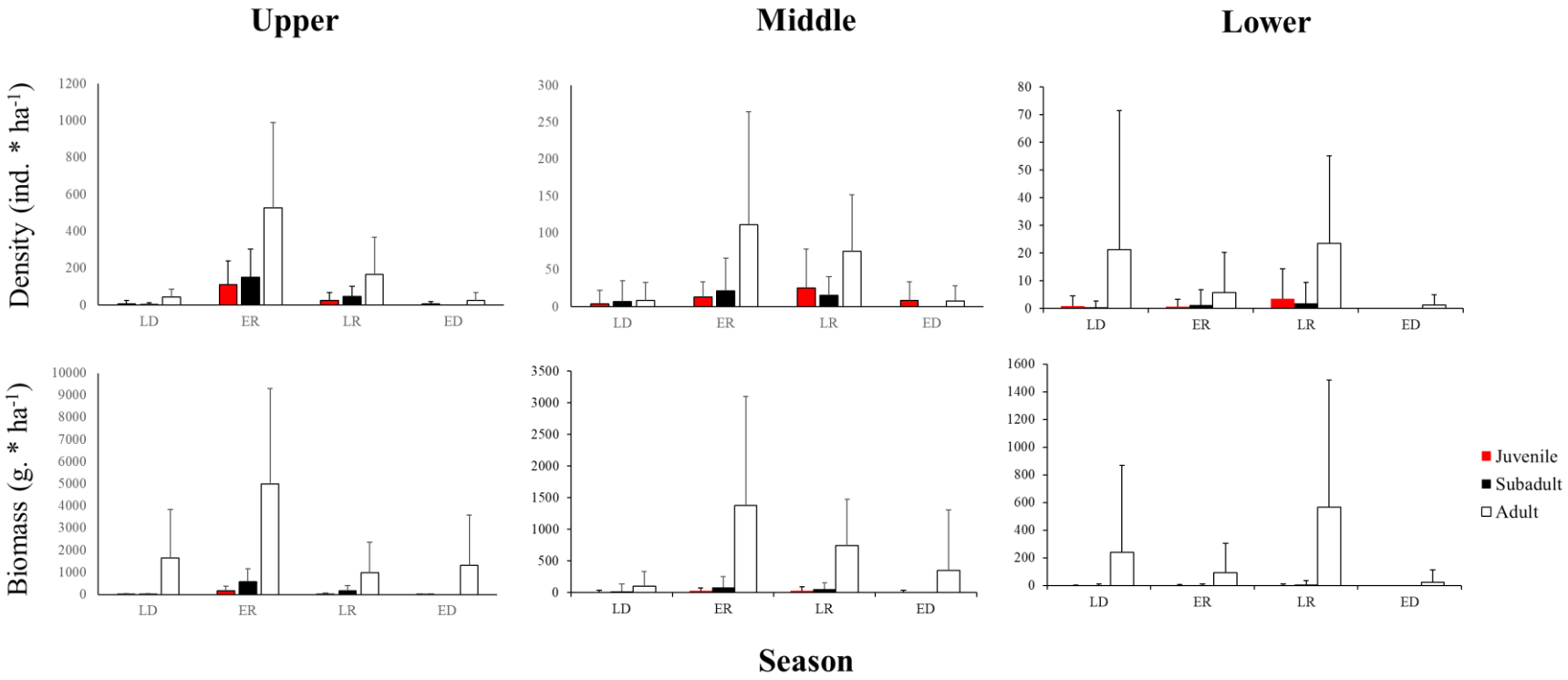
Table S7. Results of canonical correspondence analysis (CCA) using environmental parameters rainfall, water temperature, salinity, Secchi depth and dissolved oxygen (DO) and the index of relative importance (%I_{RI}) of the items ingested by *Achirus lineatus* in different ontogenetic phases. (ns: not significant) (**p <0.01).

S1.


Length vs. weight relationship
Wg = 0.00002 * Lt 2.9426
R² = 0.9842
N = 1441
Wg (g)
Lt (mm)
0
50
100
150
200
250
300
350
400


Frequency of Adults
L50= 50 mm
Lt (mm)
0.0
0.2
0.4
0.6
0.8
1.0
0
50
100
150
200

S2.


