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ÉRICA DINIZ ABRANTES GONÇALVES

ESTRUTURA E DIVERSIDADE FUNCIONAL DE COPEPODA EM DOIS ESTUÁRIOS TROPICAIS

CAMPINA GRANDE

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Dissertação de mestrado apresentada ao Programa de Pós-graduação em Ciência eTecnologia Ambiental, da Universidade Estadual da Paraíba, como requisito para obtenção do título de mestre em Ciência e Tecnologia Ambiental.

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RESUMO

Neste estudo caracterizou-se a comunidade de Copepoda quanto à estrutura e à variação da diversidade funcional ao longo do gradiente salino de dois estuários do nordeste brasileiro, Mamanguape (6º43‘02‖ S; 35º67‘46‖ W) e Paraíba do Norte (6º57‘30‘‘ S; 34º51‘30‘‘ W), em períodos distintos: seca (novembro/2013) e chuva (julho/2014).As coletas foram realizadas no sentido montante-jusante, em preia-mar, em lua cheia, em quatro zonas salinas (Z1, Z2, Z3 e Z4), com três réplicas em cada local de amostragem de cada uma das zonas salinas, com exceção da zona Z4 do estuário do Rio Paraíba do Norte (PB), onde se realizaram 3 réplicas em cada um dos lados da Ilha da Restinga. As amostras coletadas com malha de 68 µm foram triadas e os organismos identificados até ao nível taxonômico mais específico possível, procedendo-se à contagem dos mesmos. No total foram identificadas 22 espécies (duas identificadas até gênero), sendo apenas *Notodiaptomus iheringi* e *Microcyclops anceps* típicas de água doce, presentes apenas no estuário do Mamanguape, enquanto *Acartia tonsa* e *Paracalanus campaneri* exclusivas do estuário do Paraíba do Norte. As demais espécies/géneros são típicas de ambientes estuarino-costeiro-oceânico. Em ambos os estuários, a maior densidade de organismos foi observada na época da chuva, no estuário da Paraíba do Norte na Z2 e no estuário do Mamanguape na Z3. No Paraíba do Norte *Oithona brevicornis* apresentou as maiores densidades no período chuvoso, nas zonas Z2 (27 ind/L), Z3 (24 ind/L) e Z4 (12 ind/L). No Mamanguape *O. brevicornis* apresentou sua maior densidade no período chuvoso, registando-se 52 ind/L na zona Z3. Durante o período de seca observou-se a entrada de espécies costeiras-oceânicas em direção a montante, como é o caso da espécie *Oithona oswaldocruzi* que apresentou suas maiores densidades nas zonas Z1 (19 ind/L), Z2 (21 ind/L) e Z3 (25 ind/L) no estuário de Mamanguape, ao passo que no período chuvoso a maior densidade foi registrada na zona Z3 (21 ind/L). No estuário da PB do Norte, *Oithona oswaldocruzi* alcançou o valor de 38 ind / L na Z2 no período chuvoso. Para ambos os estuários, a maior biomassa (*u*g/L) foi registrada para O. *brevicornis* e *O. oswaldocruzi,* durante o período chuvoso. Quanto aos dados ambientais, os valores de nitrogênio inorgânico dissolvido (NID), no Mamanguape, variaram de 23.54 µg/L (Z4 - chuva) a 460.98 µg/L (Z4), e no Paraíba do Norte de 434.30 µg/L (Z4) à 4139.54 µg/L (Z3) na época seca, enquanto que no período de chuva a variação foi de 991.81 µg/L (Z4) a 4037.4 µg/L (Z3); o fósforo total (Pt) apresentou valores mais elevados no Paraíba do Norte (153.88 µg/L a 754.44 µg/L) quando comparado ao Mamanguape (63.33 µg/L a 284.44 µg/L); o ortofosfato (ORP) variou de 45 à 87 µg/L no Mamanguape e de 57 a 191 µg/L no Paraíba do Norte; o fósforo solúvel reativo (SRP) apresentou valores mais elevados no Paraíba do Norte, variando de 68.55 µg/L à 639.11 µg/L. A análise da Diversidade de Shannon-Wiener apresentou os valores mais elevados na Z4 em ambos os estuários, sendo no estuário do Mamanguape (H'=2,8 - período de seca) mais elevado que no estuário da Paraíba do Norte (H'= 2,5 - período chuvoso). Os resultados da PERMANOVA evidenciaram que não existe interação significativa entre a densidade do mesozooplâncton com os pontos amostrados (Pseudo-F = 1,2326; P= 0,293), os períodos de coleta (Pseudo-F = 3,4829; P = 0,026) e os estuários (Pseudo-F = 4,2619; P = 0,058), sendo significativas apenas as diferenças entre as zonas (olig, poli P = 0,004; olig, euha P = 0,016). Quanto à diversidade funcional, em ambos os estuários, o índice de riqueza funcional apresentou valores baixos. No estuário da PB do Norte os valores mais elevados foram registrados durante o período de seca nas Z2, Z3 e Z4, enquanto no Mamanguape o valor mais elevado foi registrado na Z3 no período de seca. O índice de equitabilidade funcional não apresentou grandes diferenças, concentrando o maior valor no período de seca no estuário do Mamanguape, e o menor valor observado na época seca no estuário da Paraíba do Norte (Z2). Os índices de divergência funcional foram maiores durante o período chuvoso em ambos os estuários, mas sem grandes variações. Os índices de dispersão funcional foram baixos nos dois estuários, mas foram mais representativos durante o período de seca.O estudo comprovou a existência de diferenças na diversidade de copépodes ao longo do gradiente salino de ambos os estuários.

Palavras chave: Copepoda, diversidade funcional, época seca, época chuva, Estuário do Mamaguape, Estuário Paraíba do Norte.

ABSTRACT

This study characterized the community of Copepods as the structure and variation of functional diversity along the saline gradient in two estuaries of northeastern Brazil, Mamanguape (6º43'02‖ S; 35º67'46‖ W) and North Paraiba (6º57 '30' 'S; 34º51'30' 'W), in different periods: dry (November / 2013) and rain (July / 2014). Samples were collected in the amount-downstream direction, at high tide, full moon in four saline zones (Z1, Z2, Z3 and Z4), with three replicates at each sampling site of each of the saline areas, except the Z4 area of the North Paraiba estuary (CP), which took place in three replicas on each side of the island of Restinga. Samples collected with a 68 μm mesh organisms were screened and identified to the specific possible taxonomic level, proceeding to count them. In total, they identified 22 species, only being *Notodiaptomus iheringi* and *Microcyclops anceps* typical of freshwater areas, present only in the estuary of the Mamanguape, while *Acartia tonsa* and *Paracalanus campaneri* exclusive to North Paraiba estuary. The other species/genera are typical of estuarine-coastal ocean environments. In both estuaries, the highest density of organisms was observed during the rainy season, in the estuary of North Paraiba in Z2 and Z3 in the estuary of the Mamanguape. In the estuary of North Paraiba *Oithona brevicornis* had the highest density in the rainy season in areas Z2 (27 DIN / L), Z3 (24 DIN / L) and Z4 (12 DIN / L). The estuary of the Mamanguape *O. brevicornis* presented its highest density in the rainy season, registering 52 ind / L Z3 zone. During the dry period the input was observed in coastal-pelagic species toward upstream, as in the case of *Oithona oswaldocruzi* species which showed the highest densities in the Z1 zones (19DIN / L) Z2 (21DIN / L) and Z3 (25 DIN / L) in the estuary of Mamanguape, while in the rainy season the highest density was recorded in the zone Z3 (21 ind / L). In the estuary of North Paraiba, *O. oswaldocruzi* reached the value of 38 ind / L Z2 in the rainy season. For both estuaries, the largest biomass (ug / L) was recorded for *O. brevicornis* and *O. oswaldocruzi* during the rainy season. considering environmental data, dissolved inorganic nitrogen values (DIN) ranged from 23:54μg / L (Z4 - rain) to 460.98 μg / L (Z4) at the estuary of the Mamanguape whereas at the North Paraiba estuary it ranged from 434.30 μg / L (Z4) to 4139.54 μg / L (Z3) in the dry season, whereas in the rainy season variation was from 991.81 μg / L (Z4) to 4037.4 μg / L (Z3); total phosphorus (Pt) showed higher values in the North Paraíba (153.88 μg / L to 754.44 μg / L) compared to Mamanguape (63.33 μg / L to 284.44 μg / L); orthophosphate (ORP) ranged from 45 to 87 μg / L in the estuary of the Mamanguape and 57-191 μg / L in the North Paraiba estuary; reactive soluble phosphorus (SRP) had been steepest in North Paraiba, ranging from 68.55μg / L to 639.11 μg / L. Shannon-Wiener Analysis of Diversity presented the highest values in the Z4 in both estuaries, and the estuary of the Mamanguape (H '= 2.8 - drought) higher than in the estuary of North Paraiba (H' = 2.5 - rainy season). The results of PERMANOVA showed that there is no significant interaction between the density of mesozooplankton with the sampled points (Pseudo-F = 1.2326; P = 0.293), the collection periods (Pseudo-F = 3.4829; P = 0.026) and estuaries (Pseudo-F = 4.2619, P = 0.058), with only significant differences between the zones (olig, poly P = 0.004; olig, euha P = 0.016). As to functional diversity in both estuaries, functional richness index showed low values. In the North Paraiba estuary the highest values were recorded during the dry season in Z2, Z3 and Z4, while in Mamanguape the highest value was recorded in the Z3 in the dry season. The functional evenness index showed no significant differences by concentrating the highest value in the dry season in the estuary of the Mamanguape, and the lowest value observed in the dry season in the estuary of North Paraiba (Z2). The functional divergence rates were higher during the rainy season in both estuaries, but no major changes. The functional dispersion rates were low in both estuaries, but were more representative during the dry season. The study proved the existence of differences in copepod diversity along the salt gradient of both estuaries.

