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André Ricardo de Araújo Lima

Variação sazonal, espacial e lunar do ictioplâncton e do microplástico nos diferentes habitats do estuário do Rio Goiana (Resex Acaú-Goiana PE/PB)

Recife/2015

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Tese apresentada ao programa de pós-graduação em oceanografia como requisito parcial à obtenção do título de Doutor em Oceanografia Biológica.

Orientador: Dr. Mário Barletta

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Tese submetida ao curso de Pós-Graduação em oceanografia da Universidade Federal de Pernambuco, como requisito parcial para obtenção do Grau de Doutor.

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Banca examinadora:

_____

Prof. Dr. Mário Barletta – UFPE (Orientador)

_____

Prof. Dr. Werner Ekau - ZMT

_____

Prof. Dr. André Luiz Machado Pessanha – UEPB

_____

Prof. Dr^{a}. Beatrice Padovani Ferreira – UFPE

_____

Prof. Dr. Jonas de Assis Almeida Ramos – IFPB

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RESUMO

Estuários são considerados ambientes importantes por promover refúgio, alimentação, reprodução e crescimento, além de servir como possíveis habitats de berçário para muitas espécies de peixes. Estudos sobre os padrões de movimento da comunidade ictioplanctônica dentro do ecossistema estuarino são de grande importância para entender como as espécies utilizam os recursos disponíveis para completar os seus ciclos de vida através das variações temporais e espaciais das diferentes fases ontogenéticas. Entretanto, a complexidade hidrodinâmica dos estuários não só influencia os organismos vivos, mas também materiais inanimados, tais como os detritos plásticos, atuando em sua retenção ou transporte para outros ambientes. Os detritos plásticos, associados ao aumento da urbanização das bacias hidrográficas, se originam principalmente em terra devido ao descarte impróprio, acidental ou desastres naturais. Durante seu tempo na terra, no mar ou nos estuários, os plásticos se fragmentam em microplásticos (< 5 mm). Flutuações sazonais de larvas de peixes e detritos plásticos (< 5mm) e suas quantidades em relação ao seston (organismos vivos e partículas não-vivas) foram estudadas ao longo do gradiente de salinidade do estuário do Rio Goiana (Resex Acaú-Goiana PE/PB) (Entre Abril, 2012 até Março, 2013). Além disso, a influência lunar na distribuição de larvas de peixes, zooplâncton e detritos plásticos (< 5 mm <) em canais de maré do mesmo estuário foi estudada durante um ciclo lunar (Entre Abril e Maio, 2008). Os taxa mais abundantes no canal principal foram *Rhinosardinia bahiensis* e *Harengula* sp., seguidos por *Trinectes maculatus*. Estes contaram 78,7% da captura total. Larvas de espécies marinhas (n = 15) dominaram o sistema. A flutuação sazonal da cunha salina parece regular a distribuição das larvas de peixes e de microplásticos ao longo do sistema. A densidade total de microplásticos (26,1 itens 100 m^{-3}) representou metade da densidade total de larvas (53,9 ind. 100 m^{-3}) e foi comparável com a densidade de ovos de peixes (32,4 ind. 100 m^{-3}). Plásticos moles, duros, filamentos e fragmentos tintas de barco foram encontrados nas amostras (n = 216). Suas origens são provavelmente a bacia de drenagem do rio, o mar e a pesca, incluindo a pesca de lagosta). Em algumas ocasiões, a densidade de microplásticos ultrapassou a de ictioplâncton. Durante o início da estação chuvosa, zooplâncton e larvas de peixes apresentaram densidades baixas no estuário superior. No estuário intermediário, a maior densidade de larvas de peixes coincidiu com as altas concentrações de zooplâncton. No final da estação chuvosa, o fluxo rio abaixo foi responsável pelo transporte do plâncton total e dos microplásticos para a região próxima

à costa. A maior quantidade de microplásticos foi observada durante o final da estação chuvosa (14 itens $100m^{-3}$), quando o ambiente está sob a influência de maior vazão do rio, o que induz o escoamento dos fragmentos de plásticos para o estuário inferior. No início da estação seca, a densidade total do plâncton aumenta rio acima. No final da estação seca, o "bloom" de zooplâncton no estuário inferior resultaram em altas densidades de larvas (12,74 ind. $100m^{-3}$) e ovos de peixes (14,65 ind. $100m^{-3}$), indicando que peixes marinhos utilizam a porção inferior como áreas de desova durante o verão. Além disso, *Cetengraulis edentulus*, *Anchovia clupeoides* e *R. bahiensis* foram as larvas de peixes mais abundantes (56.6%) em canais de maré da porção inferior do estuário, independente da fase da lua. A lua cheia teve influência positiva na densidade de *Gobionellus oceanicus*, *Cynoscion acoupa* e *Atherinella brasiliensis*, e a lua nova em *Ulaema lefroyi*. As luas cheia e nova também influenciaram o número de zoé e megalopa de *U. cordatus*, e protozoé e larva de camarão Caridae, bem como o número de plásticos duros e moles de ambos os tamanhos < 5 mm e > 5 mm. Micro e macroplásticos contaminaram todos os 12 canais de maré estudados. A densidade de fragmentos plásticos é similar à do terceiro táxon mais abundante, *R. bahiensis* (4,8 ind $100m^{-3}$). *C. edentulus* e *R. bahiensis* mostraram forte correlação com a lua quarto crescente, quando há menos zooplâncton. A lua quarto crescente também teve uma influência positiva nas altas densidades de micro filamentos plásticos nos canais. *Anchovia clupeoides*, *Diapterus rhombeus*, *U. lefroyi* e microplásticos duros tiveram associação com diferentes fases da lua, ocorrendo quando copépoda calanoida, larva de Caridae e zoé de *U. cordatus* foram abundantes nos canais. *Cynoscion acoupa*, *G. oceanicus* e *A. brasiliensis*, tiveram forte associação com a lua cheia, quando protozoé de Caridae e megalopa de *U. cordatus* também estavam altamente disponíveis, bem como plásticos duros e moles > 5mm, e tintas de barco e plásticos moles < 5mm. As fases da lua influenciaram a assembléia faunal e a poluição por plástico, mudando suas composições entre diferentes estágios de marés dentro dos canais da porção inferior do estuário do Rio Goiana. Esses resultados reforçam a importância do canal principal e dos canais de maré para proteção e estratégias alimentares. Além disso, a assembleia de larvas de peixes do estuário do Rio Goiana inclui muitas espécies que ocorrem no sistema como juvenis e adultos, confirmando o uso do estuário como berçário.

Palavras chave: Séston. Cunha salina. América do Sul. Zooplâncton. Larva de peixe. Microplásticos. Ciclo lunar. Estuário tropical.

ABSTRACT

Estuaries are considered important environments for promoting refuge, food, reproduction, growth and for being the nursery grounds of many fish species. Studies on the movement patterns of the ichthyoplankton in an estuarine ecosystem are of great importance for understand how the species utilize the available resources to complete their life cycles using the temporal and spatial variations of different ontogenetic phases. Although, the hydrodynamic complexity of estuaries not only influences the living organisms, but also inanimate material, such as plastics debris, acting in their retention or transportation to other environments. Plastics debris, associated to the increasing urbanization of watersheds, originate mainly on land due to improper disposal, accidental release or natural disasters. During their time at land, sea and estuaries, plastics fragment into microplastics (< 5 mm). Seasonal fluctuations of fish larvae and plastic debris (< 5mm) and their quantification relative to the seston (living organisms and non-living particles) were studied along the salinity gradient of the Goiana Estuary (Resex Acaú-Goiana PE/PB) (between April, 2012 and March, 2013). Moreover, the lunar influence on the distribution of fish larvae, zooplankton and plastic debris (> 5 mm <) in mangrove creeks of the same estuary was studied over a lunar cycle (between April and May, 2008). The most abundant taxa in the main channel were *Rhinosardinia bahiensis* and *Harengula* sp., followed by the achirid *Trinectes maculatus*. These accounted for 78.7% of total catch. Larvae of marine species (n = 15) dominated the system. Seasonal fluctuation of salt wedge seems to rule the larval fish and microplastics distribution along the system. Microplastics (26.1 items 100 m^{-3}) represented half of the total fish larvae density (53.9 ind. 100 m^{-3}) and was comparable to fish eggs density (34.2 ind. 100 m^{-3}). Soft, hard plastics, threads and paint chip fragments were found in the samples (n = 216). Their origins are probably the drainage river basin, the sea and fisheries, including the lobster fleet. In some occasions, the density of microplastics surpassed that of Ichthyoplankton. During the early rainy season, zooplankton and fish larvae presented low densities in the upper estuary. In the middle estuary, the higher density of fish larvae coincided with high zooplankton concentrations. In the late rainy season, the downstream flow was responsible for the shoreward transport of total plankton and microplastics. The highest amount of microplastics (14 items $100m^{-3}$) was observed during the late rainy season, when the environment is under influence of the highest river flow, which induces the runoff of plastic fragments to the lower estuary. In the early dry season, the turbidity drops and the density of total plankton rises upstream. In the late dry season, the bloom of

zooplankton in the lower estuary results in summer high densities of fish larvae (12.74 ind. $100m^{-3}$) and fish eggs (14.65 ind. $100m^{-3}$), indicating that marine fishes utilizes the lower portion as spawning grounds during the summer. In addition, *Cetengraulis edentulus*, *Anchovia clupeoides* and *R. bahiensis* were the most abundant fish larvae (56.6%) in mangrove creeks of the lower portion of the estuary, independent of moon phase. The full moon had positive influence on densities of *Gobionellus oceanicus*, *Cynoscion acoupa* and *Atherinella brasiliensis*, and the new moon on *Ulaema lefroyi*. The full and new moon also influenced the number of zoea and megalopa of *U. cordatus*, and protozoea and larvae of Caridae shrimp, as well as the number of hard and soft plastics, both < 5mm and > 5mm. Micro and macroplastics contaminated all twelve creeks studied. Their density is similar to the third most abundant taxa, *R. bahiensis* (4.8 ind. $100m^{-3}$). *Cetengraulis edentulus* and *R. bahiensis* showed a strong correlation with the last quarter moon, when there were less zooplankton in the creeks. Last quarter moon also had a positive influence on higher densities of micro-sized plastic threads. *Anchovia clupeoides*, *Diapterus rhombeus*, *U. lefroyi* and micro-sized hard plastics were associated to different moon phases, occurring when copepod calanoida, Caridae larvae and zoea of *U. cordatus* were abundant in the creeks. *Cynoscion acoupa*, *G. oceanicus* and *A. brasiliensis*, were strongly associated to full moon, when protozoea of Caridae and megalopa of *U. cordatus* were also highly available, as well as hard and soft plastics > 5mm, and paint chips and soft plastics < 5mm. The moon phases influenced the composition of the faunal assemblage, and plastic pollution by shifting them between different tidal stages into the mangrove creeks of the Goiana Estuary. These results reinforce the importance of the main channel and mangrove creeks for protection and feeding strategies. In addition, the larval fish assemblage of the Goiana Estuary includes many species that occurs in the system as juveniles and adults, confirming the use of the estuary as a nursery.

Key words: Seston. Salt wedge. South America. Zooplankton. Fish larvae. Microplastics. Lunar cycle. Tropical estuary.

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1. INTRODUÇÃO

O ictioplâncton estuarino pode ser residente, originário de ambientes marinhos ou de água doce, contudo, a maioria das larvas encontradas em estuários são, originalmente, espécies marinhas (Gaughan et al., 1990; Whitfield, 1990; Neira et al., 1992; Barletta-Bergan et al., 2002 *b*; Sutherland et al., 2012; Williams et al., 2012). O sucesso e a sobrevivência de muitos teleósteos marinhos, durante os estágios iniciais do seu ciclo de vida, estão relacionados à proteção contra predadores em áreas menos salinas e à alta produtividade do ambiente estuarino (Barletta-Bergan et al., 2002 *b*). Algumas outras espécies completam todo seu ciclo de vida dentro do estuário em associação com as condições de turbidez, principalmente nas áreas mais superiores do sistema (Blaber e Blaber, 1980; Neira et al., 1992; Suzuki et al., 2014). Assim, os estuários são usados por espécies de peixes como áreas de recrutamento, assentamento, alimentação e berçário (Barletta-Bergan et al., 2002 *a*, *b*; Dantas et al., 2012, 2013; Lima et al., 2011, 2013; Potter et al., 2013; Gomes et al., 2014).

As características físico-químicas dos estuários variam drasticamente, em diferentes escalas de tempo, de horas a meses (Barletta-Bergan et al., 2002*a*,*b*; Barletta et al., 2005, 2008). A variação espacial das condições estuarinas é determinada pela flutuação das marés na boca do estuário, e a descarga de agua doce provenientes da chuva ou do fluxo do rio. O encontro da água costeira, mais densa, com a água doce, cria uma estratificação na coluna d'água, conhecida como cunha salina (Kurup et al., 1998; Ramos et al., 2006; Barletta e Barletta-Bergan, 2009; Lacerda et al., 2014; Lima et al., 2014). Esta hidrodinâmica, quando combinadas com a temperatura, correntes e vento, fornece um padrão de circulação em dupla camada que afeta a distribuição e abundancia das larvas de peixes nos diferentes habitats do sistema (Moais e Morais, 1994; Blaber et al., 1997; Barletta-Bergan et al., 2002*a*,*b*; Ooi e Chong, 2011; Gomes et al., 2014; Sarpedonti et al., 2013). Estudos em estuários tropicais demonstram que a abundância do ictioplâncton é, principalmente, dirigida pela variação sazonal da salinidade e padrões de precipitação bem definidos (Moais e Morais, 1994; Blaber et al., 1997; Barletta-Bergan et al., 2002*a*,*b*; Ooi e Chong, 2011; Gomes et al., 2014; Sarpedonti et al., 2013). No estuário do rio Caeté (norte do Brasil), os padrões sazonais de precipitação e salinidade foram mais importantes para a distribuição larvas de peixes do que as variações de temperatura (Barletta-Bergan et al., 2002*b*). Para este estuário, o estuário superior foi representado pelas espécies associadas as condições de água doce, enquanto que as espécies com afinidades marinhas foram capturadas no estuário inferior (Barletta-Bergan

et al., 2002*b*). Em estuários do Indo-Oeste Pacífico (Sarawak e Sabah), variações sazonais na salinidade e precipitação também foram importantes (Blaber et al., 1997). Neta região, nos estuários com haloclinas bem definidas e mudanças sazonais no padrão de precipitação, taxa com afinidades marinhas foram os mais representativos (Blaber et al., 1997).

O padrão de circulação estuarina resulta em alta concentrações de sólidos suspensos e atua na retenção e concentração de nutrientes, contribuindo para o aumento da produtividade, e consequentemente, aumentando a sobrevivência de larvas dentro do estuário (Allen et al., 1980; Suzuki et al., 2014; Watanabe et al., 2014). Considerando que a densidade de zooplâncton está relacionada com a turbidez estuarina, estudos sugerem que o sucesso do assentamento e recrutamento de larvas e juvenis é mais alto onde os sólidos suspensos estão em maior concentração devido à alta disponibilidade de alimento (Cloern, 1987; North e Houde, 2003; Martino e Houde, 2010). Os picos de atividade alimentar podem variar diurnamente e afetar a distribuição espacial dos peixes dentro de um ambiente (Morrison et al., 2002; Willis et al., 2006; Krumme et al., 2008). Larvas de peixes planctívoras tendem a se distribuírem de acordo com a disponibilidade de suas presas, e as mudanças nas amplitudes das marés e na intensidade da luz em diferentes fases da lua podem ter efeitos marcantes nesse comportamento (Alldredge e King, 1980; Kingsford e MacDiarmid, 1988; Hampel et al., 2003; Hernández-León, 2008). O ciclo lunar determina a disponibilidade temporal e espacial dos habitats de canais de maré das florestas de manguezal. Durante as marés de quadratura, há uma parcial inundação dos canais, enquanto que durante as marés vivas, eles estão completamente inundados por um longo período (Hampel et al., 2003; Ramos et al., 2011). A intensidade da corrente também varia ao longo das fases da lua, promovendo ciclos de inundação das florestas de manguezais mais ou menos eficientes. Cada ciclo de maré traz organismos, detritos e poluentes para os habitats entremarés. Alguns organismos são adaptados a permanecerem dentro dos canais, e outros retornam para o canal principal durante as marés vazantes (Kneibe, 1997; Barletta et al., 2000; Morrison et al., 2002; Willis et al., 2006).

Apesar da importância da complexidade hidrodinâmica para os estuários, esse comportamento físico pode facilitar sua poluição por detritos marinhos (Barnes et al., 2009; Lima et al., 2014). Os plásticos são discutidos, por décadas, como sendo os principais componentes do detrito a poluir todos os habitats do ambiente marinho, desde o equador até os polos (Bergmann e Klages, 2012; Costa et al., 2011; Moore et al., 2001; Moore, 2008; Thornton e Jackson, 1998; Barnes et al., 2009). Eles se originam em terra,

onde o uso excessivo cria problemas de disposição, resultando na sua acumulação por lançamento acidental, desastre natural ou hábitos de disposição inadequados (Thompson et al., 2009; Watters et al., 2010). O transporte por ventos e ondas permite que plásticos inteiros e outros detritos entrem no ambiente marinho (Wright et al., 2013). A baixa taxa de degradabilidade e a alta flutuabilidade permitem que os plásticos viagem por longas distâncias, alcançando habitats distantes de suas fontes de origem, até mesmo áreas remotas, como ilhas oceânicas (Ivar do Sul et al., 2013) e as profundezas dos oceanos (Bergmann e Klages, 2012; Lozano e Mouat, 2009). Entretanto, durante o tempo em que passam no mar, os plásticos se fragmentam em microplásticos (< 5mm).

Os fragmentos de plásticos entram nos estuários tanto pelo escoamento superficial, quanto pelo oceano através do vento, ondas ou marés (Le Roux, 2005; Nordstorm et al., 2006). Eles também podem ser fragmentados *in situ* pela dinâmica física do ambiente (Barnes et al., 2009). Uma vez que os fragmentos de plásticos alcançam o estuário, eles serão encontrados quase que em qualquer habitat (Browne et al., 2010; Thornton e Jackson, 1998; Lima et al., 2014). Plásticos menos densos tendem a submergir no encontro águas de diferentes densidades (Cole et al., 2011); e partículas menores são transportadas e depositadas onde o fluxo de água é menos intenso, como planícies entremarés e floresta de manguezal (Costa et al., 2011). Isso sugere que os microplásticos estão disponíveis para os organismos planctônicos, estágios larvais de muitas espécies de peixes de importância econômica, suas presas naturais e para predadores maiores, promovendo a transferências dessas partículas entre diferentes níveis tróficos (Gregory, 1996; Boerge et al., 2010; Possatto et al., 2011; Dantas et al., 2012; Lima et al., 2014; Ramos et al., 2012).

No sentido de ampliar o conhecimento sobre estudos estuarinos em larvas de peixes e sua interação com os compartimentos abióticos (ex. fragmentos de plásticos), este trabalho surge como uma ferramenta para descrever como o plâncton (ictioplâncton e zooplâncton) e partículas não-vivas (microplásticos) estão distribuídas ao longo canal principal do estuário do Rio Goiana (Nordeste do Brasil - PE/PB). Neste sentido, o trabalho irá descrever a assembleia ictioplanctônica, não só taxonomicamente, mas também em termos de estrutura ecológica e uso dos recursos disponíveis, incluindo o microplástico como um item alimentar potencial, nos diferentes habitats dos estuários baseados nas variações sazonais dos parâmetros abióticos (Barletta et al., 2003; 2005; Barletta e Blaber, 2007).

Com este estudo será possível identificar os locais utilizados como berçário pelas principais espécies de peixes encontradas e comercializadas no local. Essa região é uma área de constante ocupação e ação antrópica, enfatizando a importância da identificação do papel desses habitats para a ontogenia das espécies presentes no local. Considerando que o estuário do Rio Goiana é uma reserva extrativista (Resex Acaú-Goiana), sua comparação com outros estuários do mundo, considerados preservados, visa gerar dados que contribuam para a aplicação de medidas de manejo voltadas à preservação desses habitats e para proteger essas espécies durante sua reprodução e renovação dos estoques pesqueiros.

2. OBJETIVOS

2.1. Objetivo geral

O presente trabalho tem por objetivo estudar a estrutura e a variação sazonal e espacial da comunidade ictioplanctônica em relação ao plâncton (fitoplâncton e zooplâncton) e partículas não-vivas (microplásticos) no canal principal do estuário do Rio Goiana. Além disso, estudar a influência das diferentes fases da lua na composição das larvas de peixes, detritos plásticos e zooplâncton em canais de maré da porção inferior do estuário.

2.2. Objetivos específicos

- Examinar a composição e a abundância das famílias e espécies da comunidade ictioplanctônica nos canais de maré da porção inferior e no canal principal (estuários superior, intermediário e inferior) do estuário do Rio Goiana.
- Determinar a variação sazonal do plâncton total em termos de densidade (ind. 100 m^{-3}) ao longo do gradiente de salinidade do canal principal (estuários superior, intermediário e inferior) do estuário.
- Determinar a variação sazonal e espacial de microplásticos ao longo do gradiente de salinidade do canal principal, e sua composição e quantificação em relação ao plâncton presente no sistema.
- Determinar os padrões de uso e composições de larvas de peixes, zooplâncton e detritos plásticos (ind. 100 m^{-3}) nos canais de maré do estuário de acordo com as diferentes fases da lua.

- Identificar os possíveis habitats (estuários superior, intermediário, inferior e canais de maré) utilizados como berçário pelas espécies mais abundantes em termos de densidade.

3. MATERIAIS E MÉTODOS

3.1. Área de estudo

A bacia hidrográfica do rio Goiana tem uma área de 2.878,3 km^2 e localiza-se na divisa dos estados de Pernambuco e Paraíba (7˚32' a 7˚35'S e 34˚50' a 34˚58'W) (Fig. 1). É formada pela confluência dos rios Capibaribe Mirim e Tracunhaém, originando o rio Goiana. Seu sistema estuarino é formado pelos rios Goiana e Megaó, e possui 477.600m^2. Sua cobertura vegetal é predominantemente de florestas de manguezal na faixa de influência das marés (principalmente as espécies *Rhizophora mangle*, *Laguncularia racemosa* e *Avicenia spp.*). O clima é tropical úmido do tipo As, segundo a classificação de Köppen. A temperatura média do ar é de 25 ˚C, e oscila entre 27 ˚C nos meses de verão e 24^{0}C nos meses de inverno (Barletta e Costa, 2009). Apresenta duas estações bem definidas, uma seca e outra chuvosa. Baseado nos padrões de precipitação da região, estas estações podem ser divididas em início da estação seca (setembro a novembro), final da seca (dezembro a fevereiro), início da estação chuvosa (março a maio) e final da estação chuvosa (junho a agosto).

Em 2007, o estuário do Rio Goiana tornou-se a Resex Acaú-Goiana, uma unidade de conservação federal classificada como reserva extrativista que abrange os municípios de Caaporã e Pitimbu no estado da Paraíba e Goiana em Pernambuco (Barletta e Costa 2009). Essa área estuarina abriga uma fauna rica em peixes, crustáceos e moluscos, cuja coleta assegura o sustento de grande parte da população dos aglomerados urbanos circunvizinhos, a exemplo de Goiana, Tejucopapo, São Lourenço e Carne de Vaca (PE) assim como Caaporã e Pitimbú (PB) (Fidem, 1987). A poluição hídrica de origem industrial e doméstica, além do corte e aterros de manguezais para a implantação de grandes projetos de carcinicultura, e a atividade da cana de açúcar, representam uma ameaça à sua preservação (Barletta e Costa 2009).

O canal principal foi dividido em 3 áreas de acordo com o gradiente de salinidade e a geomorfologia do estuário (Fig. 1). Sendo que a área superior, com maior influência do rio, área intermediária, e área inferior, com maior influência das águas costeiras (Barletta & Costa, 2009) (Fig. 1).

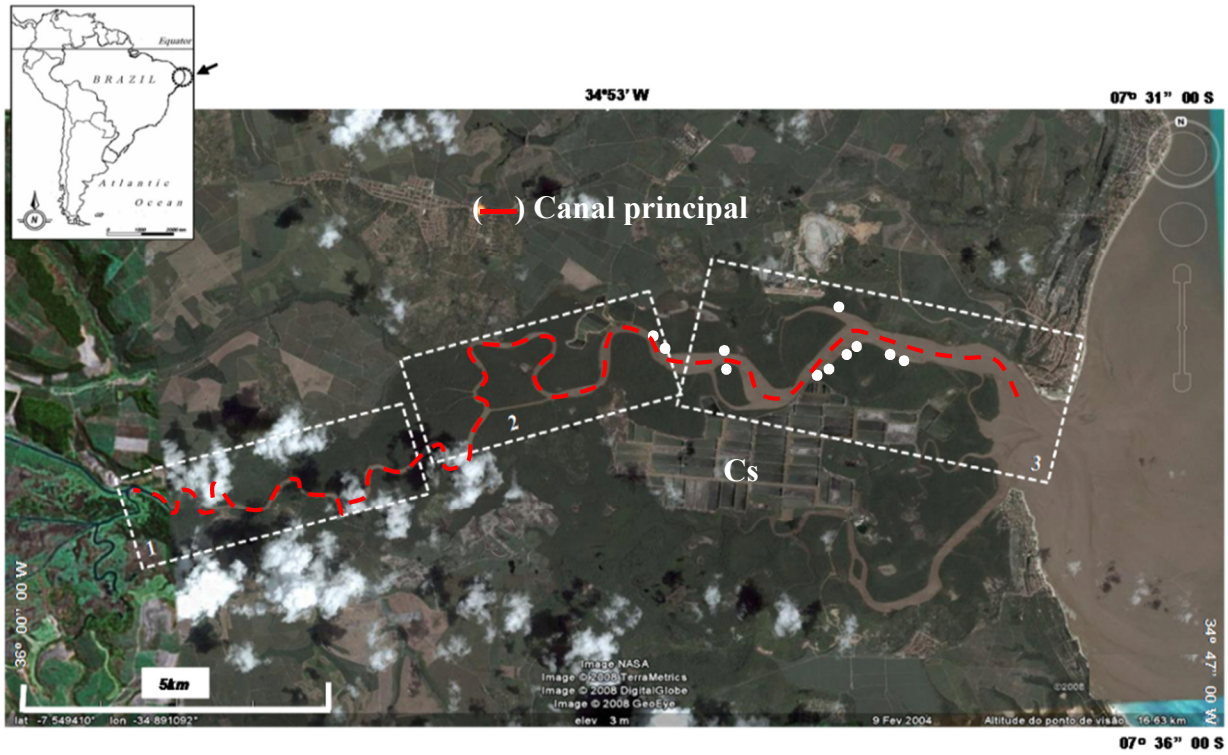

Figura 1. Estuário do Rio Goiana. Estuários (1) superior, (2) intermediário e (3) inferior, onde as coletas no canal principal serão realizadas, estão realçadas no mapa. Os pontos indicam os canais de maré da porção inferior que foram amostrados. (Cs) Empreendimento de carcinicultura desativado. Fonte: Google Earth (2014).

3.2. Métodos amostrais

Todas as amostragens realizadas neste estudo possuíam autorização para atividades com finalidade científica (ANEXO A) emita através do Sistema de Autorização e Informação em Biodiversidade (SISBIO), pelo Instituto Chico Mendes de Conservação e Biodiversidade (ICMBio).

3.2.1. Amostragem do seston no canal principal

Para as coletas de plâncton no canal principal foi utilizado o método amostral proposto por Barletta & Barletta-Bergan et al. (2009). As amostragens foram realizadas com arrastos horizontais simultâneos de superfície (0 – 1 m de profundidade) e de fundo (3 – 6 m), durante marés de quadratura, utilizando um barco de motor de polpa à uma velocidade média de 2,7 nós, com duração de 15 minutos, entre abril de 2012 a março de 2013. Foi utilizada uma rede de plâncton cônica de meia água com malha de 300 μm, diâmetro de 0,6 m e 2 m comprimento. Um fluxômetro (General Oceanics - Model 2030 Digital Series) foi posicionado no centro da rede para medir o volume de água filtrada pela rede de plâncton em cada arrasto no canal principal do estuário. Um GPS (Ensign

GPS Trimble Navigation) foi utilizado para determinar a posição das coletas. Uma ecossonda (Eagle Supra Pro D) registrou a profundidade do canal principal. Foram realizadas 6 amostras mensais (3 de superfície e 3 de fundo) em cada uma das áreas do estuário (superior, intermediária e inferior), totalizando 72 amostragens para cada um destes habitats. Antes de cada arrasto, foram coletados dados referentes às variáveis ambientais, salinidade, temperatura da água (ºC), oxigênio dissolvido (mg/L) e saturação de oxigênio dissolvido na água (%) (Wissenschaftlich Technische Werkstätten, WTW OXI 325) e transparência do disco de Secchi (cm), tanto na superfície quanto no fundo da coluna d'água. Os dados referentes à precipitação foram compilados em 2014 da estação meteorológica mais próxima, "Curado 82900", localizada em Recife-PE (INMET, 2014). As amostras foram preservadas em formol tamponado (4%).

3.2.2. Amostragem do seston nos canais de maré

As amostras de plâncton foram realizadas em 12 canais de marés da porção inferior do estuário entre abril e maio 2008, seguindo a metodologia descrita por Ramos et al. (2011). Para assegurar a detecção da influência lunar na distribuição do seston (plâncton e microplásticos), os meses de amostragem coincidiram com um estuário mais estável, durante o início da estação chuvosa (Barletta e Costa, 2009). Condições ambientais extremas, como elevadas precipitações (Junho à Agosto) ou temperatura da água (Dezembro à Fevereiro) foram evitadas (Barletta & Costa, 2009). Além disso, as amostras iniciaram após o principal período de desova, quando as larvas de peixes utilizam o estuário e as águas costeiras têm grande influência no estuário inferior (Lima et al., 2014). Os canais foram escolhidos de acordo com suas similaridades em largura e comprimento. Para cada fase da lua (quarto crescente, cheia, quarto minguante e nova) três canais diferentes foram amostrados em 3 dias consecutivos para evitar a perturbação do substrato durante as coletas dos peixes (Barletta-Bergan *et al*., 2002 *a*). Durante as luas quarto crescente e quarto minguante, as marés altas variaram de 1.8 até 2.1 m. Durante a lua nova, elas variaram de 2.4 até 2.7 m, e durante a lua cheia de 2.2 até 2.4 m. Os canais 1–3 (azuis) foram amostrados durante a lua quarto crescente, 4–6 (vermelhos) durante a lua cheia, 7–9 (verdes) durante a lua quarto minguante e 10–12 (amarelos) durante a lua nova (Fig. 2). Para cada lua, o primeiro canal amostrado era mais distante e o terceiro era o mais próximo a boca do estuário (Fig. 2) (Ramos et al., 2011). As amostras iniciaram durante o segundo pico diário de maré alta. Foi usada uma rede de tapagem retangular com 10 m de comprimento e 2 m de altura, com malha de 1000 μm para

bloquear a boca do canal de uma margem até a outra (Fig. 3). Um saco cônico (Ø 0,6 m; 500 µm), com um copo coletor no final, foi fixado a rede de tapagem. As amostras foram coletadas após 4 horas na mare baixa. Um fluxômetro (General Oceanics - Model 2030 Digital Series), acoplado a uma boia, foi posicionado na frente da rede retangular para medir o volume de água filtrada. A temperatura da água (◦ C), oxigênio dissolvido (mg l^{-1}) (Wissenschaftlich Technische Werkstätten, WTW OXI 325; www.wtw.com) e salinidade (WTW LF 197) foram registradas da superfície da agua na boca dos canais durante 4 horas consecutivas. As amostras foram preservadas em formol tamponado (4%).

