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Ecologia alimentar e distribuição espaço-temporal das diferentes fases ontogenéticas da espécie *Cynoscion acoupa* no estuário do Rio Goiana (PE/PB)

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Dissertação apresentada à Universidade Federal de Pernambuco, como parte das exigências do Programa de Pós-Graduação em Oceanografia, para obtenção do título de Mestre em Ciências na área de Oceanografia.

Orientador: Dr. Mário Barletta

Co-orientador: Dr. David V. Dantas

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RESUMO

Este estudo avalia a influência espaço-temporal nos padrões de distribuição, ecologia alimentar e ingestão de microplástico da espécie *Cynoscion acoupa* (Pescada Amarela) ao longo da sua ontogenia, no estuário do rio Goiana, localizado no nordeste do Brasil. A área de estudo se trata de um estuário tropical, com altas médias de temperatura anual e pequenas oscilações. O ambiente está submetido a um regime de mesomaré semi-diurna. As principais alterações encontradas no estuário são oriundas do ciclo sazonal, que está diretamente ligado à pluviometria, divida em quatro estações (início da chuva, fim da chuva, início da seca e fim da seeca), com o intuito de se ter uma maior precisão na avaliação dos processos abiológicos e na comunidade aquática. Os espécimes foram coletados em diferentes porções estuarinas (estuário superior, intermediário e inferior) e nos canais de maré. Simultaneamente a coleta dos dados biológicos foram obtidas informações a respeito dos parâmetros físico-químicos. Os padrões de distribuição e alimentação da espécie estudada ocorrem de forma distinta em relação à ontogenia, sazonalidade e áreas do estuário. A fase juvenil de *C. acoupa* ocupa a porção superior do estuário durante todas as estações do ano, em busca de baixos valores de salinidade, onde encontram condições ideais apara evitar predadores marinhos. As principais presas dos juvenins no estuário superior foram filamentos de microplástico (FO=63%), amphipoda (FO=28%) e mysidacea (FO=22%). Além disso, no início do período chuvoso, o estuário superior apresenta um papel crucial para o ciclo de vida da espécie, por se caracterizar como uma área berçário para *C. acoupa* (grande densidade 228,4 ind. ha^{-1} e baixa biomassa 46 g ha^{-1} de juvenis), pois a sua contribuição de indivíduos juvenis para a população adulta é muito maior que nos outros hábitats. No ambiente de berçário os juvenis se alimentaram principalmente de filamentos de microplástico (FO=48%), camarão (FO=28%), mysidacea (FO=22%) e amphipoda (FO=21%). Nas demais porções estuarinas, também são registrados indivíduos juvenis, sobretudo nos períodos chuvosos (início da chuva 115 ind ha^{-1} e fim da chuva 7,3 ind. ha^{-1}), porém em menor escala. Os subadultos de *C. acoupa* utilizam principalmente o estuário superior durante o início da seca (1,7 ind. ha^{-1}), fim da seca (1,6 ind. ha^{-1}) e início da chuva (6,5 ind. ha^{-1}) como área de alimentação. Predando no estuário superior, no início da seca, principalmente peixes não identificados (FO=50%), no fim da seca, filamentos de microplástico (FO=60%), mysidacea (FO=20%) e syllidae (FO=20%) e no início da chuva, filamentos de nylon FO=100% e peixes não identificados (FO=20%). Durante o fim do período chuvoso os indivíduos subadultos migram para o estuário intermediário (2,3 ind. ha^{-1}) para evitar grandes estresses osmorregulatórios e predaram principalmente amphipoda (FO=75%), *Cathorops spixii* (FO=50%), *Anchovia clupeoides*

(FO=25%) e peixes não identificados (FO=25%). A fase adulta de *C. acoupa* foi registrada somente nas porções mais externas do estuário inferior, por se tratar de uma espécie de hábitos costeiros quando completamente desenvolvida. Os indivíduos adultos de *C. acoupa* predaram principalmente filamentos de nylon (FO=100%), *C. spixii* (FO=18%), *Achirus lineatus* (FO=15%), S*tellifer stellifer* (FO=15%) e camarão (FO=15%). *C. acoupa* apresentou uma variação no padrão de distribuição em relação a sua ontogenia, sazonalidade e diferentes porções estuarinas. Ao longo do seu desenvolvimento ontogenético *C. acoupa* também apresentou uma alteração na sua guilda trófica, os juvenis e os subadultos foram classificados como oportunistas e os indivíduos adultos como piscívoros. A grande quantidade de fragmentos e microplástico encontrada na espécie estudada, demostra que ela é particularmente vulnerável a esse tipo de contaminante, sobre tudo os espécimes adultos, que registraram os maiores níveis de contaminação, provavelmente em razão do seu nível trófico, como predadores de topo.

Palavras chave: Oceanografia. Distribuição espaço-temporal. Ecologia alimentar. Gradiente ambiental. Berçário. Filamentos de microplástico. Predador de topo.

ABSTRACT

This study assess the spatio-temporal distribution patterns, feeding ecology and microplastic ingestion of the *Cynoscion acoupa* (Acoupa weakfish) during its ontogeny in the Goianna Estuary, located in northeast Brazil. The study area is a tropical estuary, with high mean temperatures and narrow annual temperature variations. The environment is classified as a semi-diurnal mesotidal estuary. The major environmental dynamics are caused by the seasonal regime, which is related to the rainfall, according to the pluviometry, the area was divided in four different seasons (early dry, late dry, early rainy and late rainy). Biological samples were performed in different estuarine reaches (upper, middle and lower estuary) and in tidal creeks. During the fish sampling, environmental parameters were also recorded. The distribution and feeding patters of the studied species occur in a distinctive form according to the ontogeny, seasonality and estuarine area. The juveniles of *C. acoupa* occupy the upper estuary, during all seasons, seeking low salinity that provide ideal conditions to avoid marine predators. The main juveniles prey in the upper estuary were microplastic threads (FO=63%), amphipoda (FO=28%) and mysidacea (FO=22%). Moreover in the early rainy season, the upper estuary is crucial for the life ciclo of the species, because it is a nursery ground for *C. acoupa* (high density 228.4 ind. ha^{-1} and low biomass 46 g ha^{-1} of juveniles), as a result of the higher contribution of juveniles for to the adult population than in the other habitats. In the nursery ground, the juveniles of *C. acoupa* fed mainly on plastic threads (FO=48%), penaeid shrimps (FO=28%), mysidacea (FO=22%) and amphipoda (FO=21%). In other estuarine areas juveniles were also recorded, mainly on the rainy seasons (early rainy 115 ind. ha^{-1} and late rainy 7.3 ind. ha^{-1}), however with lower densities. The subadults of *C. acoupa* inhabited mostly the upper estuary, during the early dry (1.7 ind. ha^{-1}), late dry (1.6 ind. ha^{-1}) and early rainy seasons (6.5 ind. ha^{-1}) as a feeding ground. In the upper estuary, during the early season they fed mainly on unidentified fish (FO=50%), in the late dry, plastic threads (FO=60%), mysidacea (FO=20%) and syllidae (FO=20%) and in the early rainy, plastic threads (FO=100%) and unidentified fish (FO=20%). During the late rainy season the subadults of *C. acoupa* migrated to the middle estuary (2.3 ind. ha^{-1}) to avoid osmoregulatory stress, the subadults and fed mainly on amphipoda (FO=75%), *Cathorops spixii* (FO=50%), *Anchovia clupeoides* (FO=25%) and non-identified fish (FO=25%). The adults of *C. acoupa* were recorded only in seaward areas of the lower estuary, as a result of *C. acoupa* being a coastal/marine species when fully developed. The adults preyed mostly plastic threads (FO=100%), *C. spixii* (FO=18%), *Achirus lineatus* (FO=15%), *Stellifer stellifer* (FO=15%) and penaeid shrimp (FO=15%). During the ontogenetic process, *C. acoupa* showed a trophic guild shift, the juveniles and subadults were assigned as opportunistic and the adults as psicivores. *C. acoupa* showed a variation in the distribution pattern in relation to the ontogeny, seasonality and different estuarine reaches. During the ontogenetic development, *C. acoupa* showed a trophic guild shift, the juveniles and subadults were assigned as opportunistic and the adults as psicivores. The high occurrence of microplastic threads in the species, evince that *C. acoupa* is particularly vulnerable to this contaminant, especially the adult phase that registered highest contamination levels, probably due its trophic level, as a top predator.

Keywords: Oceanography. Spatial-temporal distribution. Feeding ecology. Environmental gradient. Nursery. Microplastic debris. Top predator.

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1 INTRODUÇÃO

Estuários são ecossistemas costeiros extremamente produtivos, caracterizados como importantes áreas de alimentação, abrigo e desenvolvimento (reprodução, berçário e recrutamento) da ictiofauna (Blaber *et al*., 2000; Barletta *et al*., 2010). Estuários tropicais apresentam uma grande variabilidade em suas características bióticas e abióticas, em função das diferentes condições encontradas ao longo dos gradientes ambientais, além das produzidas pelos aspectos sazonais (Burton, 1976; Barletta *et al*., 2005; 2008). Desta forma, fatores como disponibilidade de alimento e suprimento larval são diretamente afetados, causando uma oscilação no padrão de distribuição das espécies no estuário durante seu ciclo de vida (Barletta-Bergan *et al*. 2002 a, b; Barletta *et al*., 2005; 2008; Barletta & Blaber, 2007).

O uso do estuário como berçário é um importante aspecto na ontogenia de diversas espécies, pois estes ambientes fornecem alimento em abundância e abrigo para os organismos (Beck *et al.*, 2001; Barletta-Bergan *et al.*, 2002a, b; Dantas *et al.*, 2013). Como definição, um habitat pode ser considerado berçário para uma determinada espécie, quando a quantidade de indivíduos juvenis, que são recrutados para a população adulta é maior, que o recrutamento de outros hábitats em que os juvenis ocorrem (Beck *et al.*, 2003). As famílias de espécies de peixes que utilizam os estuários como berçário são representadas pelos arídeos, clupeídeos, engraulídeos, lutijanídeos, gobídeos, centropomídeos e sciaenídeos.

Os representantes da família Sciaenidae são encontrados em águas rasas da plataforma continental de mares tropicais e temperados (Lowe-McConnell, 1962), principalmente sobre o substrato arenoso e lamoso das regiões estuarinas (Cervigón *et al*., 1993). O gênero *Cynoscion* é composto por oito espécies que são encontradas ao longo da costa leste do continente americano, em uma faixa que se estende do mar caribenho até região sul do Brasil (Cervigón *et al*., 1993).

Dentre as espécies do gênero *Cynoscion*, a pescada amarela, *Cynoscion acoupa* (Lacepèle, 1801) é uma espécie de grande importância para a atividade pesqueira, usualmente encontrada em porções estuarinas, associada a manguezais (Carpenter, 2002), especialmente durante as primeiras fases do seu ciclo de vida (Barletta-Bergan *et al.*, 2002b; Barletta *et al*., 2003).

Esta espécie também possui uma grande importância econômica, sendo apontada como a terceira espécie mais capturada na costa brasileira no ano de 2010 (20.879 ton.) (MPA, 2012). Além do aproveitamento dos organismos na alimentação, as bexigas natatórias também apresentam um alto valor comercial, sendo utilizadas como matéria-prima para fabricação de ictiocola (Carpenter, 2002). Esta espécie utiliza vários habitats do ecossistema estuarino, ressaltando os canais de marés que drenam os manguezais (Barletta *et al*., 2003) e o canal principal do estuário (Barletta *et al.*, 2005; 2008). Devido seu hábito alimentar demersal, ela é usualmente encontrada sobre os substratos lamosos desses ambientes, alimentando-se principalmente de peixes e crustáceos (Carpenter, 2002).

Neste contexto está inserido o estuário do Rio Goiana, localizado no litoral norte do estado de Pernambuco, situado na região nordeste do Brasil, onde são encontradas extensas áreas de manguezais, responsáveis por uma grande produtividade biológica para a região (Barletta & Costa, 2009). Além da sua importância ecológica, esta localidade também possui uma grande relevância no âmbito econômico, pois ela é responsável pelo sustento de atividades pesqueiras tradicionais, destacando a captura de peixes, crustáceos e moluscos (Barletta & Costa, 2009).

2 OBJETIVOS

2.1 Objetivo geral

O presente estudo visa descrever as variações espaciais e temporais do padrão de distribuição das diferentes fases ontogenéticas da espécie *C. acoupa*, no estuário do Rio Goiana. Além disso, determinar a influência dos fatores ambientais na ecologia alimentar das diferentes fases ontogenéticas da espécie, ao longo do ecossistema estuarino.

2.2 Objetivos específicos

- Investigar como a distribuição espacial e sazonal de *C. acoupa* 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 para a espécie estudada;
- descrever a variação nos itens ingeridos em função do desenvolvimento ontogenético da espécie e da variação sazonal e/ou espacial no estuário do Rio Goiana;
- descrever a ingestão sazonal de microplástico pelas diferentes fases ontogenéticas da Pescada amarela.

3 METODOLOGIA

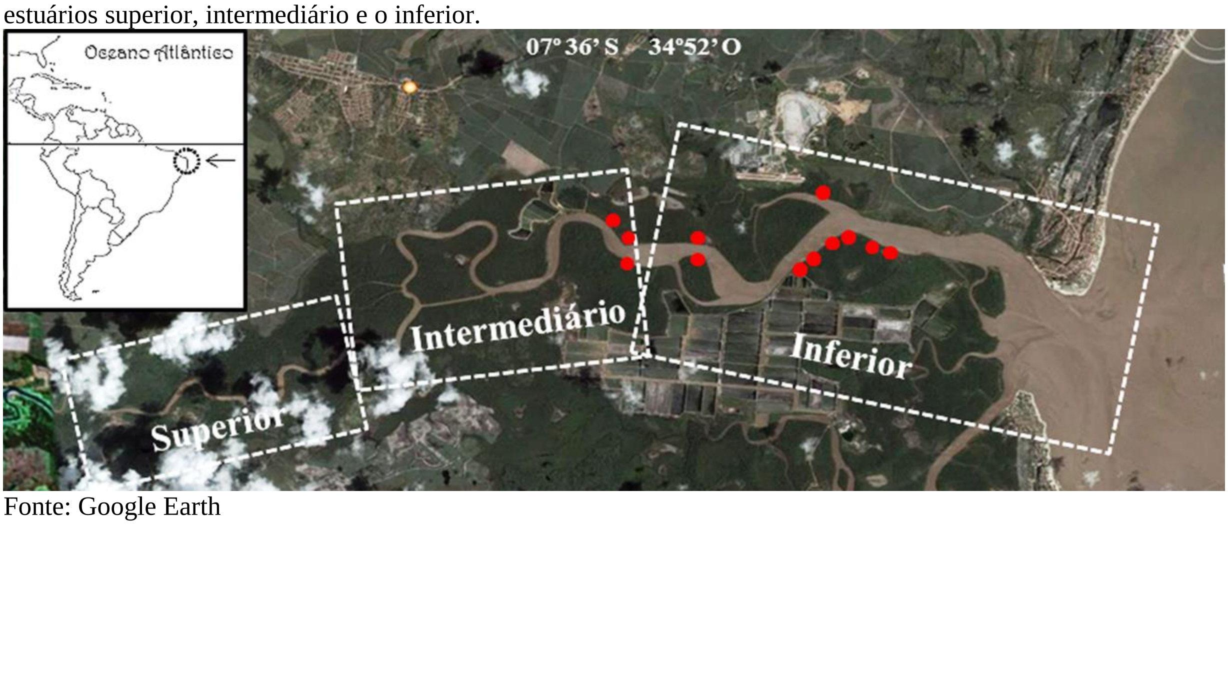
3.1 Área de estudo

O estuário do Rio Goiana está situado na divisa do estado de Pernambuco com o estado da Paraíba, na região nordeste do Brasil (Fig. 1), possui uma área total de 4700 ha, incluindo seu canal principal, a planície de inundação e a floresta de mangue que o circunda (Barletta & Costa, 2009).

O clima da região é classificado como tropical semiárido, a temperatura média do ar possui uma pequena amplitude de variação (27±2 ºC). A região é caracterizada por apresentar quatro estações, dentre elas o início do período chuvoso (Março a Maio) e o fim do período chuvoso (Junho a Agosto), assim como o início da estiagem (Setembro a Novembro) e o fim da estiagem (Dezembro a Fevereiro) (Barletta & Costa, 2009).