Upper
Middle
Lower
Density (ind. * ha-1)
Biomass (g. * ha-1)
LD
ER
LR
ED
Season
Juvenile
Subadult
Adult

S3.

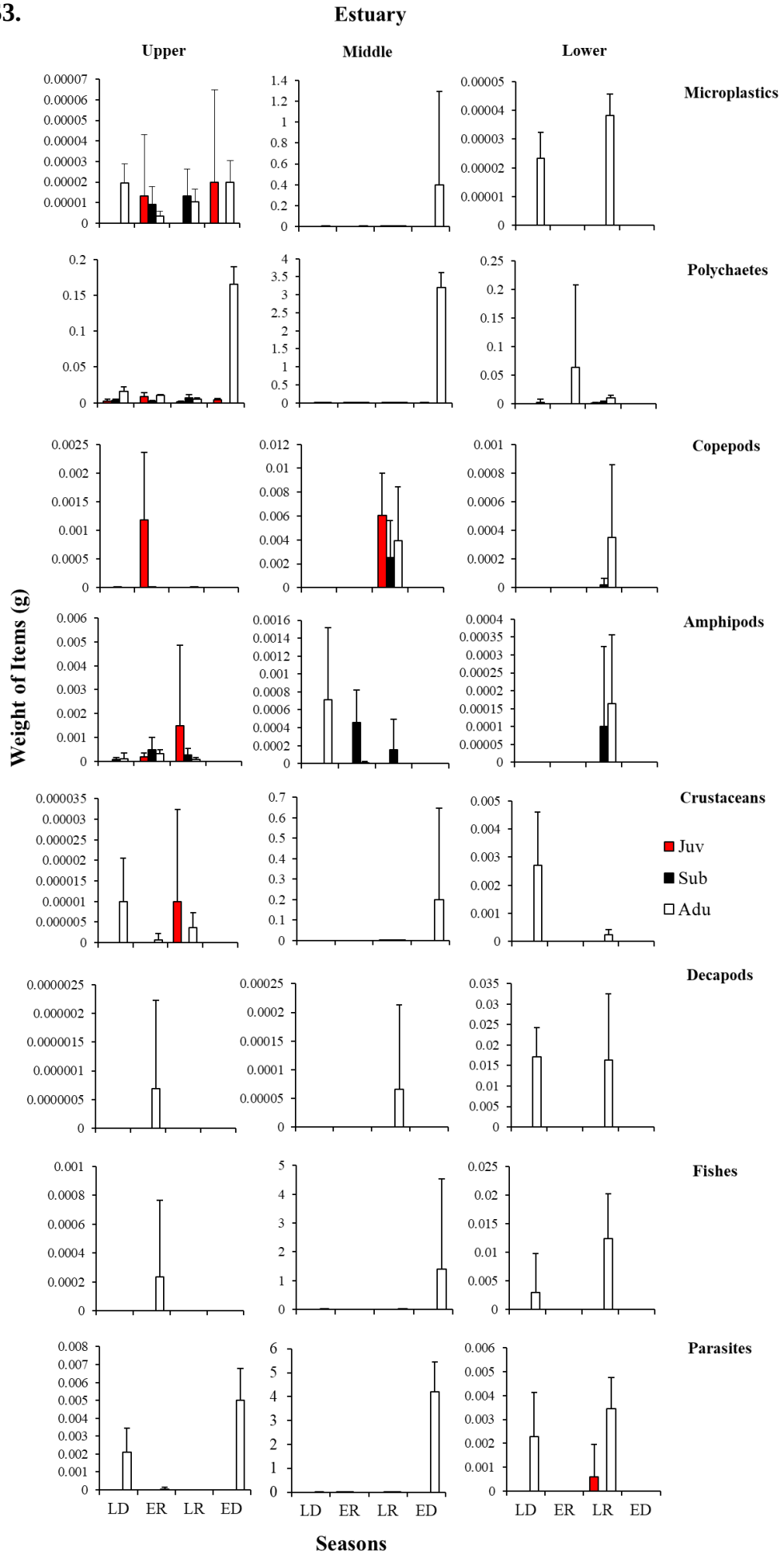

Estuary
Upper
Middle
Lower
Microplastics
Polychaetes
Copepods
Amphipods
Crustaceans
Decapods
Fishes
Parasites
Weight of Items (g)
Juv
Sub
Adu
LD ER LR ED
Seasons

Table S1.