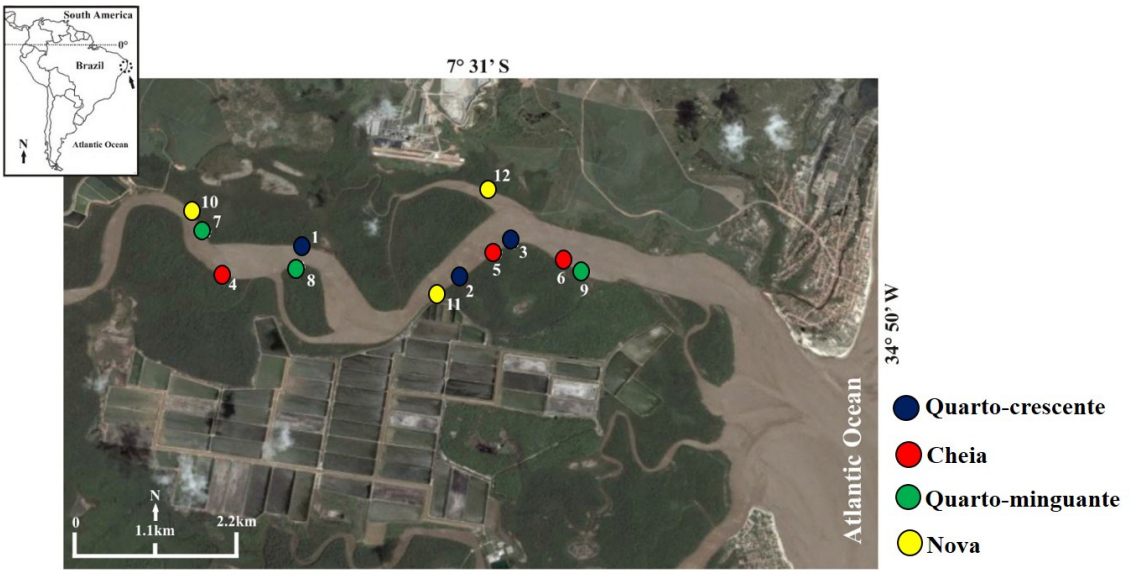

Figura 2. Porção inferior do estuário do Rio Goiana. Os círculos marcam a entrada de cada canal de maré. Amostragens: (1–3), lua quarto crescente; (4–6), lua cheia; (7–9), lua quarto minguante; (10–12), lua nova. Fonte: Google Earth (2014).

3.3. Procedimentos laboratoriais

As amostras foram divididas em alíquotas menores (100 ml) para facilitar a separação do plâncton e dos fragmentos plásticos envolvidos na matéria orgânica com o auxílio de um estereomicroscópio – ZEISS; STEMI 2000-C (x5). Larvas e ovos de peixes foram reservados da amostra e suas quantidades foram convertidas para um volume padrão de 100 m^3. Os fragmentos de plásticos flutuantes encontrados na amostra estática, foram peneirados primeiro, numa malha de 45 µ. Os microplásticos foram secados em estufa à 60°C e classificados em plásticos, filamentos plásticos e fragmentos de tinta de barco. Características como rigidez (duro ou mole) e as cores de cada item também foram registrados. O mensuramento dos microplásticos foi feito com o auxílio de uma câmera

digital (Canon-Powershot G10) acoplada a um estereomicroscópio trinocular, utilizando o software AxioVision Release 4.7.2 (calibrado com uma escala que converte os pixels da imagem em milímetros).

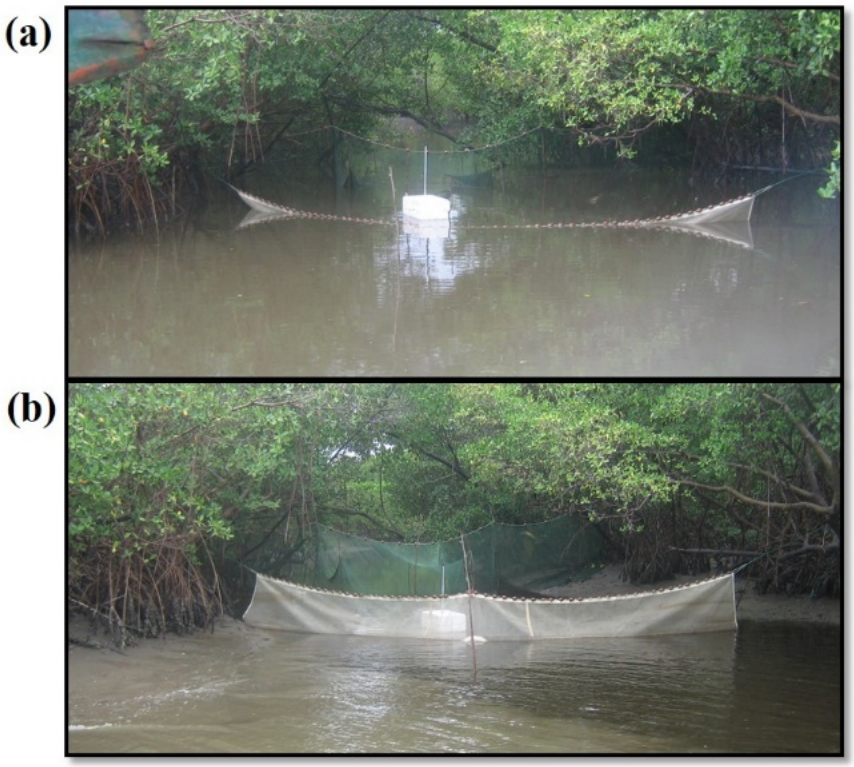

Figura 3. Rede de tapagem utilizada nas coletas de plâncton e fragmentos de plásticos nos canais de maré. Marés: (a) alta; (b) baixa. Fonte: LEGECE.

Para contar o zooplâncton, cada amostra (700 mL) foi homogeneizada e três sub amostras de 10 mL foram removidas usando um pipeta de Stempel, com reposição (Postel et al., 2000). Cada táxon de zooplâncton foi identificado até o menor nível taxonômico possível (Boltovskoy, 1981, 1999) e contados separadamente das três alíquotas para o cálculo das médias. As medias foram extrapoladas para 700 mL e depois convertidas para um volume padrão de 100 m^3. A identificação taxonômica do ictioplâncton foi baseada em séries de desenvolvimento, pela regressão de adultos e juvenis capturados na mesma região, usando caracteres comuns aos sucessivos estágios ontogenéticos iniciais (Balon, 1990). Além disso, características merísticas e os tamanhos dos estágios larvais em pré-, flexão e pós-flexão foram utilizados como critérios de identificação. A identificação das espécies seguiu Figueiredo e Menzes (1978, 1980), Menezes e Figueiredo (1980, 1985), Sinque (1980), Moser et al. (1984), Richards (2006).

O cálculo de densidade (Ind. 100 m^{-3}) de cada item do plâncton e dos detritos plásticos no canal principal usou o volume de água filtrado resultante da seguinte equação:

$$\Delta \text{ Flux} * 0{,}3 \text{ m} * 0{,}2827 \text{ m}^2 * 100 = \text{Volume de água filtrada (100 m}^3\text{)}.$$

Onde, Δ Flux é a diferença entre os valores de revolução final e inicial do fluxômetro, 0.3 m é a distância de uma revolução do fluxômetro e 0.6 m é o diâmetro da boca da rede, totalizando uma área de 0,2827 m^2 ($A = \pi r^2$).

Nos canais de maré, o volume filtrado foi calculado a partir da área da rede, da duração da amostragem e da velocidade da corrente. A área da rede resultou da distância entre os finais da rede de ~8 m devido ao posicionamento ligeiramente arqueado da rede na coluna d'água e uma profundidade estimada de ~1,5 m. A constante do fluxômetro foi de 57,56/999999.

$$\text{Distância percorrida pela água (m)} = \Delta \text{ Flux} * 57{,}56/999999$$

$$\text{Velocidade da corrente (m/s)} = \text{Distância (m)} * 100/\text{tempo (s)}$$

$$\text{Área filtrada (m}^2\text{) *velocidade da corrente (m/s) * tempo (s)} = \text{Volume (100 m}^3\text{)}$$

3.4. Análise estatística

3.4.1. Variação sazonal e especial do plâncton e do microplástico no canal principal

Três amostras de superfície e três de fundo, por área e por mês, foram consideradas como réplicas e usadas para testar as hipóteses. A análise de variância (ANOVA 3-fatores), com um nível de significância de 5 %, foi conduzida para avaliar se a distribuição de larvas e ovos de peixes (espécies), zooplâncton (grupos) e microplásticos (duro, mole, filamentos plásticos e fragmentos de tinta de barco) variaram com o espaço (estuário superior, médio e inferior), tempo (estações secas e chuvosas) e a profundidade da coluna d'água (superfície e fundo) (Zar, 1996). O teste de Cochran foi usado para verificar a homogeneidade das variâncias. Os dados originais foram Box-Cox transformados para aumentar a normalidade da distribuição (Box e Cox, 1964). O teste de Bonferroni (α = 0.05) foi usado sempre que diferenças significativas foram detectadas (Quinn e Keough, 2002).

Análises de cluster (baseadas nas matrizes de similaridade entre espécies usando distância Euclidiana ranqueadas) foram usadas para verificar como os grupos de larvas e ovos de peixes, zooplâncton e microplásticos estão distribuídos ao longo do estuário,

usando as áreas, as estações e a profundidade da coluna d'água como atributos (Clarke e Gorley, 2006).

Analises canônica de correspondência (CCA) (CANOCO for Windows 4.5) foram realizadas para detectar correlações ecológicas (ter Braak e Smilauer, 2002). Múltiplas regressões dos mínimos quadrados foram computadas com os escores derivados das médias ponderadas das densidades de larvas e ovos de peixes, zooplâncton e microplásticos (duro, mole, filamentos e tintas de barcos) e os agrupamentos do fatores (áreas, estações e profundidade) como variáveis dependentes e os parâmetros ambientais (precipitação, temperatura da água, oxigênio dissolvido e salinidade) como variáveis independentes (ter Braak, 1986; Palmer, 1993). Para evitar tendências relacionadas aos altos valores de densidade do zooplâncton, os dados foram $\log_{10}(x + 1)$-transformados. A CCA foi rodada com 100 interações com locais randomizados para facilitar o teste de Monte-Carlo entre os autovalores e as correlações espécie-ambiente para cada eixo resultante do CCA bem como aqueles esperados por chance. Com esse procedimento, um "triplot" é produzido onde as variáveis ambientais aparecem como vetores radiando da origem da ordenação. O tamanho do vetor está relacionado ao poder de relação entre a variável ambiental que o vetor representa e os grupos, para cada estação principal.

3.4.2. Influencia lunar na composição do plâncton e detritos plásticos nos canais de maré

ANOVA (1-fator) foi realizada para determinar se as densidades médias de larvas de peixes, zooplâncton e detritos plásticos variam com as diferentes fases da lua (Zar, 1996). O teste de Cochran foi usado para verificar a homogeneidade das variâncias. Os dados originais foram Box-Cox transformados para aumentar a normalidade da distribuição (Box e Cox, 1964). O teste de Bonferroni ($\alpha = 0.05$) foi usado sempre que diferenças significativas foram detectadas (Quinn e Keough, 2002).

Uma análise canônica de correspondência (CCA) (CANOCO for Windows 4.5) foi realizada para observar a relação entre as variáveis ambientais e cada fase da lua com a composição do plâncton e detritos plásticos nos canais de maré (ter Braak e Smilauer, 2002). Múltiplas regressões dos mínimos quadrados foram computadas com os escores dos locais, derivados das medias ponderadas de larvas de peixes, zooplâncton e detritos plásticos e das fases da lua como variáveis dependentes e os parâmetros ambientais (precipitação, temperatura da água, oxigênio dissolvido e salinidade) como variáveis independentes (ter Braak, 1986; Palmer, 1993).

4. ESTRUTURA DA TESE

De acordo com os objetivos e os resultados obtidos ao longo da realização do presente estudo, esta tese foi dividida em três capítulos. Cada capítulo se refere a artigos científicos e seguem as normas de publicação das revistas escolhidas para publicação.

Capítulo 1: "**Seasonal distribution and interactions between plankton and microplastics in a tropical estuary**"

Este capítulo foi submetido à revista científica Estuarine, Coastal and Shelf Science (ISSN 0272-7714). Este estudo avaliou como o plâncton (ictioplâncton e zooplâncton) e os microplásticos estão distribuídos ao longo do canal principal e se há variações ao longo do ciclo sazonal e do gradiente de salinidade do estuário do Rio Goiana.

Capítulo 2: "**Distribution patterns of microplastics within the plankton of a tropical estuary** (doi:10.1016/j.envres.2014.03.031)"

Este capítulo refere-se ao exame de qualificação do Programa de Pós-Graduação em Oceanografia realizado em setembro de 2013 e publicado na revista científica Environmental Research (ISSN 0013-9351). Este estudo avaliou se os microplásticos variam sazonalmente e espacialmente ao longo do gradiente de salinidade do estuário, e sua composição em relação a todo o plâncton presente no sistema.

Capítulo 3: "**Changes in the composition of ichthyoplankton assemblage and plastic debris in mangrove creeks relative to moon phases**"

Este capítulo foi aceito pela revista científica Journal of Fish Biology (ISSN 1095-8649). O estudo teve como objetivo quantificar as larvas de peixes, zooplâncton e detritos plásticos (ind. 100 m^{-3}) associados aos canais de maré do estuário para compreender suas distribuições de acordo com as fases da lua durante um intervalo de 30 dias.

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CAPÍTULO 1

Seasonal distribution and interactions between plankton and microplastics in a tropical estuary

Seasonal distribution and interactions between plankton and microplastics in a tropical estuary

A. R. A. Lima, M. Barletta*, M. F. Costa

Laboratório de Ecologia e Gerenciamento de Ecossistemas Costeiros e Estuarinos, Departamento de Oceanografia, Universidade Federal de Pernambuco, CEP 50740-550, Recife, Brazil

*Author to whom correspondence should be addressed: Tel. and fax: 00558121267223; email: barletta@ufpe.br

Abstract

Seasonal migration of salt wedge and rainfall were the major factors influencing the spatiotemporal distribution of ichthyoplankton and microplastics along the main channel of the Goiana Estuary. The most abundant taxa were the clupeids *Rhinosardinia bahiensis* and *Harengula clupeola*, followed by the achirid *Trinectes maculatus* (78.7% of the catch). Estuarine and mangrove larvae (*e.g. Anchovia clupeoides*, *Gobionellus oceanicus*), as well as microplastics were ubiquitous. During dryer months, the salt wedge reaches the upper estuary and marine larvae (*e.g. Cynoscion acoupa*) migrated upstream until the zones of coastal waters influence. However, the meeting of waterfronts in the middle estuary forms a barrier that retains the microplastics in the upper and lower estuary most part of the year. During the late dry season, a bloom of zooplankton was followed by a bloom of fish larvae (12.74 ind. $100m^{-3}$) and fish eggs (14.65 ind. $100m^{-3}$) at the lower estuary. During the late rainy season, the high freshwater inflow flushed microplastics, together with the biota, seaward. During this season, a microplastic maximum (14 items $100m^{-3}$) was observed, followed by fish larvae maximum (14.23 ind. $100m^{-3}$) in the lower estuary. Differently from fish larvae, microplastics presented positive correlation with high rainfall rates, being more strictly associated to flushing out/into the estuary than to seasonal variation in environmental variables. Microplastics represented half of fish larvae density. Comparable densities in the water column increase the chances of interaction between microplastics and fish larvae, including the ingestion of smaller fragments, whose shape and colour are similar to zooplankton prey.

***Keywords*:** Seston, salt wedge, rainfall, South America, zooplankton, fish larvae.

1. Introduction

The connectivity between estuarine and ocean habitats provides a great physic-chemical variability on hydrological circulation patterns, where the denser marine water flows below the river freshwater, creating a stratified water column upstream, commonly referred to as a salt wedge estuary (Kurup et al., 1998; Able, 2005; Barletta and Barletta-Bergan, 2009; Williams et al., 2012; Strydom, 2015). These mechanisms act for the retention of nutrients originated in the river basin and mangrove forest, partially supplying a diverse planktonic community, which function as the basin of the estuarine food web (Kjerfve, 1994; Beck at al., 2003; Nagelkerken et al., 2008).

Estuaries are important marine coastal ecosystems used as settlement, feeding and nursery grounds by many estuarine dependent fish species (Whitfield, 1990; Kjerfve, 1994; Able, 2005; Dantas et al., 2013; Lima et al., 2013; Potter et al., 2013; Gomes et al., 2014). Many fish species spawn in estuaries at times that ensure protection and food availability for their eggs and larvae (Cloern, 1987; North and Houde, 2003; Martino and Houde, 2010). Seasonal variations on salinity, temperature, oxygen, turbidity and availability of food resources, are the main factors influencing the spatiotemporal distribution and abundance of fish larvae and other planktonic organisms in estuaries worldwide (Blaber et al., 1997; Harris et al., 1999; Barletta-Bergan et al., 2002a,b; Hoffmeyer et al., 2009; Ooi and Chong, 2011; Williams et al., 2012).

Although, the hydrodynamic complexity of estuaries not only influences the living part of the plankton, but also inanimate material, such as plastics debris, acting in their retention or transportation to other environments (Cole et al., 2011; Costa et al., 2011; Lima et al., 2014). Plastics debris, associated to the increasing urbanization of watersheds, originate mainly on land due to improper disposal, accidental release or natural disasters (Alongi, 1998; Able 2005; Watters et al., 2010). These fragments enter estuaries by land runoff, river discharge or from the ocean (Le Roux, 2005; Nordstorm et al., 2006). However, during their time at land, sea and estuaries, plastics fragment into microplastics (< 5 mm) (Barnes et al., 2009; Thompson et al., 2009).

Plastics have been discussed as the principal marine debris to ubiquitously pollute the marine environment. Recent studies recorded high concentration of microplastics in estuarine, coastal waters and sea samples, with densities comparable to the living plankton (Collignon et al., 2012; Frias et al., 2014; Lima et al., 2014). The increasing amount of microplastics in the aquatic environment have raised concerns about their incorporation into food webs. Their small size makes them available to a wide range of

marine biota (Barnes et al., 2009; Cole et al., 2011). Microplastic ingestion has been widely reported in marine organisms, including microcrustaceans (Besseling et al., 2014), bivalves (Cauwenberghe and Janssen, 2014), amphipods (Chua et al., 2014), mysid shrimps, copepods, polychaete larvae (Setälä et al., 2014) and fishes (Boerger et al., 2010; Possatto et al., 2011; Dantas et al., 2012; Lusher et al., 2013; Sá et al., 2015). Ingested microplastics might induce gut blockage and limit food intake (Cole et al., 2013). In addition, microplastics have the capacity of adsorb persistent organic pollutants (POPs), biocides and trace metal posing a threat to the environment and organisms, such as the effects of eating contaminated fragments, consequently, reducing the nursery function of estuarine habitats (Moore, 2008; Frias et al., 2010; Turner, 2010).

Thence, this study described the spatial movement of the living plankton (ichthyoplankton and zooplankton) and non-living particles (microplastics) according to the seasonal migration of the salt wedge of the Goiana River Estuary in order to assess how environmental factors influence their distribution patterns. Whereas researches on the occurrence of microplastic in estuaries are scarce, this study also describes the possible effects of the presence of microplastics within the plankton of the estuary for fish larvae.

2. Material and methods

2.1. Study area

The Goiana Estuary has a main channel 17 km long and its floodplain covers 4700 ha in total area. It is located on the Northeast coast of Brazil (7°32'–7°35'S; 34°50'–34°58'W) and characterised by a tropical semi-arid climate (Fig. 1). The rainfall patterns define four seasons: early dry (September to November), late dry (December to February), early rainy (March to May) and late rainy (June to August) (Barletta and Costa, 2009) (Fig. 2). The Goiana Estuary is also a Resex (Acaú-Goiana PE/PB) and the fishery of fish, molluscs and crustaceans all along the year determine the subsistence of traditional populations (Barletta and Costa, 2009). The study area was divided into three portions according to the salinity gradient and the geomorphology of the estuary (Fig. 1). The upper estuary is located next to the river mouth where the width of the main channel varies from 0.05 to 0.09 km, with mean depth of 4.5 m (Fig. 1). The salinity of the upper estuary varies from 0 (late rainy) to 10 (late dry). The middle estuary has between 0.05 and 0.37 km in width, with mean depth of 4.7 m (Fig. 1). It is considered the portion at which occurs the mixing of fresh and salty waters with salinity range from 0 (late rainy)

to 21 (late dry). The lower estuary is dominated by marine waters throughout the year with a width range of 0.14 to 0.61 km and mean depth of 4.1 m (Fig. 1). The salinity of the lower estuary varies from 13 (late rainy) to 35 (late dry) in surface waters; and from 0 (late rainy) to 34 (early rainy) in bottom waters.

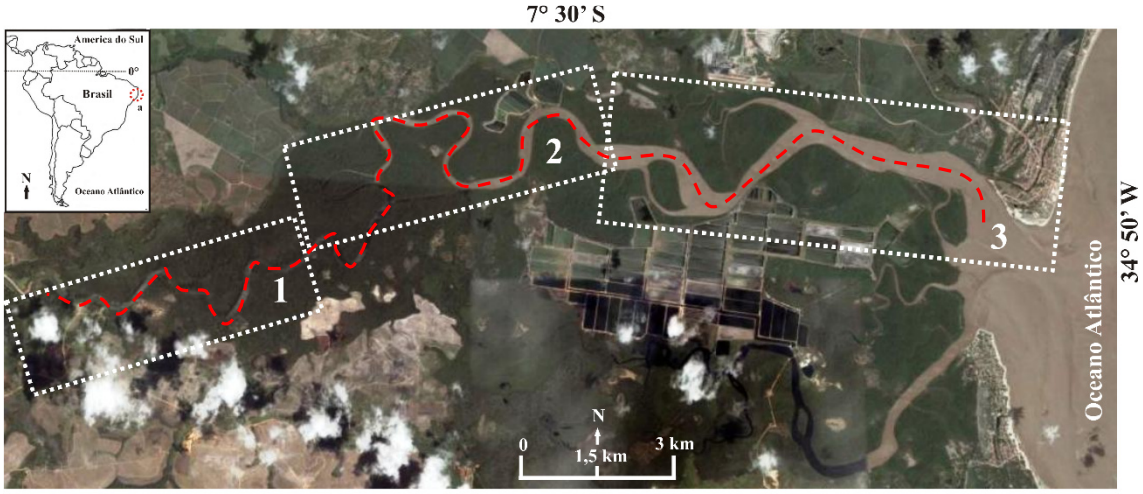

Figure 1. Goiana Estuary. ▭ = (1) upper, (2) middle and (3) lower portions of the estuary. (▬) Main channel. Source: Google Earth (2014).

2.2. Sampling

Samples were conducted in the main channel of the Goiana Estuary from April 2012 to March 2013. They were performed during neap tide cycles to avoid tide effects. Three superficial (0–1 m) and three bottom (3–6 m) water sample replicates were taken monthly in each portion of the estuary (upper, middle and lower) by towing a conical plankton net (300 µm; Ø 0.6 m; 2m long) for 15 min at an average speed of 2.7 knots, totalling 216 samples. The volume filtered per tow was calculated using a flowmeter (General Oceanics - Model 2030 Digital Series). A GPS (Ensign GPS Trimble Navigation) determined the sampling position and an echo sounder (Eagle Supra Pro D) registered the depth along the track. Water temperature (◦ C), dissolved oxygen (mg l^{-1}) (Wissenschaftlich Technische Werkstätten, WTW OXI 325; www.wtw.com) and salinity (WTW LF 197) were recorded before the beginning of each sampling, from both surface and bottom waters. Samples were preserved in buffered formalin (4%).

2.3. Laboratory procedures

Samples were divided into smaller aliquots (100 ml) to facilitate the separation of plankton and organic matter with the aid of a stereomicroscope – ZEISS; STEMI 2000-C (x5). Fish larvae, fish eggs and microplastics were totally separated from the bulk sample and their counts per unit were converted to a standard volume of 100 m^3. The

ichthyoplankton taxonomic identification was based on developmental series, working backwards from the adults and juveniles captured in the same region, using characters common to successively earlier ontogenetic stages (Balon, 1990). Species identification followed Figueiredo & Menzes (1978, 1980), Menezes & Figueiredo (1980, 1985), Sinque (1980), Moser et al. (1984), Richards (2006). To ascertain the presence of microplastics, plastic fragments were oven dried at 60 °C. Withered fragments were discarded, and the remaining were classed as plastics, paint chips or threads. For counting zooplankton, each sample was diluted to 700 mL and homogenized. Three subsamples of 10 mL were removed using a Stempel pipette, with reposition (Postel et al., 2000). Each zooplankton taxon was identified to the lowest possible taxonomic categories (Boltovskoy, 1981, 1999) and counted separately from the three aliquots to calculate the means. Mean counts were extrapolated to 700 mL and then converted to a standard volume of 100 m^3.

2.4. Statistical analysis

Three superficial and three bottom water samples per area per month were considered as replicates and were used to test the proposed hypothesis. The factorial analysis of variance (three-way ANOVA), with a 5 % level of significance, was performed to assess whether the distribution and density of the most abundant plankton groups and microplastics varied with space (upper, middle and lower estuary), time (dry and rainy seasons) and catch depth (surface and bottom) (Zar, 1996). The Cochran's test was used to check the homogeneity of variances. The original data were Box-Cox transformed (Box and Cox, 1964) to increase normality of the distribution. The Bonferroni's test (α = 0.05) was used whenever significant differences were detected (Quinn and Keough, 2002).

A canonical correspondence analysis (CCA) (CANOCO for Windows 4.5) was performed to detect ecological correlations (ter Braak and Smilauer, 2002). A multiple least-squares regression was computed with the site scores (derived from weighted average densities of fish larvae, fish eggs, zooplankton, and microplastics) as dependent variables and the environmental parameters (rainfall, water temperature, dissolved oxygen and salinity) as independent variables (ter Braak, 1986; Palmer, 1993). To avoid the effect of high density values, data were $\log_{10}(x + 1)$-transformed. The CCA was run with 100 iterations with randomized site locations to facilitate the Monte-Carlo tests between the eigenvalues and species–environment correlations for each axis that resulted from CCA as well as those expected by chance. With this procedure, a triplot is produced

where the environmental variables appear as vectors radiating from the origin of the ordination. The length of the vector is related to the power relationship between the environmental variable that the vector represents and the groups, for each main season.

3. Results

3.1. Seasonal fluctuation of environmental variables

At the beginning of the early dry season (Sep-Oct), when there are low rainfall rates, the salinity presented an increasing trend in the upstream, with wider ranges in the lower estuary (19.9 - 29.2), intermediate values in the middle estuary and lower values in the upper estuary (5.8 in bottom waters) (Fig. 2). For this period, the salt wedge was formed in the middle estuary. During this season, coastal water had low influence in the upper estuary. From November to March, the salinity of the lower and middle estuaries increased (Fig. 2). For this period (late dry season), the marine coastal water had greater influence in the main channel, causing an increase of salinity in the upper estuary, which ranged from 3.5 in surface to 11.2 in bottom waters (Fig. 2). During this period, the salt wedge reached the upper estuary. At the end of the early rainy season (Apr-May), salinity values of the lower estuary drop to 17.2 in surface and 34.2 in bottom waters (Fig. 2). Consequently, the salinity of the middle portion also decreased (4.3 - 21.2) (Fig. 2). During this period, the salinity of the upper portion decreased to 1.1, meaning that the salt wedge retreated to the middle portion because of low coastal water influence. At the beginning of the late rainy season (Jun-Jul), when precipitation reaches its highest values, the salinity of the lower estuary ranged from 0.1 in bottom to 27.9 in surface waters (Fig. 2). The salinity reached 0 in the upper estuary and 0.3 in the middle estuary (Fig. 2). During this period, the river had a greater influence in the main channel. The high flow of freshwater downstream makes the salt wedge migrates to the lower estuary. At the end of the late rainy season (Aug), rainfall drop, the salinity of the lower estuary increased to 29.9 in surface waters, and marine coastal waters initiate to influence again the middle estuary (Fig. 2).

Temperature presented a seasonal trend in the upper and middles estuaries, with higher values during the dry season (Sep-Feb) and early rainy season (Mar-May), and lower values during the late dry season (Fig. 2). For these areas, the highest temperature was observed in January in surface waters of the middle estuary (30.8 °C), and the lowest in June in surface waters of the upper portion (24 °C) (Fig. 2). For the lower estuary, temperatures were higher in bottom water during the dry season (Sep-Feb), ranging from

27 to 30 °C (Fig. 2). During the early rainy season, the higher temperature was observed in surface waters in March (32.1 °C) and in bottom waters in May (29 °C) (Fig. 2). The lowest temperatures occurred in the lower portion, coinciding with the late rainy season, ranging from 25 to 26 °C in bottom waters (Fig. 2). Whereas, dissolved oxygen did not present a corresponding seasonal trend. The highest values were observed in surface waters of the lower estuary, while the upper and middle estuaries presented lower values (Fig. 2). The lowest value was registered in bottom waters of the middle estuary in March (3.5 mg L^{-1}), while the highest was observed in May, but in surface waters of the lower estuary (8.5 mg L^{-1}) (Fig. 2).

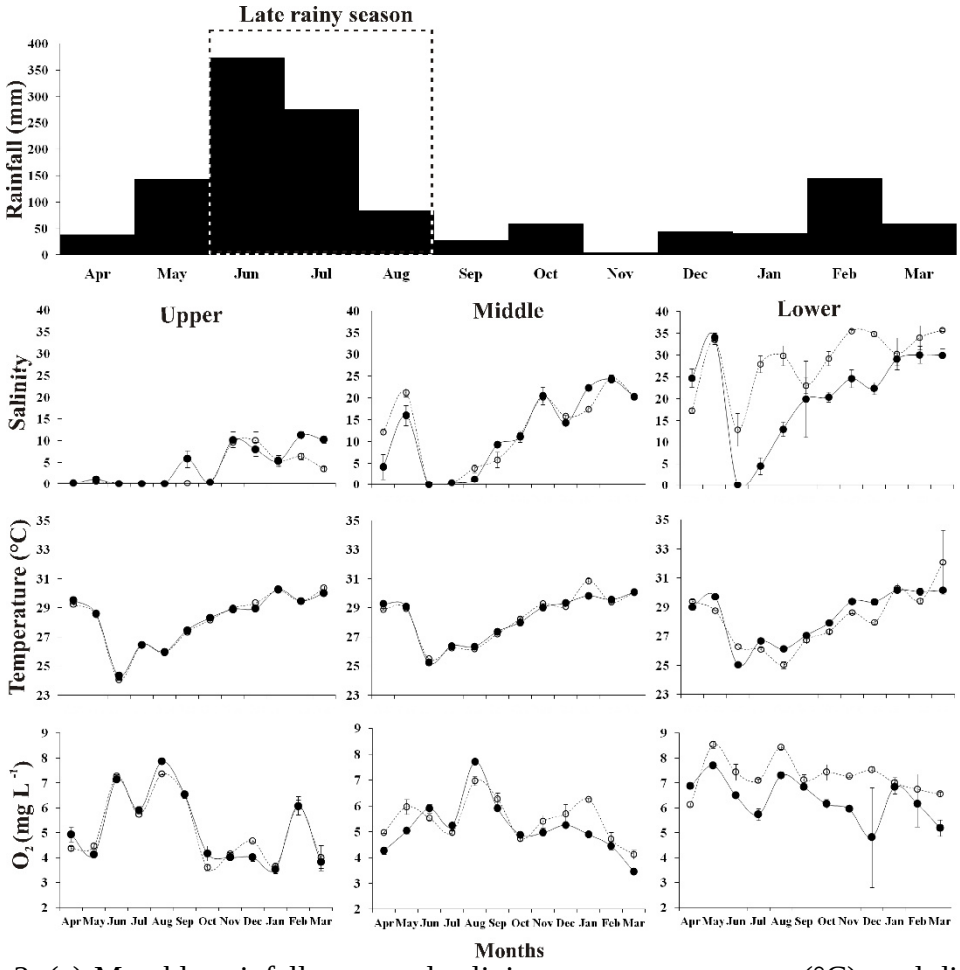

Figure 2. (a) Monthly rainfall rates and salinity, water temperature (°C), and dissolved oxygen (mg L^{-1}) means (+ S.D.) in surface (○) and bottom (●) waters for the three areas (upper, middle, lower) of the Goiana Estuary from April 2012 to March 2013.