Esse estuário possui uma grande diversidade de habitats que têm sido estudados em diversos projetos de pesquisa, como o canal principal (Dantas *et al*., 2010, Lima *et al*. 2014), canais de maré nas florestas de manguezal (Ramos *et al*., 2011), e em sua porção externa, junto à desembocadura, praias e bancos arenosos dispostos nas margens do estuário (Lacerda *et al*., 2014) (Fig. 1). Estes habitats apresentam uma grande importância ecológica (locais de alimentação, reprodução e/ou berçário) e também econômica, pois representam a fonte de sobrevivência para as comunidades ribeirinhas (Barletta & Costa, 2009).

Figura 1 - Estuário do Rio Goiana, situado na divisa do estado de Pernambuco e Paraíba, no nordeste brasileiro. As áreas marcadas por representam a entrada dos canais de maré, e as delimitadas por indicam os estuários superior, intermediário e o inferior.



Fonte: Google Earth

3.2 Métodos amostrais

Foram realizadas amostragens em diferentes habitats do estuário do Rio Goiana, no canal principal do estuário, que compreende as três porções do ecossistema (estuário superior, intermediário e inferior) e nos canais de maré, localizados nos estuários intermediário e inferior.

A coleta da ictiofauna e das variáveis abióticas foram realizadas 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).

3.2.1 Canal principal

O canal principal do estuário foi dividido de acordo com sua morfologia e salinidade em: estuário superior (salinidade < 5), intermediário (salinidade 5 - 20) e inferior (salinidade > 20) (Barletta & Costa, 2009). Seis réplicas foram realizadas mensalmente em cada área do estuário entre dezembro de 2005 e novembro de 2006. Posteriormente, para adicionar dados ao estudo da ecologia alimentar da espécie, foram realizadas coletas em um período de três meses (durante os meses do fim da chuva e do fim da seca) entre os anos de 2006 a 2008.

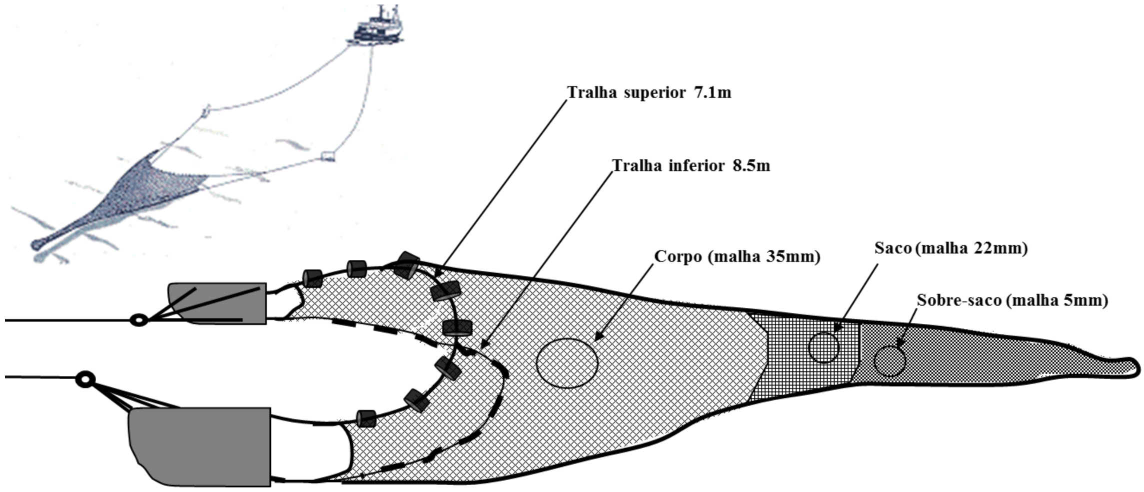
As amostragens foram realizadas por uma rede de arrasto com portas, com as dimensões de 7,72 m de abertura e 8,72 m de comprimento. A tralha superior possui um comprimento de 7,1 m e a inferior 8,5 m. A rede possui diferentes tamanhos de malha, 35 mm no corpo da rede, 22 mm no saco e 5 mm no sobre saco, os diferentes tamanhos de malha proporcionam uma maior representatividade das diferentes classes de tamanho da ictiofauna (Fig. 2).

Os arrastos foram realizados durante a maré de quadratura, para atenuar a influência da maré, em uma profundidade entre 5 e 10 metros. Durante as amostragens, foram registrados o tempo de duração (5 min), a profundidade de cada arrasto (ecossonda) e a distância percorrida (GPS) (Dantas *et al*., 2013). A área de arrasto foi estimada utilizando a seguinte equação:

$$A = D * h * X_2$$

Onde, **D** é a extensão percorrida pela rede, **h** o comprimento da tralha superior e $\mathbf{X_2}$ a fração do comprimento da tralha superior que corresponde à largura do percurso arrastado pela abertura da rede.

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



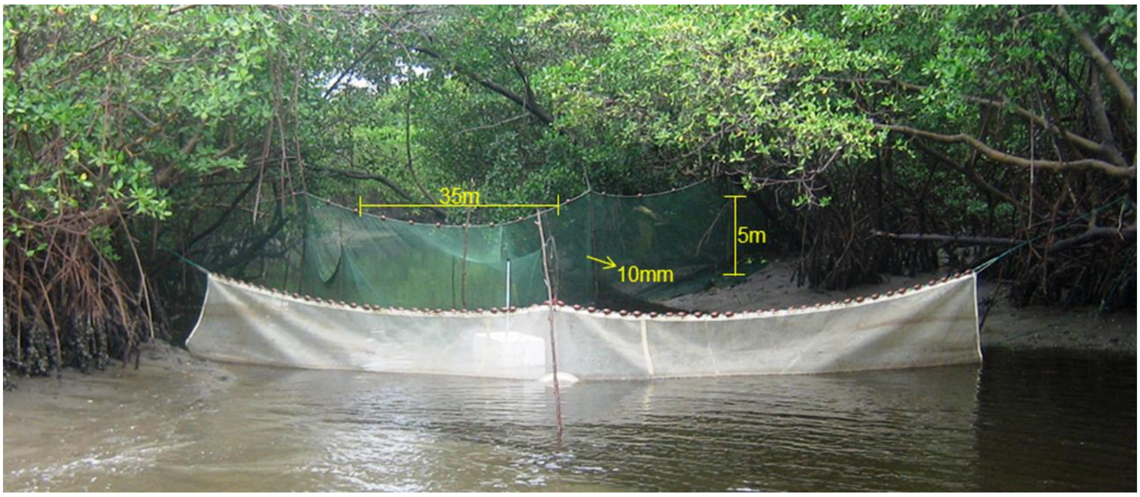
Fonte: LEGECE

3.2.2 Canais de maré

Para avaliar a importância do ecossistema de manguezal, foram realizadas amostragens nos canais de maré entre os meses de Abril e Maio de 2008 (três replicas). Os canais de maré são estruturas morfológicas, localizadas na planície de maré, que são mantidos unicamente pelo fluxo da maré.

As amostragens foram realizadas com uma rede de tapagem de 35 m de comprimento e 5 m de altura, com uma malha de 10 mm entre nós opostos (Fig. 3). A rede foi fixada na entrada dos canais de maré, por estruturas de madeira, durante o estofo de preamar, posteriormente, a despesca foi realizada na baixa-mar (Ramos *et al*., 2011).

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



Fonte: LEGECE

3.2.3 Parâmetros ambientais

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

3.3 Procedimentos laboratoriais

Após as coleta os espécimes foram etiquetados, congelados e armazenados em um banco de amostras. Em laboratório os indivíduos foram triados e identificados, em seguida foram tomadas medidas de comprimento total e padrão (mm) e peso (g).

3.3.1 Fases ontogenéticas

Os espécimes de *C. acoupa* foram divididos em diferentes classes de tamanho, de acordo com suas fases ontogenéticas, com o intuito de tornar o estudo das interações ecológicas da espécie mais preciso, já que seus hábitos de vida sofrem alterações consideráveis durante seu desenvolvimento ontogenético.

O tamanho da transformação (14 mm), obtido através da literatura (Barletta-Bergan, 1999), definiu o início da fase juvenil. O ponto de inflexão da curva de peso *vs*. comprimento (220 mm), delimitou o tamanho máximo dos juvenis e o comprimento inicial dos subadultos. Para estipular o tamanho inicial da fase adulta (340 mm), foi utilizado o comprimento da primeira maturação (L_{50}), obtido a partir da função logística, realizada com os dados gerados pela análise macroscópica das gônadas (Vazzoler, 1996) (Fig. 4). A definição das fases ontogenéticas de *C. acoupa* proposta pelo estudo, poder ser observada na tabela 1 e na figura 5.

Figura 4 – Função logística que definiu o comprimento da primeira maturação de C. acoupa em 340 mm.

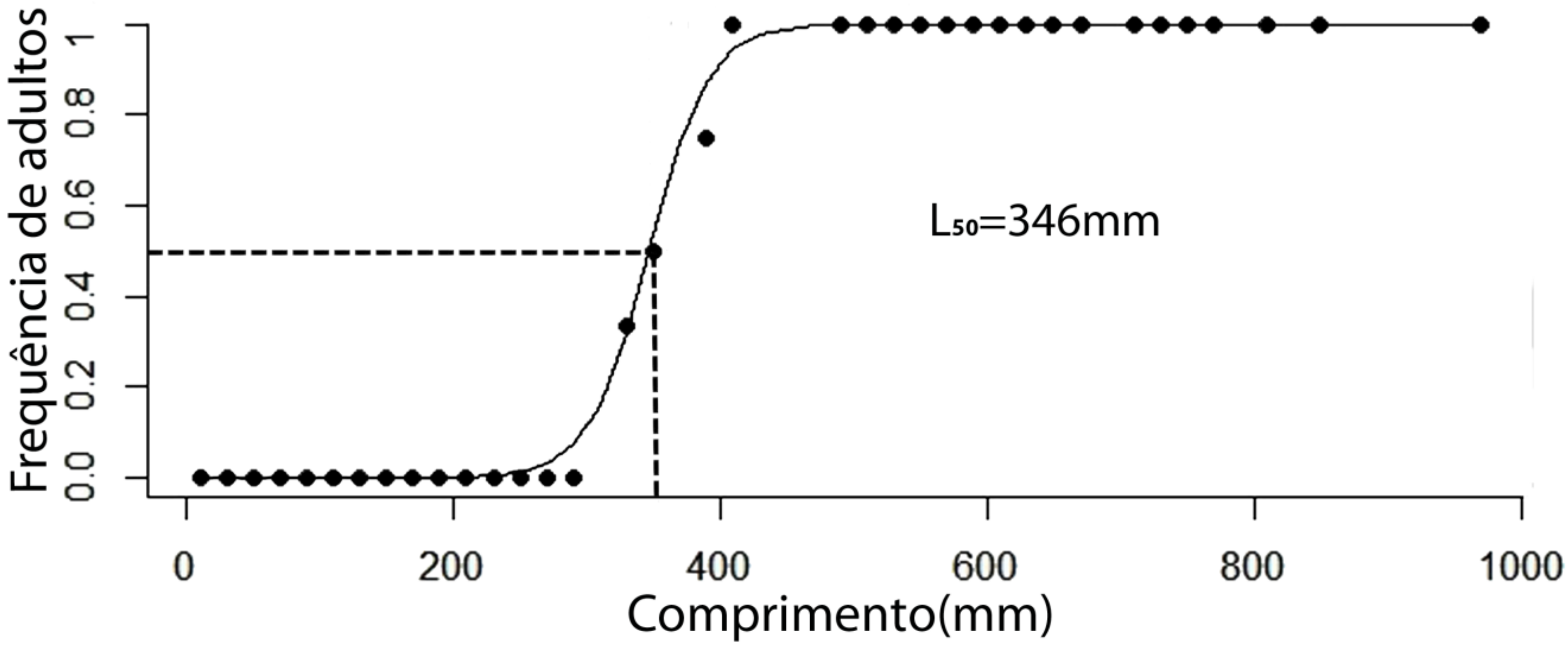
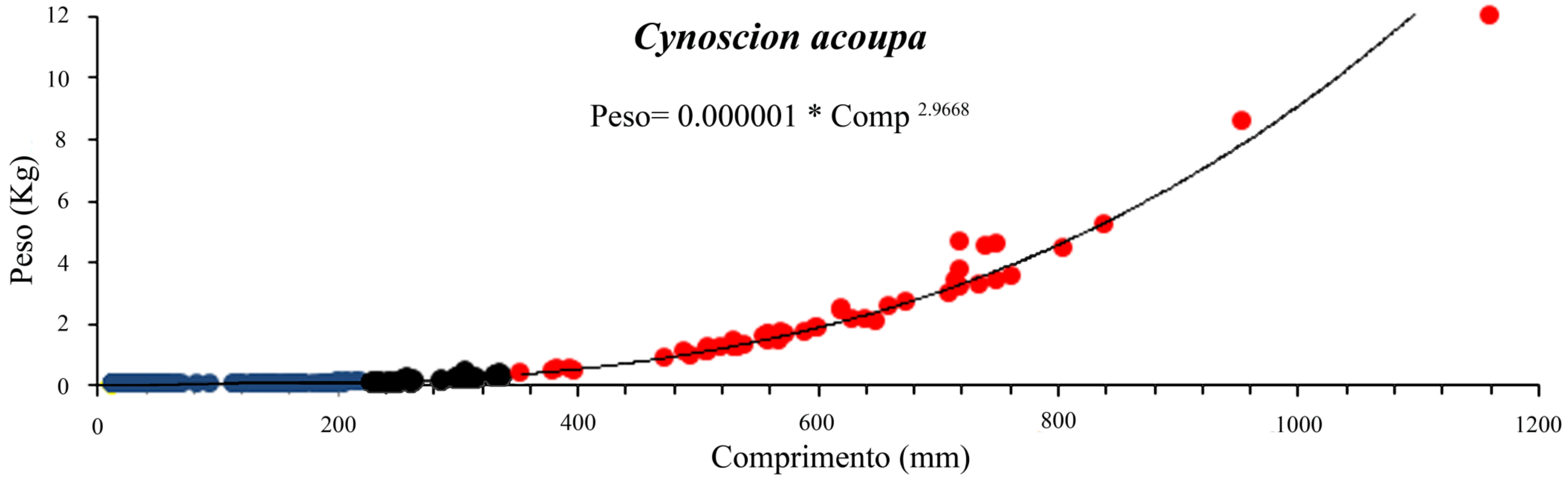


Tabela 1 - Classe de tamanho das fases ontogenéticas de *C. acoupa*.

Fases ontogenéticas de *C. acoupa*	
Juvenil	14 – 220 mm
Subadulto	220 – 340 mm
Adulto	> 340 mm

Figura 5 – Fases ontogenéticas de C. acoupa evidenciadas na relação Peso vs. Comprimento (juvenis ●, subadultos ● e adultos ●). O coeficiente de determinação, obtido pela linearização dos dados transformados por logaritmo natural foi de 98,44% (N= 839).



3.3.2 Análise de dados

Utilizando as informações obtidas referentes à área arrastada de cada amostra realizada, foi determinada a captura por unidade de área, com o intuito de estimar a densidade e a biomassa dos indivíduos, através da divisão do número e peso dos espécimes pela área de coleta (Sparre & Venema 1997), expressos da seguinte forma:

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

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

Onde, *n* é o número de indivíduos e A é a área arrastadas pela rede.

Posteriormente foi realizada a análise do conteúdo estomacal, que foi removido e triado. 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 foram lavados com água destilada, secos em papel e pesados em balança analítica.

Durante o processo de identificação do conteúdo alimentar, foi constatada a ocorrência de fragmentos de microplástico nos estômagos de *C. acoupa*. Os fragmentos de microplástico foram separados e submetidos ao processo de aquecimento em uma estufa a 70 ºC por um período de 24 h, para certificar que de fato se tratavam de um contaminante inorgânico, pois qualquer matéria orgânica submetida a esse processo seria desidratada. Quando devidamente constatada a ocorrência de microplástico, eles foram submetidos aos mesmos processos que os demais itens alimentares.

Para averiguar os principais itens alimentares utilizados pela espécie e determinar seu grau de relevância, foi utilizado o Índice de Importância Relativa (I_{IR}) (Pinkas *et al*., 1971) que consiste da seguinte equação:

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

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 em peso de cada item alimentar (Hynes, 1950; Hyslop, 1980):

$$\%F_i = (F_i \;/\; F_t) * 100$$

Onde, F_i é o número de estômagos contendo o item i e F_t é o número total de estômagos analisados.

$$\%N_i = (N_i \;/\; N_t) * 100$$

Onde, N_i é o numero do item alimentar i e N_t é o número total de itens no estômago.

$$\%P_i = (P_i \;/\; P_t) * 100$$

Onde, P_i é o peso do item alimentar *i* e P_i é peso total do itens alimentares no estômago.