			Density (ind. * ha^{-1})											
			Upper				Middle				Lower			
Phase	**Density (ind. * ha^{-1})**	**%**	**LD**	**ER**	**LR**	**ED**	**LD**	**ER**	**LR**	**ED**	**LD**	**ER**	**LR**	**ED**
Juvenile	17.1	14.0	6.7	109.8	26.3	6.2	4.2	13.3	25.7	8.8	0.89	0.65	3.4	0
Subadult	20.5	16.8	2.9	150.1	45	0	6.7	21.8	15.6	0	0.53	1.2	1.8	0
Adult	84.6	69.2	42.8	528	164.6	24.9	8.3	111.2	75.4	7.8	21.2	5.8	23.6	1.2
Total	**122.2**		**52.4**	**787.9**	**235.9**	**31.1**	**19.2**	**146.3**	**116.7**	**16.6**	**22.62**	**7.65**	**28.8**	**1.2**

			Biomass (g. * ha^{-1})											
			Upper				Middle				Lower			
Phase	**Biomass (g. * ha^{-1})**	**%**	**LD**	**ER**	**LR**	**ED**	**LD**	**ER**	**LR**	**ED**	**LD**	**ER**	**LR**	**ED**
Juvenile	25.8	2.3	10.5	179.3	26.9	7.7	8.3	28	34.1	8.9	1	1.5	3.1	0
Subadult	78.8	6.9	11.4	579.4	173.6	0	26	84.1	60.3	0	2	2.5	6.9	0
Adult	1038.7	90.9	1651	4997.6	994.2	1307	105	1381.3	743.9	356.5	242.2	95.5	566.4	24
Total	**1143.3**		**1673**	**5756**	**1195**	**1315**	**139**	**1493**	**838.3**	**365**	**245.2**	**99.5**	**576**	**24**

Table S2.

	Factors	F	df	*p*-value	*Post-hoc*
Density (ind. ha^{-1})	Area	47.34	2	*0.001*	U M L
	Season	35.37	3	*0.001*	ED LD LR ER
	Phase	32.69	2	*0.001*	Juv Sub Adu
	Area *vs*. Season	22.91	6	*0.001*	**
	Area *vs*. Phase	12.92	4	*0.001*	**
	Season *vs*. Phase	9.71	6	*0.001*	**
	Area *vs*. Season *vs*. Phase	5.44	12	*0.001*	**
Biomass (g. ha^{-1})	Area	33.26	2	*0.001*	U M L
	Season	13.74	3	*0.001*	ED LD LR ER
	Phase	69.24	2	*0.001*	Juv Sub Adu
	Area *vs*. Season	8.56	6	*0.001*	**
	Area *vs*. Phase	23.53	4	*0.001*	**
	Season *vs*. Phase	8.44	6	*0.001*	**
	Area *vs*. Season *vs*. Phase	5.39	12	*0.001*	**

Table S3.

Item	Phase	Early Dry %I_{RI}			Late Dry %I_{RI}			Early Rainy %I_{RI}			Late Rainy %I_{RI}		
		U	M	L	U	M	L	U	M	L	U	M	L
Microplastic	Juv	3.01	0	-	0	0	-	0.15	0	-	0	0.01	0
	Sub	-	-	-	0	0	-	0.20	0	-	0.94	1.78	0
	Adu	0.75	0.43	-	2.47	1.14	3.48	0.06	4.60	0	0.59	0.21	6.15
Polychaetes nd	Juv	89.94	100	-	100	0	-	77.16	96.88	-	86.08	13.57	93.24
	Sub	-	-	-	61.03	72.37	-	88.64	70.76	-	53.98	27.25	92.48
	Adu	50.91	13.06	-	54.67	54.13	0	81.54	61.48	100	95.53	63.85	15.72
Terebellidae	Juv	0	0	-	0	0	-	0	0	-	0	0	0
	Sub	-	-	-	0	0	-	0	0	-	0	3.51	0
	Adu	0	0	-	0	0	0	0	0	0	0	0	0
Syllidae	Juv	0	0	-	0	0	-	0	0	-	0	0	0
	Sub	-	-	-	0	0	-	0	0	-	0	0	0
	Adu	0	0	-	0	0	0	0.01	0.38	0	0	0	6.54

Table S3 continued...