3.2. Distribution of plankton and microplastics

In total, 71,212 fish larvae (54 ind. 100 m^{-3}) and 42,898 (32.4 ind. 100 m^{-3}) fish eggs, with mean densities of 6 and 3.6 individual 100 m^{-3}, respectively, were collected

(Table 1 and Table 2). The three-way ANOVA results showed that fish larvae differed significantly among areas and catch depth (Fig. 3 and Table 3). Zooplankton (13,564 ind. 100 m^{-3}) differed significantly among seasons and areas. (Table 3). Fish eggs and microplastics (26.1 particles 100 m^{-3}), differed among the three factors (season, area and depth) (Fig. 3 and Table 3). The interactions season *vs*. area, season *vs*. depth and area *vs*. depth were also significant for these variables ($p < 0.01$) (Fig. 3 and Table 3). Such interactions suggest that seasonality and the depth are influencing the distribution of plankton and microplastics in the main channel of the Goiana Estuary. Fish larvae and fish eggs were more abundant in the lower estuary along the year, with highest values in bottom and surface waters of the lower estuary during the late dry season, respectively ($p < 0.01$) (Fig. 3 and Table 3). Zooplankton was abundant in the entire estuary, independent of the catch depth, with highest mean density in bottom waters of the lower estuary during the late dry season ($p < 0.01$) (Fig. 3 and Table 3). Microplastics (> 0.58 to 3.88 mm) were found in the three areas of the estuary yearlong, with highest mean densities in bottom waters of the lower estuary during the late rainy season ($p < 0.01$) (Fig. 3 and Table 3).

3.3. Distribution of main ichthyoplankton

The ANOVA showed that the mean density of the 8 most frequent species differed either among season, area and/or catch depth (Fig. 4 and Table 3). In the early rainy season, fish larvae had higher densities in bottom waters of the middle estuary, where *Rhinosardinia bahiensis* (pre-flexion: 46% and post-flexion: 32.9%) was the most abundant for instance (Fig. 4 and Table 2). The most important larvae in the upper estuary were *R. bahiensis* and *Anchovia clupeoides* (pre-flexion: 52.1%) (Fig. 4 and Table 2). While, *Trinectes maculatus* (pre-flexion: 100%) and Engraulidae eggs were the most important in surface waters of the lower estuary (Fig. 4 and Fig. 5). In addition, *Cynoscion acoupa* (pre-flexion: 74.1%) presented highest density in bottom waters, and Achiridae eggs in surface waters of the lower estuary during the early rainy season, with significant differences ($p < 0.05$) (Fig. 4 and Table 3). In the late rainy season, higher densities of fish larvae were observed in surface waters of the lower estuary, where larvae of *R. bahiensis*, *A. clupeoides*, *T. maculatus* and *Cetengraulis edentulus* (pre-flexion: 55.1%) were most abundant (Fig. 4 and Table 2). *Harengula clupeola* (pre-flexion: 100%) differed significantly, with highest mean density in bottom waters of the lower estuary during this season ($p < 0.01$) (Fig. 4). Fish eggs were not found in the upper and middle estuaries during the late rainy season (Fig. 5).

Table1. Density of the plankton and microplastics from the Goiana Estuary during different seasons (ER, early rainy; LR, late rainy; ED, early dry; LD, late dry) and areas (upper, middle and lower). E, estuarine; E-M, estuarine-marine; MS, mangroves; M, marine. Sub-total densities in bold.

					Density (%)											
			Total density		Upper				Middle				Lower			
Seston	**Habitat**	**Number**	**N° 100 m^{-3}**	**%**	**ER**	**LR**	**ED**	**LD**	**ER**	**LR**	**ED**	**LD**	**ER**	**LR**	**ED**	**LD**
Fish larvae																
Rhinosardinia bahiensis	E	40 076	28.26	52.44	58.34	7.04	90.19	83.68	85.91	63.76	17.38	32.72	2.94	52.44	23.79	50.25
Harengula clupeola	M	10 572	7.20	13.37	5.83			1.55	6.34	20.20	64.68	25.85	6.33	21.13	1.73	2.62
Trinectes maculatus	M	5 988	6.99	12.98	3.24	0.12	0.14	1.32	1.32	0.43	5.68	11.04	39.97	5.84	40.43	23.98
Gobionellus oceanicus	MS	1 524	2.52	4.67	0.87	4.96	0.02	0.24	0.16	0.75	0.50	0.85	5.70	1.86	9.64	13.17
Anchovia clupeoides	E	3 969	2.38	4.41	11.43	37.16	2.05	4.92	2.85	2.19	0.48	2.90	1.09	9.41	4.02	0.85
Cynoscion acoupa	M	2 205	1.96	3.64	1.12		4.56	1.94	0.94	1.16	5.66	14.61	15.85	1.37	5.82	2.29
Cetengraulis edentulus	M	2 815	1.94	3.61	1.50	0.12	0.72	1.03	0.26	0.52	2.16	1.85	14.65	4.96	9.63	1.74
Lupinoblennius nicholsi	M	451	0.49	0.91	0.18	0.12	0.02	0.16	0.54	0.08	0.18	5.76	0.91	0.16	1.33	2.19
Achirus lineatus	E	723	0.46	0.86	0.05		0.01	0.09	0.04			0.06	4.26	0.63	0.51	1.53
Syngnathus sp.	E-M	588	0.43	0.80	4.54	13.90	0.98	2.40	0.22	1.86	0.70	1.13	2.07	0.15	0.19	0.04
Opisthonema oglinum	M	580	0.41	0.77	11.47	34.83	0.01		0.47	2.09				0.01		
Stellifer stellifer	E-M	527	0.33	0.62	0.07					6.47				1.11		
Stellifer sp.	E-M	77	0.14	0.27	0.16			1.23	0.18	0.12			0.07	0.73		
Bathygobius soporator	MS	117	0.07	0.12					0.04					0.04	1.24	0.19
Lutjanus sp.	M	102	0.06	0.11			0.19		0.02		0.03	0.06		0.07	0.04	0.30
Pseudophallus mindii	E-M	105	0.05	0.10	0.16	1.13	0.47	0.70		0.06	0.03	0.09	0.02	0.04	0.03	0.01
Achirus sp.	E	91	0.05	0.09	0.04		0.04	0.76	0.15	0.12	0.12	1.14		0.01	0.04	0.02
Larimus breviceps	E-M	71	0.04	0.07	0.43				0.15			0.37	0.04		0.05	0.13
Anchoa sp.	E-M	55	0.03	0.06					0.37				0.13		0.29	
Sphoeroides testudineus	M	27	0.02	0.04	0.03		0.16		0.01	0.03		0.35	0.02	0.04		0.01
Micropogonias sp.	M	21	0.02	0.03			0.35									
Trachinotus coralinus	E-M	5	0.01	0.02	0.51						0.04					
Eugerres brasilianus	M	6	0.01	0.01			0.02		0.01	0.13				0.01		
Guavina guavina	E	4	0.001	0.01		0.62	0.02									
Oligoplites saurus	M	3	0.001	0.001	0.04		0.02									
Pomadasys sp.	M	2	0.001	0.001			0.01			0.03						
Atherinella sp.	M	2	0.001	0.001									0.03			
Eleotris sp.	MS	2	0.001	0.001									0.03			
Strongylura timucu	M	1	0.001	0.001									0.02			
Centropomus undecimalis	M	1	0.001	0.001									0.02			
Sub-total density (A)			**53.90**		**1.71**	**0.39**	**4.36**	**1.93**	**5.06**	**2.67**	**3.49**	**1.27**	**3.77**	**14.23**	**2.71**	**12.74**
Fish eggs																
Clupeidae eggs		21749	16.40	50.61					0.28		3.88	4.03	26.93	11.46	52.51	22.70
Engraulidae eggs		20098	15.29	47.19				6.18	3.43		0.49	10.09	54.89	36.58	27.87	57.67
Achiridae eggs		1051	0.71	2.19			100.00	93.82	56.48		36.86	66.90	6.36	0.21		0.48
Sub-total density (B)			**32.40**				**0.02**	**0.01**	**0.03**		**0.09**	**0.19**	**6.73**	**3.15**	**7.09**	**14.65**

Table 1. Continued

Seston	Total density		Density (%)											
			Upper				Middle				Lower			
	N° 100 m^{-3}	%	ER	LR	ED	LD	ER	LR	ED	LD	ER	LR	ED	LD
Zooplankton														
Nauplii of Cirripedia	8223.43	60.63	0.33	1.15	10.95	10.05	19.26		83.60	92.17	25.93	26.77	74.90	46.17
Hydromedusa larvae	2762.14	20.36				2.48	44.15		7.95	2.35	55.52	9.33	10.57	36.26
Zoeae of *Ucides cordata*	848.65	6.26	63.79	76.45	76.12	77.16	12.74	10.77	5.09	2.27	2.44	7.32	3.93	1.56
Calanoid copepods	754.78	5.56	2.48	12.02	10.27	5.84	12.21	87.00	2.38	2.24	5.92	46.05	7.21	1.94
Appendicularia	721.10	5.32				1.43	11.18		0.89	0.92	9.20	0.46	2.29	11.57
Molusc larvae	102.61	0.76									0.04	1.22	0.10	2.44
Penaeidae larvae	76.71	0.57	22.60	7.94	2.08	3.03	0.12	2.00	0.03	0.05	0.06	0.67	0.31	0.01
Amphipoda spp.	38.94	0.29	10.80	2.44	0.43		0.17	0.23		0.01	0.74	0.44	0.03	
Zoeae of Euphasidae	19.01	0.14									0.06	3.65	0.50	0.03
Chaetognata	8.22	0.06									0.07	2.64		0.02
Mysis of *Lucifer faxoni*	6.25	0.05									0.03	1.31	0.17	
Isopoda spp.	2.27	0.02			0.15		0.17		0.05			0.15		
Sub-total density (C)	**13564.1**		**231**	**27.9**	**244.2**	**103.4**	**511.9**	**176.8**	**1369.3**	**3796.5**	**1248.2**	**245.6**	**1613.2**	**3996.2**
Microplastics (D)	**26.1**		**1.3**	**1.7**	**1**	**2.5**	**0.4**	**0.7**	**0.5**	**0.5**	**0.8**	**14**	**0.4**	**2.3**
Total density (A+B+C+D)	**13676.4**		**234.1**	**30**	**249.5**	**107.8**	**517.3**	**180.2**	**1373.3**	**3798.5**	**1259.4**	**276.9**	**1623.4**	**4025.9**

Table 2. Developmental stages size of the most important species catch in the main channel of the Goiana Estuary.

Larval species	Developmental stages (Length ± S.D. mm)		
	Pre-flexion	Flexion	Post-flexion
Rhinosardinia bahiensis	4.80 ± 0.63 (*n* = 18 435)	7.26 ± 0.87 (*n* = 8 416)	13.22 ± 2.53 (*n* = 13 225)
Harengula clupeola	4.09 ± 0.61 (*n* = 10 572)	-	-
Trinectes maculatus	2.54 ± 0.57 (*n* = 5 988)	-	-
Gobionellus oceanicus	2.56 ± 0.48 (*n* = 1 077)	6.65 ± 1.09 (*n* = 29)	14.04 ± 3.56 (*n* = 418)
Anchovia clupeoides	4.72 ± 0.62 (*n* = 2 068)	7.79 ± 1.19 (*n* = 779)	16.46 ± 4.43 (*n* = 1 122)
Cynoscion acoupa	2.85 ± 0.47 (*n* = 1 633)	4.44 ± 0.28 (*n* = 281)	9.35 ± 3.45 (*n* = 291)
Cetengraulis edentulus	4.35 ± 0.86 (*n* = 1 552)	7.80 ± 1.17 (*n* = 662)	15.70 ± 4.79 (*n* = 601)
Lupinoblennius nicholsi	3.12 ± 0.49 (*n* = 451)	-	-

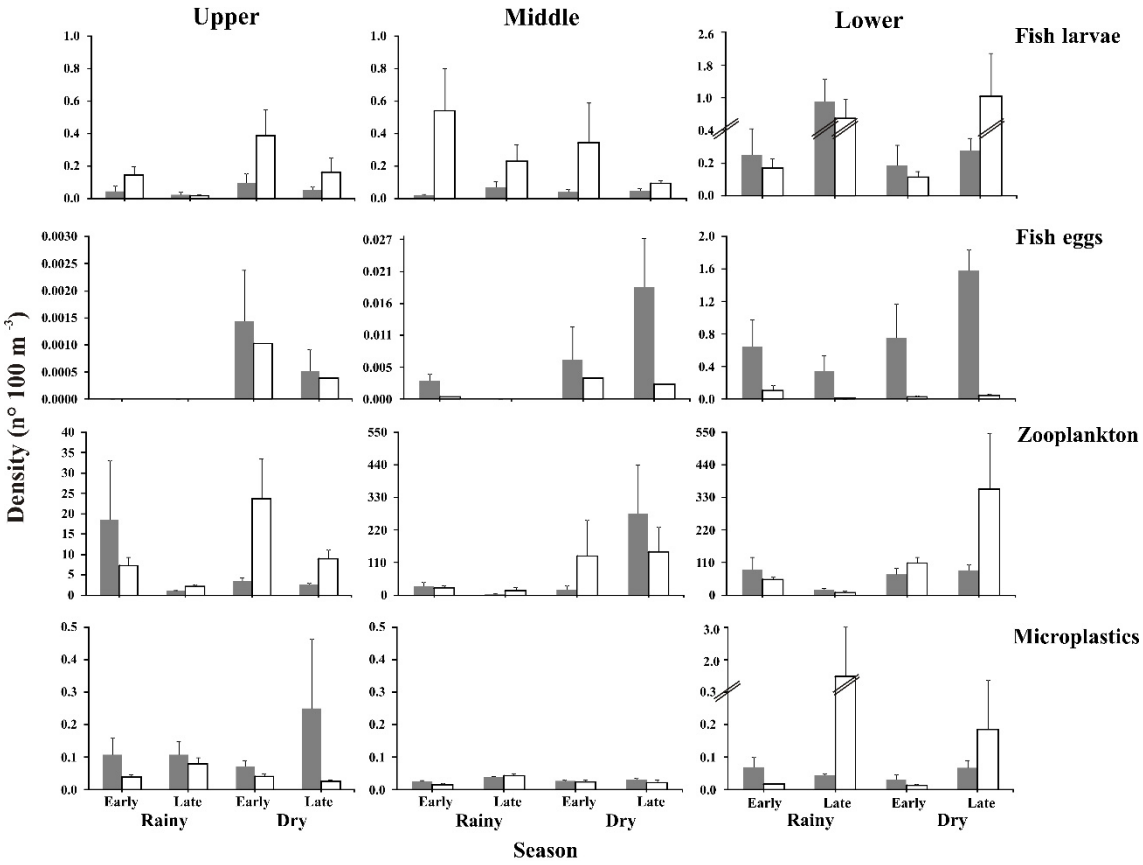

Figure 3. Total mean (+ S.E.) density of seston (fish larvae, fish eggs, zooplankton, microplastics) in different depths [(■) surface; (□) bottom] of the three areas of the Goiana Estuary (upper; middle; lower) for each season (early and late dry; early and late rainy).

In the early dry season, higher larval density was observed in the bottom waters of the upper and middle estuaries, where the most abundant larvae were *R. bahiensis* and *H. clupeola*, respectively (Fig. 4). Only Achiridae eggs were present in the upper estuary. The most important fish larvae in the upper estuary were *R. bahiensis*, *C. acoupa* and *Gobionellus oceanicus* (pre-flexion: 70%) (Fig. 4 and Table 2). Larvae of *T. maculatus* was the most abundant in surface and *R. bahiensis* in bottom waters of the lower estuary. Only Clupeidae and Engraulidae eggs were present in the lower estuary during the early dry season (Fig. 5). In the late dry season, higher density of fish larvae was observed in bottom waters of the lower portion. *R. bahiensis*, *H. clupeola*, *T. maculatus*, *G. oceanicus*, *C. acoupa* and *Lupinoblennius nicholsi* (pre-flexion: 100%) were the most abundant (Fig. 4 and Table 2). All three families of fish eggs were found in the middle estuary, however, only Engraulidae and Achiridae eggs were present in the upper portion during the late dry season (Fig. 5). In addition, the highest mean density of *R. bahiensis*, *L. nicholsi* and *G. oceanicus* occurred in bottom waters of the lower estuary during the late dry season ($p <$

0.05) (Fig. 4 and Table 3). Clupeidae and Engraulidae eggs presented their highest mean densities in surface waters of the lower estuary during the late dry season, with significant differences ($p < 0.05$) (Fig. 5 and Table 3).

Table 3. Summary of the ANOVA results for the mean density of plankton and microplastics. Analysis performed using Box-Cox transformed data. Differences among seasons, areas and water column were determined by Bonferroni's *post hoc* comparisons test. Seasons: ER, early rainy; LR, late rainy; ED, early dry; LD, late dry. Areas of the Goiana Estuary: UE, upper; ME, middle; LE, lower. Depth of water column: SUF, surface; BOT, bottom. ns, not significant; ** $p < 0.01$; * $p < 0.05$.

	Source of variance			Interactions			
Variables	**Season (1)**	**Area (2)**	**Depth (3)**	**1x2**	**1x3**	**2x3**	**1x2x3**
Fish larvae	**ns**	**UE ME LE****	**SUF BOT****	****	*****	****	**ns**
Rhinosardinia bahiensis	**ns**	**ns**	**SUF BOT****	****	**ns**	**ns**	****
Harengula clupeola	**ns**	**UE LE ME****	**SUF BOT****	**ns**	**ns**	**ns**	**ns**
Trinectes maculatus	**ns**	**UE ME LE****	**ns**	**ns**	**ns**	****	**ns**
Gobionellus oceanicus	**ns**	**UE ME LE****	**ns**	**ns**	**ns**	*****	**ns**
Anchovia clupeoides	**ns**	**ns**	**SUF BOT****	****	****	****	****
Cynoscion acoupa	**ns**	**UE ME LE****	**SUF BOT****	**ns**	**ns**	**ns**	**ns**
Cetengraulis edentulous	**ns**	**UE ME LE****	**ns**	**ns**	**ns**	**ns**	**ns**
Lupinoblennius nicholsi	**LR ED ER LD****	**UE ME LE****	**ns**	**ns**	**ns**	**ns**	**ns**
Fish eggs	**LR ER ED LD****	**UE ME LE****	**SUF BOT****	*****	**ns**	****	**ns**
Clupeidae eggs	**ns**	**UE ME LE****	**SUF BOT****	**ns**	*****	****	*****
Engraulidae eggs	**LR ER ED LD****	**UE ME LE****	**SUF BOT****	****	****	****	**ns**
Achiridae eggs	**LR ED LE ER***	**UE ME LE***	**SUF BOT****	****	****	****	****
Zooplankton	**LR ED ER LD****	**UE ME LE****	**ns**	****	**ns**	**ns**	**ns**
Microplastics	**ED ER LD LR****	**ME LE UE****	**BOT SUF****	**ns**	**ns**	**ns**	**ns**

3.4. Influence of the environmental variables in plankton and microplastc distributions

In both seasons dry and rainy, the first axes explained more than 50% of the variance of the species/microplastic-environment relation and represented the estuarine ecocline (salinity gradient) (Fig. 6a-b). The first axes of these seasons showed negative correlation with salinity ($p < 0.01$) (Fig. 6a-b and table 4). For the rainy season, the second

axis explained 28.3% and represented the seasonality (late rainy season above and early rainy season below the first axis) (Fig. 6a). For the dry season, the second axis explained 22.9% of the variance and represented depth (bottom waters above and surface waters below the first axis) (Fig. 6b).

During the rainy season, larval *Stellifer* sp., *H. clupeola*, *R. bahiensis*, *A. clupeoides*, together with calanoid copepods, paint chips, threads, soft and hard microplastics, showed positive correlations with high rainfall, in both depths of the middle and lower estuaries, during the late rainy season (Fig. 6a and table 3). *Achirus lineatus*, *C. edentulus*, *G. oceanicus*, *C. acoupa*, *L. nicholsi*, *T. maculatus* showed positive correlations with dissolved oxygen in surface waters of the lower portion during the late rainy season, and in bottom waters of the middle portion during the early rainy season (Fig. 6a and table 4). Nauplii of cirripedia, appendicularia and hydromedusa larvae showed positive correlations with salinity and temperature in both depths of the middle and lower portions during the early rainy season (Fig. 6a and table 4). *Opisthonema oglinum*, *Syngnathus* sp., zoeae of *U. cordatus*, amphipoda and Penaeidae larvae were strongly correlated with the upper portion of the estuary during the entire rainy seasons, in both depths (Fig. 6a and table 4).

During the dry season, larval *A. clupeoides*, *Syngnathus* sp., Achiridae eggs, together with zoea of *U. cordatus*, Penaeidae larvae, paint chips, threads, soft and hard microplastics, showed positive correlation with the entire dry season, in both depths of the upper estuary; and with surface waters of the middle estuary during the early dry season (Fig. 6b and table 4). *Achirus lineatus*, Engraulidae and Clupeidae eggs presented positive correlations with the dry season in surface waters of the lower estuary. *C. edentulus*, *T. maculatus*, *G. oceanicus*, *L. nicholsi*, *A. brasiliana* larvae, hydromedusa larvae and appendicularia were strongly positive correlated with salinity, dissolved oxygen, rainfall and temperature in bottom waters of the lower estuary along the dry season (Fig. 6b and table 4). *Cynoscion acoupa*, *R. bahiensis*, *H. clupeola*, calanoid copepods and nauplii of cirripedia showed correlation with the middle portion along the entire dry season (Fig. 6b and table 4).

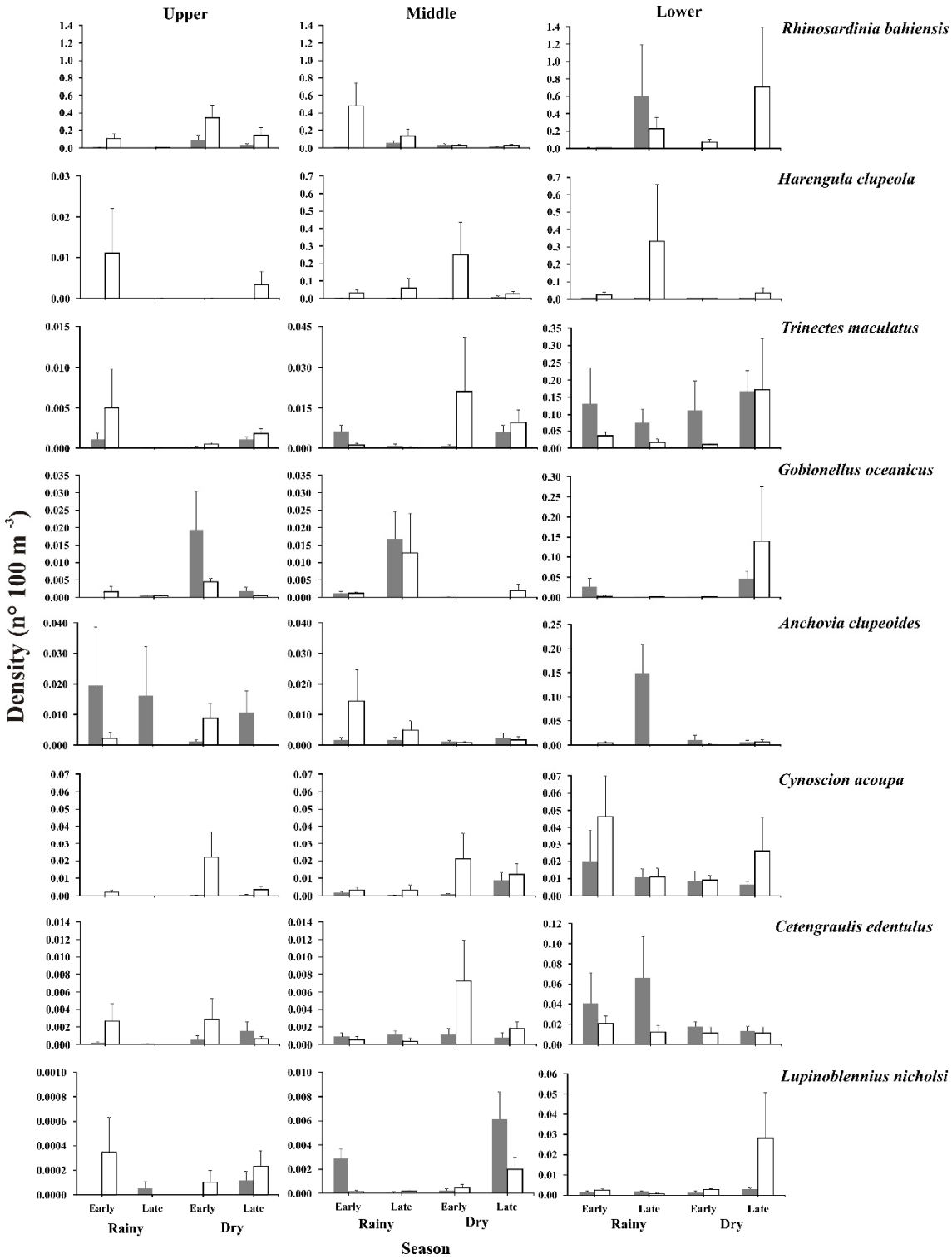

Figure 4. Total mean (+ S.E.) density of fish larvae species in different depths [(■) surface; (□) bottom] of the three areas of the Goiana Estuary (upper; middle; lower) for each season (early and late dry; early and late rainy).

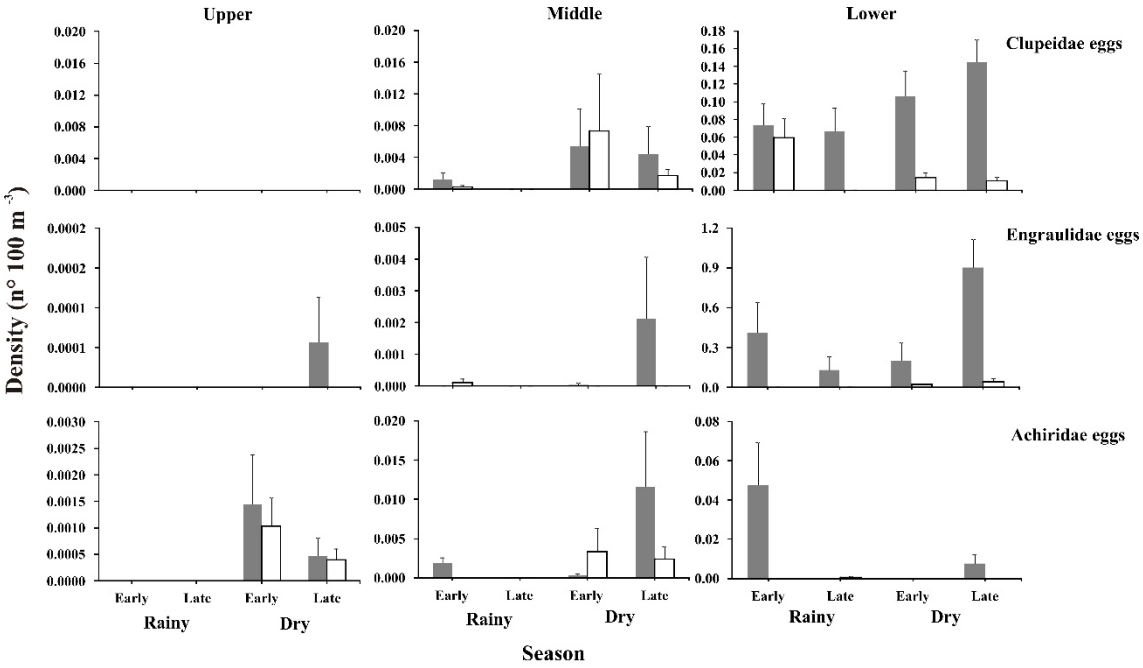

Figure 5. Total mean (+ S.E.) density of fish eggs in different depths [(■) surface; (□) bottom] of the three areas of the Goiana Estuary (upper; middle; lower) for each season (early and late dry; early and late rainy).

4. Discussion

4.1. Influence of seasonal patterns on fish larvae distribution

In the Goiana Estuary, Clupeidae and Achiridae larvae represented 66.6% and 13.9% of the ichthyofaunal assemblage, respectively. Engraulidae larvae contributed with only 8.1%. From these, most were represented by early stages of marine and estuarine fishes (> 70% in pre-flexion). Similar trends are observed in other tropical estuaries, where early Clupeidae and Engraulidae are highly abundant (Rakocinski et al., 1996; Sarpedonti et al., 2013). However, this is not so in all tropical estuaries. In Indo-West Pacific and Peninsular Malaysia estuaries, for example, Gobiidae larvae are ubiquitous with high densities (Blaber et al., 1997; Ooi and Chong, 2011).

In the Goiana Estuary, seasonal fluctuations in rainfall and salinity are the responsible for the distribution of fish larvae along the main channel. Due to a high diversity of marine larvae associated with the saline lower estuary and total absence of freshwater species, the density of larvae increase seaward, being ~1.6 times higher than in the areas upstream. In a sub-tropical well-mixed estuary (Mississippi Sound - northern Gulf of Mexico), the taxonomic diversity also increased seaward due to the abundance of coastal spawning fishes (Rakocinski et al., 1996). For this estuary, larval distribution were

positive correlated with water temperature and salinity changes due to high freshwater input during springs (Rakocinski et al., 1996). For another well-mixed temperate estuary (Lima Estuary - northwest Portugal), fish larvae were more diverse near the ocean, due to the presence of marine and the absence of freshwater species, although highest abundances occurred in upstream saltmarsh zones (Ramos et al., 2006). For this estuary, seasonal variations in temperature and precipitation were responsible for the larval distribution (Ramos et al., 2006). However, in the Caeté Estuary (tropical Northern Brazil), where fish larvae were more influenced by area, most larvae were from estuarine and freshwater species associated with freshwater conditions (*e.g. Rhinosardinia amazonica* and *A. clupeoides*), with maximum abundance in the upper estuary during the dry season (Barletta-Bergan et al., 2002b). This indicate that fish larvae of similar ecological guilds use different habitats regarding their environmental characteristics. Such features were observed in Indo-West Pacific estuaries (Sarawak and Sabah) (Blaber et al., 1997). Larger and deeper mixed estuaries, with high turbidity and strong currents had larvae associated with estuarine conditions, while smaller and shallower estuaries, with marked haloclines and seasonal changes in freshwater inflow had taxa with marine affinities (Blaber et al., 1997).

Marine and estuarine fishes spawn in the Goiana Estuary because of the high food supply provided by the system for early stages of fish larvae (~70% in pre-flexion) all along the year (Nagelkerken et al., 2008). Larvae of estuarine fish species, such as *R. bahiensis* and *A. clupeoides* and mangrove larvae of *G. oceanicus* dwelled both depths of the main channel during the entire year, supporting a wide range of salinity variation. For this estuary, during dryer months, coastal waters influence the main channel and the salt wedge reaches partially the upper estuary. Thence, marine larvae migrate in bottom flows to the upper estuary (*e.g. H. clupeola*, *T.maculatus*, *C. acoupa*, *C. edentulus* and *L. nicholsi*). Similar trends were observed in the Caeté Estuary, when in dry months the influence of coastal waters allows marine larvae to inhabit the upper estuary (*e.g. C. acoupa*, *Lycengraulis grossidens* and *Stellifer rastrifer*) (Barletta-Bergan et al., 2002b).