3.3.3. Análises estatísticas

O estudo da distribuição espacial de *C. acoupa* foi realizado através dos dados de densidade e biomassa das diferentes fases ontogenéticas para cada porção estuarina e estação do ano, referente as amostragens do canal principal do estuário realizadas entre os anos de 2005 e 2006..

Com o intuito de alcançar a normalidade dos dados (distribuição e ecologia alimentar), s dados 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, quando as premissas dos testes paramétricos foram constatadas, prosseguiu-se com as análises estatísticas.

Posteriormente, a análise de variância ANOVA (três fatores) foi utilizada para testar se a densidade e a biomassa dos indivíduos apresentaram diferenças significativas em relação aos fatores temporais (início da seca, fim da seca, início da chuva e fim da chuva), espaciais (estuário superior, intermediário e inferior) e ontogéticos da espécie (fase juvenil, subadulta e adulta). Quando os resultados da análise determinaram diferenças significativas, foi realizado testes a posteriori (Bonferroni com $\alpha < 0,05$) para definir as fontes de variância (Quinn & Keough 2002).

Para analisar a ecologia alimentar da espécie, foram utilizadas as amostragens realizadas no canal principal do estuário (2005 a 2008) e nos canais de maré. Foi realizada uma análise de variância ANOVA para os itens alimentares mais relevantes, tanto em número, quanto em peso, em relação aos fatores espaciais, sazonais e ontogenéticos.

Em seguida foi realizada uma análise de correspondência (CCA), para constatar possíveis interações ecológicas entre a ingestão dos itens alimentares e a condições ambientais do estuário (Palmer 1993).

4 ARTIGO

Artigo submetido à revista Journal of Marine Science, intitulado "Life cycle of Acoupa weakfish in a tropical estuarine ecocline: patterns of distribution, feeding ecology and interactions with microplastics".

Life cycle of Acoupa weakfish in a tropical estuarine ecocline: patterns of distribution, feeding ecology and interactions with microplastics

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ABSTRACT

Habitat distribution and feeding ecology of *Cynoscion acoupa* were studied in relation to spatial, seasonal and ontogenetic aspects in a tropical estuary. The ontogenetic phases utilized different estuarine reaches during the seasonal fluctuations. This behaviour was strongly influenced by environmental variables, mostly salinity. Juveniles used the upper estuary during all seasons as shelter, to avoid marine predators. However, in the early rainy season the upper estuary had a great importance as nursery for the species. The sub-adults used the upper estuary along the year as a feeding ground, and migrated to the middle estuary during the late rainy season to avoid osmoregulatory stress. Adults occurred only in coastal waters of the lower estuary. The tropic guild changed during the ontogeny of *C. acoupa*, juveniles fed mainly on plastic threads (FO=64%), amphipoda (FO=34%), mysidacea (FO=17%) and *Cathorops spixii* (FO=15%). They were assigned as opportunistic. Sub-adults preferred plastic threads (FO=50%) and *C. spixii* (FO=30%) and were also classed as opportunistic. Adults were piscivorous, ingesting plastic threads (FO=100%), *C. spixii* (FO=18%), *Achirus lineatus* (FO=15%), S*tellifer stellifer* (FO=15%) and penaeid shrimp (FO=15%). The human impacts on the environment were very evident in the studied species due the high contamination of their diet by microplastic. The microplastic threads were the major prey item ingested by *C. acoupa* regardless season, area or ontogenetic phase. However, the adults showed a higher ingestion in frequency, number and weight, suggesting that this contaminant might be acquired through biotransference.

Key words: Spatial-temporal distribution, environmental gradient, nursery, recruitment, microplastic debris, fishery target species.

INTRODUCTION

Tropical estuaries are one of the most productive aquatic ecosystems on Earth. The mangrove forest and river discharges provide nutrients to the environment and supply a diverse planktonic community, which function as a basin for estuarine and coastal food webs (Barletta-Bergan *et al*., 2002a; Beck *et al*., 2003). The environmental ecocline found in these systems, mainly caused by the mixture of river freshwater and marine coastal waters, creates a great variability of habitats, promoting the occurrence of many fishes, from both marine and freshwater species (Hindell and Jenkins, 2004; Barletta and Blaber, 2007; Barletta *et al*., 2005; 2008; 2010; Dantas *et al*., 2010; Lima *et al*., 2014; 2015). Estuaries are fundamental for estuarine dependent species for providing shelter, food and developmental grounds, acting as a nursery for these fishes and invertebrates (Blaber *et al*., 2000; Barletta *et al*., 2010, Lima *et al*., 2011; Dantas *et al*., 2012b; Lima *et al*., 2013).

The seasonal fluctuation of the environmental variables is responsible for the estuarine abiotic features, influencing the movement of fish assemblages within the estuary (Schelske and Odum 1961; Wagner and Austin, 1999). Seasonal variations in salinity are a major factor influencing estuarine fish movement in tropical estuaries (Barletta *et al*., 2005; 2008; Barletta and Blaber 2007; Dantas *et al*., 2010). Studies on the movement patterns of different ontogenetic stages of fish are important to determine species biological behaviours (*e.g.* migration, spawning and recruitment) and their ecological function in the ecosystem (Dantas *et al*., 2010; 2012a; 2015; Ramos *et al*., 2011; 2014; Lima *et al*., 2013; Lima *et al*. 2014; 2015).

The connectivity between estuaries and marine coastal waters provide unique habitat attributes and remarkable resources for many fish groups (Blaber *et al*., 1989; Barletta-Bergan *et al*., 2002a; Barletta *et al*., 2003; 2005). Species which belong to the Sciaenidae family are classified as estuarine dependent, usually found in sandy or muddy bottoms (Cervigón *et al*., 1993). Regarding their ecological habit as predators (Cervigón *et al*, 1993), this group plays important ecologic and economic roles, structuring the energy transfer between ecosystems (Braga *et al*., 2012) and being a major fishery resource where they occur (Chao *et al*., 2015).

The rapid decline in abundance of predators populations, is attributed mainly to the industrial (Myers and Worm, 2003) and artisanal fleets (Guebert-Bartholo *et al*., 2011), habitat degradation and loss (Barletta *et al*., 2010; Barletta *et al*., in press). Moreover, during the feeding, fishes are susceptible to ingest microplastics (< 5 mm), available in the environment (Lima *et al*., 2014). Ingestion can occur directly, caused by similarity with their usual prey (Hoss and Settle 1990), or indirectly, through trophic transfer, when a prey had previously ingrested microplastic (Browne *et al*., 2010). In estuarine systems the ingestion of microplastic is enhanced due the abundance of this contaminant (Lima *et al*., 2014; 2015). The same has been observed for a variety of demersal species (Arridae: Possatto *et al*., 2011; Gerreidae: Ramos *et al*., 2012; Sciaenidae: Dantas *et al*., 2012b).

Due to the wide distribution and economic value, the Acoupa weakfish, *Cynoscion acoupa* (Lacepède, 1801), is one of the most representative taxon of the Sciaenidae family. This species is mainly distributed in tropical western Atlantic waters, from the Caribbean Sea to South Brazil (Cervigón *et al.*, 1993). Generally, they inhabit estuaries during the earlier phases (Barletta-Bergan *et al*., 2002a; Barletta *et al*., 2003; 2005; Ramos et al., 2011). *C. acoupa* is an important commercial species for South America fisheries, with annual landings of ≈22,000 tons in Brazil (Barletta *et al*., 1998; MPA, 2012). However, for the Goiana Estuary, traditional communities capture this fish species for commercial purposes and also for subsistence (Barletta and Costa, 2009).

For South America estuaries, such as the Caeté Estuary (Eastern Amazon), the Sciaenidae was one of the most important groups for fisheries, where the Acoupa weakfish contributed to annual landings of ≈122 ton (Barletta *et al*., 1998). The adults of *C. acoupa*, in the main channel of this estuary, represented one of the most important species (11.48 ind. ha^{-1}) (Barletta *et al*., 2005), and larvae density of 3372,2 ind. 100 m^{3} $^{-1}$ (Barletta-Bergan, 2002a). In the mangrove creeks of the Caeté Estuary the adults accounted for 10^{-7} ind. ha^{-1} (Barletta *et al*., 2003) and the larvae for 10946 ind. 100 m^{-3} (Barletta-Bergan 2002b). A similar trend is also observed in the Paranaguá Estuary (tropical-subtropical southeast of South America) for this genus, where *Cynoscion leiarchus* (Cuvier, 1830) (21.8 ind. ha^{-1}) was very well represented in the local fish assemblage (Barletta *et al*., 2008).

Regarding the ecological and economic importance of *C. acoupa*, studies on their feeding ecology and pattern of use of the different habitats along its ontogeny, will serve as important tools to understand the nursery function of the Goiana Estuary for this species. Therefore, this study provides information that might help building management strategies for the conservation of this species.

Studies on the biology (*Cynoscion othonopterus* Jordan and Gilbert, 1882 in Golf of California: Erisman *et al*., 2012), habitat use (*Cynoscion leiachus* in Southeast Brazil: Barletta *et al*., 2008), fishery (Scianidae in Brazil: Chao *et al*., 2015) and feeding ecology (*Steliffer* in Northeast Brazil: Dantas *et al*., 2015) already exist for species of the Sciaenidae family. However, especially for the genus *Cynoscion*, studies associating the distribution and feeding patterns are non-existent. This study described the distribution patterns, feeding ecology and microplastic ingestion of *C. acoupa*, regarding the spatial and seasonal fluctuations of its different ontogenetic phases in the Goiana Estuary. It also assessed the habitats used by this species as nursery, reproductive and feeding grounds.

METHODS

Study area

The Goiana Estuary is located on the eastern end of South America (Fig. 1), with annual mean air temperature around 27 ºC (Barletta and Costa, 2009). The semi-diurnal tidal regime reaches 2.5 m. It has a total area of 4,700 ha and a great variety of habitats, which includes a main channel of ~ 20 kilometres (maximum depth of 6 m) and tidal creeks within a large floodplain surrounded by mangrove forests (Barletta and Costa, 2009). In addition, there are dissipative sandy beaches located in the entrance of the estuary, extending up to hundreds of meters offshore (Lacerda *et al*., 2014). The rainfall variation characterize four seasons: early rainy (March to May), late rainy (June to August), early dry (September to November) and late dry (December to February) (Barletta and Costa, 2009).

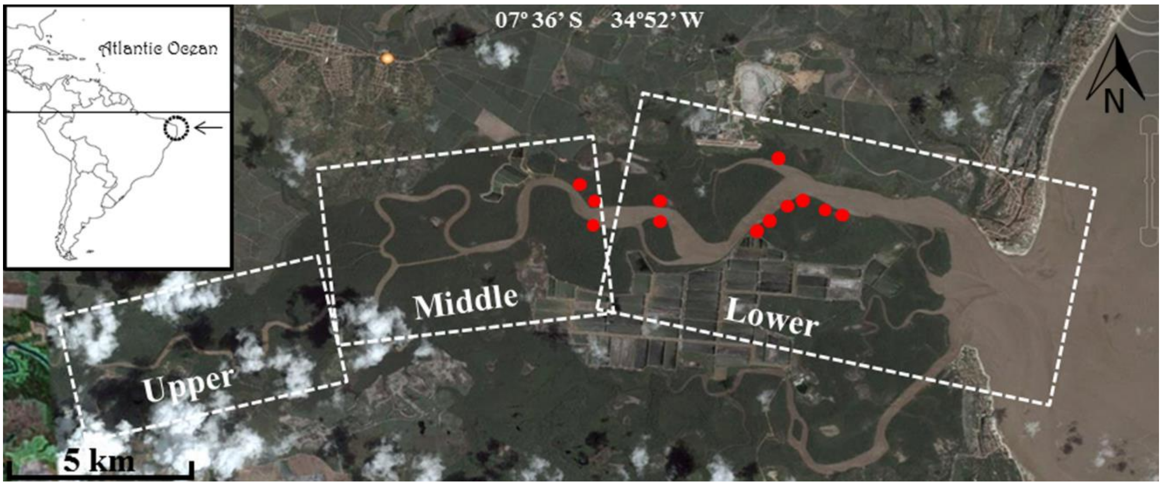

Figure 1 - Goiana Estuary divided in upper, middle and lower estuarine areas. The entrance of mangrove creeks are indicated by ●.

The estuary is occupied by small urban areas, and economic activities are developed along its margins, such as family farming along the river margin, dredging of the main channel for sand mining in the upper estuary and mining of limestone for the cement industry in the lower estuary. The major activity at the lower estuary is artisanal fishery of molluscs (*Anomalocardia brasiliana* Gmelin, 1791), crustaceans and fishes (Barletta and Costa, 2009). Due to the importance of the Goiana Estuary for traditional communities, the area became a marine protected area for sustainable use in 2007 (Resex Acaú-Goiana) (Barletta and Costa, 2009).

Sampling methods

Environmental data

Before each sampling, environmental parameters were recorded, including surface and bottom water temperature (Cº), salinity (Salinometer WTW LF 197), dissolved oxygen (DO) (mg L^{-1}) (Oximeter WTW oxi 340), Secchi depth (cm) and depth (echo sounder – Eagle Supra Pro D). Rainfall data were obtained at the nearest weather station (INMET, 2014). The sampling positions were determined by GPS.

Biological data

The samples were conducted in different habitats of the estuary (main channel and mangrove creeks) to make a more reliable model of how and when *C. acoupa* utilises this estuarine ecosystem (Fig. 1).

The main channel was divided into three areas according to the salinity gradient and morphology, upper (salinity < 5), middle (from 5 to 20) and lower estuary (salinity > 20) (Barletta and Costa, 2009). Six replicates were performed monthly in each area between December 2005 and November 2006. Trawls lasted 5 min in depths between 5 to 10 m. Samples were taken during neap tide using an otter trawl net 8.72 m long (ground rope 8.5 m and head hope 7.1 m) with 35 mm mesh size in the body and 22 mm at the codend. An additional codend cover of 5 mm was used to guarantee that more developmental stages were captured. Furthermore, trawls were made in a period of 3 months, during the late dry and late rainy season, from December 2006 to August 2008 (Dantas *et al*., 2012a).

To evaluate the importance of the mangrove creeks for this species, twelve samples were conducted at the mouth of these forest habitats, in the lower estuary (Fig. 1) (April to May/2008) (Ramos et al. 2011). Three creeks (replicates) were sampled using a fyke net of 35 m length and 5 m height, with 10 mm mesh size. The net was fixed at the entrance of the creeks during high tide, secured by wooden poles attached to the substratum (Ramos *et al*., 2011).

Furthermore, adult and sub-adult specimens were retrieved from the Goiana fishery fleet, which uses the coastal area, in the lower estuary. Data from this habitat were used only in the feeding ecology study.

Laboratory procedures

After sampling, individuals were identified to the species level (Menezes and Figueiredo, 1980; Carpenter, 2002). Specimens of *C. acoupa* were counted, weighted (g), measured (Total length mm) and had their gonads analysed.

Ontogenetic phases

For this study the specimens of *C. Acoupa* were subdivided into different ontogenetic phases: juvenile (14 – 220 mm), sub-adult (220 – 340 mm) and adult (> 340 mm). The juvenile stage was represented by specimens between the transformation length (14mm) (Balon, 1990) and the inflexion point of the length-weight equation (220mm). The length of L_{50} (340 mm) was used to distinguish sub-adults from adults. The L_{50} was calculated using the logistic regression, based on the macroscopic observation of the gonads (Appendix1) (Vazzoler, 1996). The size classes of *C. acoupa* ontogeny are described in the length-weight equation $Wg = Lt * X^{\beta_1}$ (Appendix 2).

Feeding Ecology

Stomachs of each ontogenetic phase were eviscerated and contents were removed. Then, empty stomachs were washed in distilled water to confirm that all items consumed were extracted. The feeding items were analysed using a stereomicroscope (Zeiss, Stemi 2000) and prey items were identified to the lowest taxonomic level possible (Ruppert and Barnes, 2004). Lastly, items were washed in distilled water, dried with tissue paper, counted and weighted.

Microplastic present in the stomach contents were separated from the other food items and oven dried (70 ºC), to confirm that they were indeed inorganic matter. Withered filaments were considered organic matter. After confirmation, microplastic were photographed, measured (Axiovision LE) and submitted to the same treatment of the feeding items described above (counted and weighted), being recorded as a food item category (Dantas *et al*., 2015; Ramos *et al*., 2012; Lima *et al*., 2014; 2015).