Nereis sp	Juv	3.29	0	-	0	0	-	0.35	2.11	-	0	0	0
	Sub	-	-	-	18.35	0	-	1.66	0	-	21.97	14.81	0
	Adu	30.97	31.18	-	31.52	23.48	5.44	15.33	31.51	0	2.62	14.40	21.30
Copepods nd	Juv	0	0	-	0	0	-	20.96	0	-	0	80.77	0
	Sub	-	-	-	10.90	0	-	0.05	0	-	0	10.17	4.27
	Adu	0	0	-	0	0	0	0	0	0	0.01	9.82	16.27
Cyclopoid	Juv	0	0	-	0	0	-	0	0	-	0	0	0
	Sub	-	-	-	0	0	-	0	0	-	0	0	0
	Adu	0	0	-	0	0	0	0	0	0	0	4.59	0
Calanoid	Juv	0	0	-	0	0	-	0	0	-	0	5.62	0
	Sub	-	-	-	0	0	-	0	0	-	0	40.36	0
	Adu	0	0	-	0	0	0	0	0	0	0	5.63	0
Amphipods nd	Juv	0	0	-	0	0	-	0.05	0	-	0	0	0
	Sub	-	-	-	3.53	0	-	6.35	0	-	0	1.23	3.25
	Adu	0	0	-	0	0.31	0	0	0.32	0	0	0	0.08

Table S3 continued...

Corophiidae	Juv	0	0	-	0	0	-	1.23	0	-	12.91	0	0
	Sub	-	-	-	0	0	-	3.10	26.06	-	22.95	0	0
	Adu	0	0	-	0.28	3.68	0	3.02	0	0	0.95	0	0.05
Crustaceans nd	Juv	0	0	-	0	0	-	0	0	-	0	0	0
	Sub	-	-	-	0	0	-	0	0	-	0	0	0
	Adu	0	0	-	0	0	0.76	0	0	0	0.01	0	0.14
Ostracods	Juv	0	0	-	0	0	-	0	0	-	0.23	0	0
	Sub	-	-	-	0	0	-	0	0	-	0	0.47	0
	Adu	0	0.22	-	0.37	0		0	0	0	0	0	0
Isopods	Juv	0	0	-	0	0	-	0	0	-	0	0.01	0
	Sub	-	-	-	0	0	-	0	0	-	0	0	0
	Adu	0	0	-	0	0	0.16	0	0	0	0	0.01	0.29
Mysidacea	Juv	0	0	-	0	0	-	0	0	-	0	0	0
	Sub	-	-	-	0	0	-	0	0	-	0	0	0
	Adu	0	0	-	0	0	5.73	0	0	0	0	0	0.02

Table S3 continued...

Daphinia sp	Juv	0	0	-	0	0	-	0	0	-	0	0	0
	Sub	-	-	-	0	0	-	0	0	-	0	0	0
	Adu	0	0	-	0	0	0	0	0	0	0.01	0	0.07
Decapods nd	Juv	0	0	-	0	0	-	0	0	-	0	0	0
	Sub	-	-	-	0	0	-	0	0	-	0	0	0
	Adu	0	0	-	0	0	10.89	0	0	0	0	0.19	0
Peneidae	Juv	0	0	-	0	0	-	0	0	-	0	0	0
	Sub	-	-	-	0	0	-	0	0	-	0	0	0
	Adu	0	0	-	0	0	0.36	0	0	0	0	0	0.60
Paguridae	Juv	0	0	-	0	0	-	0	0	-	0	0	0
	Sub	-	-	-	0	0	-	0	0	-	0	0	0
	Adu	0	0	-	0	0	57.54	0	0	0	0	0	0.01
Astacidae	Juv	0	0	-	0	0	-	0	0	-	0	0	0
	Sub	-	-	-	0	0	-	0	0	-	0	0	0
	Adu	0	0	-	0	0	0	0	0	0	0	0	0.21

Table S3 continued...