The distribution of fish larvae in the Goiana Estuary might also be associated to high availability of zooplankton along during the entire year (*e.g.* nauplii of cirripedia, zoeae of *U. cordatus* and calanoid copepods) (Allen et al., 1980; Suzuki et al., 2014; Watanabe et al., 2014). In the tropical Sangga Kecil Estuary (Western Peninsular Malaysia), salinity was also the most significant factor influencing the distribution of larvae (Ooi and Chong, 2011). For this estuary, late stages of Engraulidae and Clupeidae

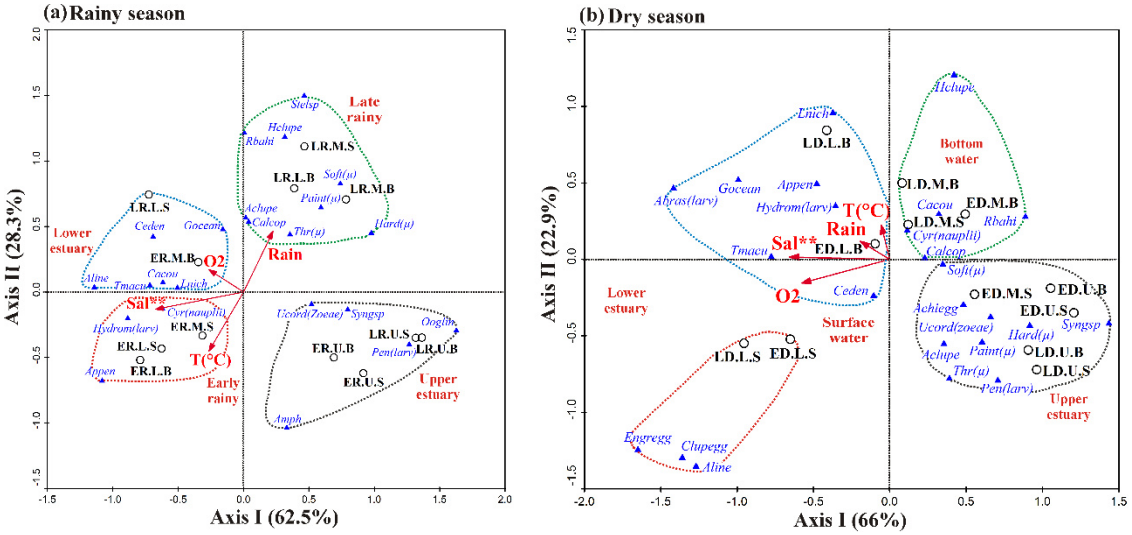

Figure 6. Canonical correspondence analysis (CCA) triplot for the ecological correlations between the plankton and the environmental variables. Circles (○) represent the three areas (U, upper; M, middle; L, lower) of the main channel of Goiana estuary in each season [(a) Rainy season: ER, early rainy; LR, late rainy and (b) Dry season: ED, early dry; LD, late dry] and depth of water column (S, surface; B, bottom). Triangles (Δ) represent the plankton [ichthyoplankton (*Aclupe*, *Anchovia clupeoides*; *Aline*, *Achirus lineatus*; *Cacou*, *Cynoscion acoupa*; *Ceden*, *Cetengraulis edentulus*; *Gocean*, *Gobionellus oceanicus*; *Hclupe*, *Harengula clupeola*; *Lnich*, *Lupinoblenius nicholsi*; *Ooglin*, *Opisthonema oglinum*; *Rbahi*, *Rhinosardinia bahiensis*; *Stelsp*, *Stellifer* sp.; *Syngsp*, *Syngnathus* sp.; *Tmacu*, *Trinectes maculatus*), zooplankton (*Abras(larv)*, *Anomalocardia brasiliana* larvae; *Amph*, amphipoda; *Appen*, appendicularia; *Copcal*, copepod calanoida; *Cyr(naupli)* cyrripedia larvae; *Hydrom*, hydromedusa larvae; *Pen(larv)* Penaeidae larvae; *Ucord(Zoea)*, Zoea of *Ucides cordata*) and microplastics (*Hard(μ)*, hard; *Soft(μ)*, soft; *paint(μ)*, paint chips; *Thr(μ)*, threads)]. The environmental variables (rainfall, dissolved oxygen, salinity, temperature) were represented by arrows. ** $p < 0.01$.

marine larvae moved to less saline shallower turbid waters with high availability of zooplankton (Ooi and Chong, 2011). In the late dry season was observed a bloom of zooplankton at the bottom of the Goiana lower estuary, followed by a maximum in ichthyoplankton density. Engraulidae and Clupeidae eggs, as well as larvae of *R. bahiensis*, *G. oceanicus* and *L. nicholsi*, presented maximum abundances at the bottom of lower estuary, indicating a spawning season for these species. During this season coastal waters penetrates farther in the Goiana upper estuary, thence, larvae of marine and

estuarine species used the entire main channel due to low salinity stratification. As such, in temperate estuaries of South Africa, early stages of estuarine larvae (*e.g.* Clupeidae and Gobiidae) peaked in abundance during warmer months, coinciding with coastal spawning and zooplankton maxima (Strydom, 2015). In the Guajará Bay Estuary (tropical northeastern Brazilian), the main spawning season occurred at the beginning of the rainy period, when larval fish density was 8 times higher than dryer months (Sarpedonti et al., 2013). In this estuary salinity never excides 1.5, although marine Sciaenidae larvae were highly abundant (Sarpedonti et al., 2013). In addition, in the Macaé Estuary (tropical Southeast Brazil), *G. oceanicus* contributed with 33% of the Gobiidae larvae and were highly abundant in dry months, such as observed in the Goiana Estuary, suggesting a similar spawning season for this species (Gomes et al., 2014).

Table 4. Summary of canonical correspondence (CCA) analysis using four environmental variables (rainfall, water temperature, dissolved oxygen, salinity) and density of fish larvae species, fish eggs, zooplankton and microplastics in the main channel of the Goiana estuary. ** $p < 0.01$.

Summary of CCA	**Rainy season**			**Dry season**		
	Axis 1	**Axis 2**	***p* value**	**Axis 1**	**Axis 2**	***p* value**
Eigenvalue	0.256	0.116		0.187	0.065	
Species-environment correlation	0.894	0.855		0.977	0.916	
Cumulative % variance						
of species data	36.8	53.6		44.8	60.5	
of species-environmental variables	62.5	90.8		66	88.9	
Correlation with environmental variables						
Rainfall	0.2829	0.6796	0.0594	-0.2862	0.2119	0.2277
Water temperature	-0.3331	-0.6593	0.5743	-0.0839	0.4087	0.0792
Dissolved oxygen	-0.3333	0.2517	0.1188	-0.8574	-0.2804	0.2079
Salinity	-0.8450	-0.1930	0.0099**	-0.9754	0.0281	0.0099**

In the late rainy season is observed another peak of larvae density in the Goiana lower estuary, with the marine larvae *C. edentulus* and *H. clupeola* being some of the most abundant. The high freshwater inflow flushed the salt wedge, together with the biota seaward (Lima et al., 2014). Although, *R. bahiensis*, *A. clupeoides* and the mangrove larvae *G. oceanicus* were found to use the upper and middle estuaries in all depths, even where salinity values reached 0. A second peak in South African estuaries was also observed during late winter rainfall, associated to the strong influence of river inflow on food availability and larval survival (Strydom, 2015). In the Kowie Estuary (temperate southeast coast of South Africa) a peak in estuarine larvae was observed in summer, also associated with high rainfall (Kruger and Strydom, 2010). Nevertheless, in the temperate

Lima Estuary, euhaline most part of the year, the winter rainfall forms a seasonal vertical stratification that decrease the abundance of fish larvae due to a decreasing in food supply, avoidance of salt water or larval flushing out of the estuary (Ramos et al., 2006)

These comparisons emphasizes that larval species might be affected differently by the seasonal fluctuation of environmental variables in accordance with their ecological guilds (Drake and Arias, 1991; Strydom et al., 2003; Potter et al., 2013). However, these comparisons must take into account differences not only in the sampling design and effort, but also in how the abiotic environment has been influenced by geomorphology, tidal amplitude, freshwater flow and anthropic factors (Blaber et al., 1997; Barletta and Barletta-Bergan, 2009; Lacerda et al., 2014).

4.2. Seasonal distribution of microplastics and the effects of their interaction with fish larvae

Microplastics might be introduced in the Goiana Estuary by the runoff of large or previously fragmented plastics from surrounded areas, such as river basin, mangrove forest and adjacent beaches due to domestic, recreational and artisanal/commercial fishery activities (Possatto et al., 2011; Ramos et al., 2012; Lima et al., 2014). Another source can be the ocean. In addition, the mangrove forest function as a pathway for microplastic contamination, due to less anthropogenic impacts (Ivar do Sul et al., 2014; Lima et al., 2014). However, studies has been suggesting that the main source of plastic fragments for the Goiana Estuary is fishery (Barletta and Costa, 2009; Dantas et al., 2012; Guebert-Bartholo et al., 2011). The weathering breakdown of large plastics will generate fragments to the size of microplastics (<5 mm), whose presence is able to cause harm to the environment and biota (Barnes et al., 2009; Thompson et al., 2009).

For the Goiana Estuary, microplastics were found everywhere during the entire year, representing half of fish larvae density. Although, during specific times microplastics surpassed the total ichthyoplankton density. Microplastics presented lowest densities in the middle estuary (2.1 items $100m^{-3}$), and were well represented in the upper and lower estuary (6.5 and 17.5 items $100m^{-3}$, respectively). It indicates that during most part of the year, when rainfall rates are low (dry season and early rainy season), the meeting of waterfronts in the middle estuary forms a barrier that does not allow the passing of microplastics from the upper to the lower estuary and also in opposite direction (Lima et al., 2014; Watanabe et al, 2014). However, during the late rainy season, the highest river flow during induces the seaward flushing of microplastics and points the

Goiana Estuary as a source of debris to the ocean (Moore et al., 2011). Microplastics presented highest mean density in bottom waters of the lower estuary, exceeding five times fish larvae density. All types of microplastics (hard, soft, paint chips and threads) presented strong positive correlation with high rainfall rates. Differently, fish larvae, whose density increased seaward, presented positive correlation with dissolved oxygen, temperature and, especially, with seasonal variations in salinity and rainfall. As such, this study indicates that microplastics drift following the main water movement, being strictly associated to flushing out/into the estuary than to seasonal variation in environmental variables (Lima et al., 2014).

Microplastics are ubiquitous available in the main channel of the Goiana estuary, negatively affecting prey-predator relations (Barnes et al., 2009; Cole et al., 2011; Wright et al., 2013). Studies have suggested that planktonic organisms, as well as their predators, can feed on microplastics and promote the trophic transfer of this class of debris throughout the food web (Possatto et al., 2011; Dantas et al., 2012; Lusher et al., 2013; Besseling et al., 2014; Chua et al., 2014; Setälä et al., 2014; Sá et al., 2015). Whereas microplastics are within the plankton of the Goiana Estuary, the main concern of this study is that the assurance of high food supply attract predators that can easily feed on plastic debris of the same size and shape as their natural prey (Barnes et al., 2009; Boerger et al., 2010; Cole et al., 2011; Wright et al., 2013). In this study, for example, flexion and pre-flexion larvae (35.75%) can prey on microplastic, especially those smaller than 1 mm (~40%), which are similar in shape and colour to zooplankton prey. Besides the effects caused by eating microplastics contaminated with persistent organic pollutants (POPs), biocides and trace metal, ingestion might cause gut blockage and induce starvation (Moore, 2008; Frias et al., 2010; Turner, 2010; Cole et al., 2013).

In this study, larval species of different ecological guilds might be affected differently by the seasonal migration of the salt wedge in the main channel of the Goiana estuary. However, microplastics remains retained in the upper and lower portion most part of the year. Meanwhile, during the late rainy season, when the environment is under influence of the highest river flow, microplastics from the upstream zones drift together with the plankton to the lower portion of the estuary, following the main water movement. This paper shows that the densities of microplastics and fish larvae have the same order of magnitude in the water column, what increase the chances of interaction between the species and this class of debris. Further studies regarding the seasonal distribution of living plankton and their interaction with non-living particles, such as microplastics, are

required to a detailed understanding on how these debris are affecting the use of South American estuaries by fish species.

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CAPÍTULO 2

Distribution patterns of microplastics within the plankton of a tropical estuary

Distribution patterns of microplastics within the plankton of a tropical estuary

A. R. A. Lima, M. F. Costa, M. Barletta[a]*

Laboratório de Ecologia e Gerenciamento de Ecossistemas Costeiros e Estuarinos, Departamento de Oceanografia, Universidade Federal de Pernambuco, CEP 50740-550, Recife, Brazil

*Author to whom correspondence should be addressed: Tel. and fax: 00550218121267223; email: mario.barletta@pq.cnpq.br

Abstract

The Goiana Estuary was studied regarding the seasonal and spatial variations of microplastics (< 5mm) and their quantification relative to the zooplankton. The total density (n $100m^{-3}$) of microplastics represented half of the total fish larvae density and was comparable to fish eggs density. Soft, hard plastics, threads and paint chips were found in the samples (n = 216). Their origins are probably the river basin, the sea and fisheries (including the lobster fleet). In some occasions, the amount of microplastics surpassed that of Ichthyoplankton. The highest amount of microplastics was observed during the late rainy season, when the environment is under influence of the highest river flow, which induces the runoff of plastic fragments to the lower estuary. The density of microplastics in the water column will determine their bioavailability to planktivorous organisms, and then to larger predators, possibly promoting the transfer of microplastic between trophic levels. These findings are important for better informing researchers in future works and as basic information for managerial actions.

***Keywords*:** estuarine trophic web, plastic ingestion, risk, physical harm

1. Introduction

Plastics have been discussed for decades as being the principal component of the marine debris polluting every habitat in the marine environment (Bergmann and Klages, 2012; Costa et al., 2011; Moore et al., 2001; Moore, 2008; Thornton and Jackson, 1998) from the equator to the poles (Barnes et al., 2009). They originate mainly on land, where excess usage creates disposal problems (Thompson et al., 2009). The durability and low recycling rates of plastic materials resulted in their accumulation after accidental release, natural disasters and inadequate disposal habits (Watters et al., 2010). Transport by winds and rivers permits entire plastic items and debris to enter the marine environment (Wright et al., 2013). Because of their low degradability rates and efficient buoyancy, plastics travel over long distances reaching habitats away from their generating sources, even remote areas as mid-ocean islands (Ivar do Sul et al., 2013) and ocean depths (Bergmann and Klages, 2012; Lozano and Mouat, 2009). However, during their time at sea, plastics fragment into microplastics (< 5mm).

Plastic fragments enter estuaries either by land runoff and from the ocean through wind, waves and the tidal flow (Le Roux, 2005; Nordstorm et al., 2006) They might also be fragmented in situ by the physical dynamics that dominate the environment (Barnes et al., 2009). Once plastic fragments reach the estuary, they will be found almost in any habitat (Browne et al., 2010; Thornton and Jackson, 1998). Low-density plastics are usually submerged when they meet water fronts (Cole et al., 2011); small particles are transported by the flow of water and are deposited in areas where the movements of water is less intense (Dalrymple et al., 1992), such as inter-tidal plains and the mangrove forest (Costa et al., 2011).

Since many microplastics are buoyant (Barnes et al., 2009; Cole et al., 2011), they will be widely available to planktonic organisms, and to a host of larval stages of many commercially important species and their natural prey (Boerger et al., 2010; Fendall and Swell, 2009; Gregory, 1996). Meanwhile, whereas estuaries are eutrophic environments, highly abundant fouling organisms, such as algae and invertebrates, may attach to buoyant microplastics and cause them to sink (Barnes et al., 2009; Browne et al., 2010; Cole et al., 2011; Moore et al., 2001). On the bottom some pieces can be buried, suffer stern physical and chemical degradation and be immobilized by sediments, remaining in the environment for a long period (Costa et al., 2011). Other pieces may enter the food chain when ingested by benthic and demersal fauna (Browne at al., 2008; Graham and Thompson, 2009; Iribarne et al., 2000; Moore, 2008; Thompson et al., 2004), including

benthophagous fish (Dantas et al., 2012; Possatto et al., 2011; Ramos et al., 2012). A third option is to be exported out to sea.

Little is known about fragmentation dispersion and deposition of microplastics in estuarine ecosystems (Browne et al., 2010; Costa et al., 2011; Thornton and Jackson, 1998). This study assessed whether microplastics vary seasonally and spatially along the salinity gradient of the main channel of the Goiana River estuary, and their relative composition in relation to the whole plankton present in the system.

2. Material and methods

2.1. Study area

The Goiana Estuary is an environment of tropical semi-arid climate located on the Northeast coast of Brazil (7°32'–7°35'S; 34°50'–34°58'W) (Fig. 1). Its main channel has 17 km and the estuarine floodplain covers 4700 ha in total area. The rainfall rates (RR) of the region allow the characterization of four seasonal periods: early dry (ED: September to November – RR: 4.2 - 58.4 mm), late dry (LD: December to February – RR: 40.7 – 145 mm), early rainy (ER: March to May – RR: 38 – 144 mm) and late rainy (LR: June to August – RR: 83.9 – 372.8 mm) (Barletta and Costa, 2009). The great diversity of habitats, including the main channel, floodplain and mangrove forest, supports a rich fauna of fish, crustaceans, and mollusks. These groups, in turn, are subjected to fishery all along the year determining the subsistence of traditional populations (Barletta and Costa, 2009). The study area was divided into three regions (upper: U, middle: M and lower: L) according to the salinity (ED- U: 0.3 – 9.8, M: 7.5 – 20.2, L: 21.5 – 30.1; LD- U: 5.4 – 9, M: 15 -24.4, L: 28.6 – 32; ER- U: 0.2 – 6.8, M: 8.1 – 20.3, L: 21 – 33.8; LR- U: 0 – 0.05, M: 0.01 – 2.5, L: 6.5 – 21.4) gradient and the geomorphology of the estuary.

2.2. Sampling

Samples (n = 216) were taken monthly during neap tide cycles from April 2012 to March 2013. Plankton samples were collected towing a conical plankton net (300 μm; Ø 0.6 m; 2m long) for 15 min, in both surface and bottom water hauls, at an average speed of 2.7 knots. A flowmeter (General Oceanics - Model 2030 Digital Series) was used to calculate the volume filtered per tow. A GPS (Ensign GPS Trimble Navigation) determined the sampling position and an echo sounder (Eagle Supra Pro D) registered the depth. Three superficial (depth between 0–3 m) and three bottom (3–6 m) water samples

replicates were taken in each reaches of estuary (U, M and L). Water temperature (◦ C), dissolved oxygen (mg l^{-1}) (Wissenschaftlich Technische Werkstätten, WTW OXI 325; www.wtw.com) and salinity (WTW LF 197) were recorded before the beginning of each sampling, from both surface and bottom waters. Samples were preserved in buffered formalin (4%) in their own estuarine water.

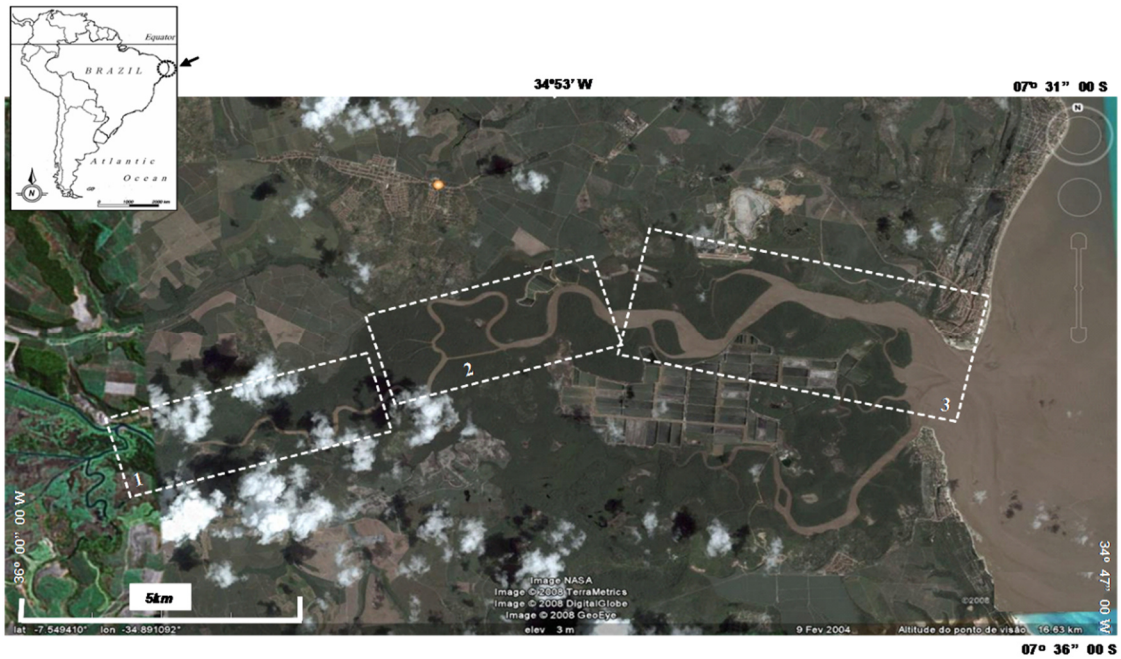

Figure 1. Goiana Estuary. ⬚ = (1) upper, (2) middle and (3) lower portions of the estuary. Source: Google Earth (2013).

2.3. Laboratory procedures

Microplastics, fish larvae and fish eggs were separated from the entire sample and then their counts were converted to a standard volume of 100 m^3. Floating plastic fragments found in the static sample, were first sieved through a 45 µ mesh. Samples were divided into small aliquots to facilitate the separation of sunk plastic fragments involved in the organic matter with the aid of a stereomicroscopy – ZEISS; STEMI 2000-C (x5). Microplastics were oven dried at 60°C and classed per type as plastic, threads and paint chips (Fig. 2). Characteristics as hard or soft plastic and the colour of each item were also registered. Occasionally, larger plastic items (> 5 mm) were found in the samples, but they were not included in the analysis. Digital measurements for microplastics were made with the aid of a digital camera (Canon-Powershot G10) attached to the trinocular stereomicroscopy and the software AxioVision Release 4.7.2 (image capturer calibrated with a millimeter scale in all micrometer zooms that converts the image pixels in millimeter). For counting the zooplankton, each sample was diluted

to 700 mL and homogenized through random movements. Three subsamples of 10 mL were removed using a Stempel pipette, with reposition (Postel et al., 2000). Each taxon was identified to large groups (Boltovskoy, 1981, 1999) and counted separately from the three aliquots to calculate the means. Means were extrapolated to the entire sample of 700 mL and their counts were also converted to a standard volume of 100 m^3.

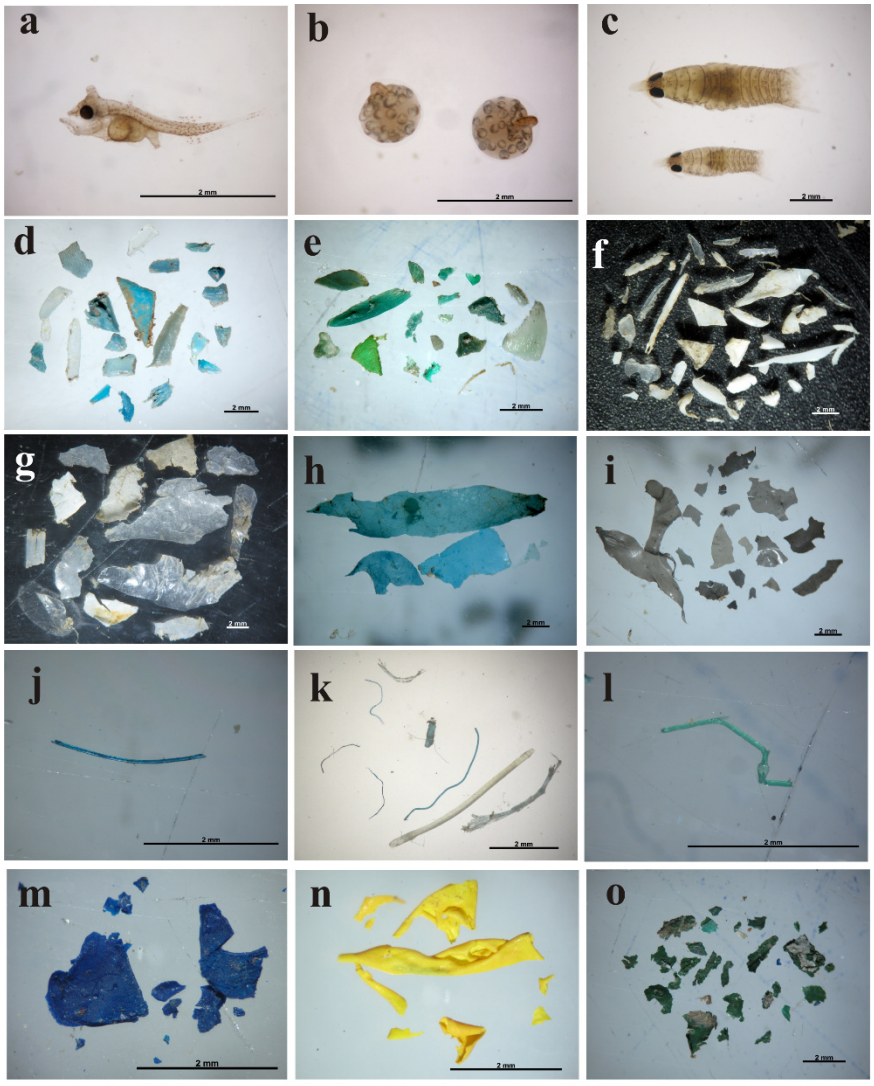

Figure 2. Examples of linving plankton and microplastics found in the main channel of the Goiana Estuary. (a) fish larvae, (b) fish eggs,(c) zooplankton (isopod), (d-f) hard plastic, (g-i) soft plastic, (j-l) threads, (m-o) paint. Images captured with a digital camera Canon PowerShot G10 coupled to a stereomicroscope - ZEISS; STEMI 2000-C.

2.4. Statistical analysis

Three superficial and three bottom water samples per area/month were considered as replicates and were used to test the proposed hypothesis. The factorial analysis of variance

(ANOVA), with a 5 % level of significance, was performed to assess whether the distribution and density of microplastics, fish larvae and fish eggs varies with space (U, M and L), time (dry and rainy seasons) and water column depth (surface and bottom) (Zar, 1996). The Cochran's test was used to check the homogeneity of variances. The original data were Box-Cox transformed (Box and Cox, 1964) to ensure it conformed to a normal distribution. Bonferroni's test ($p < 0.05$) was used whenever significant differences were detected (Quinn and Keough, 2002).

A cluster analysis, based on Euclidian distance, was used to check how microplastics, fish larvae and fish eggs are distributed along the estuary using the areas of the estuary, seasons and water column depth as attributes (Clarke and Gorley, 2006). Another similarity matrix was computed for abiotic factors (Rainfall, salinity, water temperature and O_2) considered as attributes (Clarke and Gorley, 2006). Clusters were determined based on similarity matrix using Euclidian distance, with distances calculated by group-average sorting and ranked similarities.

A canonical correspondence analysis (CCA) (CANOCO for Windows 4.5) was performed to observe ecological correlations (ter Braak and Smilauer, 2002). A multiple least-squares regression was computed with the site scores [derived from weighted averages of microplastics (soft and hard plastic, threads and paint chips), fish larvae and fish eggs and of the groups] as the dependent variables and the environmental parameters (rainfall, water temperature, dissolved oxygen and salinity) as the independent variables (ter Braak, 1986; Palmer, 1993). The CCA was run with 100 iterations with randomized site locations to facilitate Monte-Carlo tests between the eigenvalues and species–environment correlations for that each axis that resulted from CCA and those expected by chance. With this procedure, a biplot is produced where the environmental variables appear as vectors radiating from the origin of the ordination. The length of the vector is related to the relationship power between the environmental variable that the vector represents and the groups, for each season.

3. Results

3.1. Environmental variables

The four defined seasons for the region, early dry (September to November), late dry (December to February), early rainy (March to May) and late rainy (June to August) are indicated on Figure 3 for all environmental variables. Salinity and water temperature present trends related to these four seasons. When rainfall reaches its highest values, in

June, salinity drops to 0 in the upper and middle estuary, and the lower estuary shows its lowest salinity value (5) (Fig. 3b). The upper estuary always presents the lowest salinity values (0 – 9), whereas the lower estuary had the highest value (35). Water temperature showed the same trends as salinity, with the lowest value (June) in the upper estuary (24°C) and the highest (March) in the lower estuary (31°C) (Fig. 3c). Dissolved oxygen was highest in the lower estuary, while the upper and middle estuaries presented lower values (Fig. 3d). The lowest value was registered in the upper estuary in January (3.5 mg L^{-1}), while the highest was observed in May, but in the lower estuary (8 mg L^{-1}) (Fig. 3d).

The Cluster analysis distinguished two groups independent of the water column depth (Fig. 4). Group I, comprised all the three areas during the dry season (early and late) plus the early rainy season and was distinguished from the other group by lower rainfall values and higher water temperatures. Group II consisted of the three areas of the estuary during the late rainy season and was distinguished by higher rainfall values and lower waters temperatures. The first group (I) was divided into two subgroups. The first subgroup (I – A) consisted of the three areas during the early dry season, and was distinguished by the lowest rainfall values (Fig. 4). The second subgroup (I – B) comprised the three areas during the early rainy and late dry season and was distinguished from I – A by higher rainfall values. This subgroup was further subdivided into two smaller groups. The first II – B1, consisted of the lower and middle estuary during the early rainy and late dry seasons, and was distinguished by higher water temperature and salinity. The second II – B2, consisted of the upper estuary during the late dry and early rainy seasons, with lower salinity values (Fig. 4).

3.2. Distribution of microplastics items

A total of 14,724 items of microplastic (26.04 items.$100m^{-3}$) with mean size of 2.23±1.65mm were recorded from the 216 samples taken during 12 months. From these, 41.08% were soft plastic, 29.11% paint chips, 28.42% hard plastic and 1.4% threads. The most representative soft and hard plastics were white, green and blue; threads were red and blue; and paint chips were blue, green and yellow (Table 1). Large portions of the microplastics were found with signs of fouling organisms. In addition, primary plastic pellets (typically 2-5 mm in diameter) were absent in the samples. Plastics contributed with 0.18% of the whole plankton catch in the three areas (upper, middle and lower) of the main channel in the Goiana Estuary. Fish larvae and eggs contributed with 0.37 and 0.22%, respectively.