To quantify the degree of importance of each feeding item, we applied the index of relative importance (I_{RI}) (Pinkas *et al*., 1971). This index was calculated using the following equation:

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

Where, $\%F_i$ is the percentage of stomachs with the item *i* (frequency of occurrence); $\%N_i$ the number of the item *i* in the total number of stomachs analysed expressed in percentage (composition in number) and the $\%M_i$, the percentage of the item mass *i*, regarding the total weight of prey in all stomachs examined (composition in mass).

Statistical Analysis

The Levene's test was used to assert the homogeneity of variances (Levene, 1960). A three-way analysis of variance (ANOVA) was used to test if the density and biomass of the different ontogenetic stages of *C. acoupa* differed significantly in relation to temporal (seasons) and spatial (estuarine areas) factors. Moreover, the ANOVA was used to test differences in the number and mass of ingested prey items in relation to the factors area, season and ontogeny. All data were Box-Cox transformed to reach normality (Box-Cox, 1964). Whenever significant differences were observed (in distribution and diet analysis), a post hoc test was applied (Bonferroni with $\alpha = 0.05$) to identify the sources of variance (Quinn and Keough, 2003).

The Canonical Correspondence Analysis (CCA) was used to evidence ecological interactions between the prey items ingested (I_{RI} values) (dependent variables) and environmental data (independent variables). To perform the CCA, a multiple least-squares regression was computed with the site scores (derived from weighted averages of items ingested) and environmental parameters, these analyses were focused on symmetric and biplot scaling (Palmer, 1993).

A Monte Carlo Permutation Test was used to determinate which environmental parameters were significant to the variability of the biological data (Braak and Smilauer, 2002). A plot was produced, displaying the position of feeding items in each habitat/season/phase represented by geometric shapes, and the influence of environmental parameters represented by vectors.

RESULTS

Environmental parameters

The environmental parameters data refer to bottom water measurements. The estuary during the early dry season (Sep – Nov), showed a predominance of ocean waters over the river discharge. It was caused by low rainfall (58 mm), that led to an increase of salinity in the upper (3), middle (11.8) and lower estuary (27.8). Secchi depth (upper 56.3 cm, middle 72.4 cm and lower estuary 117.2 cm) and dissolved oxygen followed the same trend (upper 3 mg L^{-1}, middle 4.2 mg L^{-1} and lower estuary 6.9 mg L^{-1}). These features are a result of the entrance of marine waters, into the estuary (Fig. 2). The difference between surface and bottom waters salinity, cause a salt-wedge at the middle estuary during the early and late dry seasons.

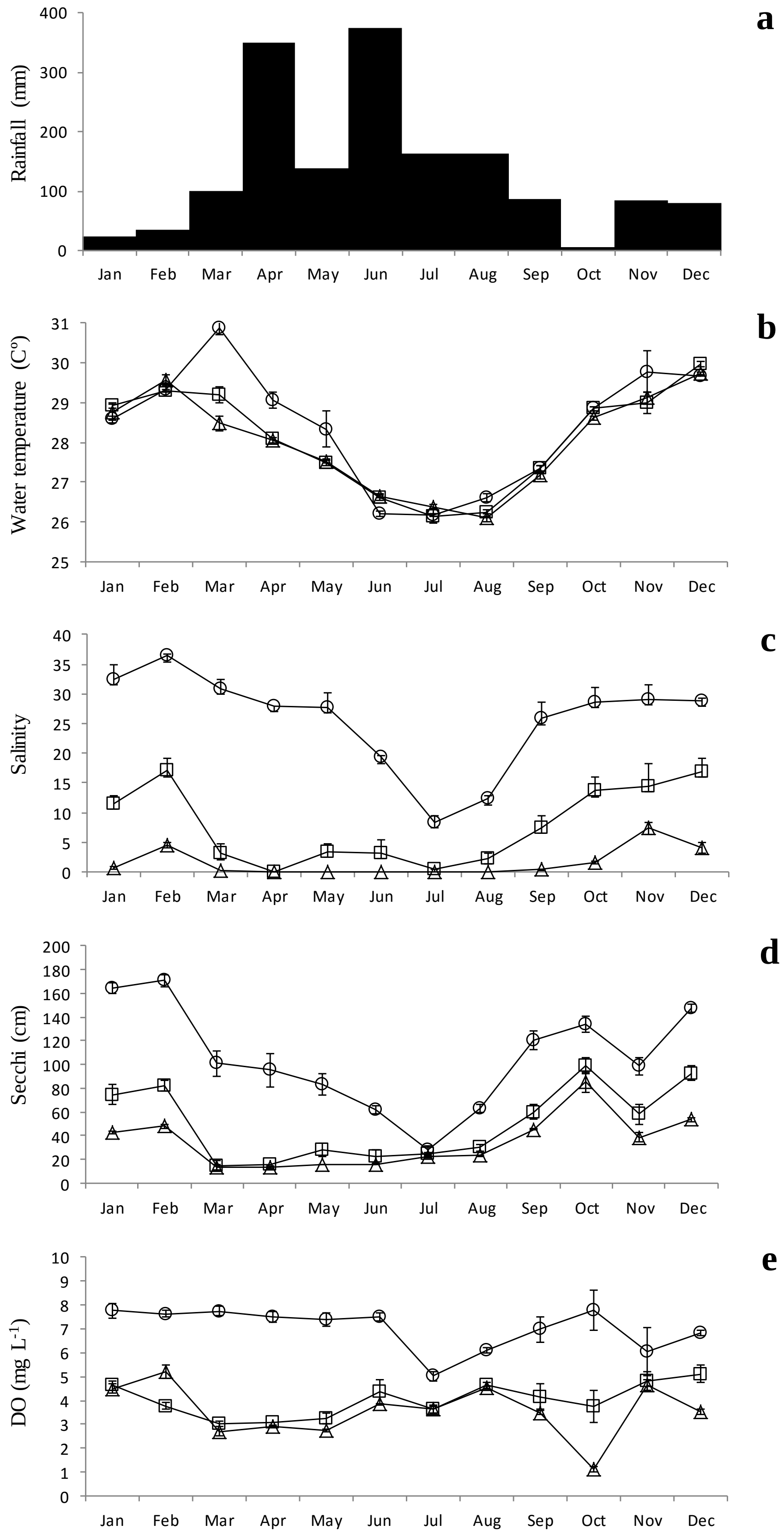

Figure 2 - (a) Total monthly rainfall (mm) and mean ± S.E. of (b) bottom water temperature, (c) bottom water salinity, (d) Secchi depth (cm) and (e) bottom water dissolved oxygen in different seasons (ED - early dry; LD – late dry; ER – early rainy; LR - late rainy) and areas (upper △, middle □ and lower ○) of the Goiana Estuary.

In the late dry season (Dec – Feb) rainfall records were lowest (46 mm), intensifying ocean influence in the estuary by raising the salinity, mainly in the middle (15.1) and lower estuary (32.5). It caused a sharp increase in Secchi depth (middle 83.2 cm and lower estuary 160.6 cm) and DO (middle 4.5 mg L^{-1} and lower estuary 7.4 mg L^{-1}) at the lower estuary (Fig. 2).

During the early rainy season (Mar – May), the increase of rainfall (196 mm), produced a higher river discharge, promoting river influence in the estuary, which caused a decline in salinity (upper 0.1, middle 2.2 and lower 28.9). In turn, river discharge brought a great quantity of suspended matter, decreasing the Secchi depth (upper 14.4 cm, middle 19.5 cm and lower 93.3 cm) and the dissolved oxygen (upper 2.7 mg L^{-1} and middle 3.12 mg L^{-1}). During the early and late rainy seasons, as a result of river higher discharge, the salt-wedge was shifted to the lower estuary (Fig. 2).

In the late rainy season (Jun – Aug) rainfall reached its peak (233 mm). Then, the estuary developed strong river-like features, producing the lowest values of salinity (upper 0.0, middle 1.9 and lower 13.5). The low values of salinity and the displacement of the salt-wedge to the lower estuary are important contributors to the formation of a highest turbidity zone, which produces high concentrations of suspended matter (Secchi depth of 51.1 cm) through flocculation (Fig. 2).

Bottom water temperature showed slight variations in relation to the estuarine areas (SE < 0.5ºC). However, it presented seasonal fluctuations within higher temperatures occurring in the early (28.3ºC) and late dry season (29.3ºC). Lower temperatures were recorded in the early (28.5ºC) and late rainy (26.3ºC) seasons, because of cloud cover, that prevents solar radiation incidence (Fig. 2).

Distribution patterns

<u>Spatio-temporal distribution patterns of the ontogenetic phases</u>

A total mean density of 27.7 ind. ha^{-1} and biomass of 287 g ha^{-1} were recorded in the Goiana Estuary for *C. acoupa*. Among them, 41.7 ind. ha^{-1} and 87 g ha^{-1} were juveniles, 2.7 ind. ha^{-1} and 648 g ha^{-1} were sub-adults. Specimens in the adult phase were not captured in the main channel of the estuary (Fig. 3) (Table 1).

The upper estuary had the highest values of density and biomass (35.4 ind. ha^{-1} and 444 g ha^{-1} respectively), independent of season and ontogeny. Indeed, the biomass of the upper estuary showed significant difference ($F=5.624$; $p<0.01$). Regarding the factor seasonal, the early rainy season presented the highest average density with interaction (116.6 ind. ha^{-1}, F=4.845; p<0.01) and average biomass (669 g ha^{-1}). The ontogeny of the species indicated that juveniles and sub-adults, are the most representative phases for density (41.7 ind. ha^{-1}, F=5.661; p<0.01) and biomass (648 g ha^{-1}, F=5.546; p<0.01), respectively (Table 1) (Appendix 3).

The interaction Season *vs*. Phase showed that the highest mean densities of juveniles, occurred in the early rainy season (228.4 ind. ha^{-1}, F= 4.595 p<0.01), especially in the upper and middle estuaries. Significance was also evinced between the interaction Area *vs*. Phase, the sub-adults registered the highest average biomass in the upper estuary (890 g ha^{-1}, F= 3.173; p<0.01), especially during the early rainy season (Table 1) (Appendix 3).

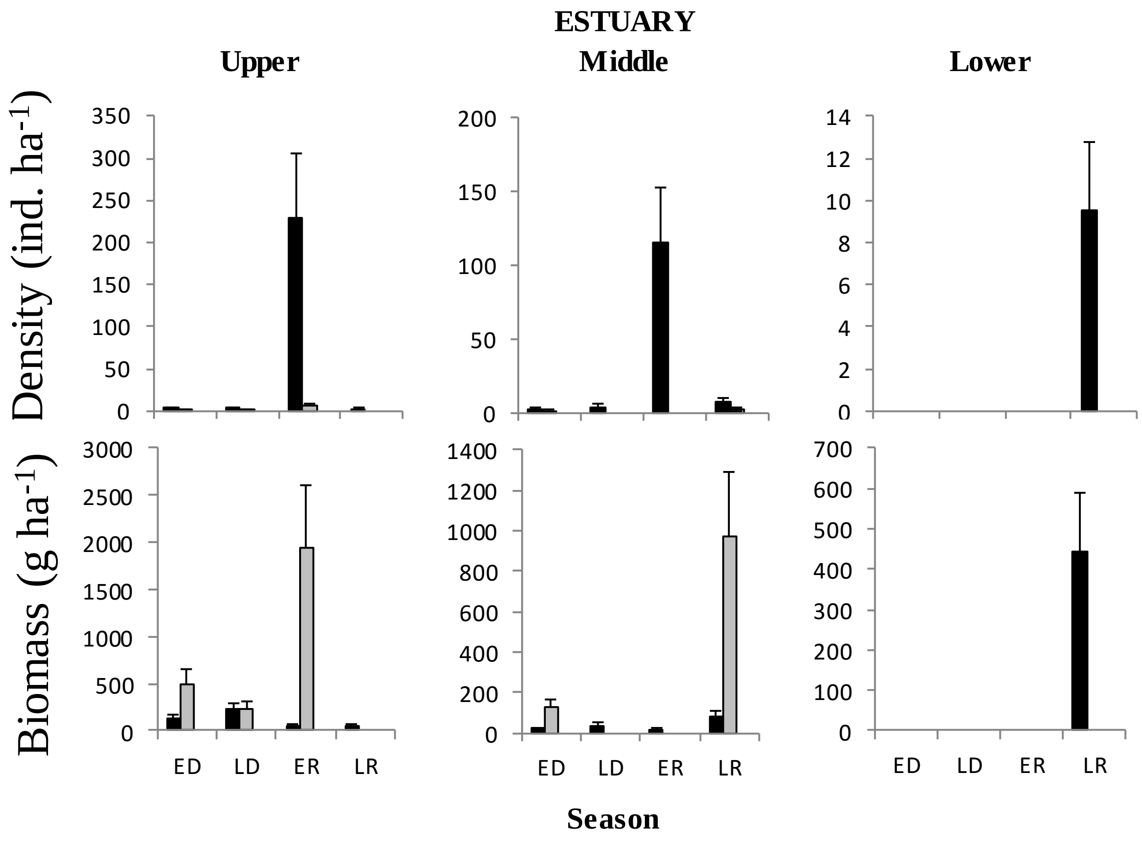

Figure 3 - Mean ± S.E. of density and biomaas of C. acoupa population in different ontogenetic phases (juveniles ■, sub-adults ■ and adults □), seasons (ED - early dry; LD – late dry; ER – early rainy; LR - late rainy) and areas (upper, middle and lower) of the Goiana Estuary.

Table 1 - Density and biomass of C. acoupa according to ontogenetic phases (Juv: juvenile, Sub: sub-adult and Adu: adult), seasons (early dry, late dry, early rainy and late rainy) and areas (upper, middle and lower) of the Goiana Estuary. (-) no capture.

Season	Phase	DENSITY (ind.ha^{-1})			BIOMASS (g ha^{-1})		
		Upper	Middle	Lower	Upper	Middle	Lower
Early Dry	Juv	3.17	2.10	-	127.8	25.0	-
	Sub	1.74	1.38	-	496.7	126.2	-
	Adu	-	-	-	-	-	-
Late Dry	Juv	3.45	3.50	-	223.7	35.0	-
	Sub	1.60	-	-	229.5	-	-
	Adu	-	-	-	-	-	-
Early Rainy	Juv	228.45	115.00	-	46.0	18.3	-
	Sub	6.50	-	-	1945.7	-	-
	Adu	-	-	-	-	-	-
Late Rainy	Juv	2.97	7.39	9.56	45.3	82.2	180.7
	Sub	-	2.30	-	-	442.6	-
	Adu	-	-	-	-	-	-
	Total	247.87	131.67	9.56	3114.6	729.2	180.7

Feeding ecology

Diet composition shifts between ontogenetic phases

From the 530 of *C. acoupa* gut contents analysed, 469 were from juveniles, 25 sub-adults and 33 adults. A total of 22 prey items were identified. The diet of *C. acoupa* consisted of fishes (FO=31.2%) (*Anchovia clupeoides, Stellifer stellifer, Stellifer brasiliensis, C. acoupa, Achirus lineatus, C. spixii, C. spixii* eggs, *Bairdiella ronchus, Diapterus rhombeus* and unidentified fishes), crustaceans (FO=57.3%) (Amphipoda, Isopoda, Mysidacea, zoeae of Brachyura, *Callinectes* sp., Copepoda and penaeid shrimp), Polychaeta (FO=16.5%) (Syllidae), Bivalve (FO=0.2%) (*Mytilidae* sp.), seaweed (FO=0.2%) and plant fragments (FO=2.9%). Moreover microplastic debris were also found and classified as microplastic threads (FO=64.2%) (Fig. 4, 5, 6, 7 and 8) (Table 2).