Keywords: copepods, functional diversity, dry season, rain season Estuary Mamanguape, North Paraiba Estuary.

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SUMÁRIO

INTRODUÇÃO

Estuário é um corpo de água semifechado, com uma livre ligação com o oceano aberto, no interior do qual há um gradiente salino que corresponde a água do mar mensuravelmente diluída pela água doce originada da drenagem continental (PRITCHARD, 1955). A palavra estuário é derivada do adjetivo latino *aestuarium* (DAY JR. *et al.* 1989), que significa maré ou onda abrupta de grande amplitude, fazendo referência a um ambiente altamente dinâmico, com mudanças constantes em resposta a forçantes naturais (MIRANDA *et al.* 2002), sendo dos habitats naturais mais produtivos e com uma elevada diversidade ecológica e de elevado hidrodinamismo (McLUSKY & ELLIOT, 2004). Esse termo é utilizado genericamente para indicar o encontro do rio com o mar, caracterizando uma foz litorânea, ou seja, um ambiente de transição entre o oceano e o continente (MIRANDA *et al.* 2002).

Dentre as comunidades que habitam estes sistemas, à que se destacar o plâncton, principalmente o zooplâncton, pela posição chave que ocupa na cadeia trófica, sendo o elo entre os produtores e os níveis tróficos superiores, e pela elevada sensibilidade que apresenta a alterações globais (GONÇALVES *et al.*, 2010a), respondendo rapidamente a essas alterações. Este grupo de organismos tem a capacidade de responder muito rapidamente a variações de fatores ambientais (SANTANGELO *et al.*, 2007; GONÇALVES *et al.*, 2010 a, b; GONÇALVES *et al.*, 2012 a, b), exercendo forte influência sobre os processos ecossistêmicos, seja pela adição ou ausência de características funcionais. A salinidade e a temperatura, por exemplo, são parâmetros ambientais que influenciam fortemente a estrutura, composição e dinâmica das comunidades estuarinas, nomeadamente o zooplâncton.

Partindo do pressuposto de que cada espécie contribui direta ou indiretamente para o funcionamento dos ecossistemas, através de características físicas, químicas e comportamentais que determinam a diversidade funcional nas comunidades, estudos têm identificado mecanismos pelos quais a diversidade interfere no funcionamento dos ecossistemas (TILMAN *et al.* 1997), sendo crescente o interesse em agrupar organismos com base nos traços funcionais que as espécies apresentam. Estes estudos defendem que o agrupamento considerando traços funcionais permite uma maior capacidade em predizer ou explicar a estrutura das comunidades e suas respostas às condições ambientais (BRASIL & HUSZAR, 2011), estando os traços vinculados às funções das espécies no ecossistema. Deste modo, medir a diversidade funcional significa determinar a diversidade de características funcionais (CIANCIARUSO *et al*. 2009), propondo ferramentas para compreender quais os componentes da biodiversidade que são responsáveis pela capacidade adaptativa dos ecossistemas, ou seja, qual a variabilidade nas respostas das espécies dentro de uma comunidade às mudanças ambientais (ELMQVIST *et al*. 2003).

Mason *et al* (2005) chegam a uma definição global da diversidade funcional como: a distribuição das espécies e abundância de uma comunidade no espaço de nicho, incluindo: a) a quantidade de espaço de nicho preenchido por espécies na comunidade (riqueza funcional); b) a regularidade da distribuição e a abundância relativa das espécies no espaço funcional (regularidade funcional) e c) o grau em que a distribuição de abundância em nichos maximiza a divergência de caracteres funcionais dentro da comunidade (divergência funcional).

A necessidade de um monitoramento das respostas de biodiversidade face às perturbações ambientais e de uma análise de como estas alterações interferem na funcionalidade dos ecossistemas (PODGAISKI *et al*. 2011) proporcionou a formação de um conjunto de estudos que procuram descrever a importância das influências recíprocas entre a riqueza de espécies e as funções ecológicas (CAMERON, 2002; DUFF, 2003; NAEEM & WRIGHT, 2003). Deste modo, a diversidade funcional mostrou ser mais interessante na avaliação e monitorização de impactos ambientais quando comparada com a riqueza de espécies (ERNST *et al.* 2006).

Devido a potencial relação entre a diversidade funcional e o funcionamento e manutenção dos processos ecológicos das comunidades (PETCHEY & GASTON, 2006) percebemos que a abordagem funcional ganha cada vez mais impulso nas pesquisas científicas, inclusive na comunidade zooplanctônica. Os primeiros estudos sobre diversidade funcional forma realizados em comunidades vegetais terrestres (e.g. TILMAN *et al.*,1997; DÍAZ & CABIDO, 2001; ROSCHER *et al*. 2012; BARBOSA *et al*. 2013; SPASOJEVIC *et al*. 2012, 2014; LAUGHLIN 2014). Atualmente encontramos trabalhos sobre diversidade funcional em fitoplâncton (PÁLFFY *et al*. 2013), zooplâncton (e.g. BARNETT *et al*, 2007, 2013; BARNETT & BEISNER, 2007; VOGT *et al*., 2013, POMERLEAU *et al*, 2015), peixes (e.g. STUART-SMITH *et al*, 2013; HITT & CHAMBERS, 2014; WIEDMANN *et al*., 2014), bactérias (e.g. ARIESYADY *et al*. 2007), macroinvertebrados bentônicos (e.g. VAN DER LINDEN *et al*. 2012. 2016a, 2016b; DOLEDEC *et al*, 1999) e mamíferos (e.g. CHILLO & OJEDA, 2012).

Embora a comunidade zooplanctônica seja de fundamental importância nas redes alimentares dos ecossistemas aquáticos, trabalhos utilizando esses organismos sob o ponto de vista funcional ainda são escassos na literatura. Barnett & Beisner (2007) relacionaram a diversidade funcional do zooplâncton com o gradiente de estado trófico em lagos canadenses, concluindo que os maiores valores de fósforo total conduziram a menores valores de diversidade funcional do zooplâncton. Litchman *et al.* (2013) listaram traços funcionais importantes para o zooplâncton, separando-os por tipo e função ecológica (alimentação, crescimento e reprodução e sobrevivência). Barnett *et al.* (2007) listaram traços funcionais das espécies de cladóceros e de copépodes da América do Norte. Mais recentemente Pomerleau *et al.* (2015) avaliaram a diversidade funcional do zooplâncton marinho do oceano Pacífico com base nos traços (i.e. tamanho corporal, ontogenia, habitat e comportamento alimentar) de 42 espécies, comparando índices de diversidade funcional com índices de diversidade taxonômica. Benedetti *et al* (2015) identificaram grupos funcionais de espécies de Copepoda a partir de traços funcionais das espécies mais representativas no Mar Mediterrâneo.

No entanto, estudos de diversidade funcional em comunidades estuarinas tropicais são escassos. Na sua maioria, os estudos da ecologia dos estuários ainda são abordados com base na composição e distribuição das espécies e impactos ambientais em suas águas e em seus entornos. No entanto, estudos que analisem as redes tróficas e façam uso de ferramentas como os isótopos estáveis e perfil em ácidos graxos (e.g. PATRÍCIO & MARQUES 2006; BAETA, 2010; BOUILLON *et al.*, 2012; GONÇALVES et al., 2012; MOURA *et al.*, 2016; TECCHIO *et al.*, 2015; VINAGRE *et al.*, 2015) ganham cada vez mais espaço nos estudos ecológicos de ambientes estuarinos, abrindo espaço para os avanços relacionados à abordagem funcional. Nicolas *et al* (2010) estudaram a diversidade funcional da comunidade de peixes de 31 estuários europeus, desde Portugal até a Escócia. Recentemente estudos no estuário de Mondego (Portugal), realizados por Van Der Linden *et al.* (2012; 2016a), utilizaram a abordagem de traços funcionais para avaliar a diversidade funcional da comunidade bentônica nesse estuário. Barnes & Hendy (2015) estudaram a diversidade funcional macrobentônica ao longo de um gradiente estuarino. No entanto, até ao momento, não temos conhecimento de estudos publicados na literatura que analisem a diversidade funcional do zooplâncton em ambientes estuarinos.

No Brasil, estudos sobre o zooplâncton estuarino foram iniciados nos anos 60 e de acordo com uma pesquisa bibliográfica realizada por Neumann-Leitão (1994), foram registradas 150 publicações relacionadas com o zooplâncton de estuários brasileiros onde 24 trabalhos referem-se à região de Cananéia-SP, 18 à região de Itamaracá- PE e 12 à Lagoa dos

Patos – RS. Nos estuários paraibanos os estudos foram iniciados em meados dos anos 70, por Singarajah (1978) que apresentou uma lista de 56 *taxa* presentes no estuário do Paraíba do Norte, onde foram registradas como predominantes as espécies *Labidocera fluviatilis, Eucalanus elongatus, Acartia longiremis, Temora stylifera, Centropages furcata, Euterpina acutifrons, Oithona* sp. *e Copilia* sp.. O autor observou dois picos distintos de sazonalidade do zooplâncton, um maior em outubro e outro em dezembro/janeiro. Pekala (1982), com o objetivo de realizar um levantamento da composição relativa da fauna planctônica ao longo do estuário do Paraíba do Norte, registrou 18 espécies de Copepoda, sendo *O. hebes* a espécie mais abundante e dominante durante todo o ano. Ainda em 1982, Nordi analisou a ecologia do zooplâncton deste estuário. Recentemente Moura *et al* (2016), quantificando e caracterizando perfis de ácidos graxos de copépodes e suas potenciais fontes alimentares provenientes de dois estuários Paraibanos – o Paraiba do Norte e o Mamanguape, verificaram que as fontes de alimento zooplanctônico do estuário do Paraíba Norte apresentaram menos qualidade trófica, com menores concentrações de ácidos graxos essenciais, possivelmente relacionada com a forte ação antrópica que se faz sentir naquele estuário.