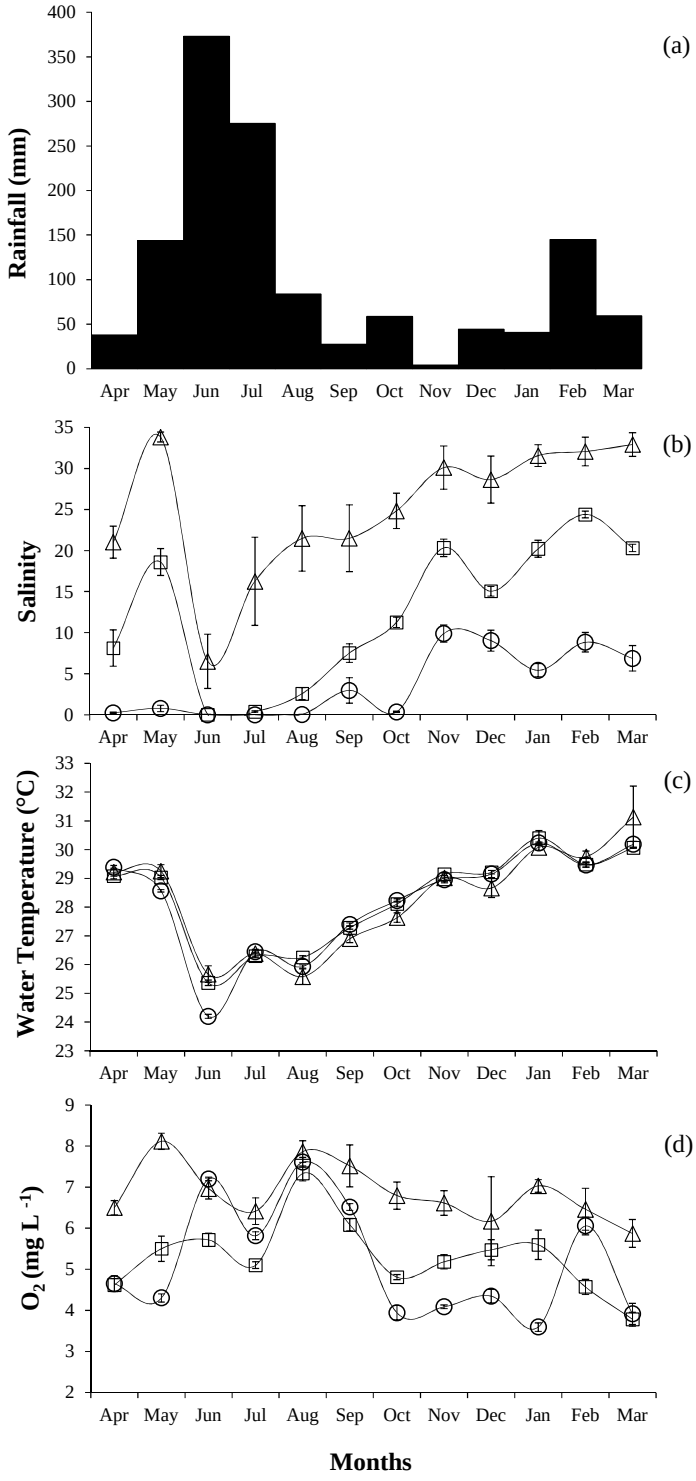

Figure 3. (a) Total monthly rainfall and means (± S.D.) of (b) salinity, (c) water temperature, and (d) dissolved oxygen in the three areas [(○) upper, (□) middle, (Δ) lower] of the Goiana Estuary from April 2012 to March 2013.

Zooplankton represented 99.2% (Table 1). The total density of microplastic represented almost half of the total fish larvae density. During the late rainy season was

observed the highest density of microplastics (5.1% of total plankton) in the lower estuary, equivalent to the density of Ichthyoplankton (5.2% of total plankton) in the same period and region of the estuary (Table 1).

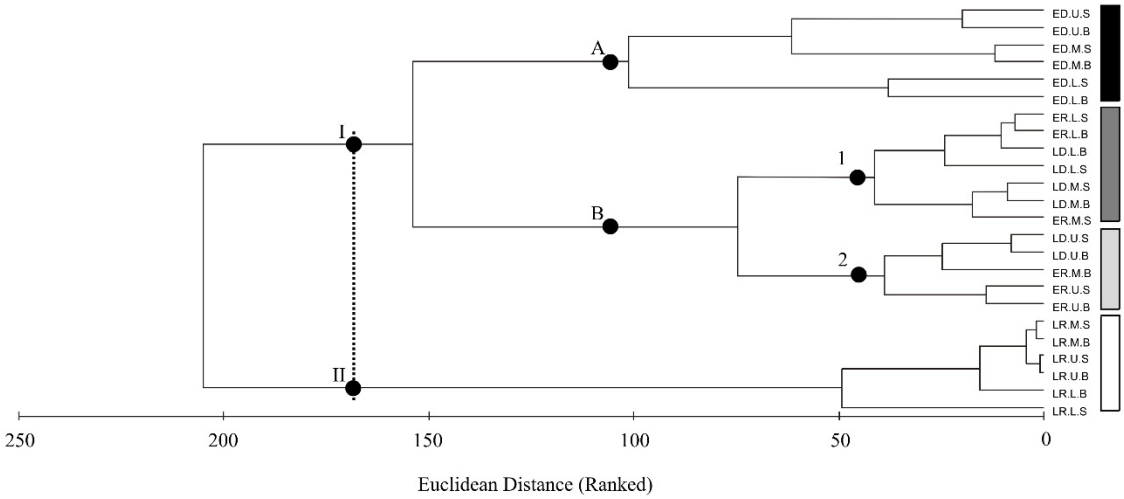

Figure 4. Cluster dendrogram based on similarities of environmental variables (rainfall, salinity, water temperature and dissolved oxygen) of the samples measured in the Goiana Estuary. Each object corresponds to the seasons (ER, early rainy; LR, late rainy; ED, early dry; LD, late dry) areas (U, upper; M, middle and L, lower estuary) and depth of water column (S, surface; B, bottom) where the samples were taken. Samples were clustered by group average of ranked Euclidean similarity index. I – II, groups; A, B, 1, 2, subgroups.

Results from the ANOVA showed that hard plastics occurred with higher densities in the upper estuary during all year (Fig. 5); but presented the highest density ($p < 0.01$) in bottom waters of the lower estuary during the late rainy season (Fig. 5 and Table 2). The lowest densities of hard plastics were observed in bottom waters of the lower estuary during the early rainy and early dry seasons (Fig. 5). In turn, soft plastics appear in highest densities in bottom waters of the lower estuary during all year, with peaks ($p < 0.01$) in the late rainy and late dry seasons (Fig. 5). Another peak of density of soft plastic was observed in the upper estuary during the late rainy season in surface waters (Fig. 5 and Table 2). The lowest densities of soft plastics were observed in bottom waters of the lower estuary during the early dry season (Fig. 5).

Threads represented the less abundant items in the whole estuary and did not significantly differ among seasons, areas and water column depth. However, peaks in the late rainy and late dry season could be observed in surface waters of the lower estuary

Table1. Density of the planktonic components (microplastics, ichthyoplankton and zooplankton) from the Goiana Estuary during different seasons (ER, early rainy; LR, late rainy; ED, early dry; LD, late dry) and areas (upper, middle and lower). The density of each item was adjusted to a standard volume of 100 m^{-3}. Bold number: sub-total densities.

			Density (%)											
	Total Density		**Upper**				**Middle**				**Lower**			
Items	**(No. 100 m^{-3})**	%	**ER**	**LR**	**ED**	**LD**	**ER**	**LR**	**ED**	**LD**	**ER**	**LR**	**ED**	**LD**
Plastic debris														
Sof plastic	10.703	0.08	0.09	2.02	0.10	0.11	0.02	0.15	0.01	<0.01	0.03	2.72	<0.01	0.02
Paint chips	7.584	0.06	0.10	0.38	0.03	1.98	0.03	0.06	<0.01	<0.01	0.02	1.12	<0.01	0.03
Hard Plastic	7.404	0.05	0.37	2.90	0.27	0.21	0.02	0.18	0.01	<0.01	0.01	1.25	<0.01	<0.01
Threads	0.364	0.00	<0.01	0.05	0.01	0.03	<0.01	0.03	<0.01	<0.01	<0.01	0.02	<0.01	<0.01
Sub-total density (A)	**26.06**		**1.33**	**1.72**	**1.01**	**2.50**	**0.37**	**0.74**	**0.47**	**0.49**	**0.8**	**13.98**	**0.39**	**2.27**
Ichthyoplankton														
Fish larvae	51.031	0.37	0.73	2.16	1.75	1.75	0.98	1.48	0.26	0.03	0.28	4.04	0.17	0.32
Eggs	30.487	0.22			0.01	0.01	0.01	0.01	0.01	0.01	0.37	1.15	0.44	0.38
Sub-total density (B)	**82**		**1.71**	**0.69**	**4.38**	**1.88**	**5.09**	**2.68**	**3.59**	**1.61**	**8.2**	**14.20**	**9.93**	**27.95**
Zooplankton														
Naupli of Cyrripedia	8223.433	60.15	0.33	0.99	10.72	9.64	19.06		83.36	92.12	25.75	24.01	74.42	45.82
Hydromedusa larvae	2762.140	20.20				2.38	43.68		7.93	2.35	55.12	8.37	10.50	35.99
Zoea of Brachyura	848.645	6.21	62.96	67.79	74.47	74.02	12.61	10.56	5.07	2.27	2.42	6.56	3.90	1.55
Copepod Calanoida	754.782	5.52	2.44	14.69	10.05	5.61	12.08	85.35	2.37	2.24	5.88	41.31	7.16	1.92
Appendicularia	721.102	5.27				1.37	11.06		0.89	0.92	9.13	0.41	2.28	11.48
Molusc larvae	102.605	0.75									0.04	1.09	0.10	2.42
Panaeidae Larvae	76.706	0.56	22.30	6.89	2.03	2.91	0.12	1.96	0.03	0.05	0.06	0.60	0.31	0.01
Amphipoda spp	38.937	0.28	10.66	2.12	0.42		0.17	0.23		0.01	0.73	0.39	0.03	
Zoea of Euphasidae	19.012	0.14									0.06	3.27	0.50	0.03
Chaetognata	8.223	0.06									0.07	2.37		0.02
Mysis *Lucifer faxoni*	6.246	0.05									0.03	1.18	0.17	
Isopoda spp	2.274	0.02			0.15		0.17		0.05			0.13		
Sub-total density (C)	**13564.1**		**231.0**	**29.7**	**244.2**	**103.4**	**511.9**	**176.8**	**1369.3**	**3796.5**	**1248.2**	**245.6**	**1613.2**	**3996.2**
Total density (A+B+C)	**13671.7**		**234.1**	**32.1**	**249.6**	**107.8**	**517.3**	**180.2**	**1373.4**	**3798.5**	**1257.1**	**273.8**	**1623.5**	**4026.4**

(Fig. 5 and Table 2). The lowest values of density of this item were observed in the middle estuary during the early dry season (Fig. 5).

Paint chips occurred in low densities in the three areas of the estuary during all year. The highest density ($p < 0.01$) of paint chips was observed in bottom waters of the lower estuary during the late rainy season (Fig. 5). Another peak ($p<0.01$) was also observed in the upper estuary during the late dry season in surface waters (Fig. 5 and Table 2).

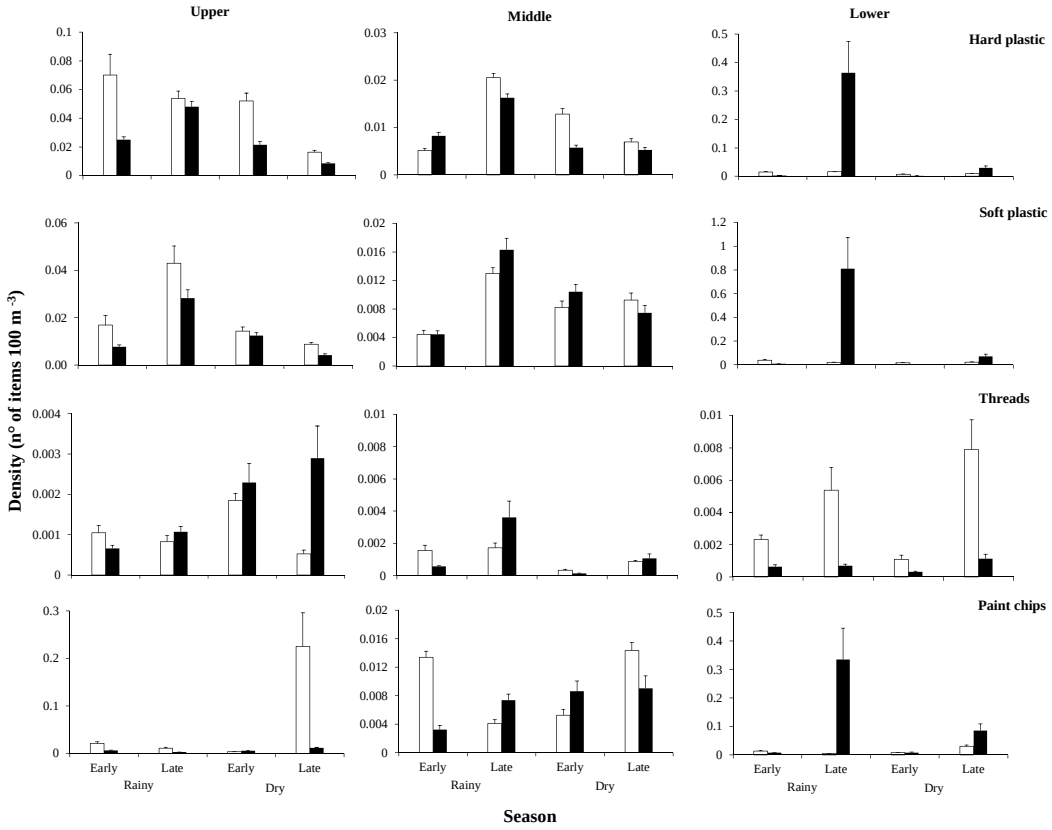

Figure 5. Total mean (± S.E.) density of microplastics (hard plastic, soft plastic, threads, paint) in the different water columns [(□) surface; (■) bottom] of the three areas of the Goiana Estuary (upper, middle, lower) for each season (early and late dry; early and late rainy).

3.3. Distribution of total microplastics and Ichthyoplankton

The ANOVA showed that the total density of microplastics, fish larvae and eggs differed significantly among seasons, areas and depth of water column (Table 2). The interactions season *vs.* area, season *vs.* water column and area *vs.* water column were also significant for these variables ($p < 0.01$) (Table 2). Such interactions suggest that

seasonality and the depth of the water column where microplastics, fish larvae and eggs are found influences the distribution of these variables in the main channel of the estuary.

Table 2. Summary of the ANOVA results for the density (n° 100 m^{-3}) of each microplastics and total of microplastic, fish larvae and fish eggs. Analysis performed on Box-Cox transformed data. Differences among seasons, areas and water column were determined by Bonferroni's test *post hoc* comparisons. Seasons: ER, early rainy; LR, late rainy; ED, early dry; LD, late dry. Areas of the Goiana Estuary: UE, upper; ME, middle; LE, lower. Water column: SUF, surface; BOT, bottom. ns, not significant; ** $p < 0.01$.

	Source of variance			**Interactions**		
Variables	**Season (1)**	**Area (2)**	**Water column (3)**	**1x2**	**1x3**	**2x3**
Hard plastic	** **LD ED ER LR**	** **ME LE UE**	ns	ns	ns	**
Soft plastic	** **ED ER LD LR**	** **ME UE LE**	ns	ns	ns	ns
Threads	ns	ns	ns	ns	ns	ns
Paint chips	** **ED LR ER LD**	ns	ns	ns	ns	ns
Total of microplastics	** **ED ER LD LR**	** **ME LE UE**	** **SUF BOT**	**	**	**
Total of fish larvae	ns	** **UE ME LE**	** **SUF BOT**	**	**	**
Total of fish eggs	** **LR ER ED LD**	** **UE ME LE**	** **SUF BOT**	**	**	**

The highest density ($p < 0.01$) of total microplastics occurred in bottom waters of the lower estuary during the late rainy season (Fig. 6 and Table 2). The lowest densities of microplastics were observed in the lower estuary during the early rainy and early dry seasons (Fig. 6). Fish larvae differed among areas and water column, but did not differ among seasons (Table 2). Fish larvae presented higher values of density mainly in the lower estuary with peaks ($p < 0.01$) in the late rainy season in surface waters and in the late dry seasons in bottom waters (Fig. 6). The highest densities of fish eggs were observed in the lower estuary, being more abundant in surface waters, with a peak ($p < 0.01$) during the late dry season (Fig. 6 and Table 2).

In the upper estuary microplastics occurred with higher density than fish larvae during the late dry season in surface water, while in the bottom microplastics occurred with higher density than fish larvae in the late rainy season (Fig. 6). In the middle estuary microplastics and fish larvae occurred in the same proportion along the year in surface waters, however in the bottom fish larvae presented higher densities than microplastics (Fig. 6). For the lower estuary, microplastic, fish larvae and fish eggs occurred in the same proportion in the early rainy season in surface waters, while in the bottom microplastics presented higher density than fish eggs and fish larvae in the late rainy season (Fig. 6).

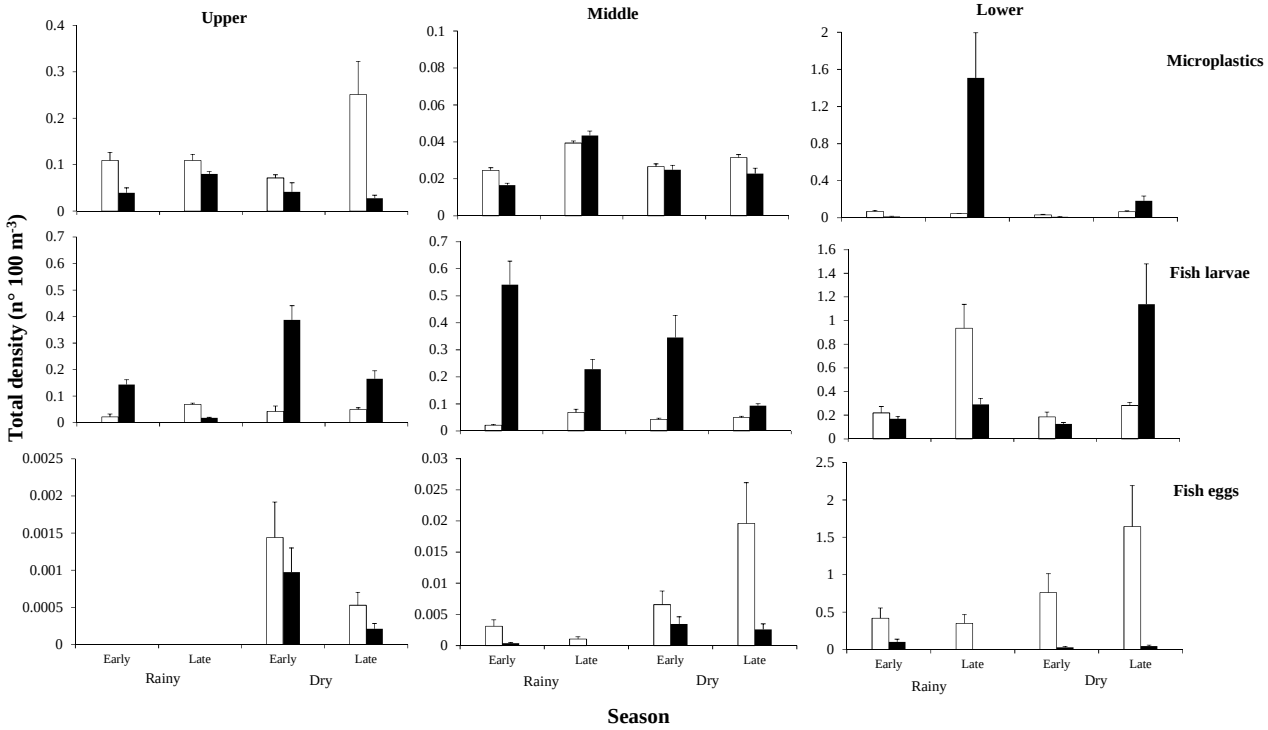

Figure 6. Total mean (± S.E.) density of plankton (microplastics, fish larvae, fish eggs) in different depths [(□) surface; (■) bottom] of the three areas of the Goiana Estuary (upper, middle, lower) for each season (early and late dry; early and late rainy).

Cluster analysis distinguished two main groups (Fig. 7). Group I consisted of fish eggs and was distinguished by their higher densities in the lower estuary, mainly in the late dry season. Group II comprised fish larvae, soft and hard plastics, and paint chips and was distinguished from the other group because they were well represented along the three areas of the estuary and presented a peak in the lower estuary during the late rainy season. This group was divided into two subgroups (Fig. 7). The first sub-group (II – a) consisted of plastic items with lower densities in the upper and middle estuary. The

second subgroup (II – b) comprised the fish larvae and was distinguished from the first subgroup by their higher densities in the upper and middle estuary.

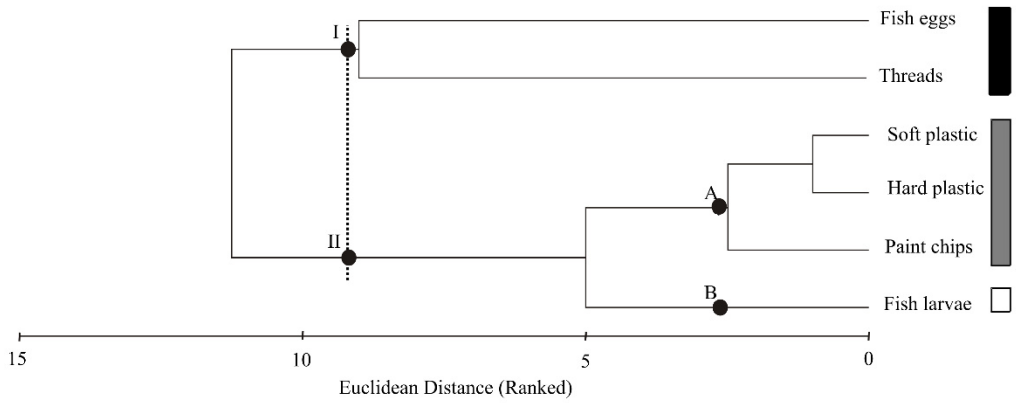

Figure 7. Cluster dendrogram based on similarities on the composition of the plankton (microplastics and ichthyoplankton) in the Goiana Estuary using the areas, seasons and water column as attributes. Samples were clustered by group average of ranked Euclidean similarity index. I – II, groups; a, b, subgroups.

3.4. Correlation of microplastics, fish larvae and fish eggs with environmental variables

The CCA was performed to determine the influence of environmental variables on the distribution pattern of microplastic, fish larvae and fish eggs in the main channel of the Goiana estuary (Fig. 8 and Table 3). The first axis explained 69.1% and the second axis explained 28.1% of the variance of the species-environment relation. The first axis represents the estuarine gradient. The lower estuary is represented by the right side of the figure and the upper the left side. The second axis represents the seasonality. The first axis showed positive correlation with dissolved oxygen ($p < 0.01$) and salinity ($p < 0.01$). Soft and hard plastic showed significant correlation with high rainfall value in the three areas of the estuary during the late rainy season, in surface and bottom waters. Paint chips and threads also showed correlation with rainfall in the upper estuary during the early and late dry and early rainy season in both surface and bottom waters. Moreover, paint chips and threads showed correlation with the middle estuary during the early and late dry and late rainy seasons in surface waters. Fish larvae showed correlation with high temperatures in the middle estuary during the early and late dry seasons in bottom water and during the early rainy season in both surface and bottom water. Fish eggs were strongly correlated with the lower portion of the estuary during the early and late dry seasons and early- rainy season in surface waters, and then during the early dry and early

rainy season in bottom waters. The factors Salinity, temperature and dissolved oxygen showed correlation with fish eggs.

4. Discussion

4.1. Sources of microplastics and environmental contamination

Microplastic contamination spreads throughout the whole system of the Goiana Estuary along the entire year. Soft and hard plastics were well represented in the upper and lower estuary. However, in the middle estuary they occurred in lower density values during all year.

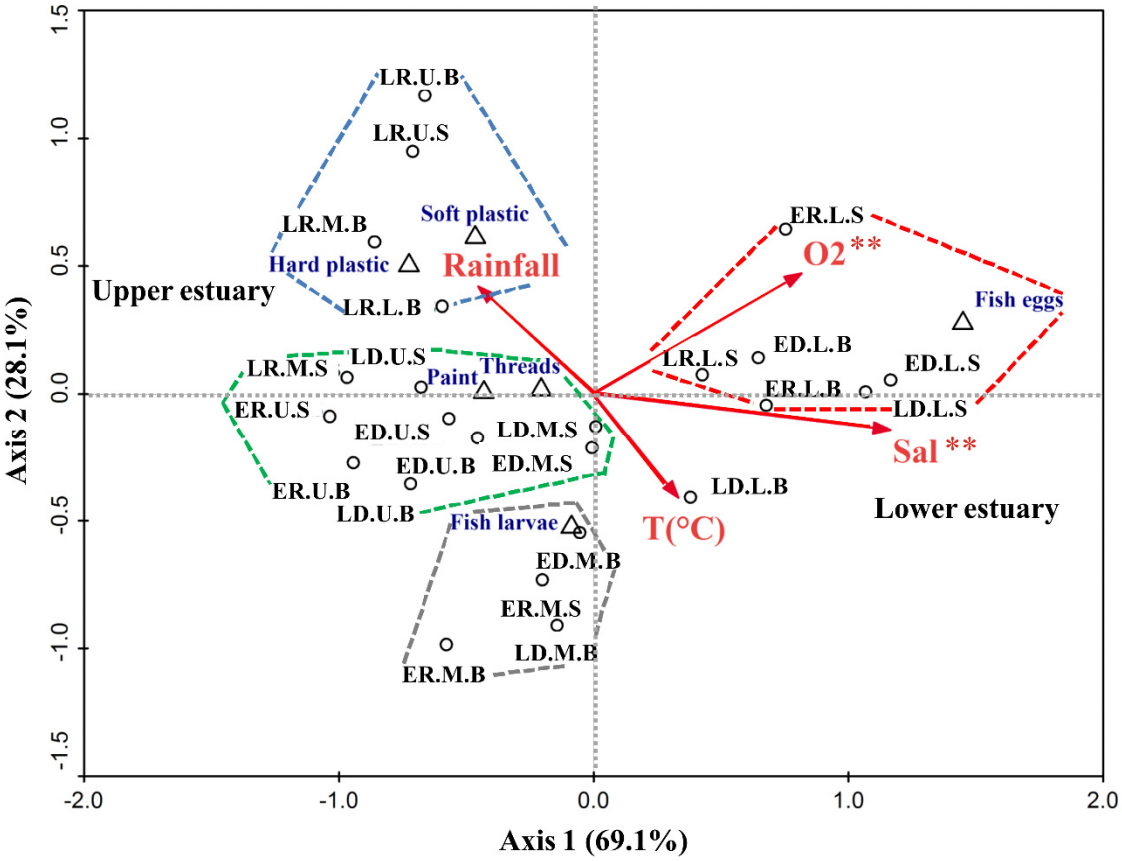

Figure 8. Canonical correspondence analysis (CCA) triplot for the ecological correlations between microplastics, fish larvae and fish eggs and the environmental variables. Circles (○) represent the three areas (U, upper; M, middle; L, lower) of the main channel of Goiana estuary in each season (ER, early rainy; LR, late rainy; ED, early dry; LD, late dry) and depth of water column (S, surface; B, bottom). Triangles (Δ) represent microplastics (soft and hard plastic, threads and paint chips), fish larvae and fish eggs. The environmental variables (rainfall, dissolved oxygen, salinity, temperature) were represented by arrows. * $p < 0.05$.

Table 3. Summary of canonical correspondence (CCA) analysis using four environmental variables (rainfall, water temperature, dissolved oxygen, salinity) and density of microplastics (soft and hard plastic, threads and paint chips), fish larvae and fish eggs groups in the main channel of the Goiana estuary. * $p < 0.05$; ** $p < 0.01$

	Axis 1	**Axis 2**	***p* value**
Eigenvalue	0.07	0.028	
Species-environment correlation	0.808	0.773	
Cumulative % variance of species data	35.1	49.3	
Cumulative % variance of species-environmental variables	69.1	97.2	
Rainfall	-0.3506	0.3610	0.089
Water temperature	0.2564	-0.3519	0.128
Dissolved oxygen	0.6306	0.4049	0.009**
Salinity	0.8996	-0.1206	0.009**

It is already known that during the dry season (stable hydrological conditions) the upper and middle portion of the estuary is a transition region between fresh water and marine costal water, generating turbulence and creating stratification in the water column of the middle portion of the Goiana Estuary (Dantas et al., 2010). It seems that this physical behaviour does not allow the passing of microplastics from the upper to the lower estuary. In addition, transport in the opposite direction (upstream) seems equally difficult. This suggests that the origin of microplastics might be associated to different sources. The origin of microplastics from the upper estuary is, probably, associated to the river basin (Costa et al., 2011; Ramos et al. 2012). Furthermore, it is also possible that microscopic size plastic items, associated with river basin contamination, are also polluting the environment. For example, primary plastics used in the formulation of facial cleansers and cosmetics, generally in the form of micro spheres, may enters the estuary by sewage transport, after domestic use (Cole et al., 2011; Fendall and Swell, 2009). On the other hand, microplastics found in the lower estuary seem to have a local and/or marine origin associated to coastal villages/harbours fishery activities in the lower estuary and adjacent waters (Barletta and Costa, 2009). For example, mussel pickers, by digging the sediment, can cause the exhumation of buried plastic items (Costa et al., 2011).

In the case of the Goiana Estuary, fishery has been appointed as the main source of plastic fragments (Dantas et al., 2012; Guebert-Bartholo et al., 2011; Ramos et al., 2012). The constant activity of fishermen during the maintenance of gears (*e.g.*, nets mending) generate microplastics (Browne et al., 2010; Cole et al., 2011). Ropes, handlines or nets,

when left behind or lost by fishermen, will degrade in the environment and release threads and fragments to the size of microplastics (<5 mm) (Possatto et al., 2011; Ramos et al., 2012). Threads had their highest density in the lower estuary in surface waters, but they were well represented in the whole system during the entire year. For this estuary, threads have received special attention taking into account their predominance over other types of fragments found in the gut of demersal fish (Dantas et al., 2012; Possatto et al., 2011; Ramos et al., 2012). In fact, 20% of juvenile Ariidae catfishes that use this estuary, as nursery ground, had ingested threads (Possatto et al., 2011). Moreover, 13.4% of Gerreidae mojarras were found to have threads in their stomachs (Ramos et al., 2012). Rencently, 8% of Scianidae drums were found with blue threads in their gastrointestinal contents (Dantas et al., 2012). In this study, at the middle estuary, threads presented higher density values during the late rainy season when compared with the other times of the year. Such fact coincided with the temporal pattern of ingestion of blue thread by adult drums that live in the Goiana Estuary, which had also ingested the highest amount of these fragments in the same area during the same season (Dantas et al., 2012).

Paint chips sink to the sediment and contaminate the benthic environment. As the other microplastics, they have the capacity of adsorb persistent organic pollutants (POPs), posing a threat to coastal environments (Barletta et al., 2012; Frias et al., 2010; Moore, 2008). Their presence is harmful also because they increase the level of biocides and trace metal found in their formulation (Bellinger and Benham, 1978; Turner, 2010). Large quantities of paint chips are generated during boat maintenance and cleaning (*e.g.*, paint chip scraping) (Tuner, 2010). However, for this study it seems to be directly related to the period of the year. During the rainy season, lobster and prawns are the most profitable catch and 53% of fishers are dedicated to its capture (Guebert-Bartholo et al., 2011). Consequently, the number of boats increases in the region making the environment more prone to contamination. The open-season of lobsters coincided exactly with the time in which paint particles reached their highest density in the lower estuary during the late rainy season in bottom waters. The off-season of lobsters occurs during the late dry and early rainy seasons (Guebert-Bartholo et al., 2011) when the amount of paint chips is at its lowest levels in the estuary.

For this study, the highest amount of microplastics was observed during the late rainy season (from June to August) in the lower estuary, when the environment reaches its highest level of rainfall. This period is characterized by an increased river flush into the estuary. The high fresh water discharge in the lower portion makes the environmental

variables (salinity and water temperature) similar to those of the upper estuary, so the stratification of the water column moves from the middle to the lower estuary (Dantas et al., 2010). It is hypothesised here that in rainy periods microplastics generated in the river basin migrate together with the biota to the lower portion of the estuary, following the river flow. Moreover, the stream of rainy water flowing through land on the adjacent areas of the estuary (mangrove forest, flood plains and beaches) may induce the runoff and the resurrection of previously fragmented microplastics to the estuary. Such fact emphasizes the idea of Moore et al., 2011, which asserted that the river is an important source exporting microplastic to the sea.