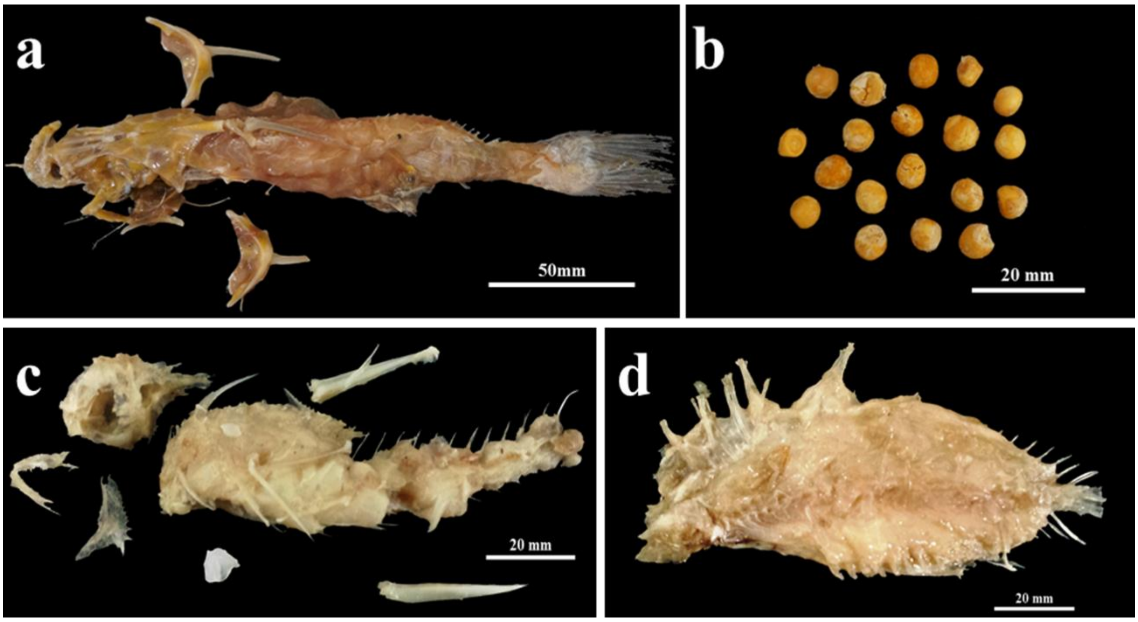

Figure 4 - Prey items consumed by *C. acoupa*. (a) *C. spixii*; (b) *C. spixii* eggs; (c) *B. ronchus*; (d) *A. lineatus*.

For all ontogenetic phases, the most frequent item were the microplastic threads (FO=64.2%), followed by Amphipoda (FO=33%), *C. spixii* (FO=16.5%) and Mysidacea (FO=16.2%). For the juvenile phase, the most abundant item were microplastic threads (FO=64.4%), Amphipoda (FO=34.3%), Mysidacea (FO=17.2%) and *C. spixii* (FO=15.4%) (Fig. 6), while sub-adults consumed mostly microplastic threads (FO=50%), *C. spixii* (FO=35%) and unidentified fishes (FO=15%) (Fig. 6). The most frequent item preyed by adults were microplastic threads (FO=100%), *C. spixii* (FO=18.1%), *A. lineatus* (FO=15.1%), *S. stellifer* (FO=15.1%) and penaeidae shrimp (FO=15.1%) (Fig. 6).

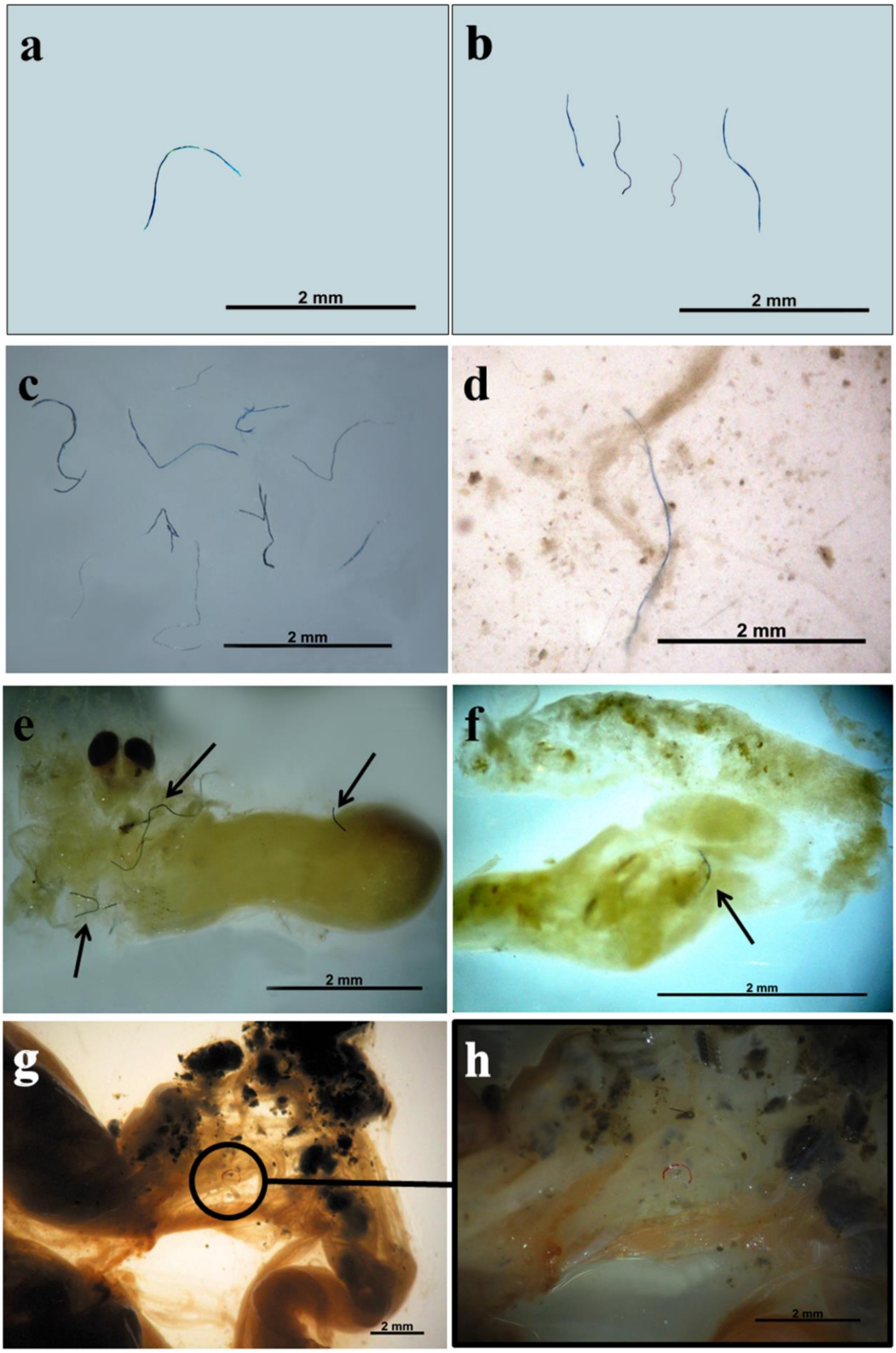

Figure 5 - Microplastic found in *C. acoupa* stomachs. (a) Microplastic threads; (b) Multi-coloured microplastic threads; (c) Accumulation of microplastic threads in the stomach of an individual of *C. acoupa*; (d) Microplastic threads attached to prey itens, perifiton and (e) penaeid shrimp; (f) Microplastic threads in stomach of larvae; (g) Microplastic threads in juvenile specimen intestine (zoom 10X) and (h) (zoom 20X).

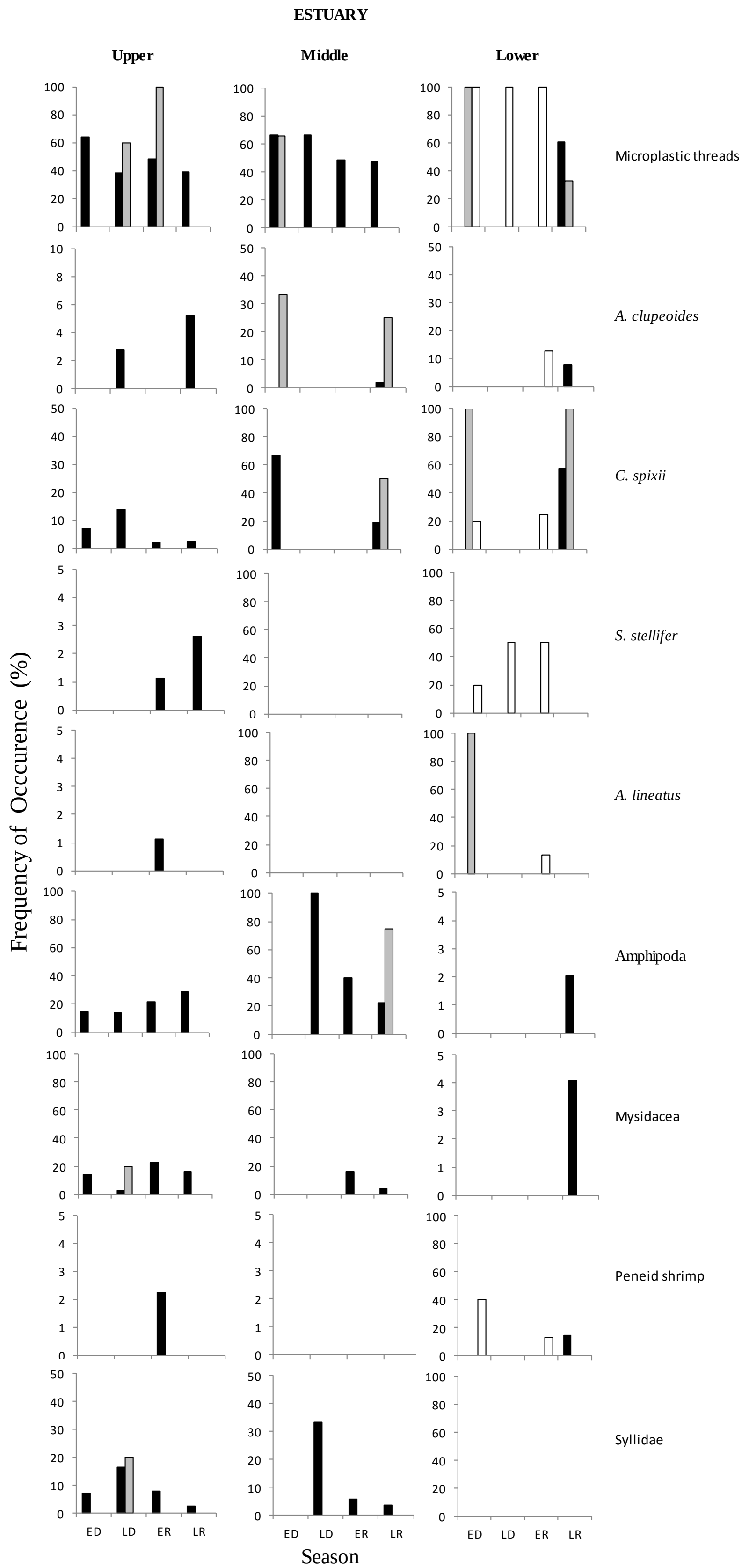

Figure 6 - Frequency of Occurrence in percentage (FO%) of prey items consumed by *C. acoupa* in different ontogenetic phases (juveniles ■, sub-adults ■ and adults □), seasons (ED - early dry; LD – late dry; ER – early rainy; LR - late rainy) and areas (upper, middle and lower) of the Goiana Estuary.

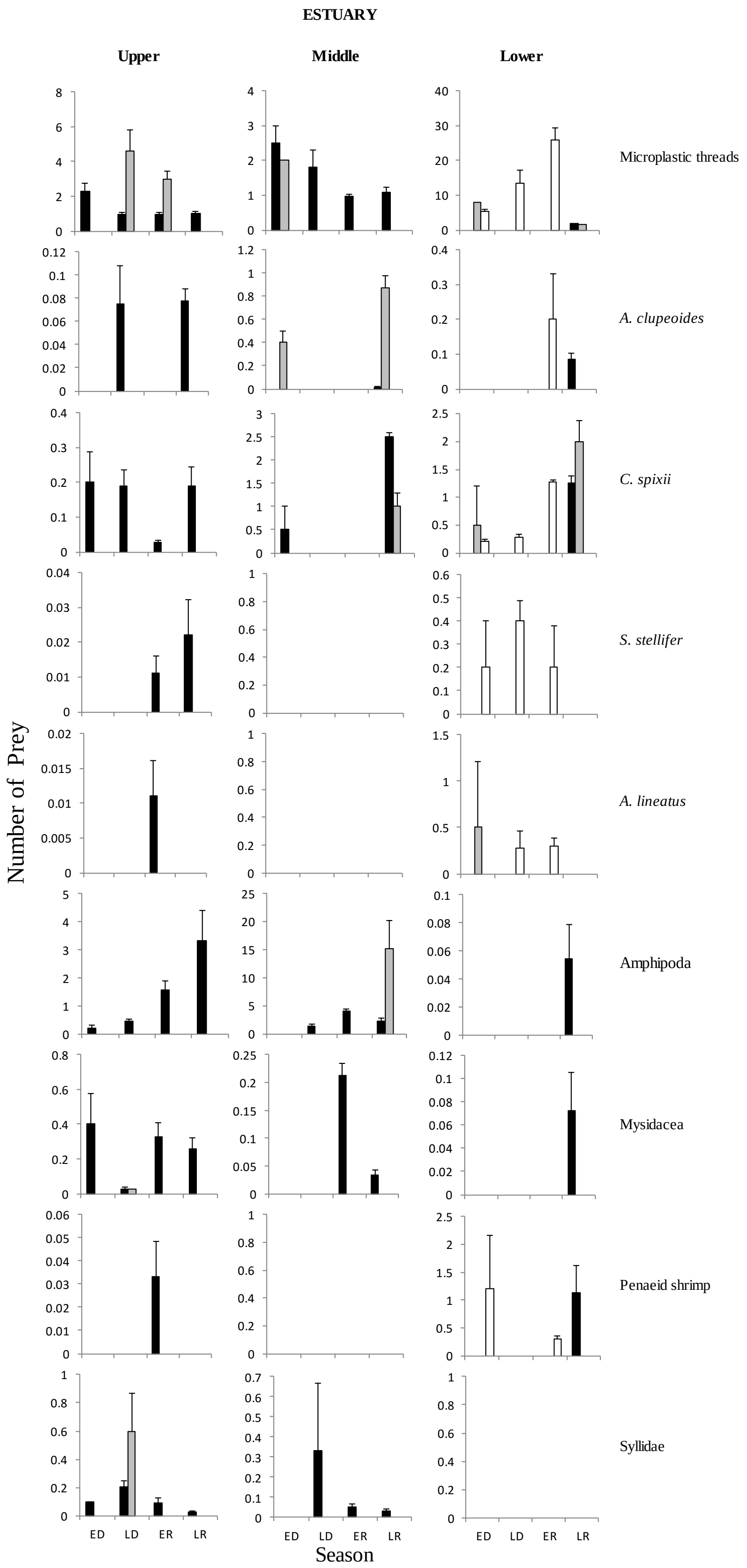

Figure 7 - Mean ± S.E. of number of prey items consumed by *C. acoupa* in different ontogenetic phases (juveniles ■, sub-adults ■ and adults □), seasons (ED - early dry; LD – late dry; ER – early rainy; LR - late rainy) and areas (upper, middle and lower) of the Goiana Estuary.