Embora o conceito de diversidade funcional seja relativamente simples de entender, um número crescente de estudos revelou que medi-la é uma tarefa complexa. Assim, princípios básicos da diversidade, como equitabilidade ou uniformidade, dispersão, entre outros, serviram como base para o desenvolvimento de índices de diversidade funcional, havendo o consenso de que a diversidade funcional pode ser compreendida em diferentes componentes ligeiramente análogos à diversidade de espécies: riqueza, dispersão, similaridade e divergência funcionais (MASON *et al*., 2005, GERISCH *et al.*, 2012, VAN DER LINDEN *et al.*, 2012).

Atualmente variados são os métodos utilizados para medir e quantificar a diversidade funcional nos ecossistemas, nomeadamente através de índices que permitem determinar a medição dos traços relativos à riqueza, equitabilidade e divergência funcional. A entropia quadrática de Rao (RAO, 1982) incorpora tanto a abundância relativa de espécies quanto a medida de diferenças funcionais entre pares de espécies. Walker *et al* (1999) propuseram uma medida de diversidade funcional (FAD, *functional attribute diversity*), obtida diretamente das características funcionais das espécies e baseadas na distância de pares de espécies num espaço multidimensional. Segundo Petchey & Gaston (2006), essas medidas violam o princípio da monotonicidade e consideram, erroneamente, que as distâncias entre os pares de espécies são independentes, levando a uma estimativa inflacionada da diversidade funcional.

Petchey & Gaston (2002, 2006) apresentaram uma medida de diversidade funcional (FD, *functional diversity*) que consiste na soma dos comprimentos dos "braços" de um dendrograma funcional, ou seja, um dendrograma gerado a partir de uma matriz de “espécies ×traços funcionais” (CIANCIARUSO, 2009). Petchey *et al*. (2004) compararam as medidas de diversidade, riqueza de espécies, riqueza de grupos funcionais (FGR), diversidade de atributos funcionais (FAD) e diversidade funcional (FD), concluindo que as últimas explicam melhor as diferenças funcionais da comunidade, e que a FD, por não ser influenciada pela riqueza de espécies, se torna um melhor previsor do funcionamento de uma comunidade.

Mason *et al.* (2005), foram os primeiros que popularizaram a distinção dos principais componentes da diversidade funcional *(functional richness* - FRic, *functional evenness* - FEev, *functional divergence* - FDiv), propondo medidas estimadas com base em características individuais e argumentando que a diversidade funcional não pode ser sintetizada por um número único, mas sim por suas facetas complementares. O FRic representa a quantidade do espaço funcional ocupado por uma assembleia de espécies, fornecendo informações que podem estar relacionadas com a produtividade e variabilidade ambiental; FEev corresponde ao quão regularmente as abundâncias das espécies são distribuídas no espaço funcional, onde um valor elevado indica uma distribuição regular e um valor baixo pode ser interpretado como uma sobre-representação de determinados traços (MASON *et al.*, 2005; SCHLEUTER *et al.*, 2010); FDiv define o quão distante as abundâncias das espécies estão do centro do espaço funcional.

Villegér *et al.* (2008), por sua vez, propõem medidas que incorporam múltiplos traços funcionais e quantificam como os traços funcionais ponderados pela densidade estão espalhados ao longo de um volume multidimensional de características e são independentes da riqueza de espécies e entre si. Segundo Mouchet *et al*. (2010), questões como a influência da biodiversidade sobre o funcionamento do ecossistema, que buscam esclarecer quais facetas da biodiversidade possuem maior influência sobre os processos do ecossistema e em que condições ambientais isso acontece, deve combinar as medidas de Riqueza Funcional (FRic - *Functional Richness*, VILLÉGER *et al.*, 2008), Divergência Funcional (FDiv - *Functional Divergence*, VILLÉGER *et al.*, 2008) e Equitabilidade Funcional (FEev - *Functional Evenness*, VILLÉGER *et al.*, 2008). Essas medidas/indices são independentes entre si, mas também complementares na representação das propriedades de uma comunidade, devendo ser consideradas simultaneamente (GERISCH *et al.*, 2012) e, tomadas em conjunto, descrevem a distribuição das espécies e suas abundâncias dentro do espaço funcional (MOUCHET *et al*. 2010).

Recentemente Laliberté & Legendre (2010), propuseram a dispersão funcional (*Functional Dispersion* - FDis) como um novo índice multidimensional de diversidade funcional, e está intimamente relacionado com a entropia quadrática de Rao. É baseada em dispersão multivariada (ANDERSON *et al.*, 2006), sendo caracterizada pela distância média de cada *taxon* ao centróide de todos os *taxa* na comunidade.

Neste estudo pretendemos contribuir para colmatar a falta de conhecimento sobre a ecologia funcional do mesozooplâncton (Copepoda) de sistemas estuarinos tropicais. Para isso, selecionámos dois estuários com diferentes níveis de impacto humano: um sob forte ação antropogênica – estuário do Rio Paraíba do Norte –, e outro localizado numa Área de Protecção Ambiental (Area de Protecção categoria 5, IUCN) – estuário do Mamanguape. Coletámos mesozooplâncton ao longo do gradiente salino de cada estuário, em duas estações do ano (época da chuva e época seca). Esperamos que a diversidade funcional seja mais elevada na estação do ano, zonas e sistema estuarino que apresentam menor impacto antrópico.

PERGUNTA: A diversidade funcional será tão mais elevada nas estações do ano, zonas e sistemas estuarinos quanto menor for o impacto antrópico?

OBJETIVO GERAL: Avaliar a diversidade funcional do mesozooplâncton de dois estuários do estado da Paraíba.

OBJETIVOS ESPECÍFICOS

- Avaliar a diversidade funcional do mesozooplâncton através da análise dos traços funcionais da comunidade;
- Compreender como os parâmetros físicos e químicos da água influenciam na diversidade funcional e na estrutura das comunidades mesozooplanctônicas.
- Comparar a diversidade funcional entre os estuários com diferentes pressões antrópicas.

HIPÓTESE: Espera-se que a diversidade funcional seja mais elevada na estação do ano, zonas e sistema estuarino que apresentam menor impacto antrópico.

MATERIAL E MÉTODOS

a) Locais de coleta

Os locais de amostragem foram dois estuários tropicais, Mamanguape (6º43‘02‖ S; 35º67‘46‖ W) e Rio Paraíba do Norte (6º57‘30‘‘ S; 34º51‘30‘‘ W), localizados no estado da Paraíba, Brasil (Figura 1). O estuário do Mamanguape é o segundo maior estuário do Estado da Paraíba, localizado dentro de uma área de proteção ambiental ("APA", IUCN categoria de proteção V), cujo objetivo principal é proteger os habitats costeiros e o peixe-boi marinho *Trichechus manatus* Linnaeus, 1758. À beira da área do mangue existem extensos campos de cana-de-açúcar e áreas de aquicultura dedicadas à produção de crustáceos e uma comunidade ribeirinha de cerca de 66.000 habitantes. O estuário do Paraíba do Norte está localizado numa área urbana com aproximadamente 1.000.000 habitantes. Na proximidade do estuário existem campos agricolas, áreas de aquicultura e o Porto de Cabedelo. O estuário tem aproximadamente 22 km de extensão. A foz do rio tem 2,2 km de largura, com uma profundidade média de três metros, exceto perto do porto, onde tem 11 m (MOURA *et al*., 2016).

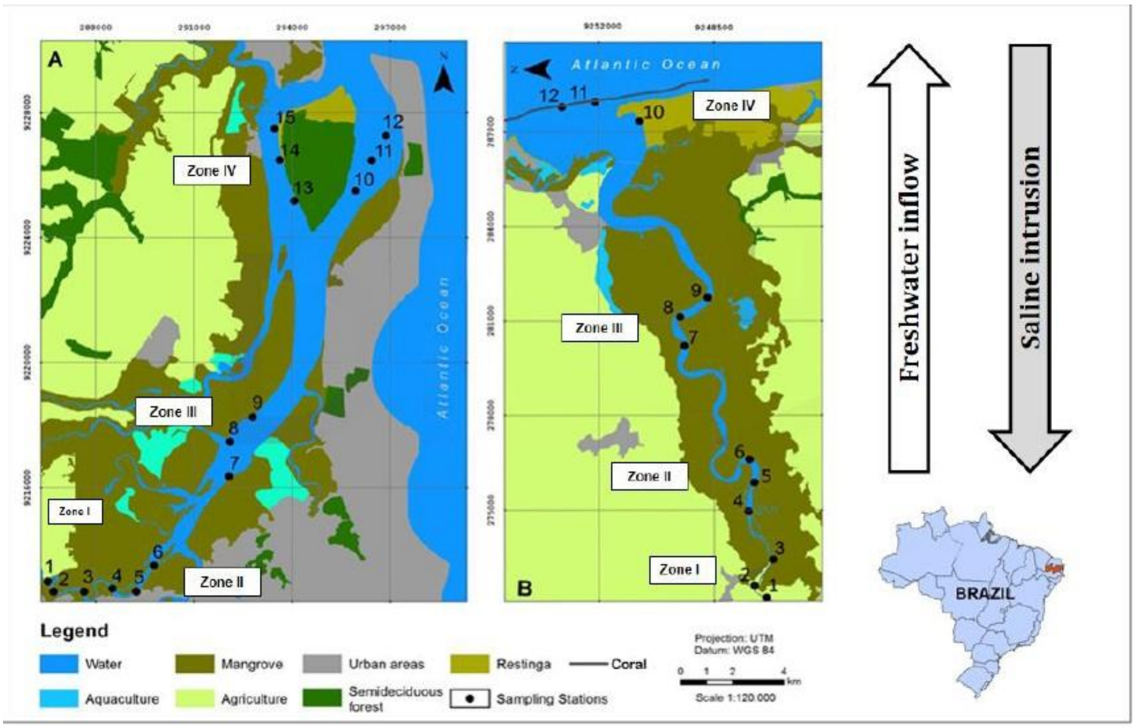

Figura 1 – Localização geográfica dos Estuários do Paraíba do Norte (A) e do Mamanguape (B), com a representação das zonas e pontos de coleta.