Aratus pisonii	Juv	0	0	-	0	0	-	0	0	-	0	0	0
	Sub	-	-	-	0	0	-	0	0	-	0	0	0
	Adu	0	0	-	0	0	0	0	0	0	0	0	1.65
Fishes nd	Juv	0	0	-	0	0	-	0	0	-	0	0	0
	Sub	-	-	-	0	0	-	0	0	-	0	0	0
	Adu	0	3.22	-	0	0.14	0	0	0	0	0	0.21	0.89
Mirophys punctatus	Juv	0	0	-	0	0	-	0	0	-	0	0	0
	Sub	-	-	-	0	0	-	0	0	-	0	0	0
	Adu	0		-	0	0	1.52	0.02	0	0	0	0	0
Gobionellus oceanicus	Juv	0	0	-	0	0	-	0	0	-	0	0	0
	Sub	-	-	-	0	0	-	0	0	-	0	0	0
	Adu	0	0	-	0	0	0	0	0	0	0	0	2.48
Cathorops spixii	Juv	0	0	-	0	0	-	0	0	-	0	0	0
	Sub	-	-	-	0	0	-	0	0	-	0	0	0
	Adu	0	0	-	0	0	0	0	0	0	0	0	0.10

Table S3 continued...

Cathorops spp egg	Juv	0	0	-	0	0	-	0	0	-	0	0	0
	Sub	-	-	-	0	0	-	0	0	-	0	0.10	0
	Adu	0	0	-	0	0	0	0	0	0	0	0	0
Plant fragments	Juv	3.01	0	-	0	0	-	0.10	0	-	0.77	0	0
	Sub	-	-	-	2.89	27.63	-	0	2.09	-	0	0	0
	Adu	2.22	0.23	-	0.46	0.29	0.19	0.01	1.36	0	0.02	0.93	5.04
Seaweed	Juv	0.75	0	-	0	0	-	0	0	-	0	0	0
	Sub	-	-	-	3.31	0	-	0	0.65	-	0.16	0	0
	Adu	0	0	-	0	0.07	0	0	0.35	0	0	0.07	0.19
Nematocera nymph	Juv	0	0	-	0	0	-	0	0	-	0	0.02	0
	Sub	-	-	-	0	0	-	0	0	-	0	0	0
	Adu	0	0	-	0.55	0	0	0	0	0	0.25	0.08	0.01
Parasites	Juv	0	0	-	0	0	-	0	1.01	-	0	0	6.76
	Sub	-	-	-	0	0	-	0	0.43	-	0	0.33	0
	Adu	15.16	51.66	-	9.68	16.77	13.92	0	0	0	0	0.01	22.21

Table S4.

	Factors	F	df	*p*-value	*Post-hoc*
Microplastics	Area	2.62	2	*0.07*	*ns*
	Season	5.29	3	*0.001*	ED ER LD LR
	Phase	26.48	2	*0.001*	Juv Sub Adu
	Area *vs*. Season	1.57	6	*0.15*	*ns*
	Area *vs*. Phase	0.43	4	*0.78*	*ns*
	Season *vs*. Phase	3.52	6	*0.001*	**
	Area *vs*. Season *vs*. Phase	2.62	12	*0.001*	**
Polychaetes	Area	75.49	2	*0.001*	U M L
	Season	31.72	3	*0.001*	ED LD ER LR
	Phase	32.79	2	*0.001*	Juv Sub Adu
	Area *vs*. Season	8.91	6	*0.001*	**
	Area *vs*. Phase	2.09	4	*0.08*	*ns*
	Season *vs*. Phase	9.17	6	*0.001*	**
	Area *vs*. Season *vs*. Phase	0.18	12	*0.001*	**
Copepods	Area	5.68	2	*0.001*	UL M
	Season	17.92	3	*0.001*	ED LD ER LR
	Phase	0.82	2	*0.44*	*ns*
	Area *vs*. Season	12.04	6	*0.001*	**
	Area *vs*. Phase	1.80	4	*0.13*	*ns*
	Season *vs*. Phase	0.59	6	*0.73*	*ns*
	Area *vs*. Season *vs*. Phase	2.36	12	*0.001*	**
Amphipods	Area	10.17	2	*0.001*	U M L
	Season	8.56	3	*0.001*	ED LD LR ER
	Phase	3.14	2	*0.04*	*ns*
	Area *vs*. Season	4.14	6	*0.001*	**
	Area *vs*. Phase	0.42	4	*0.78*	*ns*
	Season *vs*. Phase	0.71	6	*0.64*	*ns*
	Area *vs*. Season *vs*. Phase	1.21	12	*0.28*	*ns*
Crustaceans	Area	0.10	2	*0.89*	*ns*
	Season	4.80	3	*0.001*	ER ED LD LR
	Phase	6.54	2	*0.001*	Juv Sub Adu
	Area *vs*. Season	1.39	6	*0.22*	*ns*
	Area *vs*. Phase	2.17	4	*0.07*	*ns*
	Season *vs*. Phase	1.76	6	*0.11*	*ns*
	Area *vs*. Season *vs*. Phase	1.81	12	*0.05*	*ns*