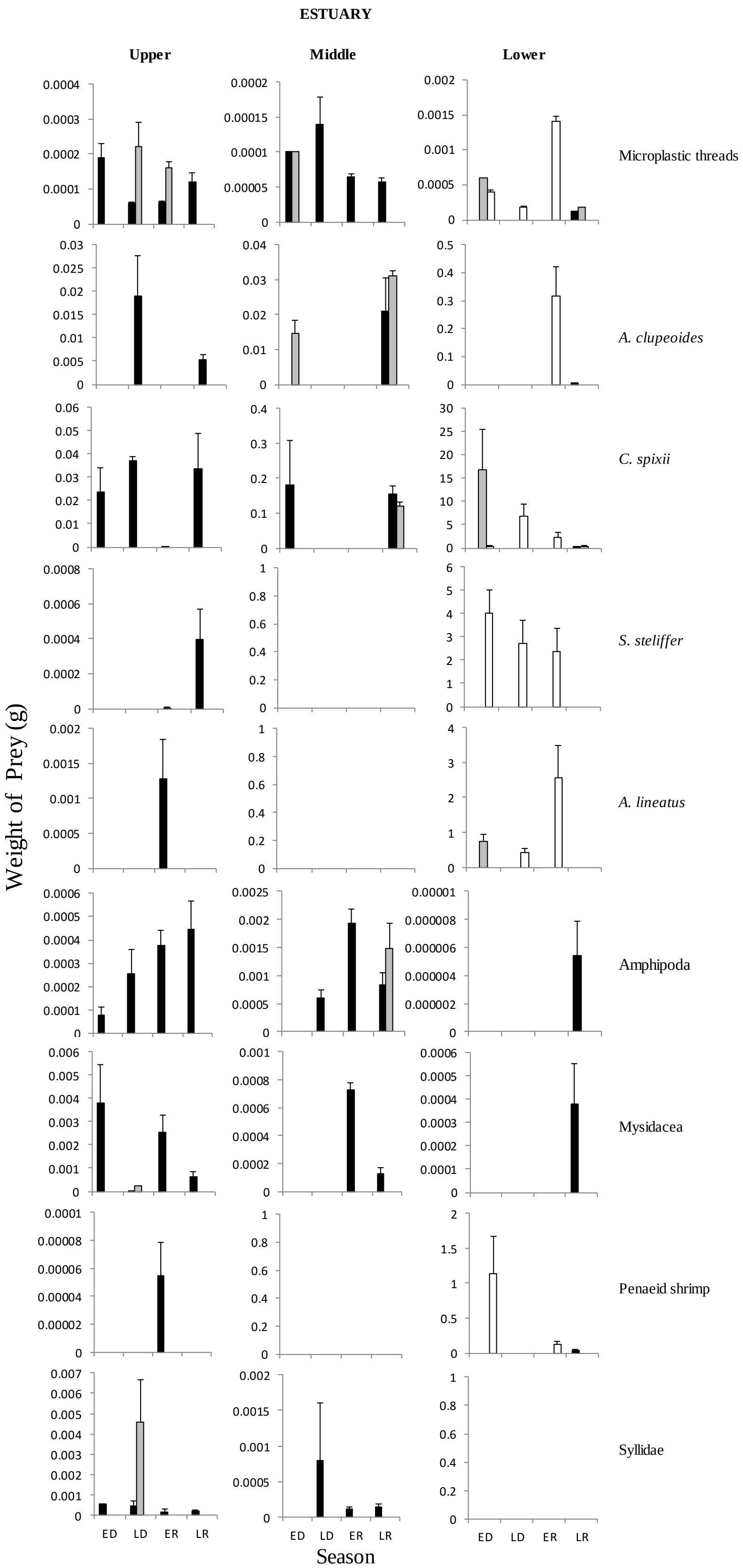

Figure 8 - Mean ± S.E. of weight (g) of prey items consumed by *C. acoupa* in different ontogenetic phases (juveniles ■, sub-adults ■ and adults □) seasons (ED - early dry; LD – late dry; ER – early rainy; LR - late rainy) and areas (upper, middle and lower) of the Goiana Estuary.

Table 2 - Diet composition of *C. acoupa* expressed as percentage of the Index of Relative Importance (IRI%), according to ontogenetic phases (Juv: juvenile, Sub: sub-adult and Adu: adult), seasons (early dry, late dry, early rainy and late rainy) and areas (upper, middle and lower) of the Goiana Estuary. (-) no capture.

Prey Item	Phase	Early Dry %I_{RI} U	Early Dry %I_{RI} M	Early Dry %I_{RI} L	Late Dry %I_{RI} U	Late Dry %I_{RI} M	Late Dry %I_{RI} L	Early Rainy %I_{RI} U	Early Rainy %I_{RI} M	Early Rainy %I_{RI} L	Late Rainy %I_{RI} U	Late Rainy %I_{RI} M	Late Rainy %I_{RI} L
Plastic threads	Juv	59.70	32.13	-	46.27	49.21	-	32.74	13.64	-	15.53	15.84	27.52
	Sub	0	33.37	43.48	50.02	-	-	70.81	-	-	-	38.63	15.45
	Adu	-	-	70	-	-	73.62	-	-	78.22	-	-	-
***A. clupeoides* (Fish)**	Juv	0	0	-	2.18	0	-	0	0	-	3.34	0.04	0.38
	Sub	0	66.63	0	0	-	-	0	-	-	-	12.98	0
	Adu	-	-	0	-	-	0	-	-	0.43	-	-	-
***C. spixii* (Fish)**	Juv	8.67	67.87	-	37	0	-	0.33	0	-	4.93	47.30	65.05
	Sub	0	0	0	0	-	-	0	-	-	-	45.49	84.06
	Adu	-	-	1.74	-	-	13.90	-	-	4.34	-	-	-
***C. acoupa* (Fish)**	Juv	0	0	-	0	0	-	0	0.06	-	0	0	0.09
	Sub	0	0	0	0	-	-	0	-	-	-	0	0
	Adu	-	-	0	-	-	0	-	-	0.27	-	-	-
***S. stellifer* (Fish)**	Juv	0	0	-	0	0	-	0.01	0	-	0.30	0	0
	Sub	0	0	0	0	-	-	0	-	-	-	0	0
	Adu	-	-	12.99	-	-	2.13	-	-	1.96	-	-	-
***A. lineatus* (Fish)**	Juv	0	0	-	0	0	-	0.73	0	-	0	0	0
	Sub	0	0	11.73	0	-	-	0	-	-	-	0	0
	Adu	-	-	0	-	-	1.25	-	-	2.18	-	-	-
***B. ronchus* (Fish)**	Juv	0	0	-	0	0	-	0	0	-	0	0	0
	Sub	0	0	0	0	-	-	0	-	-	-	0	0
	Adu	-	-	0	-	-	4.86	-	-	10.56	-	-	-
***D. rhombeus* (Fish)**	Juv	0	0	-	0	0	-	0	0	-	0	0	0
	Sub	0	0	0	0	-	-	0	-	-	-	0	0
	Adu	-	-	0	-	-	4.86	-	-	10.56	-	-	-
***C. spixii* egg (Fish)**	Juv	0	0	-	0	0	-	0	0	-	0	0	0
	Sub	0	0	0	46.84	-	-	0	-	-	-	0	0
	Adu	-	-	0	-	-	3.93	-	-	0	-	-	-
Non identified fish	Juv	1.74	0	-	0	0	-	0.37	1.32	-	1.93	3.83	1.22
	Sub	100	0	44.78	0	-	-	9.96	-	-	-	2.90	0
	Adu	-	-	1.95	-	-	0.28	-	-	0	-	-	-
Amphipoda (Crustacean)	Juv	3.33	0	-	9.64	19.44	-	26.79	80.81	-	69.30	31.91	0.02
	Sub	0	0	0	0	-	-	0	-	-	-	0	0
	Adu	-	-	0	-	-	0	-	-	0	-	-	-
Mysidacea (Crustacean)	Juv	3.28	0	-	0.13	0	-	33.75	3.66	-	4.08	0.21	0.06
	Sub	0	0	0	0.74	-	-	0	-	-	-	0	0
	Adu	-	-	0	-	-	0	-	-	0	-	-	-
Penaeid Shrimp (Crustacean)	Juv	22.76	0	-	0	0	-	0.11	0	-	0	0	5.26
	Sub	0	0	0	0	-	-	0	-	-	-	0	0
	Adu	-	-	13.27	-	-	0	-	-	0.27	-	-	-
Zoeae of Brachyura	Juv	0	0	-	0	0	-	4.03	0.22	-	0	0.14	0
	Sub	0	0	0	0	-	-	0	-	-	-	0	0
	Adu	-	-	0	-	-	0	-	-	0	-	-	-
***Callinectes sp.* (Crustacean)**	Juv	0	0	-	0	0	-	0	0	-	0	0	0
	Sub	0	0	0	0	-	-	0	-	-	-	0	0
	Adu	-	-	0	-	-	0	-	-	1.33	-	-	-
Copepoda (Crustacean)	Juv	0	0	-	0	0	-	0	0.00	-	0.43	0.65	0
	Sub	0	0	0	0	-	-	0	-	-	-	0	0
	Adu	-	-	0	-	-	0	-	-	0	-	-	-
***Mytilidae sp.* (Crustacean)**	Juv	0	0	-	0	0	-	0	0	-	0	0	0.08
	Sub	0	0	0	0	-	-	0	-	-	-	0	0
	Adu	-	-	0	-	-	0	-	-	0	-	-	-
Isopoda (Crustacean)	Juv	0	0	-	0	0	-	0	0.00	-	0	0	0
	Sub	0	0	0	0	-	-	0	-	-	-	0	0
	Adu	-	-	0	-	-	0	-	-	0	-	-	-
Syllidae (Polychaeta)	Juv	0.52	0	-	4.78	31.35	-	1.01	0.26	-	0.14	0.09	0
	Sub	0	0	0	2.40	-	-	0	-	-	-	0	0
	Adu	-	-	0	-	-	0	-	-	0.02	-	-	-
Seaweed	Juv	0	0	-	0	0	-	0	0	-	0	0	0
	Sub	0	0	0	0	-	-	0	-	-	-	0	0
	Adu	-	-	0	-	-	0	-	-	0.16	-	-	-
Plant Fragments	Juv	0	0	-	0	0	-	0.14	0.02	-	0	0	0.32
	Sub	0	0	0	0	-	-	19.23	-	-	-	0	0.49
	Adu	-	-	0	-	-	0	-	-	0.02	-	-	-

Spatio-temporal patterns of ingested items for ontogenetic phases

Acording to the ANOVA the most important feeding items of *C. acoupa* diet are presented with in the combination of factors; season, estuarine area and ontogenetic phase (Appendix 4 and 5).

For the juveniles of the upper estuary the most important items, with interactions (season *vs.* area *vs.* phase) were Amphipoda in number (F= 3.030; p<0.01) during the early rainy (1.5 ind.) and late rainy seasons (3.3 ind.). This phase at the middle estuary in the early rainy season recorded interactions for Amphipoda in number (4 ind., F= 3.030; p<0.01) and weight (0.002g, F=2.596; p<0.01), and for Mysidacea in number (0.2 ind., F=1.958; p<0.05). During the late rainy season interactions occurred for *C. spixii* in number (0.4 ind., F= 3.094; p<0.01). Likewise, the juveniles of the lower estuary in the late rainy season showed interactions for the feeding item *C. spixii* in number (1.2 ind., F= 3.094; p<0.01) (Fig. 7 and 8).

Sub-adults of the middle estuary in the late rainy, recorded interactions for the number of items *A. clupeoides* (0.8 ind., F=2.551; p<0.01) and Amphipoda (15.2 ind., F= 3.030; p<0.01). In the lower estuary (late rainy), this same phase presented interactions in number for *C. spixii* (1.0 ind., F= 3.094; p<0.01) (Fig. 7 and 8).

C. acoupa adults were only captured in the lower estuary. During the early rainy season, they recorded interactions in number and weight for the items microplastic threads (25.9 ind., F= 9.498; p<0.01) (0.0014 g, F= 8.187; p<0.01) and *A. lineatus* (0.3 ind., F=2.747; p<0.01) (2.57 g, F=2.686; p<0.01), and also in number for *C. spixii* (1.2 ind., F= 3.094; p<0.01). This phase in the lower estuary, during the early dry season, showed interactions in number and weight for penaeid shrimp (1.2 ind., F= 2.499; p<0.01) (1.13 g, F= 2.504; p<0.01). The adults of the lower estuary in the late dry season, showed interactions in number for *A. lineatus* (0.3 ind., F=2.747; p<0.01), and in weight for *C. spixii* (6.81 g, F=6.497; p<0.01) (Fig. 7 and 8).

Influence of the environmental variables on the diet patterns

The CCA indicates the correlation of the most important prey items ingested by each ontogenetic phase and the environmental variables (seasonality and estuarine area) (Fig. 9) (Appendix 6). The first axis explained 62.5% and represents the salinity gradient of the estuary, it was positively correlated with the salinity (p<0.01). The second axis explained 27.5% and represents seasonality, it presented a negative correlation with temperature (Fig. 9) (Appendix 6). The first axis was mainly formed by salinity, dissolved oxygen and Secchi depth, while in the second axis the major variable was bottom water temperature (Appendix 6).

The item microplastic threads was placed in the interception of the axis, due its occurrence in all seasons, areas and ontogenetic phases of *C. acoupa* diet, as expected by the frequency of occurrence of this prey item (Fig. 6). During the early rainy season, juveniles and sub-adults of the upper and middle estuary showed positive correlations with Syllidae (polychaeta) and the crustaceans Amphipoda and Mysidacea, as showed by ANOVA (Appendix 4 and 5). The juveniles and sub-adults were positive correlated with the fishes *C. spixii* and *A. clupeoides*, during the late rainy season, corroborating the ANOVA (Appendix 4 and 5). Adults of the lower estuary, in the late dry and early rainy seasons, were strong and positively correlated with the fishes *S. stellifer* and *A. lineatus*.

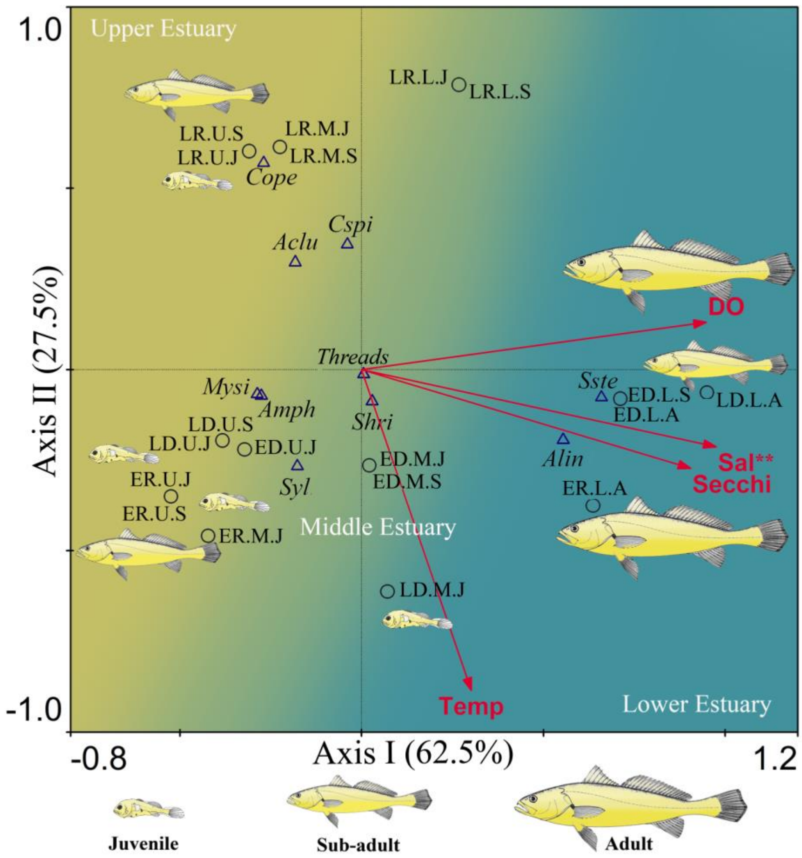

Figure 9 - Canonical correspondence analysis (CCA) triplot for correlations between environmental variables and the index of relative importance (IRI%) of prey items ingested by *C. acoupa*. The environmental parameters (Temp: Temperature; Sal: Salinity; DO: Dissolved oxygen; Secchi: Secchi depth) are represented by arrows (*$p < 0.05$). Prey items (Threads: microplastic threads; *Aclu*: *A. clupeoides*; *Cspi*: *C. spixii*; *Sste*: *S. stellifer*; *Alin*: *A. lineatus*; Amph: Amphipoda; Mysi: Mysidacea; Shri: Penaeid shrimp; Cope: Copedoda; Syl: Polychaeta Syllidae) are represented by △. The combination between seasons (ED: Early dry; LD: Late dry; ER: Early rainy; LR: Late rainy), areas (U: Upper; M: Middle; L: Lower) and ontogenetic phases (J: Juveniles; S: Sub-adults; A: Adults) are represented by ○.

DISCUSSION

The habitat use and the feeding ecology of *C. acoupa* assumed different patterns depending on spatial, seasonal and ontogenetic fluctuations. Previous studies had identified this type of variations in Ariidae (Dantas *et al.*, 2012a) and Scianidae species (Jaureguiza *et al.*, 2006; Dantas *et al.*, 2015). Environmental variables, such as salinity, have been cited as the major influence in estuarine fish assemblages distribution (Blaber *et al.*, 1989; Barletta and Dantas, 2015). Likewise, the seasonality is another important factor, because the increase of rainfall leads to the increase of river discharge and hence the salinity, turbidity, temperature and the position of the salt-wedge (Blaber *et al.*, 1989; Barletta *et al.*, 2005, 2008; Barletta and Dantas, 2015).