Segundo a classificação de Köeppen o clima da bacia do rio Mamanguape é do tipo Aw'i, ou seja, quente e úmido com chuvas de outono e inverno. Sua evapotranspiração média anual varia entre 1100 a 1600 mm e precipitações médias anuais entre 700 e 1600 mm (CERHPBa, 2004). Na bacia do Rio Paraíba do Norte, região do Baixo Curso do Rio Paraíba, vigora o clima do tipo Aw', isto é, úmido. A temperatura varia entre 20 e 24 ºC (valores mínimos) e 28 a 33ºC (valores máximos) nos meses de novembro e dezembro. A estação chuvosa começa em fevereiro e termina em julho, com o máximo de precipitação em abril, maio e junho, enquanto a estação seca ocorre de agosto a janeiro, com máxima de seca de outubro a dezembro (CERHPB b, 2004).

Foram realizadas duas coletas em cada estuário, uma no período de seca (novembro/2013) e a outra no período de chuva (julho/2014), no sentido montante-jusante, em preia-mar, em lua cheia, e em quatro zonas salinas (Z1, Z2, Z3 e Z4), com três réplicas em cada local de amostragem, com excepção da zona Z4, no estuário do Rio Paraíba do Norte, onde se realizaram 3 réplicas em cada um dos lados da Ilha da Restinga (Fig. 2).

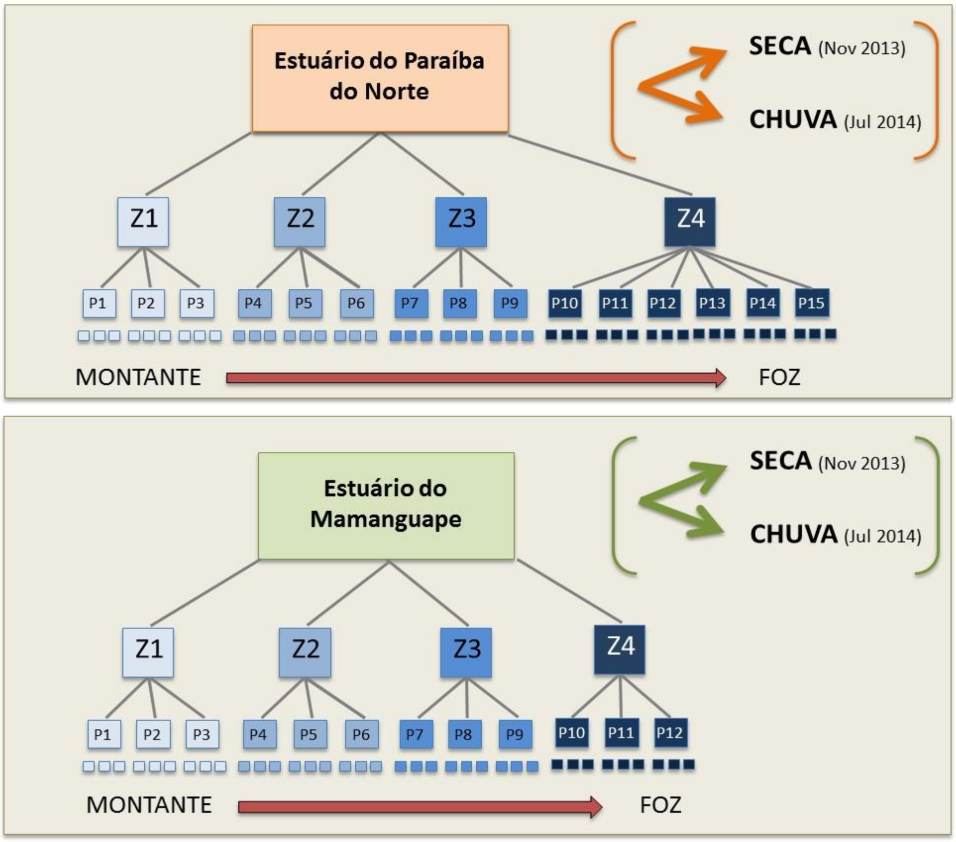

Figura 2 - Desenho amostral das coletas

b) Amostragem do zooplâncton

O zooplâncton foi coletado em maré-viva, preia-mar, com uma rede com abertura de malha de 68 µm. Para tanto, foram realizados arrastos horizontais com 5 minutos de duração. Posteriormente, as amostras foram glicosadas e preservadas em formaldeído a 4%. Procedeu-se à triagem, contagem e identificação dos indivíduos, sempre que possível, até ao nível de espécie, com auxílio de literatura especializada (BOLTOVSKOY, 1999).

c) Amostragem das variáveis ambientais

Para mensuração da salinidade, turbidez, temperatura da água (ºC), pH, Oxigénio dissolvido (mg/L) e a condutividade elétrica (µS / cm) utilizou-se uma sonda multiparamétrica (Horiba/L-50). Mediu-se a transparência, mensurada através do disco de Secchi, tendo também se medido a profundidade. Amostras de água de superfície e do fundo foram coletadas com uma garrafa de Niskin para detectar eventuais focos de perturbação orgânica: análise de PO_4 (LIMNOLOGISK METODIK, 1992), NO_3 (STRICKLAND & PARSONS, 1972), NO_2 (STRICKLAND & PARSONS, 1972), NH_4 (LIMNOLOGISK METODIK, 1992), Clorofila *a* (PARSONS *et al*., 1985), Matéria Orgânica Particulada e Matéria Orgânica Dissolvida.

d) Traços Funcionais

Os traços funcionais analisados foram selecionados para que se pudesse ter o máximo de informações referentes à esses organismos: peso seco e comprimento máximo que foram calculados a partir do zooplâncton amostrado, e a migração vertical diária (DVM), a forma do corpo, a distribuição, a alimentação, a distribuição vertical, o nível trófico e o modo de reprodução foram derivados da literatura. O peso seco foi estimado como 10% do peso húmido (o peso úmido foi estimado a partir do biovolume, assumindo que 106 μm^3 corresponde a 1 g de peso fresco) (PACE & ORCUTT, 1981)

e) Análise de Dados

Para cada amostra foi calculada a biomassa de copépodes coletados, utilizando regressões comprimento-peso de acordo com a literatura (ARA, 2001; AZEVEDO *et al*, 2012; DUMONT, *et al*, 1975). Logo após, os dados foram logaritmizados na base dois.

Para calcular a diversidade de espécies foi utilizado o Índice de Diversidade de Shannon-Wiener (SHANNON, 1948) (H'). Os valores obtidos foram comparados com a classificação proposta por Valentin *et al*. (1991):

H' > 3,0 bit. ind –1: alta diversidade

$3{,}0 \leq H' < 2{,}0$ bit. ind –1: média diversidade
$2{,}0 \leq H' < 1{,}0$ bit. ind –1: baixa diversidade
$H' < 1{,}0$ bit. ind –1: muito baixa diversidade

Análises de Variância Permutacional PERMANOVA (*Permutational Multivariate Analysis of Variance*) (ANDERSON, 2001) foram realizadas para testar diferenças significativas quanto aos padrões de distribuição de densidade da comunidade de zooplâncton entre estuários (radom, nível 2), períodos sazonais (fixed, nível 2), pontos (radom, nível 15) e zonas (fixed, nível 4). Termos significativos foram investigados com 999 permutações e um nível de significância de α <0,05. Como medida de similaridade, foi usada a distância euclidiana para os parâmetros biológicos. Todas as análises foram realizadas com os softwares Primer 6 Beta (CLARKE & GORLEY, 2006) e Permanova (McARDLE & ANDERSON, 2001).

f) Diversidade Funcional

Calculou-se a diversidade funcional das comunidades consideradas, por zona, estuário e época do ano, seguindo as indicações de Schleuter *et al*. (2010), abrangendo as três facetas complementares da diversidade funcional: Riqueza Funcional (FRic - *Functional Richness)* (VILLÉGER *et al.*, 2008), Divergência Funcional (FDiv - *Functional Divergence)* (VILLÉGER *et al.*, 2008) e Equitabilidade Funcional (FEev - *Functional Evenness*) (VILLÉGER *et al.,* 2008), além da Dispersão Funcional (FDis - LALIBERTE' & LEGENDRE, 2010). Outro índice calculado foi a CWM, ou Média Ponderada da Comunidade, que é o valor médio das características de todas as espécies presentes na comunidade, (LAVOREL *et al*. 2008), obtida tomando o valor médio dos traços de uma dada espécie, ponderado pela sua abundância relativa dentro da comunidade e somados em todas espécies (GARNIER *et al*., 2014).

RESULTADOS

Variáveis Ambientais

O estuário de Mamanguape apresentou os maiores valores de salinidade em relação ao Rio Paraíba em amostras pontuais nos dois períodos de coleta, variando de 14,9 a 36,53 na época seca, e entre 0,4 a 34,3 na época das chuvas seguindo o gradiente halino. No estuário do Paraíba, a salinidade variou entre 5,73 a 34,5 na estação seca, e na época das chuvas, a variação foi de 1,6 (Z4) a 29,2 (Z3) (Tabela 1). A temperatura em ambos os estuários variou de 27 a 29 ºC, e o pH de neutro a levemente alcalino, tanto no período seco quanto no período chuvoso (Tabela 1). Quanto aos dados pluviométricos mensais para a época de estudo, o estuário do Mamanguape apresentou pluviometria de 32,6 mm no período de seca e 57,7 mm no período chuvoso, ao passo que o Paraíba do Norte apresentou a menor pluviometria na época da seca (17 mm) e a maior durante o período chuvoso (111 mm) (AESA, 2015)

Os maiores valores de turbidez (NTU) foram registrados na Z3 no estuário do Mamanguape durante o período de seca (101.73 NTU), e na Z1 no estuário da PB do Norte também no período seco (74.7 NTU). No geral, Mamanguape apresentou valores mais elevados de turbidez, apresentando suas águas mais turvas no período seco. Quanto a transparência da água, mensurada através do disco de Secchi, foi possível perceber que não houve diferenças significativas entre os dois ambientes, onde no Mamanguape variou de 0.5 m a 1.3 m e no Paraíba do Norte de 0.57 m a 1.1 m, bem quanto aos sólidos totais dissolvidos (TDS).