Decapods	Area	23.73	2	*0.001*	U M L
	Season	12.56	3	*0.001*	ED ER LR LD
	Phase	38.33	2	*0.001*	Juv Sub Adu
	Area *vs*. Season	12.07	6	*0.001*	**
	Area *vs*. Phase	23.73	4	*0.001*	**
	Season *vs*. Phase	12.56	6	*0.001*	**
	Area *vs*. Season *vs*. Phase	12.07	12	*0.001*	**
Fishes	Area	2.73	2	*0.06*	*ns*
	Season	2.48	3	*0.06*	*ns*
	Phase	11.31	2	*0.001*	Juv Sub Adu
	Area *vs*. Season	2.31	6	*0.03*	*ns*
	Area *vs*. Phase	2.73	4	*0.03*	*ns*
	Season *vs*. Phase	2.48	6	*0.02*	*ns*
	Area *vs*. Season *vs*. Phase	2.31	12	*0.001*	**
Parasites	Area	0.13	2	*0.87*	*ns*
	Season	7.02	3	*0.001*	ER LR LD ED
	Phase	67.28	2	*0.001*	Juv Sub Adu
	Area *vs*. Season	12.17	6	*0.001*	**
	Area *vs*. Phase	1.30	4	*0.27*	*ns*
	Season *vs*. Phase	13.04	6	*0.001*	**
	Area *vs*. Season *vs*. Phase	9.91	12	*0.001*	**

Table S5.

	Factors	F	df	*p*-value	*Post-hoc*
Polychaetes	Area	23.35	2	*0.001*	UL M
	Season	60.97	3	*0.001*	LD LR ER ED
	Phase	122.31	2	*0.001*	Juv Sub Adu
	Area *vs*. Season	27.89	6	*0.001*	**
	Area *vs*. Phase	19.41	4	*0.001*	**
	Season *vs*. Phase	68.5	6	*0.001*	**
	Area *vs*. Season *vs*. Phase	29.35	12	*0.001*	**
Copepods	Area	5.16	2	*0.001*	U L M
	Season	5.91	3	*0.001*	ED LD ER LR
	Phase	0.62	2	*0.53*	*ns*
	Area *vs*. Season	5.96	6	*0.001*	**
	Area *vs*. Phase	0.29	4	*0.87*	*ns*
	Season *vs*. Phase	0.31	6	*0.92*	*ns*
	Area *vs*. Season *vs*. Phase	0.45	12	*0.93*	*ns*
Amphipods	Area	1.99	2	*0.14*	*ns*
	Season	1.22	3	*0.30*	*ns*
	Phase	0.02	2	*0.97*	*ns*
	Area *vs*. Season	1.06	6	*0.38*	*ns*
	Area *vs*. Phase	0.78	4	*0.53*	*ns*
	Season *vs*. Phase	1.07	6	*0.37*	*ns*
	Area *vs*. Season *vs*. Phase	0.96	12	*0.48*	*ns*
Crustaceans	Area	0.93	2	*0.39*	*ns*
	Season	0.95	3	*0.41*	*ns*
	Phase	1.15	2	*0.31*	*ns*
	Area *vs*. Season	1.02	6	*0.41*	*ns*
	Area *vs*. Phase	0.92	4	*0.44*	*ns*
	Season *vs*. Phase	0.95	6	*0.45*	*ns*
	Area *vs*. Season *vs*. Phase	1.02	12	*0.42*	*ns*
Decapods	Area	4.97	2	*0.001*	U M L
	Season	1.68	3	*0.17*	*ns*
	Phase	5.00	2	*0.001*	Juv Sub Adu
	Area *vs*. Season	1.67	6	*0.13*	*ns*
	Area *vs*. Phase	4.97	4	*0.001*	**
	Season *vs*. Phase	1.68	6	*0.12*	*ns*
	Area *vs*. Season *vs*. Phase	1.67	12	*0.07*	*ns*