The absence of adult specimens of *C. acoupa* in the main channel of the estuary, also reported by Barletta *et al.* (2005), suggest that this ontogenetic phase does not frequently use this area, instead, they prefer coastal waters. That is the reason why adults were only captured by fishers activity focused on fish stocks from coastal waters. The diet of adults always consisted of fishes and penaeid shrimps, principally ingest during the early rainy season, likely due to the increase of river discharge, leading to the shift of the salt-wedge to the lower estuary, causing high turbidity levels which favoured the occurrence of demersal fish species (*C. spixii*, *S. stellifer* and *A. lineatus)*, major prey items of *C. acoupa*.

Lima *et al.* (2015) recorded the highest density of larval *C. acoupa* in the lower estuary. Hence, it is probable that adults breed and spawn in the coastal waters adjacent to the estuary. Then, larvae take advantage of the flooding tide currents to enter the estuary, seeking favourable conditions for development, such as high availability of food and shelter (Barletta-Bergan *et al.*, 2002b).

Juveniles occur principally in the upper estuary, as reported by Barletta *et al.* (2005), because of high turbidity associated to low salinity (stressful conditions for marine predators), where they suffer low predation pressure and there are many microhabitats to be used as shelter (Barletta *et al.*, 2010). The upper estuary, especially during the early rainy season is therefore notably important for the life cycle of this species. The high density and the low biomass of juveniles recorded confirms that this estuarine area, in this particular season, is the nursery ground of *C. acoupa*. They consumed a wide range of prey items, including fish (pelagic and demersal), plankton and benthos (micro and macrobenthos) in the upper estuary. This strategy is common among early stages of estuarine fishes (Cyrus and Blaber, 1983; Gning *et al., 2008*).

During the early and late rainy seasons juveniles also inhabit the middle estuary, and in the late rainy season even the lower estuary. This habitat shift occurs as a result of the environmental changes caused by the variation in river discharge, that is increased during the rainy seasons. The presence of such vulnerable phase in the lower estuary (late rainy) is explained by the salt-wedge occurrence and the highest turbidity zone in this area, that generate favourable conditions of salinity and turbidity for this ontogenetic phase (Laegdsgaard and Johnson, 2001; Barletta *et al*., 2005). Similarly these characteristics are optimal for juveniles of *C. spixii*, that use the lower estuary (late rainy) as a nursery ground (Dantas *et al*., 2012a). Therefore, juveniles of *C. acoupa* use this area as feeding grounds, due to the high density of their main prey.

The sub-adults also preferred habitats with river-like characteristics, occurring mainly at the upper estuary during all seasons. In the late rainy season they migrate to the middle estuary seeking less stressful salinity conditions. Despite the high energy spent on osmoregulation control, the sub-adults opt to use the upper and middle estuary in search of a food, preying mostly on Amphipods, *A.clupeoides* and *C. spixii*, hence hastening their growth rates.

When *C. acoupa* becomes fully developed (Lt $\geq$ 340 mm), they migrate to coastal waters and assume a marine behaver. Therefore, this species could be classed as marine estuarine dependent (Blaber and Blaber, 1980; Blaber *et al.*, 1989), a marine species which relies on the estuary to complete its life cycle (nursery and development).

The trophic guild of this species varied according to the ontogenetic phase. Juveniles exhibit an opportunistic behaviour, preying on a wide variety of feeding items, like fish species, planktonic and benthonic crustaceans. Sub-adults were also opportunistic. However, the range of prey items ingested were narrower, indicating a guild transition, from opportunistic to piscivore. Lastly, adults were classified as piscivore, preying mainly on fishes and macrobenthos (Elliot *et al.*, 2007).

<u>Anthropogenic threats</u>

The most ingested item in number was not a proper prey. The major diet item was an evidence of human interference on the estuarine food weeb: microplasticplastic threads. This contaminant occurred in specimens throughout estuarine areas, seasons and ontogenetic phases. In fact, the Goiana estuary waters are highly contaminated by microplastics, their densities being comparable to half of the fish larvae density (Lima *et al.*, 2014). Althourgh, previous studies reported the contamination of demersal species by this type of plastic debris (Possatto *et al.*, 2011; Dantas *et al.*, 2012b; Ramos *et al.*, 2012). However, none recorded such a vast occurrence of microplastic in a fish diet, > 50% (Possatto *et al*., 2011; Boerger *et al*., 2010; Dantas *et al*., 2012b; Lusher *et al.*, 2013). Microplastic threads were found in 64% of the *C. acoupa* stomachs during the presente study.

The high FO, number and weight of microplastic threads in stomachs of *C. acoupa*, might be related to its trophic position. Top predators rely on a great amount of prey (biomass) to supply their energy requirements. In turn, these prey may be previously contaminated, enhancing the possibility of ingestion of microplastic via biotransference process. This might be the reason why the highest amount of ingested microplastic occurred in the adult phase. Whereas this phase consumed a greater number of prey items, they are more susceptible to this type of contamination. Indeed studies reported that fish are capable of transfer microplatic through the trophic chain (Eriksson and Burton, 2003). However, this contaminant is aparently not biocumulated in the process (Santana *et al.*, 2015), evincing that microplastic threads found in the studied species probably came from the digestive tract of their prey and are likely to have high occurrences in other top predators as well.

Other explanation for the high occurrence of threads in stomachs of *C. acoupa* in the lower estuary, mainly during the rainy season, is the intensification of the lobster and prawns fisheries, leading to an increase in number of fishing vessels at surrounding coastal waters. The maintenance of gears, ropes, handlines and nets provide a large quantity of plastic threads to the lower estuary, as a result of the fragmentation process (Guebert-Bartholo *et al.,* 2011; Possatto *et al.*, 2011).

Despite microplastic threads being only one type o microplastic in the water column of the Goiana estuary, accounting for only 1.4% (Lima *et al.,* 2014), it was the major sort of microplastic ingested by *C. acoupa*. It is probably caused by direct consumption of microplastic threads by the whole food web (invertebrates and fish, including *C. acoupa*), during the foraging process, fish possibly confound microplastic threads with prey items, such as polychaeta parapodias (Possatto *et al.*, 2011), ingesting large amounts of this contaminant, due its aplenty in the estuary (Lima *et al.*, 2014).

The Acoupa weakfish use the estuary in a complex pattern (Fig. 10). The habitat utilization varies according to season and ontogenetic phase. In turn, they are linked by the fluctuation of environmental parameters (mainly salinity) and the availability of feeding items, regarding quantitative but mostly qualitative features. Thus, ecosystem needs to be preserved as a whole, because this species depends on the diversity of estuarine reaches to complete its life cycle. Likewise, their prey populations also need protection, mainly because *C. acoupa* is a top predator, relying on a variety of prey from different trophic positions, and therefore, is more vulnerable to food web disturbances (Carpenter *et al.*, 1985). Indeed, this species is already classified as near threatened by the Brazilian red list assessment of Scianidae (Chao *et al.,* 2015).

Studies describe the trophic group of carnivorous as the most threatened species by the habitat loss, through dredging process (Barletta *et al., in press*) and industrial fishery (Myres and Worm, 2003). Therefore, research on top predators populations, including all its ontogenetic phases, from larvae to adults, are urgent to establish their ecology, fishery stocks, pollutants contaminations and dredging vulnerability. This data will provide remarkable information to create management strategies to ensure the health status of these species.

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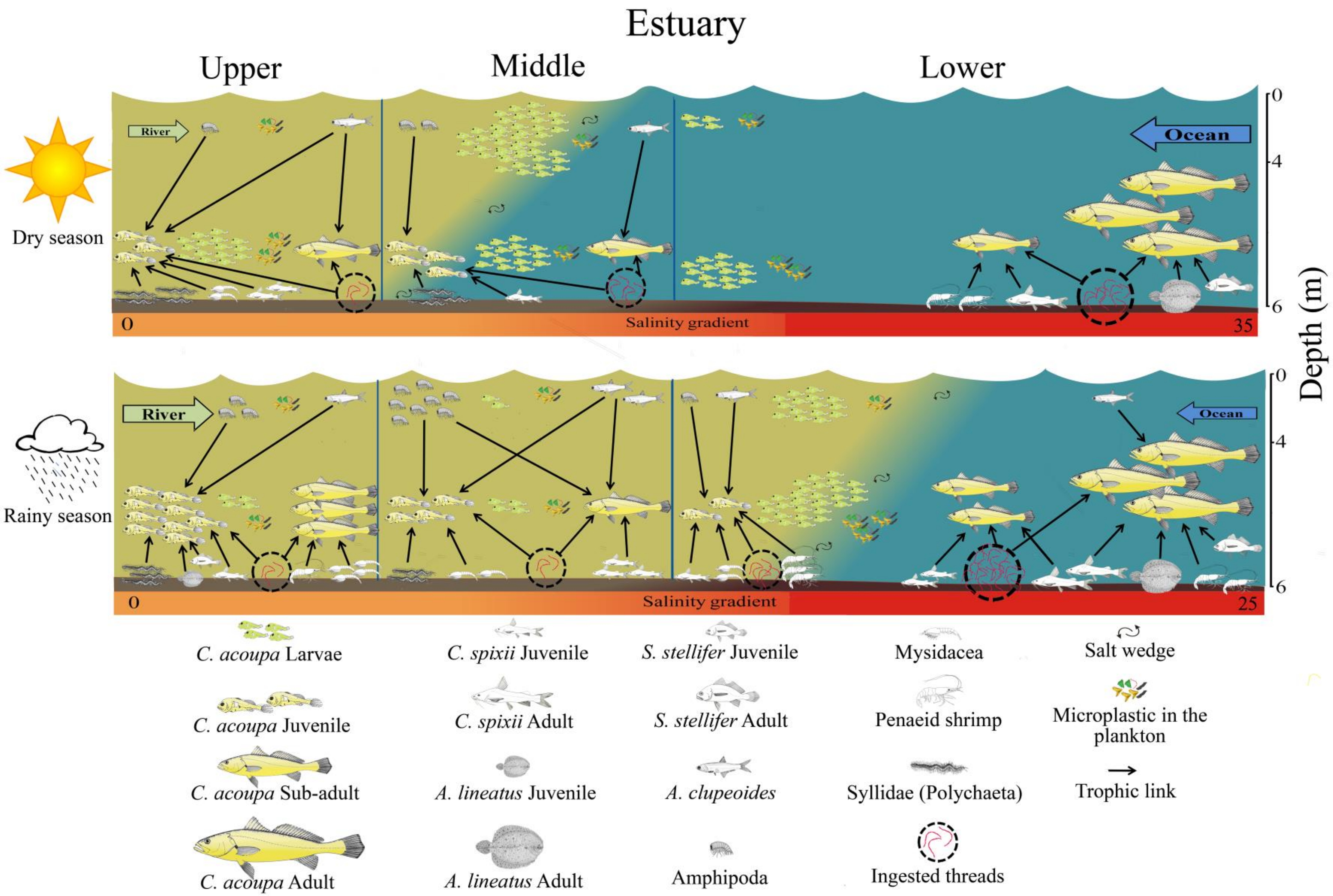

Figure 10 - Conceptual model of *C. acoupa* distribution and feeding ecology in the Goiana estuary, regarding seasonal, spatial and ontogenetic flutuations. Microplastic in the plankton and *C. acoupa* larvae data were retrieved from Lima *et al*. (2015).

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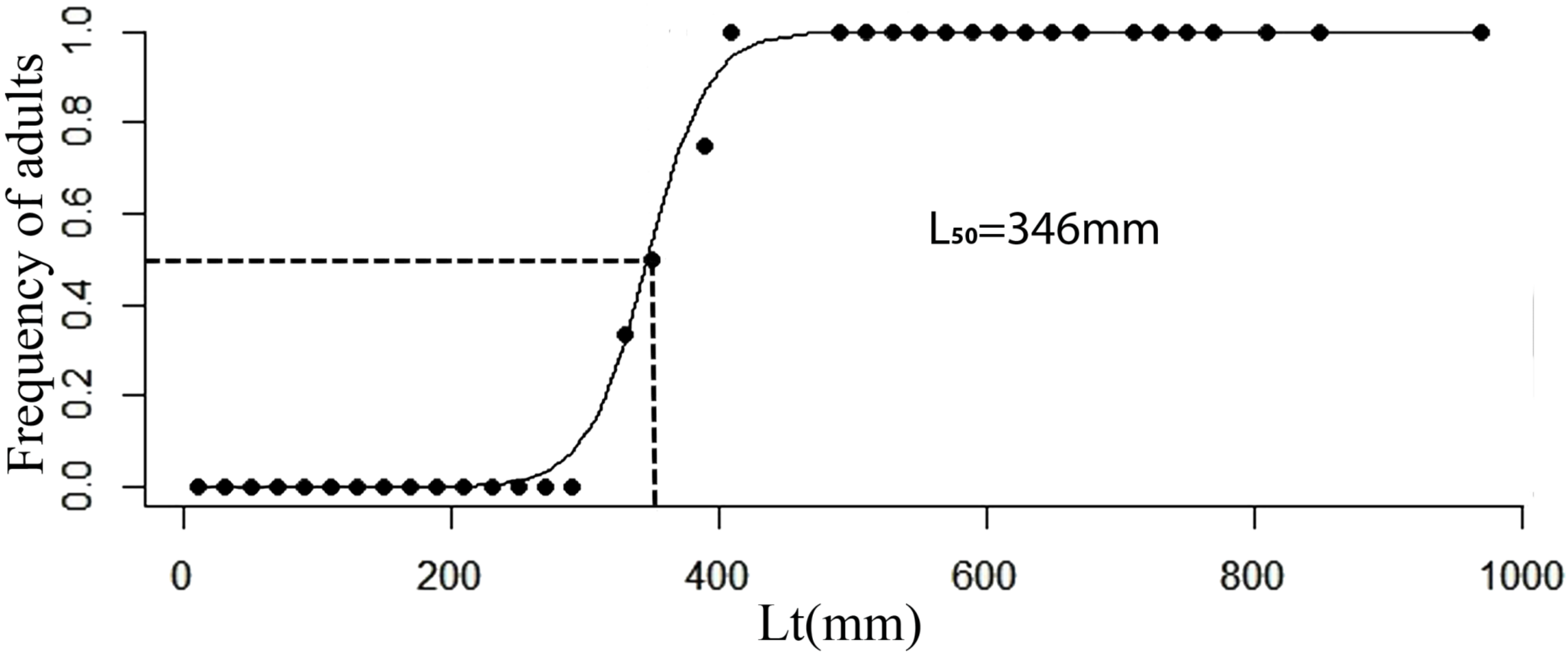
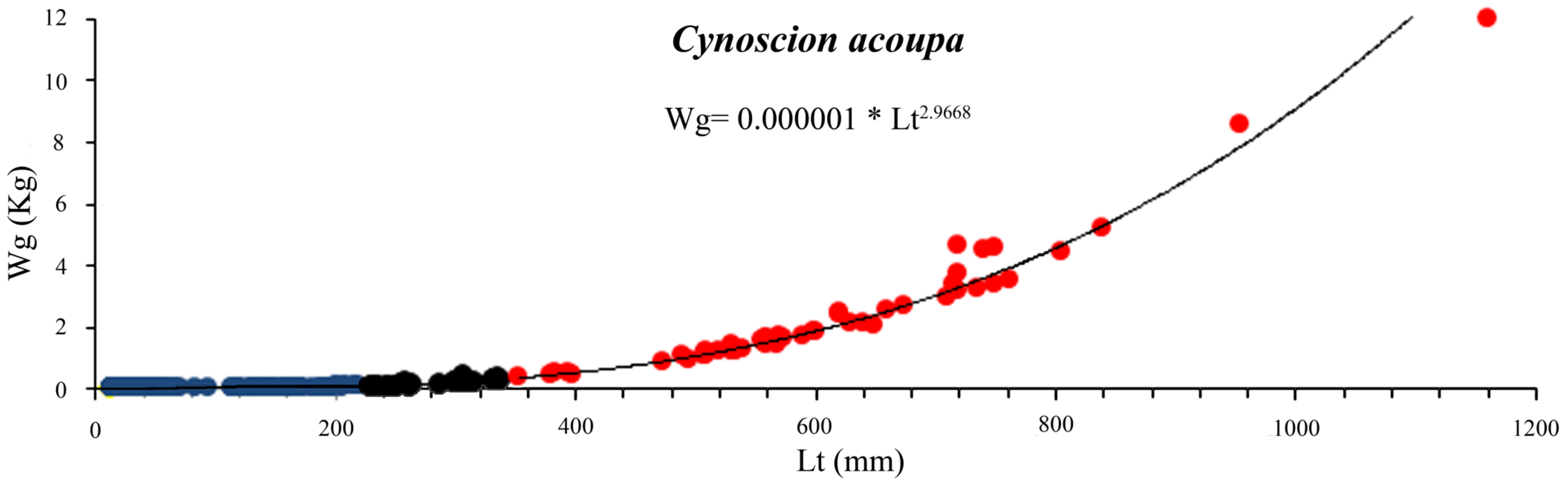
APPENDICES

Appendix 1 - Logistic curve of the frequency of adult in relation to length of individuals (N= 744).