Os valores de nitrogênio inorgânico dissolvido (NID), no Mamanguape variaram de 23.54 μg/L (Z IV – época da chuva) a 460.98 μg/L (Z IV – época seca), e no Paraíba do Norte os valores foram mais elevados, variando na época seca de 434.30 μg/L (Z IV) a 4139.54 μg/L (Z III) e na época da chuva de 991.81 μg/L (Z IV) a 4037.4 μg/L (Z III); o fósforo total (Pt) apresentou valores mais elevados no estuário da Paraíba do Norte (153.88 μg/L a 754.44 μg/L) quando comparado ao Mamanguape (63.33 μg/L a 284.44 μg/L); o ortofosfato (ORP) variou de 45 a 87 μg/L no Mamanguape e de 57 a 191 μg/L no Paraíba do Norte; o fósforo solúvel reativo (SRP) apresentou valores mais elevados no Paraíba do Norte, variando de 68.55 μg/L a 639.11 μg/L.

Tabela 1: Variáveis ambientais (média e desvio padrão) no período seco (novembro / 2013) e no período chuvoso (julho / 2014) para os estuários do Rio Mamanguape e do Rio Paraíba do Norte, Nordeste, Brasil

	Estuário do Mamanguape							
	Período seco				Período chuvoso			
	Zona 1	Zona 2	Zona 3	Zona 4	Zona 1	Zona 2	Zona 3	Zona 4
Profundidade (m)	1.23 ± 0.3	2.416 ± 0.72	4.25 ± 2.26	2.65 ± 1.53	0.8 ± 0.31	1.73 ± 1.1	3.81 ± 0.75	1.63 ± 0.71
Transparência (m)	0.93 ± 0.3	1.3 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.9 ± 0.1	0.6 ± 0.1	0.83 ± 0.15	1 ± 0.52
Salinidade	14.93 ± 1.1	20.66 ± 1.46	29.46 ± 0.68	36.53 ± 0.11	0.43 ± 0.06	3.46 ± 1.86	13.3 ± 1.23	34.3 ± 2.34
Temperatura (ºC)	28.1 ± 0.005	20.66 ± 0.01	29.05 ± 0.15	28.54 ± 0.09	26.81 ± 0.4	27.23 ± 0.32	27.34 ± 0.15	27.16 ± 0.2
pH	8.49 ± 0.07	8.53 ± 0.07	8.83 ± 0.05	9.39 ± 0.11	7.79 ± 0.25	7.21 ± 0.13	7.58 ± 0.23	7.65 ± 0.45
Condutividade (µS/cm)	24.56 ± 1.65	33.06 ± 2.11	45.5 ± 0.98	55.1 ± 0.17	0.91 ± 0.04	6.30 ± 3.14	22.13 ± 1.86	52.03 ± 3.15
Turbidez (NTU)	55.93 ± 0.7	61.5 ± 22.4	101.73 ± 23.74	93 ± 22.41	26.8 ± 6.3	31.86 ± 6.84	32.16 ± 24.47	36.93 ± 24.49
TDS (g/L)	15.23 ± 1.00	20.2 ± 1.31	27.76 ± 0.58	33.03 ± 0.11	0.58 ± 0.03	3.98 ± 1.96	19.33 ± 5.06	31.4 ± 1.65
NID(µg/L)	249.79 ± 70.69	263.60 ± 30.04	324.89 ± 116.23	460.98 ± 160.02	244.54 ± 231.10	136.69 ± 53.55	157.53 ± 13.06	23.54 ± 8.72
SRP (µg/L)	51.33 ± 10.0	83.55 ± 38.63	56.88 ± 6.93	43.55 ± 29.87	82.44 ± 11.71	106.88 ± 15.75	89.11 ± 3.85	63.55 ± 6.94
P total(µg/L)	284.44 ± 284.84	113.33 ± 11.547	131.11 ± 10.71	115.55 ± 27.14	131.11 33.72	163.33 ± 17.32	131.11 ± 19.25	63.33 ± 13.33
Cl-a (µg/L)	5.24 ± 4.02	2.24 ± 1.18	2.54 ± 0.68	0.89 ± 1.55	1.49 ± 0.26	2.69 ± 1.56	1.64 ± 0.52	0.74 ± 0.69

	Estuário do Paraíba do Norte							
	Período seco				Período chuvoso			
	Zona 1	Zona 2	Zona 3	Zona 4	Zona 1	Zona 2	Zona 3	Zona 4
Profundidade (m)	2.7 ± 1.3	1.66 ± 0.29	2.73 ± 0.07	6 ± 1.45	2.08 ± 0.14	2.40 ± 0.43	1.82 ± 0.58	2.92 ± 1.11
Transparência (m)	0.66 ± 0.05	0.66 ± 0.2	1.1 ± 0.1	0.88 ± 0.17	0.63 ± 0.06	0.73 ± 0.06	0.57 ± 0.06	0.92 ± 0.24
Salinidade	5.73 ± 2.2	8.73 ± 4.69	21.06 ± 0.68	3.45 ± 0.09	0.16 ± 0.11	0.78 ± 0.19	1.83 ± 0.18	2.92 ± 0.27
Temperatura (ºC)	29.15 ± 0.08	29.37 ± 0.24	29.17 ±0.23	28.17 ± 0.17	27.6 ± 0.07	27.22 ± 0.04	27.38 ± 0.07	27.44 ± 0.18
pH	8.45 ± 0.03	8.45 ± 0.11	8.41 ± 0.04	8.95 ± 0.07	6.88 ± 0.31	7.43 ± 0.13	7.68 ± 0.31	8.35 ± 0.1
Condutividade (µS/cm)	10.063 ± 3.60	14.92 ± 7.52	29.93 ± 7.39	52.46 ± 1.21	3.1 ± 2.06	13.47 ± 3.11	29.67 ± 2.6	45.1 ± 3.68
Turbidez (NTU)	74.7 ± 9.02	68.86 ± 12.423	24.23 ± 5.54	45.3 ± 8.46	51.97 ± 22.49	18.77 ± 3.38	16.40 ± 7.26	19.67 ± 3.47
TDS (g/L)	1.13 ± 0.6	9.26 ± 4.63	20.5 ± 0.62	31.48 ± 0.71	1.97 ± 1.28	8.36 ± 1.93	18.27 ± 1.5	27.42 ± 2.1
NID(µg/L)	489.54 ± 170.2	1948.35 ± 1525.6	4139.54 ± 443.01	434.30 ± 186.45	2533.3 ± 1640.6	3835.6 ± 165.7	4037.4 ± 439.79	991.81 ± 819.54
SRP (µg/L)	358 ± 58.97	466.88 ± 157.49	594.66 ± 14.53	68.55 ± 26.86	405.78 ± 77.63	639.11 ± 25.46	616.89 ± 220.01	174.67 ± 76.71
P total(µg/L)	434.44 ± 270.17	668.88 ± 25.24	710 ± 18.55	153.88 ± 21.43	561.11 ±59.75	754.44 ± 94.59	650 ± 250.07	224.4 ± 65.48
Cl-a (µg/L)	44.19 ± 18.71	18.27 ± 15.28	4.49 ± 1.18	1.27 ± 1.04	7.34 ± 3.49	2.4 ± 1.13	2.55 ± 2.56	1.72 ± 0.6

(TDS: sólidos totais dissolvidos; NID: nitrogênio inorgânico dissolvido; SRP: fósforo solúvel reativo; P total: fósforo total; Cl-a: clorofila-a).

Zooplâncton

Foram identificadas oito famílias de copépodes nos estuários em estudo, tendo sido identificadas 20 espécies e duas identificadas até ao gênero. Registrou-se a presença de *Acartia lillijerboji, Acartia tonsa, Paracalanus crassirostris, Parvocalanus scotti, Paracalanus campaneri, Paracalanus nanus, Parvocalanus* sp., *Pseudodiaptomus richardi, Pseudodiaptomus marshi, Pseudodiaptomus acutus, Temora turbinata, Notodiaptomus iheringi, Oithona hebes, Oithona oculata, Oithona rígida, Oithona brevicornis, Oithona oswaldocruzi, Oithona nana, Oithona* sp., *Microcyclops anceps, Euterpina acutifrons*, e ainda formas de nauplii e copepoditos de Harpacticoida, Calanoida e Cyclopoida (Tabela 5). Dessas 22 espécies, apenas *N. iheringi* e *M. anceps* são típicos de água doce, e estiveram presentes apenas no estuário do Mamanguape. A grande maioria das espécies são típicas de ambientes estuarino, costeiro e/ou oceânico.

Algumas dessas espécies estiveram presentes em apenas um dos estuários, como *A. tonsa* e *P. campaneri* que foram exclusivas do estuário da PB do Norte. A espécie exótica, *T. turbinata* foi encontrada tanto no estuário do Mamanguape (época seca) quanto no estuário da PB (época da chuva).

Tabela 2 - Espécies de copépodes identificados nos estuários do Rio Paraíba do Norte e de Mamanguape, e seus respectivos traços funcionais. * indica traços derivados de gênero; **indica traços derivados de família ou ordem.