Due to variations in sampling methods and effort, as well as differences in geomorphology and tidal range of the regions, care is needed to compare coastal ecosystems regarding the density of microplastics. The mean density of microplastics per $100m^3$ found in the Goiana Estuary, varied from 0.031 to 0.26 items.$100m^{-3}$ in surface waters, considering that this is a long time study (one year). These values are much lower than that found in a short time study (lesser number of samples and replicates) of coastal pelagic ecosystems of the Northeast Pacific ocean (Southeast Bering sea) with mean density varying from 0.4 to 19 items.$100m^{-3}$ (Doyle et al., 2011).

The total density of microplastics for the Goiana estuary varied from 7.13 items.$100m^{-3}$ during the dry period to 19 items.$100m^{-3}$ during the rainy period. The lower microplastic density levels of the Goiana estuary are also identified when compared to a study in the coastal ocean near Long Beach (California), next to the mouth of San Gabriel River, that varied from 1,000 items. $100m^{-3}$ during the dry period to 6,000 items.$100m^{-3}$ after a storm when land-based runoff was extensive, however, this is a study of only one sample per station and period (Moore et al., 2002). In addition, the density of microplastics on the surface of the Goiana estuary was 4.3 items.$100m^{-3}$ during the dry period and 3.5 items.$100m^{-3}$ during the rainy period. While in the bottom it increased from 2.8 during the dry period to 15.3 items.$100m^{-3}$ during the rainy period. When compared with Santa Monica Bay (southern California), another short time study, the studied estuary also present smaller density levels. In association with urban runoff, enhanced quantities from <100 to 1,800 items.$100m^{-3}$ in the surface nearshore were also observed after a late storm event (Lattin et al., 2004). However, in the bottom offshore, it decreased from 600 during the dry period to 130 items.$100m^{-3}$ after the storm (Lattin et al., 2004).

4.2. Contribution of microplastics to the composition of estuarine zooplankton

Whereas most studies on plastic debris take into consideration how large items accumulates on estuarine shorelines or river-beach interfaces, mainly on muddy and sandy plains (Araújo and Costa, 2007; Browne et al., 2010; Cordeiro and Costa, 2010; Thornton and Jackson, 1998; Williams and Simmons, 1997), the contamination of estuarine planktonic habitats (main channel) by microplastic had not yet been examined.

The main concern of this study is that microplastics are found everywhere along the main channel of the Goiana Estuary sharing the habitat with planktonic organisms during the entire year. Also, a large amount of microplastics were found with fouling organisms, evidencing that such fragments are within the estuary for a long time. Worldwide, estuaries function as nursery habitats for a wide range of fish and invertebrates (Dantas et al., 2013; Lima et al., 2011; Lima et al., 2013). It is expected that Ichthyoplankton (fish larvae and fish eggs) and zooplankton are found in large quantities in these systems. When compared with the amount of microplastic, fish larvae presented higher densities along space and time. Fish eggs presented the same pattern being more abundant in the lower estuary. However, this is not a rule for the entire year. In specific times, the amount of microplastic surpassed Ichthyoplankton in density. The distribution of microplastics in the water column is not well understood, but one of the reasons for the sinking of plastic particles in estuaries is biofouling (Barnes et al., 2009; Browne et al., 2010; Cole et al., 2011; Moore et al., 2001). The cyclic behaviour of microplastics makes them available to planktivores, filter feeders and suspension feeders inhabiting different depths of the water column (Wright et al., 2013).

During the rainy season in the surface waters of the upper estuary, total density of microplastic was almost two times higher than fish larvae. In the early dry season, microplastics and fish larvae appear with the same density, but in the late dry season the amount of microplastic was six times higher than fish larvae. In the late rainy season in bottom waters, the amount of microplastic was also higher than that of fish. In addition, the amount of microplastic was five times higher than fish larvae during the late rainy season in bottom waters of the lower estuary. Such items, when within a dynamic environment suffer fragmentation to a small right size to cause harm to the biota in different ways. The fact is that the density and the small size of microplastic in the water column will determine the bioavailability of these items to lower and higher trophic organisms (Boerger et al., 2010; Wright et al., 2013).

The darkness and turbidity of the estuary may alter the relation prey-predator, negatively affecting the ability of zooplankton organisms to ingest natural prey. Microplastic enters the food web being first ingested by zooplankton and small fish (Boerger et al., 2010; Wright et al., 2013; Ivar do Sul et al., 2013). Through indirect ingestion, some fish preying on smaller fish or zooplankton that had ingested microplastic will contaminate their predators (Possatto et al., 2011). This trophic transfer might facilitate the transport of microplastic to other environments, especially when estuarine species are preyed by riverine or marine fish visiting the estuary (Cole et al., 2013; Possatto et. al. 2011; Wright et al., 2013).

5. Conclusion

Plastic fragments in their different compositions, forms and colours are an arising concern regarding contamination in estuaries and their adjacent areas. Most of these items are introduced in the estuary by direct runoff of previously fragmented microplastics (including micro spheres from cosmetic products). Another source is the weathering breakdown of large plastic items generated during domestic (e.g. tubs, flasks and bottles), artisanal or commercial fishery (gear and boat maintenance) or recreational activities (snacking packaging) in the river basin or beaches in surrounded areas of the estuary.

For the Goiana Estuary, the density of microplastics represents half of the total density of fish larvae, what is a large amount. They are found in every habitat of the main channel being bioavailable for planktonic organisms and many vertebrates in surface and bottom water during the entire year. Lower trophic organisms feeding on small fragments therefore represent a vector for microplastic transfer through the food chain and other environments. Moreover, microplastic contaminants, such as biocides and trace metals of paint chips, represent a threat through bioaccumulation and biomagnification, thus, being available to human population that uses estuarine food resources.

Most studies in plankton take only in consideration the living portion and left behind the non-living portion, including microplastics. Even if the attention is given, the results probably will be inconsistent because the sampling methods were not planned or the samples already are very disturbed by previous analysis/triage for a contamination insight. If microplastics are found everywhere in the marine environment and we know about the importance of preserve environments from this type of contamination, the scientific community must dedicate special attention to plankton samples. When sampling methods are planned, better results are reached, including seasonal and spatial

variations according to the dynamics of the environmental variables of the system chosen. Thus, attaching consistent knowledge, we can potentially provide information on how microplastics are negatively affecting marine habitats and solve this environmental problem.

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CAPÍTULO 3

Changes in the composition of ichthyoplankton assemblage and plastic debris in mangrove creeks relative to moon phases

Changes in the composition of ichthyoplankton assemblage and plastic debris in mangrove creeks relative to moon phases

A. R. A. Lima[1], M. Barletta[1]*, M. F. Costa[1], J. A. A. Ramos[1, 2], D. V. Dantas[1, 3], A. K. S. Justino[1], G. V. B. Ferreira[1]

[1]Laboratório de Ecologia e Gerenciamento de Ecossistemas Costeiros e Estuarinos, Departamento de Oceanografia, Universidade Federal de Pernambuco, CEP 50740-550, Recife, Brazil, [2]Instituto Federal de Educação, Ciência e Tecnologia da Paraíba-Campus Cabedelo, Rua Santa Rita de Cássia, s/n, Jardim Jericó, Cabedelo, Paraíba, CEP 58310-000, Brazil, [3]PPGOAm/Universidade Federal do Espírito Santo-DCAB-CEUNES, BR 101 Norte, Litorâneo, São Mateus, CEP 29932-540, Brazil.

*Author to whom correspondence should be addressed: Tel. and fax: 00558121267223; email: barletta@ufpe.br

ABSTRACT

Lunar influence on the distribution of fish larvae, zooplankton and plastic debris in mangrove creeks of the Goiana Estuary, Brazil, was studied over a lunar cycle. *Cetengraulis edentulus*, *Anchovia clupeoides* and *Rhinosardinia bahiensis* were the most abundant fish larvae (56.6%), independent of moon phase. The full moon had a positive influence on the abundance of *Gobionellus oceanicus*, *Cynoscion acoupa* and *Atherinella brasiliensis*, and the new moon on *Ulaema lefroyi*. Full and new moon also influenced the number of zoeae and megalopae of *U. cordatus*, protozoeae and larvae of Caridea shrimp, and the number of hard and soft plastic debris, both < 5mm and > 5mm. Micro- and macroplastics were present in samples from all twelve creeks studied, at densities similar to the third most abundant taxon, *R. bahiensis*. *Cetengraulis edentulus* and *R. bahiensis* showed a strong positive correlation with the last quarter moon, when there was less zooplankton available in the creeks and higher abundance of microplastic threads. *Anchovia clupeoides*, *Diapterus rhombeus*, *U. lefroyi* and hard microplastics were positively associated with different moon phases, occurring when calanoida copepods, Caridea larvae and zoeae of *U. cordatus* were highly available in the creeks. *Cynoscion acoupa*, *G. oceanicus* and *A. brasiliensis*, were strongly associated with full moon, when protozoeae of Caridea and megalopae of *U. cordatus* were also highly available, as were hard and soft macroplastics, paint chips (< 5mm) and soft microplastics. The results reinforce the role of mangrove creeks as nursery habitats. The moon phases influenced the distribution of fish larvae species, zooplankton and plastic debris by changing their

compositions and abundances in the mangrove creeks of the Goiana Estuary when under the influence of different tidal current regimes.

Key words: fish larvae, microplastics, lunar cycle, northeast Brazil, tropical estuary

INTRODUCTION

Estuarine mangroves are important nursery habitats for the early stages of most teleost fishes that spawn within these systems by providing food sources and protection from predators (Blaber *et al*., 1989; Laroche *et al*., 1997; Barletta-Bergan *et al*., 2002*a*,*b*; Barletta *et al*., 2003; Hampel *et al*., 2003; Krumme *et al*., 2008). Studies of fish assemblages in mangrove environments such as Tulear lagoon, Madagascar (Laroche *et al*., 1997), Embley Estuary, Australia (Blaber *et al*., 1989), Caeté Estuary, north Brasil (Barletta-Bergan *et al*., 2002*a*; Barletta *et al*., 2003; Krumme *et al*., 2008) and Westercheld Estuary, southwest Netherlands (Hampel *et al*., 2003) report that mangrove creeks are one of the most important and productive habitats of estuarine systems. The organic compounds provided to the sediments by the falling leaves of the mangrove forest, function as food sources for detritivorous organisms such as shellfish, bacteria and fungi (Robertson & Alongi, 1992; Tzeng & Wang, 1992; Yáñez Arancibia *et al*., 1993; Nagelkerken *et al*., 2008). These organisms are of vital importance for the plankton and the early stages of many fishes and invertebrates, as well as juvenile and adult fishes, benefiting from the energy transfer from estuaries to the sea (Dittmar, 1999).

Feeding activity may vary diurnally and affect the spatial distribution of fishes within the environment (Morrison *et al*., 2002; Willis *et al*., 2006; Krumme *et al*., 2008). Planktivorous fish larvae tend to be distributed according to the availability of their prey, and changing tidal amplitudes and light intensity during different moon phases can have marked effects on this behaviour (Alldredge & King, 1980; Kingsford & MacDiarmid, 1988; Hampel *et al*., 2003; Hernández-León, 2008). The lunar cycle determines the temporal and spatial availability of mangrove creek habitats. At neap tides, less creek area is flooded, while at spring tides, they are completely flooded for a longer period (Hampel *et al*., 2003; Ramos *et al*., 2011). Current intensity also varies with moon phase, promoting cycles of more or less efficient flooding and flushing. Each tidal cycle brings organisms to the intertidal habitats, some are adapted to remain within the creek, and others return to the main channel on ebb tides (Kneibe, 1997; Barletta *et al*., 2000; Morrison *et al*., 2002; Willis *et al*., 2006).

Tidal flood pulses can also bring plastic debris from land to the estuary, and creeks are possibly an important pathway of such pollution from the mangrove forest to the main channel of the estuary (Lima *et al*., 2014). Plastics pollution originates principally on land, where improper disposal, accidents and disasters cause it to reach coastal environments and the sea (Thompson *et al*., 2009; Watters *et al*., 2010). The marine environment is probably a secondary source of plastic debris to mangrove creeks during flood tides. Once exposed to environmental processes, most larger plastics (> 5mm) fragment into microplastics (< 5mm) (Barnes *et al*., 2009; Wright *et al*., 2013; Lima *et al*., 2014). For the Goiana River Estuary, Brazil, microplastics were found in the main channel in densities comparable to those of fish eggs and half of the density of fish larvae (Lima *et al*., 2014). Plastic debris buoyancy makes them as available to transport and predators as planktonic organisms. As microplastics share the habitats with fish and invertebrate larvae, they also may be ingested and initiate trophic transfer of both plastics and absorbed organic pollutants (Barnes *et al*., 2009; Fendall & Swell, 2009; Cole *et al*., 2011; Possatto *et al*., 2011; Dantas *et al*., 2012; Ramos *et al*., 2012).

Limited research on estuarine fish movements over short temporal and spatial scales has been done (Lin & Shao, 1999; Morrison *et al*., 2002; Hampel *et al*., 2003; krumme *et al*., 2008; Ramos *et al*., 2011; Lacerda *et al*., 2014). Studies of estuarine fish assemblages in space and time, usually consider timescales of months to years, but rarely examine lunar cycles, or periods of days to weeks. This is the scale at which the environment and its resources are most strictly coupled, and might be related to feeding, shelter, avoidance of predators and other behaviors (Morrison *et al*., 2002; Hampel *et al*., 2003; Lacerda *et al*., 2014). The present study quantifies fish larvae, zooplankton and pelagic plastic debris associated with mangrove creeks of the Goiana Estuary, to assess their distribution and relations over a short time span with respect to moon phases.

MATERIAL AND METHODS

STUDY AREA

The Goiana estuary, northeast Brazil (7°32'–7°35' S; 34°50'–34°58' W), is characterized by a tropical, semiarid climate (Fig. 1). This estuary has diverse habitats including the main channel, flood plain, creeks and the mangrove forest (Barletta & Costa, 2009). Mangrove trees, mainly *Rhizophora mangle* L., *Laguncularia racemosa* (L.) C.F. Gaertn and *Avicennia* spp., grow around the main channel and the creeks to form a flooded forest of 4,700 ha (Barletta & Costa, 2009). The flooded forest is subject to a semi-diurnal tidal regime, with amplitudes ranging from -0.1 to 2.7 m (Barletta & Costa,

2009). The main channel can be divided into upper, middle and lower estuary according to the different salinity ranges and channel morphology (Barletta & Costa, 2009).

Figure 1. Lower portion of the Goiana Estuary. Circles mark the entrance of each mangrove creek. Sampling: (1–3), first quarter; (4–6), full; (7–9), last quarter; (10–12), new moon. Source: Google Earth. Image accessed on 26th November 2014.

SAMPLING METHODS

Plankton samples were taken from twelve mangrove creeks of the lower estuary (1 < salinity < 35) during 30 days in April and May 2008 (Fig. 1). To assure the detection of the lunar influence on the seston distribution, the sampling months coincided with a more stable estuary, during the early rainy season (Barletta & Costa, 2009). Extreme environmental condition as highest precipitation (June to August) or water temperature (December to February) were avoided (Barletta & Costa, 2009). In addition, sampling began after the main spawning period, when fish larvae use the estuary and coastal waters have a greater influence on the lower estuary (Lima et al., *in press*; Lima et al., 2014). The 12 creeks were chosen according to similarity in width and length. For each moon phase, three creeks (replicates) were randomly sampled on 3 consecutive days to avoid bottom disturbance during the deployment of the nets (Ramos et al., 2011). During first and last quarter moon, high tides varied from 1.8 to 2.1 m. During new moon, they ranged from 2.4 to 2.7 m, and during full moon from 2.2 to 2.4 m. Creeks 1–3 were sampled

during first quarter moon, 4–6 during full moon, 7–9 during last quarter moon and 10–12 during new moon (Fig. 1). The first creek sampled was the farther and the third was the closest to the mouth of the estuary (Fig. 1) (Ramos et al., 2011). Sampling began during the second daily peak of high tide, using a rectangular 1000 μm mesh trap net (10 x 2m) to block the creek mouth from one margin to the other. A conical cod end (Ø 0.6 m; 500 μm) was positioned in the middle of the net, forming a collecting jar. Samples were retrieved after ~4 hours at low tide. During low tide, the depth varied among creeks (completely empty to *c.* 10 cm of water). To ensure that all fishes were caught, a drag-net of 2 m × 1 m with a mesh size of 500 μm was trawled from the beginning to the end of the creek. Water temperature (◦ C), dissolved oxygen (mg l^{-1}*)* (Wissenschaftlich Technische Werkstätten, WTW OXI 325; www.wtw.com) and salinity (WTW LF 197) were recorded from surface waters at the mouth of the creeks during four consecutive hours. Water flow was measured hourly using a General Oceanics flowmeter with a low-speed rotor mounted near the net. Samples were immediately fixed in 4% buffered formalin.

LABORATORY PROCEDURES

Samples were divided into smaller aliquots (100 mL) to facilitate the separation of plankton and plastic debris from the organic matter, which was made with the aid of a stereomicroscope – ZEISS; STEMI 2000-C (x5). Fish larvae and plastic debris (Fig. 2) were totally separated from the entire bulk sample and their counts were corrected to a standard volume of 100 m^3. The ichthyoplankton taxonomic identification was based on developmental series, working backwards from the adults and juveniles captured in the same region, from characters common to successively earlier ontogenetic stages (Balon, 1990) (Table II). Species identification followed Figueiredo & Menzes (1978, 1980), Menezes & Figueiredo (1980, 1985), Sinque (1980), Moser *et al*. (1984), Richards (2006).

For counting the zooplankton, three subsamples of 10 mL were removed from a diluted 700 mL sample for each creek, using a Stempel pipette, with subsequent reposition (Postel *et al*., 2000). Each zooplankton taxon from the three aliquots was counted separately to calculate a mean (± S.D.). Mean counts were then extrapolated to 700 mL and, as ichthyoplankton and plastics corrected to a standard volume of 100 m^3. Zooplankton were identified to the lowest possible taxonomic categories (Boltovskoy, 1981, 1999) (Fig. 2).

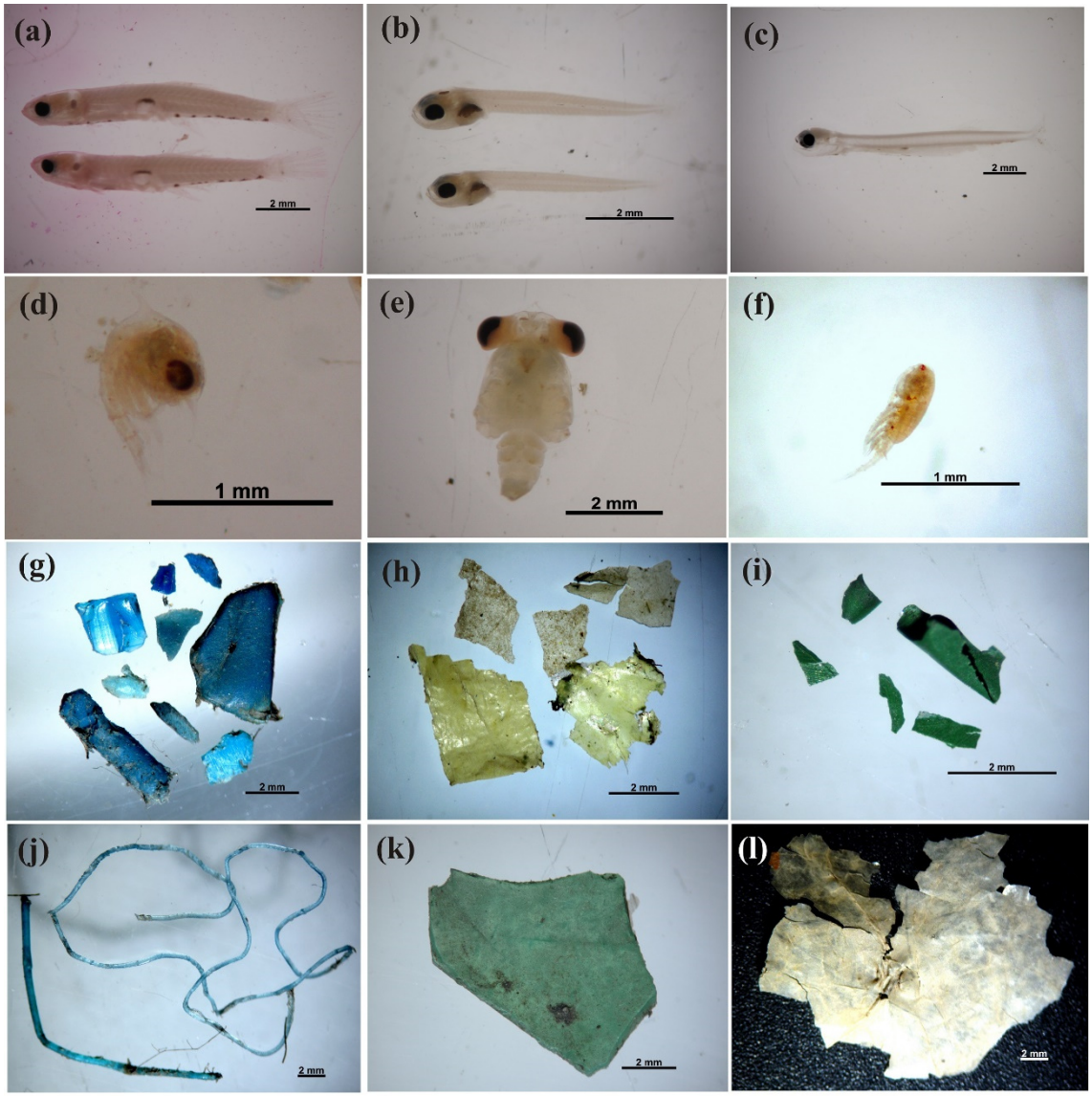

Figure 2. Examples of plankton and plastic debris found in the mangrove creeks of the Goiana Estuary. Fish larvae: (a) *Gobionellus oceanicus*, (b) *Atherinella brasiliensis*, (c) *Anchovia clupeoides*; zooplankton: (d) zoea of *Ucides cordatus*, (e) Megalopa of *U. cordatus*, (f) copepod calanoida; microplastics: (g) blue hard plastics, (h) yellow soft plastics, (i) green paint chips; Macroplastics: (j) blue threads, (k) green hard plastic, (l) white soft plastic. Images captured with a digital camera Canon PowerShot G10 coupled to a stereomicroscope - ZEISS; STEMI 2000-C.

STATISTICAL ANALYSIS

One-way ANOVA was performed to determine whether fish larvae, zooplankton and plastic debris mean densities vary with different moon phases (Zar, 1996). The Cochran's test was used to check the homogeneity of variances. The original data were Box-Cox transformed (Box & Cox, 1964) to reach a normal distribution. Bonferroni's

test ($P < 0.05$) was used whenever significant differences were detected with ANOVA (Quinn & Keough, 2002).

A canonical correspondence analysis (CCA) (CANOCO for Windows 4.5) was performed to observe the relationship power between the environmental variable and the groups, for each moon phase (ter Braak & Smilauer, 2002). Multiple least-squares regression was performed with the site scores, derived from weighted averages of fish larvae, zooplankton and plastic debris, as the dependent variables and the environmental parameters (water temperature, dissolved oxygen and salinity) as the independent variables (ter Braak, 1986; Palmer, 1993). This test was computed with 100 iterations with randomized site locations to facilitate Monte-Carlo tests between the eigenvalues and species–environment correlations. The result is shown as a triplot, where the environmental variables appear as vectors radiating from the origin of the ordination.

RESULTS

ENVIRONMENTAL VARIABLES

Significant differences were not detected for the fluctuation of environmental variables during the lunar cycle for flood and ebb tide. Salinity (6.2 – 24.6), temperature (27.4 – 30.4° C) and dissolved oxygen (2.6 – 6.3 mg L^{-1}) declined from flood to ebb tide during both new and full moon. During first and last quarter moons there are no clear trends for temperature or salinity, but dissolved oxygen presented lower values during ebb than during flood tide (Fig. 3). The environmental variables showed wider ranges during spring tides (full and new moons), when compared to neap tides (first and last quarter moons), possibly because of the increased upstream volume of marine water and flushing intensity.

DISTRIBUTION OF THE PLANKTON ACCORDING TO MOON PHASE

In total, 14 320 fish larvae (29.97 ind. $100m^{-3}$) were collected from the twelve creeks (Table I and Fig. 2). The most abundant taxa were the Engraulidae *Cetengraulis edentulus* (Cuvier 1829) (40.12%) and *Anchovia clupeoides* (Swainson 1839) (29.79%), followed by the Clupeidae *Rhinosardinia bahiensis* (Steindachner 1879) (16.49%). In total, 4 372 individual plastic debris (4.77 items $100m^{-3}$) were also recorded. Microplastics (300µm to < 5mm) represented 38% and macroplastics (> 5mm to 181mm) were 62% of the total catch in number (Table I and Fig. 2). Fish larvae represented $5.99^{e}10^{-4}$ % and plastic debris $9.54^{e}10^{-5}$ % of the whole plankton density. Zooplankton contributed to 99.9% of the catch (Table I and Fig. 2).

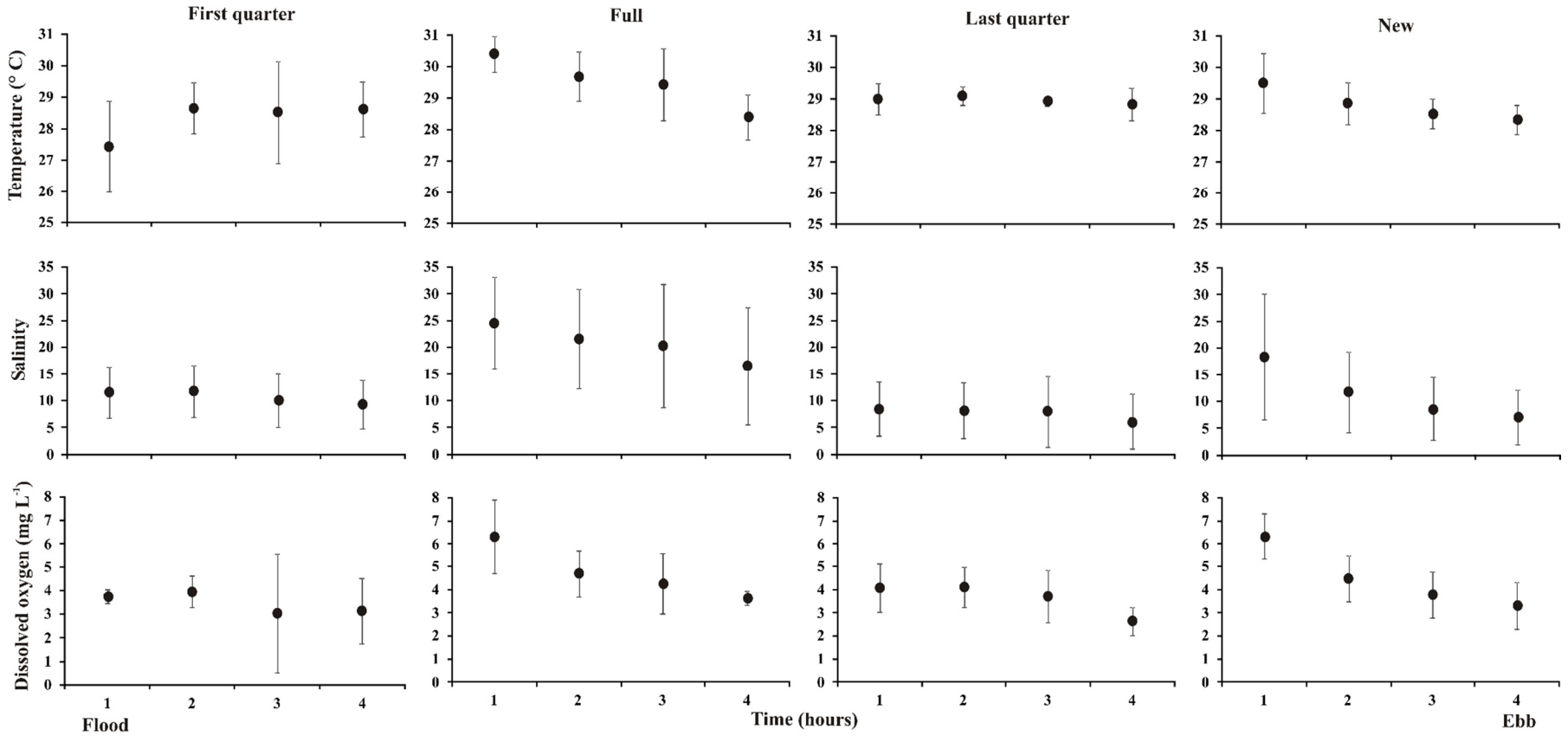

Figure 3. Means (± S.D.) of water temperature, salinity and dissolved oxygen during the following four hours after flood tide on each moon phase (first quarter, full, last quarter and new).

TABLE I. Density of the main plankton components (fish larvae, zooplankton, and plastic debris) from the mangrove creeks of the Goiana Estuary during different moon phases. E, estuarine; E-M, estuarine-marine; MS, mangroves; M, marine. Sub-total densities in bold.