Appendix 2 - Ontogeny of *C. acoupa* along in the length-weight equation (juveniles ●, sub-adults ● and adults ●). The coefficient of determination obtained from the linearization of Log transformed data was 98.44% (N= 839).



Appendix 3 - Summary of the ANOVA for density and biomass of *C. acoupa* with the factors season, area and ontogenetic phase. Differences among factors were determined by Bonferroni's Test, post hoc comparisons. (F: F-values; df: degree of freedom; p-value) (Seasons ED: early dry; LD: late dry; ER: early rainy; LR: late rainy) (Areas of the Goiana Estuary U: upper; M: middle; L: lower) (Phases Juv: juveniles; Sub: sub-adult; Adu: adult) (ns: not significant) (**p < 0.01).

	Density					Biomass			
Factors	***F***	***df***	***p*-value**	**Post-hoc**	**Factors**	***F***	***df***	***p*-value**	**Post-hoc**
Season	4.846	3	0.002	** ED LD **ER** LR	Season	0.211	3	0.889	ns
Area	2.135	2	0.119	ns	Area	5.625	2	0.004	** **U** M L
Phase	5.661	2	0.004	** **Juv** Sub Adu	Phase	5.546	2	0.004	** Juv **Sub** Adu
Season *vs.* Area	1.937	6	0.073	ns	Season *vs.* Area	2.071	6	0.055	ns
Season *vs*. Phase	4.595	6	0.001	**	Season *vs*. Phase	0.787	6	0.580	ns
Area *vs.* Phase	1.803	4	0.127	ns	Area *vs.* Phase	3.174	4	0.013	**
Season *vs.* Area *vs.* Phase	1.764	12	0.051	ns	Season *vs.* Area *vs.* Phase	1.302	12	0.213	ns

Appendix 4 - Summary of the ANOVA for number of prey itens ingested by *C. acoupa* with the factors season, area and ontogenetic phase. Differences among factors were determined by Bonferroni's Test, post hoc comparisons. (F: F-values; df: degree of freedom; p-value) (Seasons ED: early dry; LD: late dry; ER: early rainy; LR: late rainy) (Areas of the Goiana Estuary U: upper; M: middle; L: lower) (Phases Juv: juveniles; Sub: sub-adult; Adu: adult) (ns: not significant) (**p < 0.01; *p < 0.05).

		Itens in number			
	Factors	***F***	***df***	***p*-value**	**Post-hoc**
					**
Microplastic threads	Season	8.665	3	0.001	ED LD **ER** LR
					**
	Area	9.080	2	0.001	U M **L**
					**
	Phase	5.137	2	0.007	Juv Sub **Adu**
	Season *vs.* Area	7.235	6	0.001	**
	Season *vs*. Phase	8.411	6	0.001	**
	Area *vs.* Phase	14.179	4	0.001	**
	Season *vs.* Area *vs*. Phase	9.498	12	0.001	**
					**
A. clupeoides	Season	5.178	3	0.002	ED LD ER **LR**
	Area	0.262	2	0.770	ns
	Phase	1.136	2	0.324	ns
	Season *vs*. Phase	3.637	6	0.002	**
	Area *vs.* Phase	3.643	4	0.007	**
	Season *vs.* Area *vs*. Phase	2.552	12	0.004	**
					**
C. spixii	Season	22.297	3	0.000	ED LD ER **LR**
					**
	Area	4.818	2	0.009	U M **L**
					**
	Phase	10.334	2	0.001	**Juv** Sub Adu
	Season *vs.* Area	6.342	6	0.001	**
	Season *vs*. Phase	9.357	6	0.001	**
	Season *vs.* Area *vs*. Phase	3.094	12	0.001	**
S. stellifer	Season	0.562	3	0.641	ns
	Area	1.396	2	0.251	ns
	Phase	1.396	2	0.251	ns

Appendix 4 continued.

		Itens in number			
	Factors	***F***	***df***	***p*-value**	**Post-hoc**
A. lineatus	Season	3.239	3	0.024	* ED LD ER **LR**
	Area	2.256	2	0.035	* U M **L**
	Phase	2.256	2	0.045	* Juv Sub **Adu**
	Season *vs.* Area	2.256	6	0.041	*
	Season *vs*. Phase	2.256	6	0.041	*
	Area *vs.* Phase	2.747	4	0.031	*
	Season *vs.* Area *vs*. Phase	2.747	12	0.002	**
Amphipoda	Season	10.828	3	0.001	** **ED** LD ER LR
	Area	23.934	2	0.001	** U M **L**
	Phase	77.939	2	0.001	** **Juv** Sub Adu
	Season *vs.* Area	3.855	6	0.001	**
	Season *vs*. Phase	8.312	6	0.001	**
	Area *vs.* Phase	17.419	4	0.001	**
	Season *vs.* Area *vs*. Phase	3.030	12	0.001	**
Mysidacea	Season	3.754	3	0.012	* ED LD **ER** LR
	Area	6.946	2	0.001	** **U** M L
	Phase	22.540	2	0.001	** **Juv** Sub Adu
	Season *vs*. Phase	5.611	6	0.001	**
	Area *vs.* Phase	4.326	4	0.002	**
	Season *vs.* Area *vs*. Phase	1.958	12	0.032	**
Penaeid shrimp	Season	3.078	3	0.030	* ED LD **ER** LR
	Area	2.458	2	0.089	ns
	Phase	2.214	2	0.113	ns
	Season *vs.* Area				
	Season *vs*. Phase	3.609	6	0.002	**
	Season *vs.* Area *vs*. Phase	2.500	12	0.005	**
Syllidae	Season	1.990	3	0.118	ns
	Area	11.401	2	0.001	**U** M L
	Phase	14.657	2	0.001	** **Juv** Sub Adu
	Season *vs.* Area	2.925	6	0.010	*
	Area *vs.* Phase	6.735	4	0.001	**

Appendix 5 - Summary of the ANOVA for weight of prey itens ingested by *C. acoupa* with the factors season, areas and ontogenetic phase. Differences among factors were determined by Bonferroni's Test, post hoc comparisons. (F: F-values; df: degree of freedom; p-value) (Seasons ED: early dry; LD: late dry; ER: early rainy; LR: late rainy) (Areas of the Goiana Estuary U: upper; M: middle; L: lower) (Phases Juv: juveniles; Sub: sub-adult; Adu: adult) (ns: not significant) (**p < 0.01; *p < 0.05).

		Itens in weight			
	Factors	***F***	***df***	***p*-value**	**Post-hoc**
					**
Microplastic threads	Season	6.411	3	0.001	ED LD **ER** LR
					**
	Area	7.671	2	0.001	U M **L**
					**
	Phase	3.161	2	0.045	Juv Sub **Adu**
	Season *vs.* Area	5.928	6	0.001	**
	Season *vs*. Phase	7.369	6	0.001	**
	Area *vs.* Phase	11.185	4	0.001	**
	Season *vs.* Area vs. Phase	8.187	12	0.001	**
A. clupeoides	Season	1.553	3	0.203	ns
	Area	1.428	2	0.243	ns
	Phase	1.583	2	0.209	ns
	Season *vs.* Area	2.494	6	0.025	*
	Season *vs*. Phase	2.777	6	0.014	*
	Area *vs.* Phase	2.524	4	0.044	*
	Season *vs.* Area vs. Phase	2.129	12	0.018	*
					**
C. spixii	Season	21.587	3	0.001	ED LD ER **LR**
					**
	Area	14.070	2	0.001	U M **L**
					**
	Phase	7.157	2	0.001	**Juv** Sub Adu
	Season *vs.* Area	8.346	6	0.001	**
	Season *vs*. Phase	13.656	6	0.001	**
	Season *vs.* Area vs. Phase	6.498	12	0.001	**
S. stellifer	Season	0.660	3	0.578	ns
	Area	1.990	2	0.140	ns
	Phase	1.990	2	0.140	ns

Appendix 5 continued.

		Itens in number			
	Factors	***F***	***df***	***p*-value**	**Post-hoc**
					*
A. lineatus	Season	2.749	3	0.005	ED LD **ER** LR
					*
	Area	2.624	2	0.006	U M **L**
					*
	Phase	2.624	2	0.005	Juv Sub **Adu**
	Season *vs.* Area	2.624	6	0.019	*
	Season *vs*. Phase	2.624	6	0.019	*
	Area *vs.* Phase	2.686	4	0.034	*
	Season *vs.* Area vs. Phase	2.686	12	0.003	**
					*
Amphipoda	Season	3.445	3	0.018	ED LD ER **LR**
					**
	Area	10.589	2	0.001	U **M** L
					**
	Phase	8.901	2	0.001	**Juv** Sub Adu
	Season *vs.* Area	2.302	6	0.038	*
	Season *vs*. Phase	2.952	6	0.010	**
	Area *vs.* Phase	3.772	4	0.006	**
	Season *vs.* Area vs. Phase	2.596	12	0.004	**
Mysidacea	Season	0.657	3	0.580	ns
	Area	2.551	2	0.082	ns
	Phase	3.882	2	0.023	**Juv** Sub Adu
Penaeid shrimp	Season	2.294	3	0.080	ns
	Area	3.047	2	0.051	ns
	Phase	1.780	2	0.172	ns
	Season *vs.* Area	2.174	6	0.049	*
	Season *vs*. Phase	3.823	6	0.001	**
	Season *vs.* Area vs. Phase	2.505	12	0.005	**
Syllidae	Season	0.986	3	0.401	ns
	Area	1.588	2	0.208	ns
	Phase	0.725	2	0.486	ns

Appendix 6. Summary of canonical correspondence analysis using environmental parameters (salinity, water temperature, dissolved oxygen and Sechhi depth) and the index of relative importance (IRI%) of prey items ingested by the different ontogenetic phases of C. acoupa in the Goiana Estuary. (** p < 0.01).

C. acoupa	Axis 1	Axis 2	*p*-value	
Eigenvalue	0.247	0.108		
Species-environmental correlation	0.852	0.645		
Cumulative % variance of species data	15.9	22.9		
Cumulative % variance of species environmetal relation	62.5	90.0		
Correlation with environmental variables:				
Water temperature (ºC)	0.257	-0.570	0.176	ns
Salinity	0.830	-0.137	0.002	**
Dissolved oxygen (mg L^{-1})	0.807	0.083	0.932	ns
Secchi depth (cm)	0.771	-0.175	0.926	ns

5 CONCLUSÃO

O estuário do rio Goiana apresenta uma dinâmica típica dos estuários tropicais, altas médias de temperatura, com uma baixa variabilidade anual, conferindo à salinidade o papel de principal fator influente neste ambiente. De fato, as principais alterações registradas ocorrem em função deste parâmetro, que influencia diretamente a turbidez da água e a quantidade de oxigênio dissolvido.

A salinidade por sua vez, é derivada do regime pluviométrico. Desta forma, durante os períodos chuvosos, as águas fluviais são mais influentes no ambiente estuarino, em razão da maior descarga do rio, resultando em características favoráveis aos organismos mais adaptados a condições lacustres. Quando as taxas de precipitação sofrem um declínio, no período de seca, as águas costeiras incidem com maior influência sobre o estuário, resultando em uma mudança completa da dinâmica dos processos estuarinos.

O padrão de distribuição espacial de *C. acoupa* variou de acordo com os aspectos sazonais, espaciais e ontogenéticos. As condições ambientais (temperatura, salinidade, turbidez e OD) e ecológicas (recursos alimentares, predação e competição intra e interespecíficas) consideradas favoráveis a cada uma das diferentes fases ontogenéticas da espécie, são muito divergentes. Como resposta a esses fatores, *C. acoupa* adotou diferentes estratégias de sobrevivência ao longo da sua ontogenia.

Os indivíduos adultos, não foram capturados no estuário, sugerindo que esta fase, não costuma utilizar o ambiente estuarino, principalmente em razão dos baixos valores de salinidade, que provocariam grandes perdas energéticas no controle osmorregulatório. Desta forma os adultos de *C. acoupa*, provavelmente se reproduzem e desovam nas águas costeiras, na foz do rio, e as fases larvais, posteriormente, entram no estuário para realizar seu desenvolvimento. A fase adulta foi classificada na guilda trófica piscívora, se alimentando principalmente de filamentos de microplástico, *A. clupeoides, A. lineatus* e camarão.

A fase juvenil utiliza todas as porções estuarinas, entretanto tem uma preferência por águas com baixa salinidade, sendo assim, são encontradas principalmente no estuário superior, e durante os períodos chuvosos, nos estuários intermediário e inferior. O estuário superior, durante o início da chuva, se mostrou crucial para o ciclo de vida de *C. acoupa* foram registradas uma grande densidade e baixa biomassa de indivíduos juvenis, indicando esse habitat estuarino como área berçário para a espécie estudada. Os juvenis de *C. acoupa* foram classificados como oportunistas, se alimentando de uma grande variedade de presas como filamentos de microplástico, amphipoda, mysidacea e *C. spixii*.

Os indivíduos subadultos de *C. acoupa* registraram grande ocorrência no estuário superior, durante todas as estações. No fim da chuva também ocuparam o estuário intermediário. Os subadultos optaram por habitar águas com baixa salinidade em busca de áreas de alimentação e proteção contra predadores marinhos. Os subadultos de *C. acoupa* foram classificados como oportunistas, porém com uma menor variedade de presas, quando comparado aos juvenis, e se alimentaram principalmente de filamentos de microplástico, *C. spixii* e amphipoda.

O presente estudo fornece informações muito pertinentes a respeito da espécie *C. acoupa*, que podem ser empregadas no gerenciamento ambiental, no planejamento pesqueiro e em decisões socioeconômicas direcionadas a comunidade ribeirinha.

Para que o ciclo de vida da pescada amarela seja seguramente completado, o ambiente estuarino precisa ser preservado como um todo. Além disso, precisam ser levadas em considerações suas particularidades sazonais, pois os mais variados habitats exercem diferentes funções ao longo do regime sazonal.

Algumas diretrizes precisam ser tomadas imediatamente, no intuito de garantir a saúde da população de *C. acoupa*. O estuário superior, durante os meses de março a maio, necessita ter uma atenção redobrada em relação aos distúrbios externos. Por se tratar do ambiente berçário para *C. acoupa*, ao mesmo tempo em que ele é o habitat mais importante para a espécie, também é caracterizado como o habitat mais vulnerável a distúrbios externos, em decorrência da fragilidade dos indivíduos juvenis de *C. acoupa* que compõem este ambiente. Justamente nesse período, as usinas de cana de açúcar, localizadas no estuário superior despejam os resíduos da produção no estuário, que por sua vez podem causar grandes alterações nos parâmetros físico-químicos e até mesmo a formação de condições anóxicas, ameaçando um momento crucial do desenvolvimento da espécie.

C. acoupa apresenta um ciclo de vida complexo e provavelmente muito demorado, em razão do grande tamanho corporal atingido pela espécie. De fato, o tamanho de maturação da espécie é muito elevado, o que provoca uma preocupação em relação à pressão que a atividade pesqueira exerce sobre esse grupo. No estudo foram utilizados indivíduos capturados pela atividade pesqueira, e foi constada a ocorrência de diversos espécimes que foram capturados antes de atingirem a maturação sexual. A prática de se explorar espécimes que estão abaixo do comprimento da primeira maturação, pode provocar consequências desastrosas para o estoque pesqueiro local, por impossibilitar a manutenção das populações das espécies alvo da pesca.

Por fim, também é necessário que seja mantida a saúde das populações que servem de presa para *C. acoupa*, pois elas têm um papel muito importante ao fornecer energia para os níveis tróficos superiores. Desta forma, diversas espécies, muitas vezes menosprezadas por não se tratarem de espécies de grande apelo econômico como os bagres, apresentam um papel crucial na cadeia trófica dos ecossistemas estuarinos, sendo à base de sustentação de espécies predadoras de topo como *C. acoupa*, que necessitam de grandes taxas energéticas para completar seu desenvolvimento. Portanto a comunidade das presas da Pescada Amarela, também precisam ser cuidadosamente gerenciadas, com o intuito de manter a saúde dessas espécies de grande importância ecológica, viabilizando o equilíbrio trófico local.

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