Species	Mean dry weight† (µg)	Max length† (µm)	DVM	Body-Shape	Distribution	Feeding	Vertical distribuition	Trophic position	Reproduction mode
Acartia lilljerboji	*3.112*	1178.13	yes ^{*1}	cigar-shaped body	Estuarine-Coastal 5	Ambush-Stationary Suspension ^{*7}	Epipelagic **11	Omnivore **10	Broadcast spawning **16, 17
Acartia sp.	*3.719*	949.66	yes ^{*1}	cigar-shaped body	Estuarine-Coastal-Oceanic*5	Ambush-Stationary Suspension ^{*7}	Epipelagic **11	Omnivore **10	Broadcast spawning **16, 18
Acartia tonsa	*2.024*	939.08	yes ^{*1}	cigar-shaped body	Estuarine-Coastal-Oceanic 5	Ambush-Stationary Suspension ^{*7}	Epipelagic **11	Omnivore **10	Broadcast spawning **16, 19
Notodiaptomus iheringi	*7.226*	1193.83	yes ^{*2}	bullet shaped	Freshwater 6	StationarySuspension ^{**8}	none reported	Omnivore 8	none reported
Parvocalanus crassirostris	*2.154*	637.68	no ^{1}	bullet shaped	Coastal 5	StationarySuspension ^{7}	Epipelagic ^{**3}	Herbivore *11	Broadcast spawning ^{*3}
Parvocalanus scotti	*3.912*	794.76	no^{*1}	bullet shaped	Estuarine-Coastal 5	StationarySuspension ^{*7}	Epipelagic ^{**3}	Herbivore **11	Broadcast spawning ^{*3}
Parvocalanus campaneri	*2.923*	791.71	no^{*1}	body elongated	Oceanic 5	StationarySuspension ^{**7}	Epipelagic ^{**3}	Herbivore **11	Broadcast spawning ^{*3}
Parvocalanus nanus	*3.266*	795.21	no^{*1}	body elongated-oval shaped	Oceanic *5	StationarySuspension ^{**7}	Epipelagic ^{**3}	Herbivore **11	Broadcast spawning ^{*3}
Parvocalanus sp.	*3.077*	749.072	no^{*1}	body elongated	Oceanic 5	StationarySuspension ^{*7}	Epipelagic ^{**3}	Herbivore **11	Broadcast spawning ^{*3}
Pseudodiaptomus richardi	*8.651*	1254.97	yes ^{3}	body elongated-oval shaped	Estuarine 5	Ambush-StationarySuspension	Epipelagic-mesopelagic ^{3}	Omnivore-Herbivore ^{3}	Broadcast spawning *18
Pseudodiaptomus marshi	*6.561*	1147.32	yes ^{*3}	body elongated-oval shaped	Estuarine 5	Ambush-StationarySuspension	Pelagic-benthopelagic ** ^{11}	Omnivore *13	Broadcast spawning *18
Pseudodiaptomus acutus	*8.257*	1204.51	yes ^{*3}	body elongated-oval shaped	Estuarine 5	Ambush-StationarySuspension	Pelagic-benthopelagic ** ^{11}	Omnivore *13	Broadcast spawning *18
Temora turbinata	*14.603*	1329.89	no ^{1}	oval body	Coastal-oceanic 5	StationarySuspension 7	Epipelagic-bathypelagic ^{**11}	Herbivore 11	Broadcast spawning *18
Microcyclops anceps	*1.956*	658.87	none reported	oval body	Freshwater 6	Grasping *9	none reported	Omnivore-Herbivore **14	none reported
Oithona brevicornis	*2.094*	630.84	yes **4	slender, tapered body	Estuarine 5	Ambush *5	Epipelagic 5	Herbivore **11	Egg-brooding sac *19
Oithona oswaldocruzi	*1.264*	625.27	yes **4	slender, tapered body	Coastal-oceanic 5	Ambush *5	Epipelagic 5	Herbivore **11	Egg-brooding sac *19
Oithona rigida	*1.089*	583.50	yes **4	slender, tapered body	Estuarine-Coastal 5	Ambush *5	Epipelagic 5	Herbivore **11	Egg-brooding sac *19
Oithona nana	*1.348*	574.24	yes **4	slender, tapered body	Coastal-oceanic 5	Ambush *5	Epipelagic 5	Herbivore **11	Egg-brooding sac *19
Oithona oculata	*1.882*	629.63	yes **4	slender, tapered body	Estuarine-Coastal 5	Ambush *5	Epipelagic 5	Herbivore **11	Egg-brooding sac *19
Oithona hebes	*1.778*	653.14	yes **4	slender, tapered body	Estuarine-Coastal 5	Ambush *5	Epipelagic 5	Herbivore **11	Egg-brooding sac *19
Oithona sp.	*1.071*	591.75	yes **4	slender, tapered body	Estuarine-Coastal-Oceanic *5	Ambush *5	Epipelagic 5	Herbivore **11	Egg-brooding sac *19
Euterpina acutifrons	*1.227*	753.20	no ^{*1}	fusiform body	Coastal*5	SurfaceFeeding 10	Epipelagic ^{3}	Herbivore **15	Broadcast spawning 18

(1) Shimode & Shirayama (2004); (2) SILVA et al, (2009); (3) Richardson et al (2013); (4) Mackas et al (2005); (5) Boltovskoy (1999); (6) Dumont et al (1975); (7) Barton et al (2013); (8) Barnett & Beisner (2007); (9) Williamson & Reid (2001); (10) Thorp & Covich (2010); (11) Bradford-Grieve (2002 onwards); (12) Tafe & Griffiths (1983); (13) Stemberger (1979); (14) Jerling & Wooldridge (1994); (15) Woods (1993); (16) Mauchline (1998); (17) Niehoff (2007); (18) Kiorbe & Sabatini (1995); (19) Dvoretsky & Dvoretsky (2009).

Densidade e Biomassa

Ocorreram diferenças quanto à densidade de organismos nos dois períodos amostrados. Em ambos os estuários, a maior densidade de organismos foi observada na época de chuva, no PB na Z2 e no Mamanguape na Z3. No Paraíba do Norte, a espécie *O. brevicornis* apresentou maiores densidades no período chuvoso, nas zonas Z2 (27 ind/L), Z3 (24 ind /L) e Z4 (12 ind /L), zonas estas que apresentaram baixa salinidade no período indicado (figura 4). No Mamanguape, *O. brevicornis* apresentou sua maior densidade no período chuvoso, registando-se 52 ind / L na Z3 (figura 5). Durante o período de seca observou-se a entrada de espécies costeiras-oceânicas em direção a montante, como é o caso da espécie *Oithona oswaldocruzi*, que apresentou suas maiores densidades nas zonas Z1 (19 ind / L), Z2 (21 ind / L) e Z3 (25 ind / L) no estuário de Mamanguape, ao passo que no período chuvoso a maior densidade foi registrada na zona Z3 (21 ind / L). No PB do Norte, *O. oswaldocruzi* registou uma densidade de 38 ind /L na zona Z2 no período chuvoso. Quanto ao gênero *Pseudodiaptomus*, as espécies encontradas foram *P. richardi*, *P. marshi* e *P. acutus*.

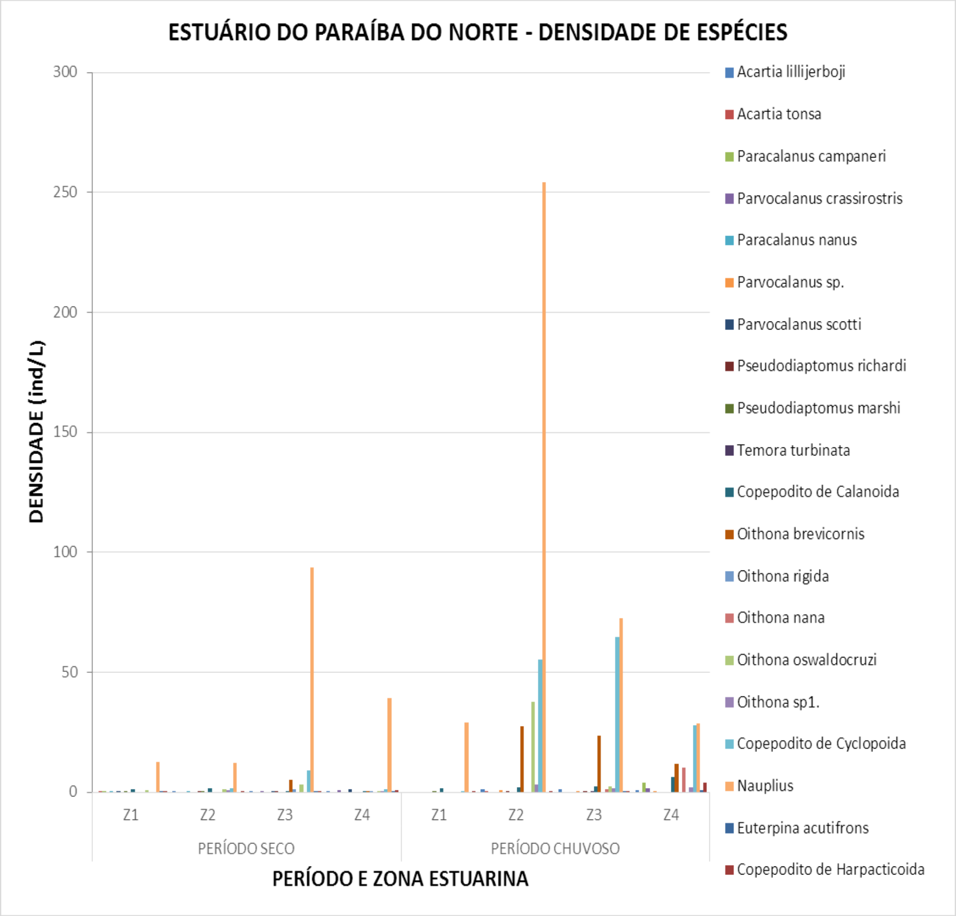

Figura 3. Densidade de Copepoda no estuário do Paraíba do Norte nos dois períodos de coleta