		Number (N)		**Density (n 100m^{-3})**		**Moon phase density (%)**			
Ichthyoplankton	**Habitat**	**N**	**%**	**Density**	**%**	**First quarter**	**Full**	**Last quarter**	**New**
Cetengraulis edentulus	M	5746	40.12	13.53	45.16	17.44	29.31	58.51	30.72
Anchovia clupeoides	E	4267	29.79	8.16	27.23	48.81	27.31	20.54	40.79
Rhinosardinia bahiensis	E	2362	16.49	4.80	16.01	25.39	14.68	18.26	9.38
Gobionellus oceanicus	MS	536	3.74	1.00	3.35	2.88	11.89	0.20	3.36
Diapterus rhombeus	M	458	3.19	0.87	2.90	0.17	3.81	1.68	5.83
Ulaema lefroyi	E-M	307	2.14	0.62	2.06	0.25	4.43	0.30	4.81
Cynoscion acoupa	M	189	1.32	0.41	1.35	0.91	4.66	0.17	1.29
Atherinella brasiliensis	M	109	0.76	0.23	0.78	0.32	2.62	0.15	0.75
Ctenogobius smaragdus	E	38	0.26	0.06	0.21		0.02		1.05
Stellifer rastrifer	M	157	1.09	0.03	0.11	2.68			
Centropomus sp.	M	30	0.21	0.03	0.11	0.46	0.21	0.01	0.19
Stellifer sp.	E-M	16	0.11	0.03	0.10				0.51
Eleotris pisonis	MS	12	0.08	0.03	0.09	0.02	0.28		0.16
Citharichthys arenaceus	M	7	0.05	0.02	0.07		0.19		0.16
Bathygobius soporator	MS	14	0.09	0.02	0.07	0.02	0.10		0.23
Gobionellus sp.	MS	5	0.03	0.02	0.06	0.02	0.03	0.09	
Mugil sp.	M	14	0.09	0.02	0.05				0.26
Stellifer stellifer	E-M	8	0.05	0.01	0.04		0.07	0.02	0.07
Sphoeroides testudineus	M	8	0.05	0.01	0.04	0.46	0.03	0.02	0.02
Achirus lineatus	E	13	0.09	0.01	0.04	0.12	0.03		0.13
Stellifer brasiliensis	E-M	5	0.03	0.01	0.04		0.18		
Etropus longimanus	M	8	0.05	0.01	0.03				0.14
Eucinostomus sp.	M	2	0.01	0.003	0.01		0.04		
Coryphopterus glaucofraenum	M	1	0.007	0.002	0.007		0.03		
Ctenogobius stigmaticus	E	2	0.01	0.002	0.007				0.04
Dormitator sp.	M	1	0.007	0.002	0.007			0.01	
Symphurus tessellatus	M	1	0.007	0.001	0.004				0.02
Bairdiella ronchus	E-M	1	0.007	0.001	0.004				0.02
Hyporhamphus unifasciatus	M	1	0.007	0.001	0.004				0.02
Sphyraena barracuda	M	1	0.007	0.001	0.004				0.02
Gobiomorus dormitor	M	1	0.007	< 0.001	0.001	0.02			
Total of fish larvae		**14320**		**29.9**		**1.3**	**6.2**	**16.5**	**6.1**

TABLE I. Continued

	Number (N)		Density (n 100m^{-3})		Moon phase density (%)			
Zooplankton	**N**	**%**	**Density**	**%**	**First quarter**	**Full**	**Last quarter**	**New**
Zoeae of *Ucides cordatus*	4287392640	91.87	4586823	91.69		10.27	0.21	98.11
Calanoida Copepods	328424670	7.03	343433	6.86	98.41	78.79	91.71	1.15
Protozoeae of Caridea	24134600	0.52	38192.52	0.76		8.01	0.833	0.27
Caridea larvae	15981560	0.34	20037.53	0.40	1.41	1.28	6.12	0.31
Megalopae of *Ucides cordatus*	8 890310	0.19	11478.13	0.23	0.017	1.32	0.65	0.15
Insects	510320	0.01	822.67	0.02	0.046	0.15	0.29	0.006
Megalopae of Portunidae	452860	0.01	530.38	0.011	0.006	0.02		0.01
Anomalocardia brasiliana larvae	274090	0.006	395.46	0.008	0.008	0.04		0.006
Isopoda	153270	0.003	311.35	0.006	0.068	0.05	0.17	0.002
Amphipoda Gammaridae	175500	0.004	232.25	0.005	0.039	0.02	0.02	0.003
Polychaeta	138320	0.003	162.15	0.003		0.02		0.002
Tanaidacea	3480	<0.003	7.68	<0.003		0.002		
Total of zooplankton	**4666531620**		**5002427**		**23375.9**	**315503**	**19808.5**	**4643739**
Microplastics (< 5mm)								
Soft	933	56.14	1.991	58.67	65.18	69.43	30.16	55.33
Hard	516	31.05	0.921	27.13	17.08	22.58	28.50	36.22
Paint chips	101	6.08	0.264	7.78	8.00	7.38	15.82	3.81
Threads	112	6.74	0.218	6.42	9.74	0.61	25.52	4.64
Total of microplastics	**1662**		**3.4**		**0.2**	**1.6**	**0.6**	**0.9**
Macroplastics (> 5mm)								
Soft	2 213	81.66	1.08	78.64	33.87	83.15	93.33	76.95
Hard	418	15.42	0.15	10.98	59.68	5.40	3.04	8.91
Paint chips	77	2.84	0.14	10.06	6.45	10.90	3.62	14.14
Threads	2	0.07	<0.01	0.32		0.56		
Total macroplastics	**2710**		**1.4**		**0.1**	**0.8**	**0.2**	**0.3**
Total debris	**4372**		**4.7**		**0.3**	**2.4**	**0.8**	**1.2**
Total density	**4666551 974**		**5002465**		**23377.7**	**315513**	**19826.3**	**4643747**

Results from ANOVA showed that the mean number of fish species differed significantly among moon phases ($P < 0.05$), with highest mean during new moon (Table II and Fig. 4). However, the mean number of fish larvae did not significantly differ, but the highest values were observed during last quarter and new moon. Densities of fish larvae and zooplankton did not differ among moon phases, however, their highest means occurred during last quarter and new moon, respectively ($P > 0.05$) (Table II and Fig. 4). The highest mean density of plastic debris occurred during the full moon, microplastic being more abundant during full and new moon and macroplastic during full moon ($P < 0.05$) (Table II and Fig. 4).

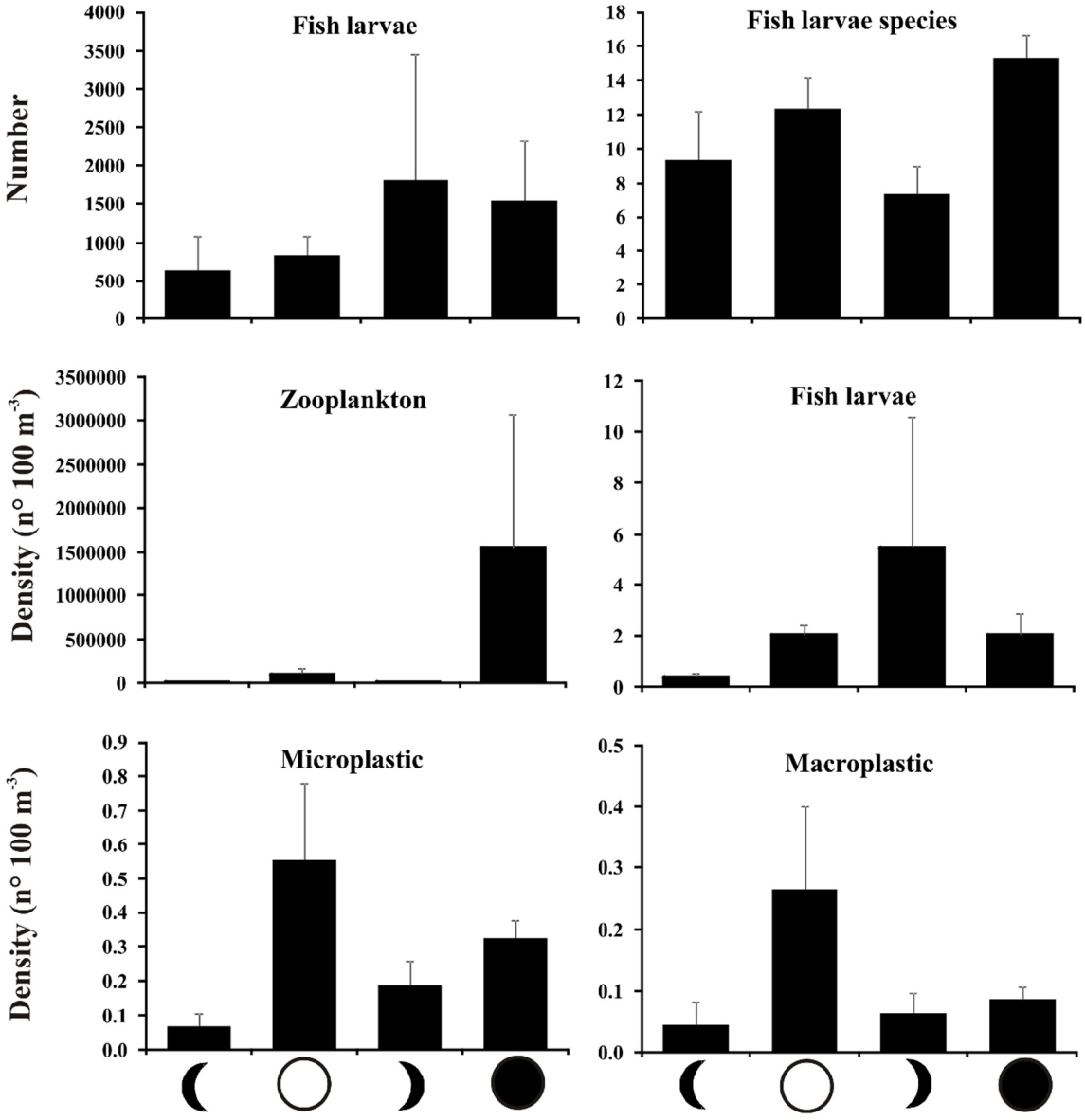

Figure 4. Mean (± S.E.) of number of fish larvae and species, and mean densities (± S.E.) of fish larvae, zooplankton, microplastic and macroplastic in the mangrove creeks of the Goiana Estuary in relation to moon phase (◖, first quarter; ○, full; ◗, last quarter; ●, new).

TABLE II. Summary of ANOVA results for the mean density of total plankton, fish larvae, zooplankton and plastic debris. Analysis performed using Box-Cox transformed data. Differences among moon phases were determined by Bonferroni's *post hoch* comparisons test. Moon phases: Fi, first quarter; Fu, full moon; La, last quarter; Ne, new moon. * $p < 0.05$; ** $p < 0.01$.

Variables	Source of variance
Plankton	Moon phases
N of individual	Ns
N of species	*La Fi Fu Ne
Total fish larvae	Ns
Total zooplankton	Ns
Total plastic debris	*Fi La Ne Fu
Total microplastics	*Fi La Ne Fu
Total macroplastics	*Fi La Ne Fu
Fish larvae	
Cetengraulis edentulus	Ns
Anchovia clupeoides	Ns
Rhinosardinia bahiensis	Ns
Gobionellus oceanicus	**La Fi Ne Fu
Diapterus rhombeus	Ns
Ulaema lefroy	*Fi La Fu Ne
Cynoscion acoupa	*Fi La Ne Fu
Atherinella brasiliensis	*Fi La Ne Fu
Zooplankton	
Calanoida Copepods	Ns
Megalopae of *Ucides cordatus*	**Li La Ne Fu
Zoeae of *Ucides cordatus*	**Li La Ne Fu
Protozoeae of Caridea	**Li La Ne Fu
Caridea larvae	*La Li Ne Fu
Microplastics (< 5mm)	
Threads	Ns
Hard	*Fi La Ne Fu
Soft	**Fi La Ne Fu
Paint chips	Ns
Macroplastics (> 5mm)	
Threads	Ns
Hard	**La Li Ne Fu
Soft	*Fi La Ne Fu
Paint chip	Ns

DISTRIBUTION OF FISH LARVAE ACCORDING TO MOON PHASE

Larvae of *C. edentulus A. clupeoides, R. bahiensis,* the Sciaenidae *Cynoscion acoupa* (Lacepède 1801) and the Atherinopsidae *Atherinella brasiliensis* (Quoy & Gaimard 1825) were captured in pre-, flexion and post-flexion stages (Table II). Whereas, the Gobiidae *Gobionellus oceanicus* (Cuvier, 1829), the Gerreidae *Diapterus rhombeus* (Cuvier, 1829) and *Ulaema lefroyi* (Goode 1874) were captured only in the post-flexion stage (Table III). Results from ANOVA showed that densities of *C. edentulus, A. clupeoides, R. bahiensis* and *D. rhombeus* did not differ among moon phases ($P > 0.05$). However, their highest mean density occurred during the last quarter moon (Table II and Fig. 5). Densities of *G. oceanicus, C. acoupa* and *A. brasiliensis* significantly differed among moon phases, with highest mean density during full moon (Table II and fig. 5). Densities of *U. lefroyi* (Goode 1874) also significantly differed among moon phases, with highest mean density during new moon (Table II and Fig. 5).

TABLE III. Developmental stages size of the most important fish larvae species catch in the mangrove creeks of Goiana Estuary.

	Developmental stages (Length ± S.D. mm)		
Species	**Pre-flexion**	**Flexion**	**Post-flexion**
Cetengraulis edentulus	5.01 ± 0.63 (*n* = 84)	8.81 ± 1.05 (*n* = 587)	15.36 ± 3.72 (*n* = 5 075)
Anchovia clupeoides	6.82 ± 0.93 (*n* = 53)	9.16 ± 0.58 (*n* = 1 016)	16.65 ± 4.06 (*n* = 3 198)
Rhinosardinia bahiensis	6.50 ± 1.17 (*n* = 267)	8.57 ± 0.28 (*n* = 285)	10.64 ± 1.77 (*n* = 1 810)
Gobionellus oceanicus			11.79 ± 4.20 (*n* = 536)
Diapterus rhombeus			11.62 ± 1.60 (*n* = 458)
Ulaema lefroyi			14.56 ± 3.24 (*n* = 307)
Cynoscion acoupa	4.50 ± 0.34 (*n* = 54)	5.84 ± 0.54 (*n* = 84)	10.78 ± 3.55 (*n* = 51)
Atherinella brasiliensis	4.62 ± 0.33 (*n* = 12)	5.92 ± 0.56 (*n* = 45)	9.89 ± 2.68 (*n* = 52)

DISTRIBUTION OF ZOOPLANKTON ACCORDING TO MOON PHASE

The ANOVA showed that densities of calanoid copepods did not differ among moon phases, although they were higher during the full moon ($P > 0.05$) (Table II and Fig. 5). Densities of megalopae of the crab *Ucides cordatus* L. 1763 and protozoeae of Caridea shrimp differed significantly, with peaks during the full and new moons ($P < 0.01$) (Table II and fig. 5). Zoeae of *U. cordatus* and Caridea larvae also differed significantly, with highest mean densities during the new moon (Table II and Fig. 5).

DISTRIBUTION OF PLASTIC DEBRIS ACCORDING TO MOON PHASE

Four main types of plastic debris were identified for each size class: soft plastic, hard plastic, paint chips and plastic threads (Table 1). Results from ANOVA showed that

the microplastic threads and paint chips did not differ among moon phases ($P > 0.05$). However the highest mean density of threads occurred during the last quarter moon and paint chips during full and last quarter moon (Table II and Fig. 6). Hard microplastics were equally abundant during full, last quarter and new moon, with lowest mean density during the first quarter moon ($P < 0.05$) (Table II and Fig. 6). Soft microplastics mean densities were highest during full and new moon ($P < 0.01$) (Table II and Fig. 6). The macroplastic threads and paint chips did not differ among moon phase either ($P > 0.05$); their highest mean densities occurred during first quarter and full moon, respectively (Table II and Fig. 6). On the other hand, hard and soft macroplastics mean densities were highest during full moon, although only significantly higher during first quarter for the soft macroplastics (Table II and Fig. 6).

CORRELATION AMONG PLANKTON COMPONENTS, MOON PHASES AND THE ENVIRONMENTAL VARIABLES

A CCA was performed to determine the influence of environmental variables on the distribution of the fish larvae, zooplankton and plastic debris in the mangrove creeks through moon phases (Table IV and Fig. 7). Fish larvae and zooplankton were studied separately to avoid obtaining results biased towards zooplankton, thus masking any trends for fish larvae [Fig. 7 (a)–(b)]. In addition, plastic debris were added in both models. For all graphs, the first axes represents the moon phases and explained more than 75% of the variance of the species/plastic-environment relation. The axes did not show correlation with the environmental variables. Both micro- and macroplastic were positively correlated with full moon, except for the microplastic threads, which were more correlated with last quarter moon (Fig. 7). *C. edentulus* and *R. bahiensis* showed positive correlation with last quarter moon, when salinity was lowest. Larval *G. oceanicus*, *C. acoupa* and *A. brasiliensis*, and protozoeae of Caridea and megalopae of *U. cordatus* showed positive correlations with full moon, when salinity and water temperature were highest [Table IV and Fig. 7 (a)–(b)]. *D. rhombeus* and zoeae of *U. cordatus* showed strong positive correlation with new moon. They were grouped together with *A. clupeoides*, *U. lefroyi*, calanoid copepods and caridean larvae because of their representative abundance in the four moon phases, but mainly during full and new moons [Fig. 7 (a)–(b)].

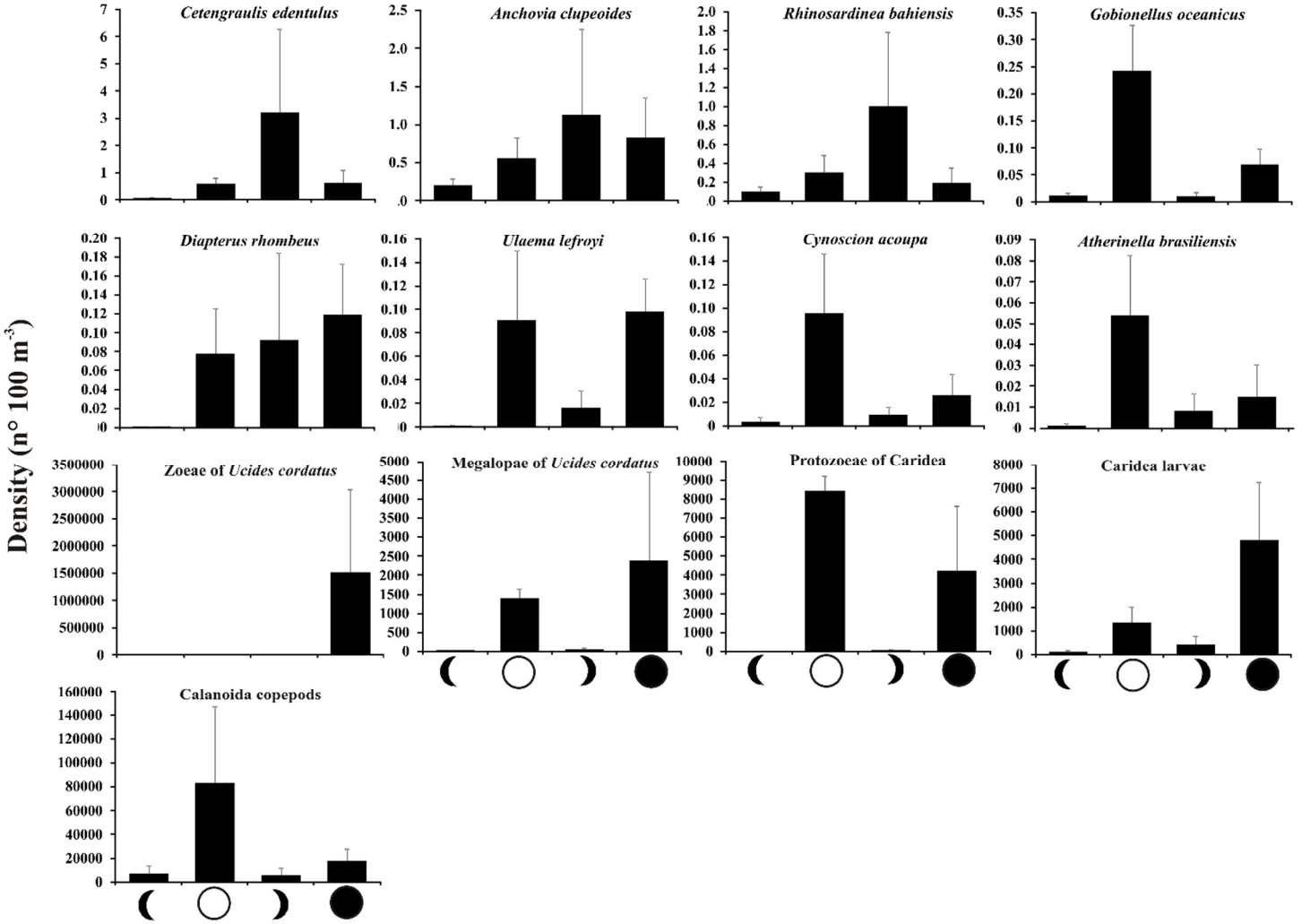

Figure 5. Mean densities (± S.E.) of the most important species of fish larvae and groups of zooplankton caught in the mangrove creeks of the lower portion of the Goiana Estuary in relation to moon phase (☾, first quarter; ○, full; ☽, last quarter; ●, new).

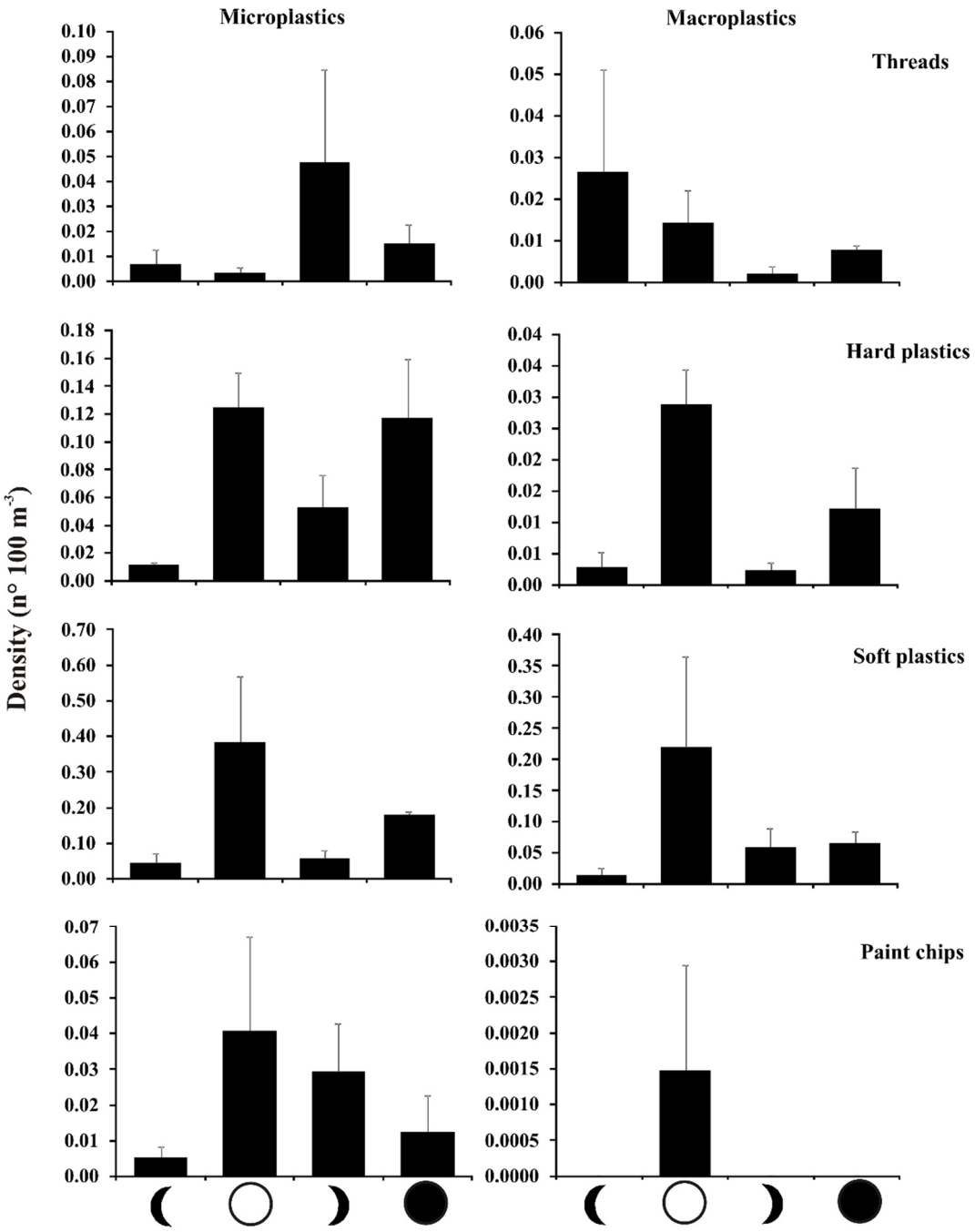

Figure 6. Mean densities (± S.E.) of plastics debris (micro and macroplastics) in the mangrove creeks of the lower portion of the Goiana Estuary in relation to moon phase (☾, first quarter; ○, full; ☽, last quarter; ●, new).

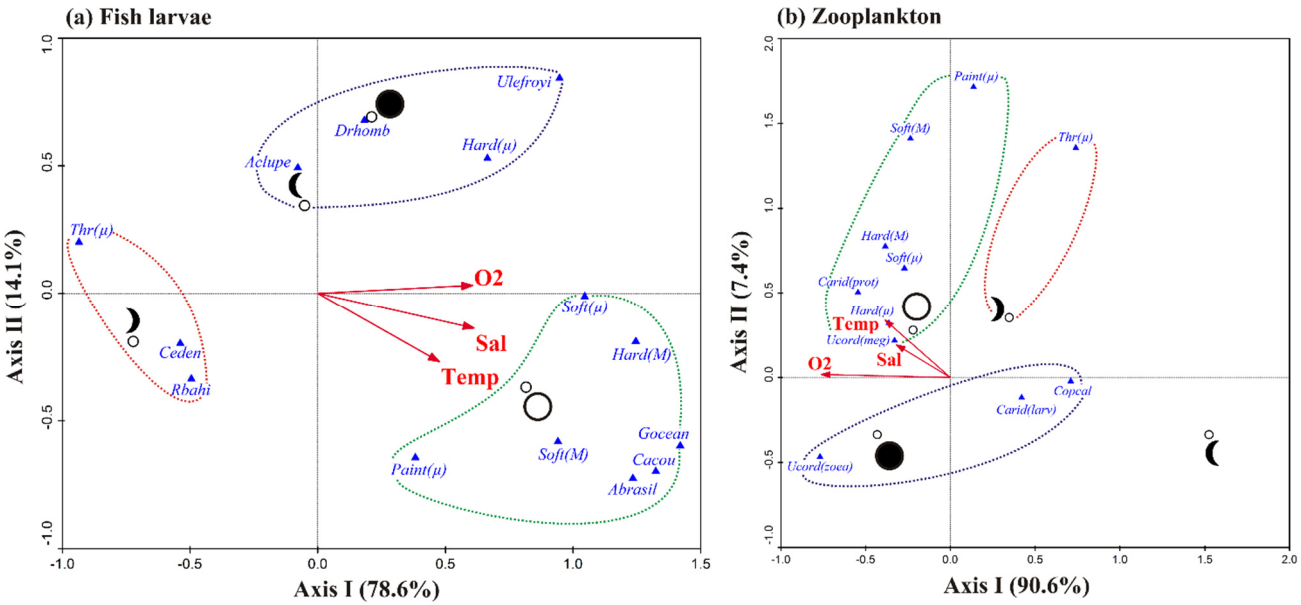

Figure 7. Canonical correspondence analysis (CCA) triplot for the ecological correlations between plastics debris and (a) fish larvae, (b) zooplankton, and the environmental variables. Circles (○) represent moon phases (◖, first quarter; ○, full; ◗, last quarter; ●, new). Triangles (Δ) represent the plankton: Ichthyoplankton (*Abrasil*, *Atherinella brasiliensis*; *Aclupe*, *Anchovia clupeoides*; *Cacou*, *Cynoscion acoupa*; *Ceden*, *Cetengraulis edentulus*; *Drhomb*, *Diapterus rhombeus*; *Gocean*, *Gobionellus oceanicus*; *Rbahi*, *Rhinosardinia bahiensis*; *Ulefroyi*, *Ulaema lefroyi*). Zooplankton (*Carid(larv)*, larvae of Caridae shirimp; *Carid(prot)*, protozoea of Caridea; *Copcal*, calanoida copepods; *Ucord(meg)*, megalopae of *Ucides cordatus*; *Ucord(zoea)*, zoeae of *U. cordatus*). Microplastics (*Hard(μ)*, hard; *Soft(μ)*, soft; *Paint(μ)*, paint chips; *Thr(μ)*, threads). Macroplastics (*Hard(M)*, hard; *Soft(M)*, soft)] The environmental variables (dissolved oxygen, salinity, temperature) are represented by arrows.

DISCUSSION

LARVAL FISH ASSEMBLAGES FROM MANGROVE CREEKS OF THE GOIANA ESTUARY

Tropical Atlantic mangrove habitats are reported to have low species richness (Robertson & Alongi, 1992). This was true for the larval fish assemblages of the mangrove creeks of the Goiana Estuary, where 8 species dominated the habitat, although 31 species were reported. Similar findings were made for other estuarine larval fish populations, such as in the Caeté Estuary (North Brazil) (Barletta-Bergan *et al.*, 2002*a*) and in the Colorado lagoon, Alamitos Bay (Southern California) (Allen & Horn, 1975), where 54 and 23 species were registered, respectively.

In this study, 74.2 % of the most abundant larval fish are estuarine resident species that spawn and hatch within the estuary where they also complete their life cycle. Only 25.8 % are larvae from marine spawners that probably use the mangrove and the estuary for short periods. The relative low contribution of marine larvae might be associated with adult marine spawners avoidance of mangrove areas due to wide salinity variations. The creeks chosen are located in the lower portion of the estuary, it would be expected a certain contribution of marine fish larvae among the most abundant species. The sampling period (April to May) was the early rainy season, when the density of marine fish larvae is low in the lower estuary because they move to the upper estuary (next to the river mouth) (Lima et al., *in press*; Lima et al., 2014). The peaks in fish larvae and zooplankton density in the main channel of the Goiana estuary occurs during the late dry season (December to February) in its lower portion (Lima et al., *in press*; Lima et al., 2014). A similar conclusion was reported for the mangrove creeks of the Caeté Estuary, where only 2 % of larvae were of marine spawners (Barletta-Bergan *et al*., 2002*a*), and in a salt-marsh nursery in North Carolina, where stenohaline species were rarely found (Weinstein, 1979). Moreover, most fish larvae in Goiana mangrove creeks were in the post-flexion stages (Table III). It suggests that early stages used the main channel during the sample period, and later stages use the creeks for feeding and protection.

Adult and juvenile fishes species from the mangrove creeks of the Goiana Estuary (Ramos *et al*., 2011) were also recorded as larvae in this study. Some were among the most abundant (*C. edentulus*, *A. clupeoides* and *R. bahiensis*) and others were less abundant [the Sciaenidae *Bairdiella ronchus* (Cuvier 1830), the Hemiramphidae *Hyporhamphus unifasciatus* (Ranzani 1841) and the Tetraodontidae *Sphoeroides testudineus* L. 1758] (Ramos *et al*., 2011). Some species were found only in the larval phase in the estuary, such as the Gerreidae *U. lefroyi* and the Sciaenidae *Stellifer rastrifer* (Jordan 1889) and *S. stellifer* (Bloch 1790). Other fish species were reported using the creeks only during the juvenile and adult phases, such as the Lutjanidae *Lutjanus jocu* (Bloch & Schneider 1801), the Engraulidae *Lycengraulis grossidens* (Spix & Agassiz 1829), and the Haemulidae *Pomadasys croco* (Cuvier 1830) (Ramos *et al*., 2011). Both studies linked the higher abundance of these species during full and new moon to higher tidal amplitudes (Ramos *et al*., 2011). Thus, fish species may utilize mangrove creeks during different phases of their life cycle or even being there for just a short time.

A study regarding the seasonal and temporal variability of ichthyoplankton in the mangrove creeks of the Caeté Estuary also identified *A. clupeoides*, *C. acoupa* and *R.*

bahiensis as common species (Barletta-Bergan *et al*., 2002*a*). In addition, it noted the presence of later phases of fish species that were found as larvae in creeks of the Goiana Estuary (*e.g*. *U. lefroyi*, *G. oceanicus* and S. *testudineus*) (Barletta-Bergan *et al*., 2002a). This study, among others, reinforce the importance of mangrove creeks as nursery habitats, and also for protection from predators and for feeding strategies of later life history stages (Blaber *et al*., 1989; Laroche *et al*., 1997; Barletta-Bergan *et al*., 2002*a*; Hampel *et al*., 2003; Barletta *et al*., 2003; Krumme *et al*., 2008).

TABLE IV. Summary of canonical correspondence analysis (CCA) using three environmental variables (water temperature, dissolved oxygen and salinity), the moon phases and the densities of fish larvae, zooplankton and plastic debris in the mangrove creeks of the Goiana estuary. Ns, non-significant.