Fishes	Area	0.75	2	*0.47*	*ns*
	Season	0.8	3	*0.49*	*ns*
	Phase	1.81	2	*0.16*	*ns*
	Area *vs*. Season	1.15	6	*0.33*	*ns*
	Area *vs*. Phase	0.75	4	*0.55*	*ns*
	Season *vs*. Phase	0.8	6	*0.56*	*ns*
	Area *vs*. Season *vs*. Phase	1.15	12	*0.32*	*ns*
Parasites	Area	4238.2	2	*0.001*	U L M
	Season	4405.9	3	*0.001*	ER LR LD ED
	Phase	4868.2	2	*0.001*	Juv Sub Adu
	Area *vs*. Season	4299.7	6	*0.001*	**
	Area *vs*. Phase	4233.6	4	*0.001*	**
	Season *vs*. Phase	4422.7	6	*0.001*	**
	Area *vs*. Season *vs*. Phase	4300.8	12	*0.001*	**

Table S6.

	Factors	F	df	*p*-value	*Post-hoc*
Blue	Area	6.49	2	*0.002*	U M L
	Season	5.75	3	*0.001*	ED LD ER LR
	Phase	24.23	2	*0.001*	Juv Sub Adu
	Area *vs*. Season	2.67	6	*0.02*	*
	Area *vs*. Phase	0.91	4	*0.45*	*ns*
	Season *vs*. Phase	3.02	6	*0.01*	*
	Area *vs*. Season *vs*. Phase	1.42	12	*0.17*	*ns*
White	Area	7.76	2	*0.001*	U M L
	Season	0.61	3	*0.60*	*ns*
	Phase	3.60	2	*0.03*	Sub Juv Adu
	Area *vs*. Season	1.28	6	*0.27*	*ns*
	Area *vs*. Phase	3.58	4	*0.010*	*
	Season *vs*. Phase	1.26	6	*0.28*	*ns*
	Area *vs*. Season *vs*. Phase	0.87	12	*0.57*	*ns*
Red	Area	0.32	2	*0.72*	*ns*
	Season	0.78	3	*0.50*	*ns*
	Phase	9.19	2	*0.001*	Juv Sub Adu
	Area *vs*. Season	0.99	6	*0.43*	*ns*
	Area *vs*. Phase	0.87	4	*0.48*	*ns*
	Season *vs*. Phase	0.63	6	*0.70*	*ns*
	Area *vs*. Season *vs*. Phase	1.54	12	*0.12*	*ns*
Black	Area	1.08	2	*0.34*	*ns*
	Season	1.26	3	*0.29*	*ns*
	Phase	1.34	2	*0.26*	*ns*
	Area *vs*. Season	0.38	6	*0.88*	*ns*
	Area *vs*. Phase	0.68	4	*0.60*	*ns*
	Season *vs*. Phase	1.41	6	*0.22*	*ns*
	Area *vs*. Season *vs*. Phase	0.84	12	*0.60*	*ns*
Green	Area	0.78	2	*0.46*	*ns*
	Season	3.12	3	*0.03*	*ns*
	Phase	3.12	2	*0.05*	*ns*
	Area *vs*. Season	0.78	6	*0.58*	*ns*
	Area *vs*. Phase	0.78	4	*0.54*	*ns*
	Season *vs*. Phase	3.12	6	*0.001*	*
	Area *vs*. Season *vs*. Phase	0.78	12	*0.66*	*ns*

Table S7.

A. lineatus	Axis I	Axis II	*p*-value	
Eigenvalues	0.2205	0.0919		
Species-environmental correlation	0.8227	0.7003		
Cumulative % variance of species data	24.8	35.1		
Cumulative % variance of species environmental relation	58.8	83.3		
Correlation with environmental variables:	Explains %	pseudo-F	*p*-value	
Rainfall	9.4	2.1	0.08	*ns*
Water temperature	6	1.3	0.274	*ns*
Salinity	23.9	6.3	0.013	*
Secchi depth	23	6	0.049	*ns*
DO mg l^{-1}	19.2	4.8	0.02	*