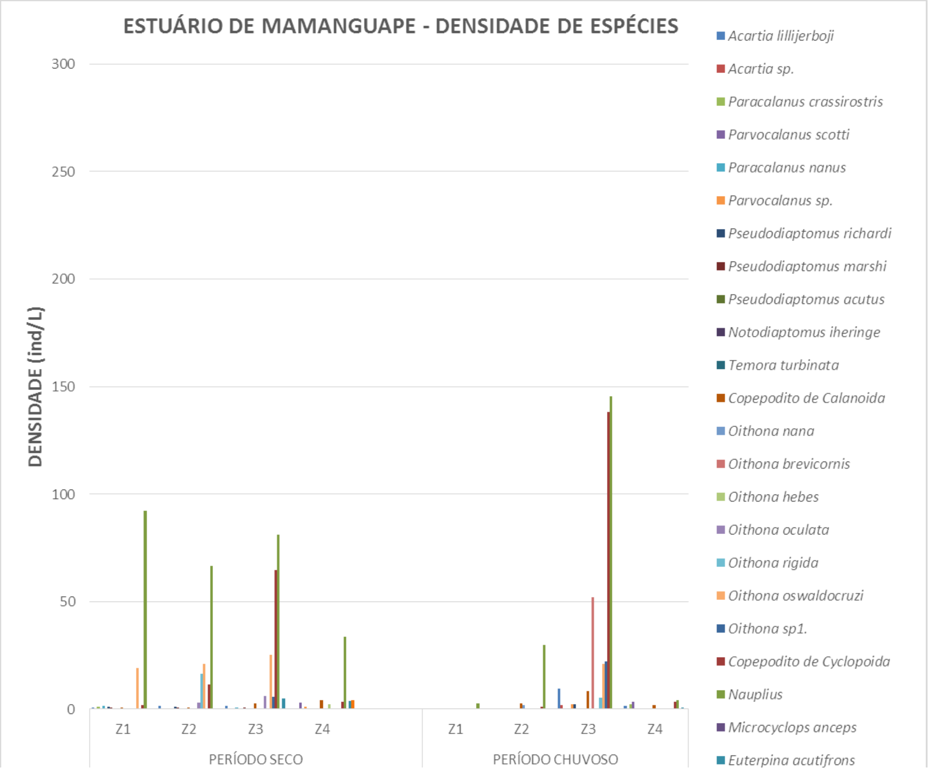

Figura 4. Densidade de Copepoda no estuário do Mamanguape nos dois períodos de coleta.

Os resultados da densidade de copépodes nesses ambientes supreendem por sua reduzida riqueza quando retiramos dos cálculos as formas de nauplii e de copepoditos. A figura 5 mostra que durante o período chuvoso, em ambos os estuários, registraram-se os maiores valores para a densidade de espécies, tendo o período seco do Paraíba do Norte apresentado os resultados menos variáveis.

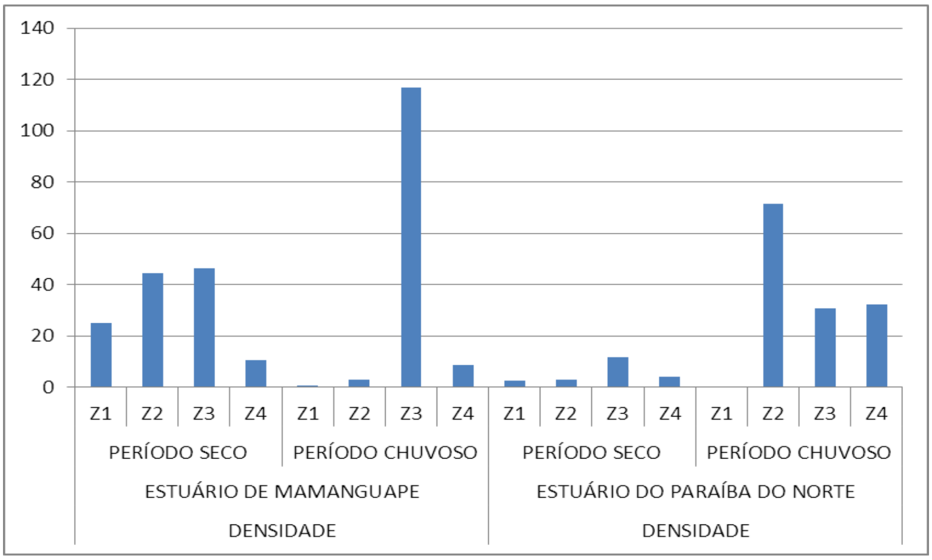

Figura 5: Resultado da densidade de espécies de copépodes excluindo as formas de nauplii e copepoditos

Para a biomassa (*ug*/L) no estuário do Paraíba do Norte, o período de chuva registrou os maiores valores dessa variável no presente estudo (figura 7). A maior biomassa de organismos foi para a espécie O. *brevicornis* nas zonas Z2 (57 *ug* /L), Z3 (49 *ug* /L) e Z4 (24 *ug* /L), seguida da espécie *O. oswaldocruzi*, que apresentou biomassa de 39 *ug* /L na zona Z2. O período seco, por sua vez, registrou valores reduzidos de biomassa em todas as zonas analisadas. Quanto ao estuário da Barra de Mamanguape a maior biomassa foi registrada para a espécie *O. brevicornis* (109 *ug*/L) durante o período chuvoso (Figura 8). Seguida desta aparece a espécie *O. oswaldocruzi* nas zonas Z1 (36 *ug*/L), Z2 (40 *ug*/L) e Z3 (48 *ug*/L).

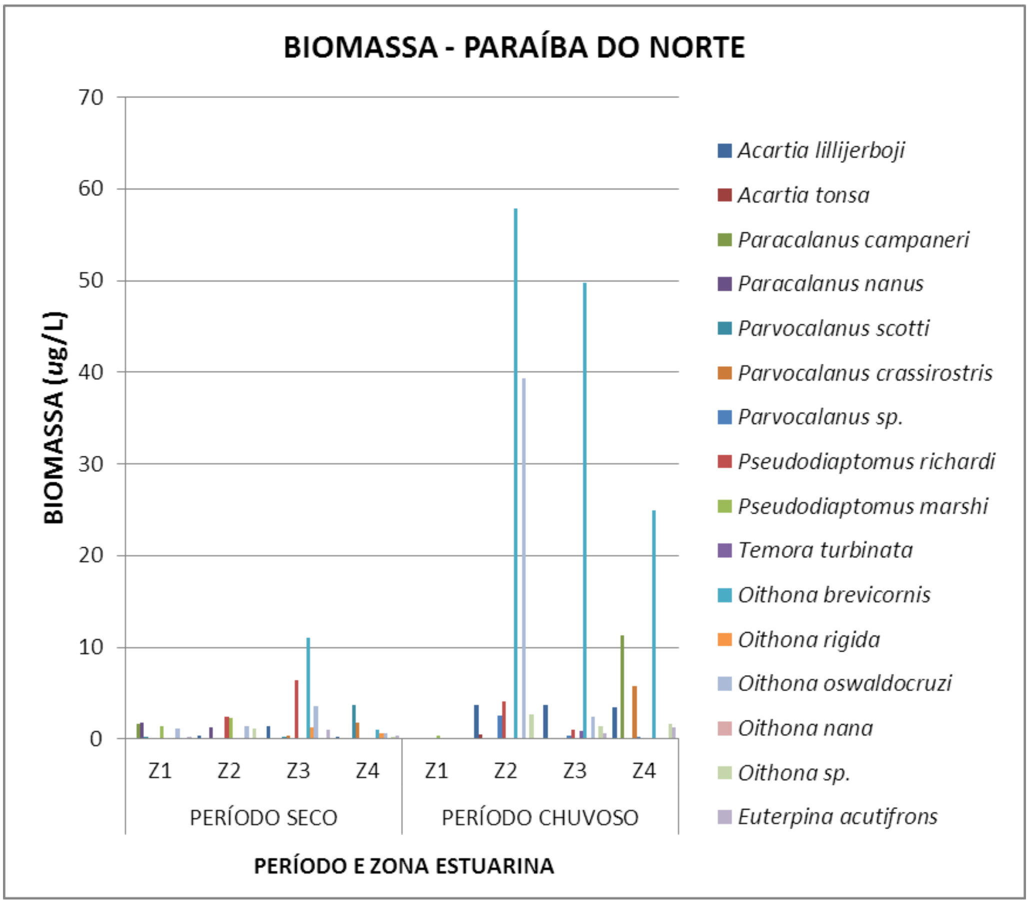

Figura 6. Biomassa de Copepoda no estuário do Paraíba do Norte nos dois períodos de coleta.