Summary of CCA	**Fish larvae/plastic**			**Zooplankton/plastic**		
	Axis 1	**Axis 2**	***p* value**	**Axis 1**	**Axis 2**	***p* value**
Eigenvalue	0.176	0.032		0.13	0.011	
Species-environment correlation	1	1		1	1	
Cumulative % variance						
of species data	78.6	92.7		90.6	98	
of species-environmental variables	78.6	92.7		90.6	98	
Correlation with environmental variables						
Water temperature	0.7556	-0.6400	0.3168^{Ns}	-0.4247	0.6937	1^{Ns}
Dissolved oxygen	0.9368	0.0750	1^{Ns}	-0.8380	0.0338	0.18^{Ns}
Salinity	0.9450	-0.3268	0.1683^{Ns}	-0.3504	0.3926	0.52^{Ns}

MOON PHASE INFLUENCE ON THE COMPOSITION OF LARVAL FISH ASSEMBLAGES

From the 31 fish species found in the mangrove creeks of the Goiana Estuary, larval *C. edentulus*, *A. clupeoides* and *R. bahiensis* were the most abundant, independent of moon phase, representing 57% of the total catch. However, full moon had a strong positive influence on catches of *G. oceanicus*, *C. acoupa* and *A. brasiliensis*, and new moon on *U. lefroyi*. Full and new moon also influenced the composition of zoeae and megalopae of *U. cordatus*, and protozoeae and larvae of Caridea shrimp, as well as the proportions of hard and soft plastics, both micro and macro sizes, possibly because of the increased tidal amplitudes and water flow. In a previous study in mangrove creeks of the Goiana Estuary, moon phase was shown to strongly influence the pattern of use of the mangrove creek by juvenile and adult fishes and to change their numbers and biomass for

feeding, protection or nursery proposes during different tidal amplitudes (Ramos *et al*., 2011). For example, the full and new moon influenced the pattern of use of the creeks of *A. clupeoides*, and the new moon influenced the Centropomidae *Centropomus pectinatus* Poey 1860 and the Belonidae *Strongylura timucu* (Walbaum 1792) by increasing abundances during higher tides (Ramos *et al*., 2011). Along sandy beaches at the mouth of the Goiana Estuary (southern shores), higher abundance of fishes (*e.g. L. grossidens* and the Mugilidae *Mugil* sp.) and crustaceans [e.g. the Portunidae crab *Callinectes danae* Smith 1869 and the Penaeidae shrimp *Litopenaeus schmitti* (Burkenroad, 1936)] occurred during first and last quarter moon (Lacerda *et al*., 2014). For this study, when tidal forces are weaker and the environmental variables more stable, conditions are better for the occupation of sandy beaches habitat (Lacerda *et al*., 2014). In addition, larvae of marine species (*e.g. C. edentulus*, *C. acoupa*, *A. brasiliensis* and *Mugil* sp.) that inhabit sandy beaches were found now in the mangrove creeks, emphasizing the use of both habitats for different purposes, and more specially the later as a nursery ground (Lacerda *et al*., 2014).

Other studies have also detected the influence of moon phases on the distribution and composition of larval fish assemblages. In the main channel of the Caeté Estuary, larvae of *C. acoupa* showed higher densities during new moon, the Sciaenidae *Stellifer microps* (Steindachner 1864), *A. clupeoides* and the Achiridae *Apionichthys durmerili* Kaup 1858 during first quarter moon, whereas the Auchenipteridae *Pseudauchenipterus nodosus* (Bloch 1794) and the Gobiidae *Microgobius meeki* Evermann & Marsh 1899 showed higher densities during full moon (Barletta & Barletta-Bergan, 2009). The faunal density of the most common species from an intertidal salt marsh creek of the Weterschelde Estuary (Northwest Netherlands) changed during the semi-lunar regime, with higher total densities during spring tides (Hampel *et al*., 2003). For this creek, a mysid shrimp was most abundant during spring tide, whereas several invertebrate taxa and a fish species were highly abundant during neap tides (Hampel *et al*., 2003). All these studies support our initial hypothesis that moon phases influence the pattern of use of the creeks by changing the number and composition of the larval fish assemblages and zooplankton in the mangrove creeks of the Goiana Estuary.

OCCURRENCE OF PLASTIC DEBRIS IN MANGROVE CREEKS

The present study is the first to describe how moon phases influence not only fish larvae and zooplankton, but also the amounts and diversity of plastic debris in mangrove creeks. Differently from the main channel, where plastic debris were numerically

comparable to ichthyoplankton (Lima *et al*., 2014), in the mangrove creeks plastic debris items were 6.3 times less abundant than fish larvae when total densities are compared. However, micro- and macroplastics occurred in all the twelve creeks, and their density is the same as the third most abundant fish taxon, *R. bahiensis*. Comparisons of the amounts of macroplastics (> 5 mm) from the main channel of the Goiana Estuary and those of the creeks, shows that larger plastics are more abundant in the mangrove creeks (Lima *et al*., 2014). It is possible that larger plastics accumulate in the mangrove forest, fragment into microplastics through the dynamics of ebb and flood tides, and during high tides are transported to the main channel of the estuary (Araújo & Costa, 2007; Browne *et al*., 2010; Cordeiro & Costa, 2010; Lima *et al*., 2014). In the Goiana Estuary, fisheries is pointed as the main potential source of plastic debris due to the high availability of identifiable specific items as threads (Guebert-Bartholo *et al*., 2011; Possatto *et al*., 2011; Dantas *et al*., 2012; Ramos *et al*., 2012; Lima *et al*., 2014).

The ubiquitous and continuous availability of these debris, mixed with the biota of mangrove creeks and plankton of the main channel, may negatively affect prey-predator relations (Boerger *et al*., 2010; Ivar do Sul *et al*., 2013; Wright *et al*., 2013). During higher tides on full and new moons, sea water entering the estuary allows juvenile and adult coastal fishes to inhabit the lower part of the main channel and mangrove creeks for protection and feeding (Barletta-Bergan *et al*., 2002*a,b*; Barletta *et al*., 2003; Ramos *et al*., 2011). When, in a dark and turbid intertidal habitat, fishes might easily feed on plastic debris of the same size and shape as their natural prey. Such was confirmed by examining the gut contents of Ariidae catfishes (Possatto *et al*., 2011), Gerreidae mojarras (Ramos *et al*., 2012) and Sciaenidae drums (Dantas *et al*., 2012). The ingestion of non-digestible items, such as plastics, may block the alimentary canal and induce starvation (Cole *et al*., 2013). In addition, plastics have the capacity of adsorb persistent organic pollutants (POPs), biocides and trace metal posing a threat to the environment such as sublethal effects of eating contaminated plastic (Moore, 2008; Frias *et al*., 2010; Turner, 2010). This, potentially, reduce survivorship and, consequently, reduce the nursery value of the mangrove creek habitat.

SHIFTS IN THE COMPOSITION OF FISH LARVAE, ZOOPLANKTON AND PLASTIC DEBRIS RELATIVE TO MOON PHASES

Lunar cycles associated with other environmental variables influence species composition due to the displacement of different water masses and their associated plankton, even over short periods of time (Alldredge & King, 1980; Kingsford &

MacDiarmid, 1988; Hernández-León, 2008). During full and new moon, tidal amplitude can be of up 2.8 m at the study site, while during first and last quarter moons it can be up to 2.1 m. It seems that during first and last quarter moons, flooding does not reach a sufficient height to reach the whole mangrove forest; and ebbing does not drain as efficiently the main channel and lower estuary. As such, it might also influence the amount of nutrients in the creeks and decrease productivity (Nagelkerken *et al*., 2008). Thence, the density of zooplankton and most larval fish species decreased. Moreover, this hydrodynamism seems to decrease plastic sources and displacement during these lunar phases, and less plastic debris enter the mangrove creeks from the lower estuary.

Scatter plots show that, during the first quarter moon, the lowest mean densities of zooplankton in the creeks coincided with the lowest densities of fish larvae. In addition, plastics debris also presented lower densities during this lunar phase, except the macroplastic threads. This suggests that when there is low availability of food, larvae utilize other areas as feeding grounds. However, this is not always so in Goiana mangrove creeks. Even with no detectable significant difference, the mean densities of the most abundant species, *C. edentulus, A. clupeoides* and *R. bahiensis*, were highest during last quarter moon, coinciding with a positive correlation with this lunar phase. During this moon phase, mean densities of zooplankton and plastic debris were low, except microplastic threads that had a positive correlation with last quarter moon. It suggests that, mainly later stages (> 8 mm) of these fish species take advantage of slower tidal flows, utilizing the creeks during neap tide for protection, and explore other habitats of the estuary as feeding grounds during spring tide.

Species that utilize mangrove creeks take advantage of stronger flushing and higher water levels during full and new moon, when there are more prey and non-living particles into the creeks (Barletta-Bergan *et al*., 2002*a*; Hampel *et al*., 2003; Barletta & Barletta-Bergan, 2009). During full and new moons, flooding fills completely the mangrove forest and the ebbing efficiently drains the main channel and lower estuary. This hydrodynamism might increase the quantity of nutrients in the creeks, rising the productivity (Nagelkerken *et al*., 2008). It ensures higher food availability for most larval fish species, which in turn present higher densities in the creeks. In addition, the sources of plastics increase during these lunar phases and plastic debris from land, river and sea enter the mangrove creeks of the lower Goiana estuary. Post-flexion larvae of *D. rhombeus* were abundant during full, last quarter and new moon. However, post-flexion larvae of *U. lefroyi* and hard microplastics had their highest densities during full and new

moon. All of these had their abundance positively associated with new moon, occurring when calanoid copepods, caridean larvae and zoeae of *U. cordatus* were abundant in the creeks. On the other hand, different larval stages of *C. acoupa* and *A. brasiliensis*, and later stages of *G. oceanicus* presented highest densities during full moon, coinciding with a positive correlation with this lunar phase, when protozoeae of Caridea and megalopae of *U. cordatus* were highly available. During this moon phase, hard and soft macroplastics, paint chip (micro) and soft microplastics were also the most detected in the creeks.

Size and shape similarity between zooplankton and plastics can be cause of concern since the presence of food resources attract predators to these environments and put them under risk of plastic ingestion (Boerger *et al*., 2010; Ivar do Sul *et al*., 2013; Wright *et al*., 2013). Most larval species in this study (80.22%) are in later developmental stages (post-flexion), and during feed can prey on microplastic, especially those smaller than 2 mm (42.2%), which are similar in shape and colour to zooplankton prey. However, the problem of ingestion is not exclusively associated with fish larvae. Mangrove creeks are also used by larger fish that can feed on plastic debris, in the water column, on the bottom, or already consumed by fish larvae (trophic transfer). This paper shows that the chances of interaction between these species and this class of debris are real, and quite high.

Mangrove creeks of the Goiana lower estuary always flood during high tides and ebb during low tides, remaining partially inundated independent of moon phase. For this reason, they function as a nursery during the entire lunar cycle by providing food and protection for different assemblages of larval fish (Blaber *et al*., 1989; Laroche *et al*., 1997; Barletta-Bergan *et al*., 2002*a*; Hampel *et al*., 2003; Barletta *et al*., 2003; Krumme *et al*., 2008). In addition, this short time study suggests that environmental variables do not significantly influence the number of fish larvae and zooplankton in this time scale. This emphasizes that plankton composition instead is positively correlated to moon phases and their associated tidal amplitudes. The changes in the abundance of different fish species and zooplankton are associated to the use of Goiana mangrove creeks during specific moon phases, as feeding and/or protection grounds. Plastic debris also presented changes in abundance and composition of its total loads relative to moon phases. Their higher densities during full moon seems to be associated to higher flooding and ebbing, due to more efficient flooding and flushing of the forest soil, as well as adjacent areas within the estuary. Further studies regarding the seasonal patterns of use of mangrove creeks by larval fish assemblages, and their interaction with other environmental abiotic

compartments, are required to a detailed understanding of the nursery function of South American mangroves for fish species.

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CONCLUSÕES

Conclusões

No estuário do Rio Goiana, seguindo uma tendência em estuários tropicais do mundo, a flutuação sazonal da salinidade e a maior vazão do rio durante os meses de alta precipitação são responsáveis pela distribuição das larvas de peixes. Neste estuário há um alto número de espécies marinhas que habitam, principalmente, o estuário inferior, e total ausência de espécies de água doce, contribuindo para o baixo número de espécies no estuário superior. 66,6% das larvas foram representadas por clupeídeos sendo *Rhinosardinia bahiensis* a espécie mais abundante, seguida por *Harengula clupeola.* Os engraulídeos contribuíram com apenas 8,1% da ictiofauna, onde as larvas de *Anchovia clupeoides* e *Cetengraulis edentulus* foram as mais representativas. Muitas das larvas também ocorrem no sistema como juvenis, confirmando o uso do estuário com um habitat de berçário.

Larvas de espécies estuarinas (ex. *R. bahiensis, Anchovia clupeoides, Gobionellus oceanicus*), bem como os microplásticos foram bem representativos em todo canal principal do estuário durante todo ano. Durantes os meses mais secos (início da chuva e início e final da seca), a cunha salina é alcança o estuário superior, permitindo que larvas de imigrantes marinhos (*H. clupeola, Trinectes maculatus, Cynoscion acoupa, C. edentulus* e *Lupinoblennius nicholsi*), que foram abundantes no estuário inferior, alcancem o estuário superior até a zona de influência de águas costeiras (Fig. 1). As larvas de espécies estuarinas, como *R. bahiensis* e *A. clupeoides*, foram as mais representativas no mesmo período. A baixa densidade de larvas de peixes, durante o início da estação chuvosa, no estuário superior também pode estar associada às baixas densidades de zooplâncton. No estuário médio, zooplâncton apresentaram altas densidades, estando mais disponível para a alimentação de larvas de peixes. Entretanto, o encontro de aguas de diferentes densidades no estuário médios forma uma barreira que retém os microplásticos nos estuários superior e inferior nos meses secos.

No final da estação seca, um bloom de zooplâncton foi seguido por uma bloom de larvas (12.74 ind. $100m^{-3}$) e ovos de peixes (14.65 ind. $100m^{-3}$) no estuário inferior. (Fig. 1). As larvas de *R. bahiensis, H. clupeola, T. maculatus, G. oceanicus, C. acoupa* e *L. nicholsi* foram as mais representativas. Esse padrão de distribuição de organismos do plâncton indica que peixes marinhos utilizam a porção inferior do estuário do Rio Goiana como área de desova durante o final da estação seca.

No final da estação chuvosa, quando o estuário recebe o maior fluxo de água do rio, a cunha salina migra para o estuário inferior (Fig. 1). Este fluxo de agua doce rio

abaixo parece ser responsável pelo transporte de microplásticos, juntamente com as larvas e ovos de peixes para as regiões mais costeiras. Nessa estação, os microplásticos apresentaram sua máxima densidade suas densidades (14 items $100m^{-3}$), comparável com a máxima densidade de larvas de peixes (14.23 ind. $100m^{-3}$) no estuário inferior.

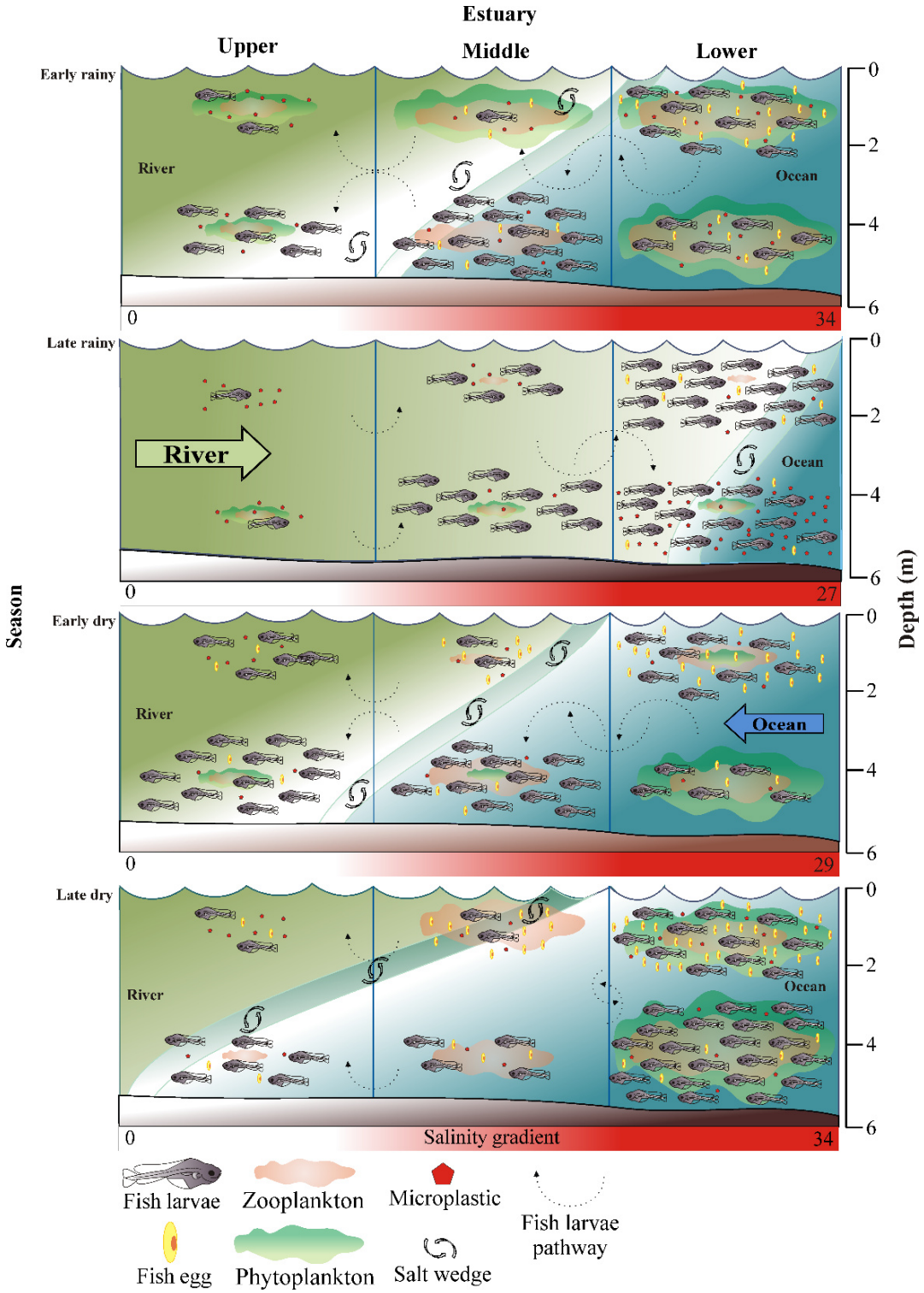

Figure 1. Modelo conceitual para a distribuição sazonal e espacial de larvas, ovos de peixes e microplásticos no estuário do Rio Goiana.

Diferentemente das larvas de peixes, os microplásticos apresentaram uma correlação positiva com altas taxas de precipitação, sendo mais associados a fluxos para dentro ou para fora do estuário do que a variações sazonais nas variáveis ambientais. Quando os padrões de distribuição do plâncton e dos microplásticos são comparados, verifica-se que os organismos seguem um padrão que se assemelha a uma cadeia alimentar, procurando regiões com condições favoráveis à alimentação. Enquanto que o padrão de águas de diferentes densidades não permite a passagem dos microplásticos do estuário superior para o inferior, e na direção oposta (rio acima). Esta observação enfatiza a ideia de que, mesmo o plâncton não sendo capaz de "nadar" contra as correntes, eles realizam migrações, procurando fluxos favoráveis que os levem para áreas que suportem suas guildas ecológicas, seja para evitar a predação, ou para alcançar áreas de melhor suprimento alimentar.

Nos canais de maré do estuário inferior foram reportadas 31 espécies, onde 8 são dominantes. 74,2% das espécies de larvas mais abundantes desovam em áreas de manguezal, ou completam seus ciclos de vida dentro do estuário. Somente 25,8% foram espécies marinhas. A baixa contribuição de larvas marinhas pode estar associada ao ato de evitar áreas de manguezal devido à alta turbidez e ampla variação de salinidade. Adultos e juvenis que são reportados como usuários dos canais de maré do estuário do Rio Goiana também aparecem como larvas neste estudo, Alguns estão entre os mais representativos, *C. edentulus*, *A. clupeoides* e *R. bahiensis*; e outros menos abundantes como *Bairdiela ronchus*, *Hyporhamphus unifasciatus* e *Sphoeroides testudineus*. Algumas espécies só são encontradas como larvas, como *Ulaema lefroyi*, *Stellifer rastrifer* e *S. stellifer*. Outras larvas abitam os canais somente durante as fases juvenis e adultas, como *Lutjanus jocu*, *Lycengraulis grossidens* e *Pomadasys croco*. A alta abundância dessas espécies durante as luas cheia e nova está relacionada as altas amplitudes da maré.

A lua cheia teve influência positiva sobre *G. oceanicus*, *C. acoupa* e *Atherinella brasiliensis*, e a lua nova sobre *U. lefroyi*. A lua cheia e nova, também influenciou o número de zoé e megalopa de *Ucides cordatus*, e protozoé e larvas de camarão Caridae, bem como o número de plásticos duros e moles, tanto os < 5 mm, quanto os > 5mm. *C. edentulus* e *R. bahiensis* mostraram forte correlação com a lua quarto crescente, quando havia menos zooplâncton nos canais e maior abundância de micro filamentos plásticos. *A. clupeoides*, *Diapterus rhombeus*, *U. lefroyi* e microplásticos duros mostraram associação com diferentes fases da lua, ocorrendo quando copepoda Calanoida, larvas de

Caridae e zoé de *U. cordatus* foram abundantes nos canais. *C. acoupa*, *G. oceanicus* e *A. brasiliensis*, foram fortemente associadas à lua cheia, quando protozoé de Caridae e megalopa de *U. cordatus* também foram altamente disponíveis, assim como plásticos duros e moles > 5mm, e tintas de barco e plásticos moles < 5mm. O estudo enfatiza a importância dos canais de maré como áreas de berçário por promover fontes alimentares e proteção para muitas espécies de larvas de teleósteos. Este estudo sugere que as variáveis ambientais não influenciam significativamente o número de larvas de peixes e zooplâncton numa curta escala de tempo. Isto enfatiza que a composição do plâncton é positivamente correlacionada com as fases da lua e suas amplitudes de maré associadas. As mudanças na abundancia de diferentes larvas e zooplâncton estão associadas ao uso dos canais de mare do rio Goiana durante uma fase da lua específica, como áreas de alimentação e/ou proteção. Os detritos plásticos também apresentaram mudanças em suas abundancies e composições totais em relação as fases da lua. Suas maiores densidades durante a lua cheia parecem estar relacionadas a maior inundação e escoamento do solo da floresta de manguezal, bem como das áreas adjacentes ao estuário (Fig. 2).

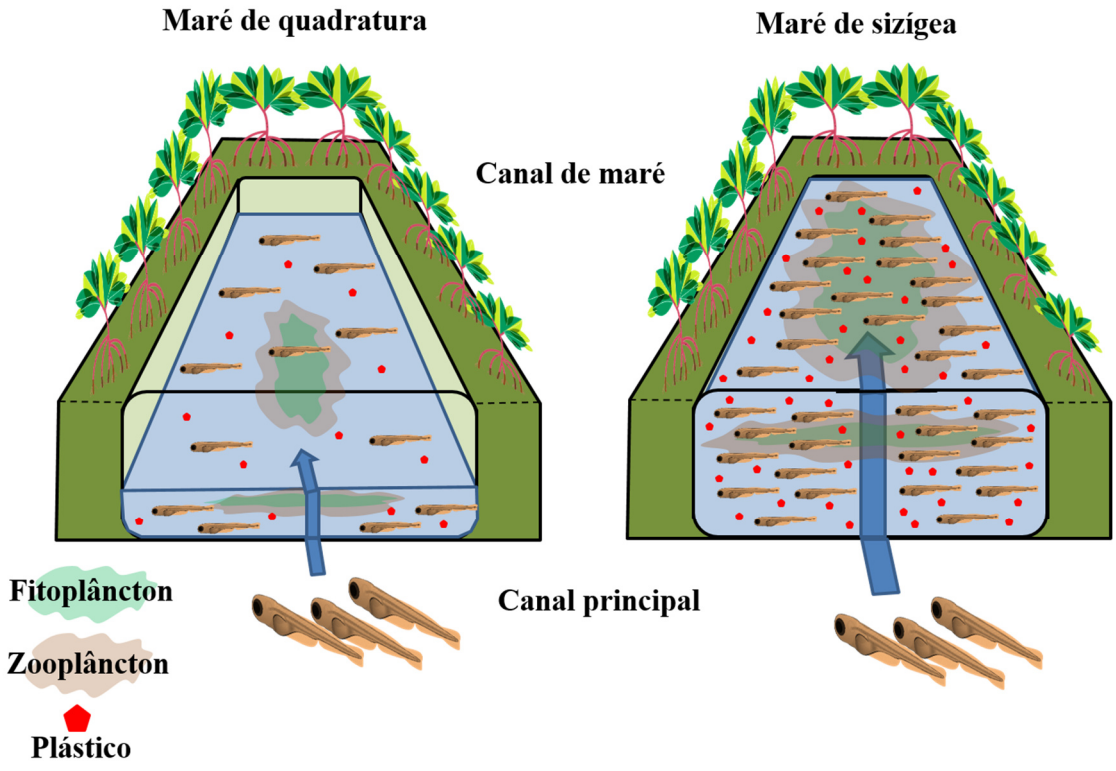

Figure 2. Modelo conceitual para a composição do plâncton e do microplástico nos canais de maré da porção inferior do estuário do Rio Goiana em relação as fases da lua.

No canal principal do estuário do Rio Goiana, a densidade de microplásticos representou metade da densidade total de larvas de peixes, o que é uma grande quantidade. Eles são encontrados em todos os habitats do canal principal e estão biodisponível para organismos planctônicos e muitos vertebrados em águas de superfície e de fundo durante todo o ano. Entretanto, nos canais de maré, um estudo mênsal detectou que os detritos plásticos foram cerca de 6,3 vezes menos abundantes do que as larvas de peixes quando suas densidades totais são comparados. Quando a quantidade de macroplásticos no canal principal a nos canais de maré é comparada, é possível notar que itens maiores são mais abundantes nos canais de maré. Por este motivo, é possível que itens maiores estejam se acumulando no manguezal, se fragmentando em microplásticos através da dinâmica das marés vazante e enchente, e, durante as marés altas, são transportados para o canal principal do estuário. Os micro e macroplásticos contaminaram todos os doze canais, e a sua densidade é semelhante a do terceiro táxon mais abundantes, *R. bahiensis*. Densidades comparaveis na coluna d'água aumenta as chances de interação entre os microplásticos e as larvas de peixes, incluindo a ingestão de fragmentos menores, cujas cires e formas são similareas às das presas zooplanctônicas. Organismos de níveis tróficos inferiores que se alimentam dos pequenos fragmentos representam, portanto, um vetor para a transferência de microplástico através da cadeia alimentar para outros ambientes. Além disso, os contaminantes do microplástico, como biocidas e metais traço de lascas de tinta, representam uma ameaça através da bioacumulação e biomagnificação, assim, estando disponíveis para a população humana que utiliza recursos alimentares estuarinos.

Fragmentos de plástico em suas diferentes composições, formas e cores são uma preocupação decorrente sobre contaminação em estuários e suas áreas adjacentes. A maioria desses itens são introduzidos no estuário pelo escoamento direto de microplásticos anteriormente dispersos (incluindo micro esferas de produtos cosméticos). Outra fonte é a quebra, pelo intemperismo, de itens plásticos grandes, gerados durante uso doméstico (por exemplo, sacolas, frascos e garrafas), artesanal ou pesca comercial (equipamentos e manutenção de barcos), ou atividades recreativas (embalagens de lanches) na bacia do rio ou praias adjacentes ao estuário.

No sentido de ampliar o conhecimento sobre estudos estuarinos em larvas de peixes, este trabalho surge como uma ferramenta para descrever a assembleia ictioplanctônica do estuário do Rio Goiana, não só taxonomicamente, mas também em termos de estrutura ecológica e uso dos recursos disponíveis (zooplâncton, fitoplâncton,

microplásticos e matéria orgânica particulada) nos diferentes habitats dos estuários baseados nas variações sazonais dos parâmetros abióticos. Com este estudo é possível identificar os locais utilizados como berçário para as principais espécies de peixes encontradas e comercializadas no local. Essa região é uma área de constante ocupação e ação antrópica, enfatizando a importância da identificação do papel desses habitats para a ontogenia das espécies presentes no local, e sua comparação com outros estuários do mundo, considerados preservados, visando gerar dados que contribuam com a aplicação de medidas de manejo voltada a preservação desses habitats e para proteger essas espécies durante sua reprodução e renovação dos estoques pesqueiros.

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ANEXO A

Autorização para atividades com finalidade científica

ANEXO A: Autorização para atividades com finalidade científica

Ministério do Meio Ambiente - MMA
Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio
Sistema de Autorização e Informação em Biodiversidade - SISBIO

Autorização para atividades com finalidade científica

Número: 11050-1	Data da Emissão: 20/01/2010 10:10

Dados do titular

Nome: Mário Barleta	CPF: 583.587.909-10
Título do Projeto: Ecologia de Ecossistemas Aquáticos: Interação: Rio, Estuário e Mar	
Nome da Instituição : UFPE - UNIVERSIDADE FEDERAL DE PERNAMBUCO	CNPJ: 24.134.488/0001-08

Cronograma de atividades

#	Descrição da atividade	Início (mês/ano)	Fim (mês/ano)
1	Produtividade pesqueira no Estuário do Rio Goiana e áreas adjacentes	01/2010	03/2012
2	Marcação e captura de espécies anádromas que utilizam o Estuário do Rio Goiana	01/2010	03/2012
3	Estudo sobre contaminação por metais pesados em invertebrados e vertebrados do Estuário do Goiana	01/2010	03/2012
4	Estudo sobre composição de dióticos (Conectividade entre peixes do estuário)	01/2010	03/2012
5	Fluxo de energia (cyclopiancion e peixes) entre diferentes habitats do Estuário do rio Goiana	01/2010	03/2012
6	Mangueiral, capim marinho e recifes artificiais: interação entre habitats e as espécies de peixes	01/2010	03/2012

De acordo com o art. 33 da IN 154/2009, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto.

Observações e ressalvas

1	As atividades de campo exercidas por pessoa natural ou jurídica estrangeira, em todo o território nacional, que impliquem o deslocamento de recursos humanos e materiais, tendo por objeto coletar dados, materiais, espécimes biológicos e minerais, peças integrantes da cultura nativa e cultura popular, presente e passa da, obtidos por meio de recursos e técnicas que se destinem ao estudo, à difusão ou à pesquisa, estão sujeitas a autorização do Ministério de Ciência e Tecnologia.
2	Esta autorização não exime o titular e a sua equipe da necessidade de obter as anuências previstas em outros instrumentos legais, bem como do consentimento do responsável pela área, pública ou privada, onde será realizada a atividade.
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6	Esta documento não dispensa o cumprimento da legislação que dispõe sobre acesso a componente do patrimônio genético existente no território nacional, na plataforma continental e na zona econômica exclusiva, ou ao conhecimento tradicional associado ao patrimônio genético, para fins de pesquisa científica, bioprospecção e desenvolvimento tecnológico.
7	Em caso de pesquisa em UNIDADE DE CONSERVAÇÃO, o pesquisador titular desta autorização deverá contactar a administração da unidade a fim de CONFIRMAR AS DATAS das expedições, as condições para realização das coletas e de uso da infra-estrutura da unidade.
8	As atividades contempladas nesta autorização abrangem espécies brasileiras constantes de listas oficiais (de abrangência nacional, estadual ou municipal) de espécies ameaçadas de extinção, sobreexplotadas ou ameaçadas de sobreexploração.

Outras ressalvas

1	ESTA AUTORIZAÇÃO PERMITE A REALIZAÇÃO DAS ATIVIDADES NELA LISTADAS NA RESERVA EXTRATIVISTA MARINHA ACAÚ-GOIANA (PB/PE).

Equipe

#	Nome	Função	CPF	Doc. Identidade	Nacionalidade
1	Monica Ferreira da Costa	Vice-líder do grupo de pesquisa	000.259.227-42	05396333-4 IFP-RJ	Brasileira
2	David Vallenge Duntas	[illegible]	[illegible]	[illegible]	[illegible]
3	André Ricardo de Araújo Lima	aluno de mestrado PPG em Oceanografia	064.070.294-57	5303216 SSP-PE	Brasileiro
4	Jonas de Assis Almeida Ramos	aluno de mestrado ppg Oceanografia	047.495.344-04	6343058 SSP-PE	Brasileiro
5	Jacqueline Santos da Silva	aluna de doutorado ppg oceanografia	007.801.004-03	5481351 SSP/PE SSP/PE-PE	Brasileiro
6	Rodrigo Lima Guerra de Moraes	aluno de mestrado PPG em oceanografia	035.220.944-50	4349887 SSP-PE	Brasileira
7	Carlos Henrique Figueiredo Lacorte	colaborador	294.209.348-02	27666637-x SP-SP	Brasileiro

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