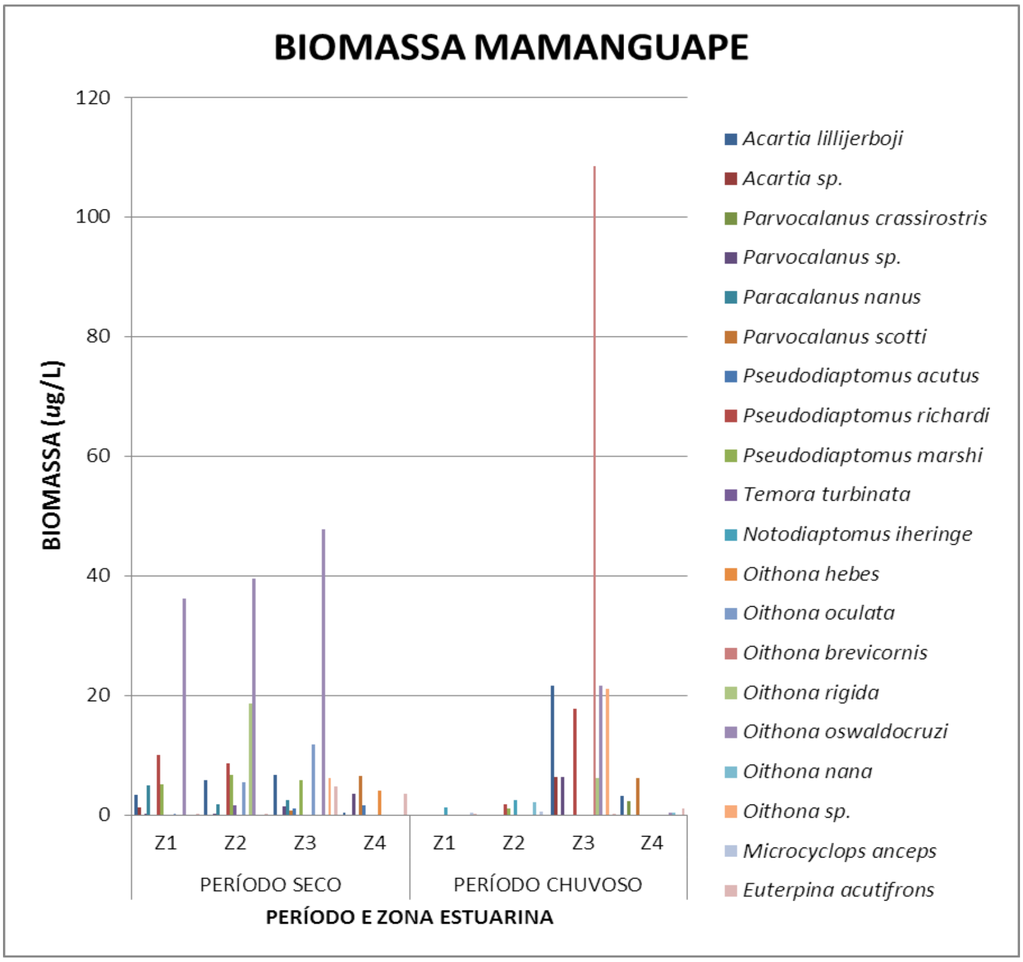

Figura 7. Biomassa de Copepoda no estuário do Mamanguape nos dois períodos de coleta.

Diversidade de Shannon-Wiener e PERMANOVA

A análise da Diversidade de Shannon-Wiener, que combina em um único valor numérico informações relativas à riqueza e à distribuição dos organismos, apresentou os valores mais elevados na Z4 em ambos os estuários, sendo no Mamanguape (H'=2,8), durante o período de seca, mais elevado que no Paraíba (H'= 2,5) durante o período chuvoso (Fig. 8).

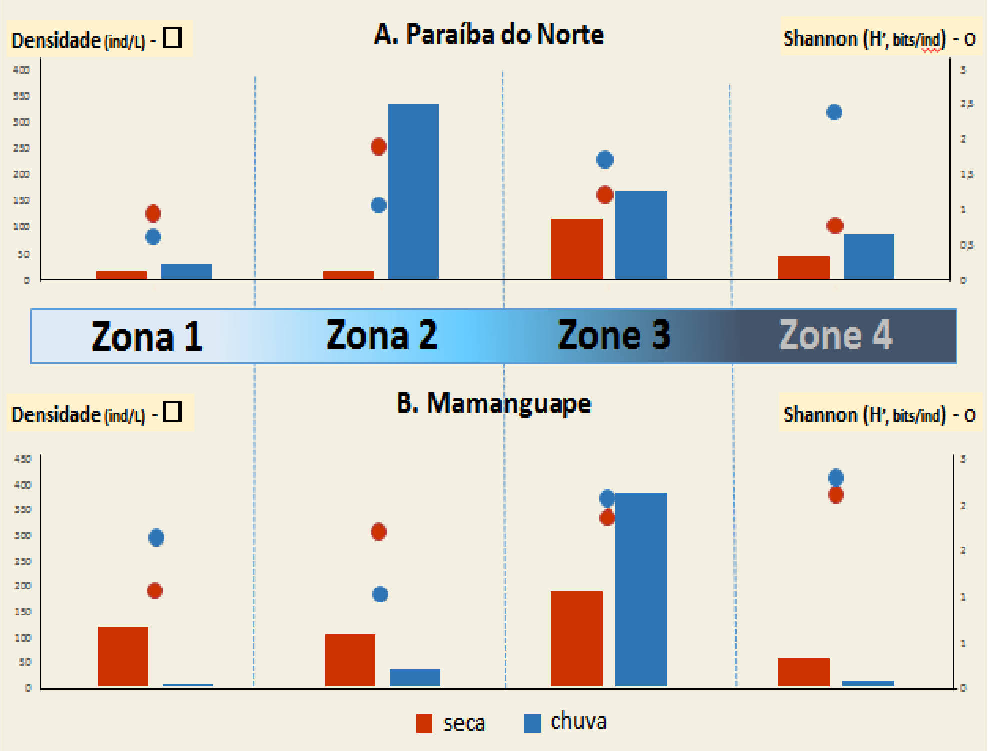

Figura 8 - Resultados de Densidade e Diversidade de Shannon para os estuários do Rio Paraíba do Norte e do Mamanguape ao longo do gradiente estuarino nos períodos

Os resultados da PERMANOVA (tabela 2) evidenciaram que não existe diferença significativa entre a densidade do mesozooplâncton com os pontos amostrados (Pseudo-F = 1,2326; P= 0,293), os períodos de coleta (Pseudo-F = 3,4829; P = 0,026) e os estuários (Pseudo-F = 4,2619;P = 0,058), sendo significativas apenas as diferenças entre as zonas (olig, poli P = 0,004; olig, euha P = 0,016).

Tabela 3: Resultados da PERMANOVA baseada na matriz de distância euclidiana densidade do mesozooplâncton analisada nos estuários do Rio Paraíba do Norte e do Mamanguape.

Fatores	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
Es	1	1,8038	1,8038	4,2619	0,058	998	0,056
Pe	1	1,2846	1,2846	2,9654	0,083	996	0,091
Zo	3	4,1159	1,372	3,4829	**0,026**	997	0,036
Po	14	7,3041	0,52172	1,2326	0,293	997	0,317

Es= estuários; Pe= períodos; Zo= zonas; Po= pontos. Em negrito destacam-se os resultados que foram estatisticamente significativos para p< 0,05.

CWM

Os resultados da CWM demonstraram que nas zonas a montante do estuário de Mamanguape, durante o período de seca, os traços mais representativos foram a presença de migração vertical diária (DVM), de distribuição epipelágica e costeira-oceânica, herbivoria e alimentação tipo emboscada, de forma do corpo cônico-esbelto e reprodução com ninhada de ovos, e nas áreas mais a jusante a DVM foi ausente, de distribuição epipelágica e estuarino-costeira, de alimentação estacionária-suspensívora e herbivoria, de corpo em forma de bala, e reprodução com transmissão de desova. No período de chuva, nas zonas a montante, o corpo foi em forma de bala, DVM presente, distribuição dulçaquicola, omnivoria e alimentação estacionária-suspensívora, e nas zonas a jusante a DVM esteve ausente, distribuição epipelágica e estuarina-costeira, herbivoria e alimentação estacionária-suspensívora e corpo em forma de bala (Tabela 4).

No estuário do Paraíba do Norte, no período de seca, nas áreas a montante, a DVM esteve presente, a forma do corpo foi cônico-esbelto, de distribuição epipelágica e estuarina, de alimentação tipo emboscada, herbivoria e reprodução com ninhada de ovos. Nas áreas a jusante, a DVM esteve ausente, o corpo foi em forma de bala, distribuição epipelágica e estuarina-costeira, nível trófico herbívoro e alimentação estacionária suspensívora e reprodução com transmissão de desova. No período de chuva, nas zonas a montante, a DVM esteve presente, a forma do corpo foi alongado-oval, de distribuição estuarina, pelágico-bentopelágico, omnivoria e alimentação emboscada-estacionária-suspensívora e reprodução tipo transmissão de desova. Na parte a jusante do estuário, a DVM também esteve presente, a forma do corpo predominante foi cônica-alongada, distribuição epipelágica estuarina, herbivoria e alimentação tipo emboscada, e reprodução por ninhada de ovos (Tabela 5).

Tabela 4 – Resultados da CWM para o Estuário da Barra de Mamanguape, nos períodos de seca e de chuva.

ESTUÁRIO DA BARRA DE MAMANGUAPE - PERÍODO DE SECA							
	DVM	Corpo	Distribuição	Alimentação	Distribuição Vertical	Nível trófico	Reprodução
Z1	sim	esbelto-cônico	Costeiro-oceânico	Emboscada	Epipelagico	Herbivoro	ninhada de ovos
	sim	esbelto-cônico	Costeiro-oceânico	Emboscada	Epipelagico	Herbivoro	ninhada de ovos
	sim	esbelto-cônico	Costeiro-oceânico	Emboscada	Epipelagico	Herbivoro	ninhada de ovos
Z2	sim	esbelto-cônico	Estuarino-costeiro	Emboscada	Epipelagico	Herbivoro	ninhada de ovos
	sim	esbelto-cônico	Costeiro-oceânico	Emboscada	Epipelagico	Herbivoro	ninhada de ovos
	sim	esbelto-cônico	Costeiro-oceânico	Emboscada	Epipelagico	Herbivoro	ninhada de ovos
Z3	sim	esbelto-cônico	Costeiro-oceânico	Emboscada	Epipelagico	Herbivoro	ninhada de ovos
	sim	esbelto-cônico	Costeiro-oceânico	Emboscada	Epipelagico	Herbivoro	ninhada de ovos
	sim	esbelto-cônico	Costeiro-oceânico	Emboscada	Epipelagico	Herbivoro	ninhada de ovos
Z4	não	forma de bala	Estuarino-costeiro	Estacionário-suspensívoro	Epipelagico	Herbivoro	transmissão de desova
	não	forma de bala	Estuarino-costeiro	Estacionário-suspensívoro	Epipelagico	Herbivoro	transmissão de desova
	não	forma de bala	Estuarino-costeiro	Estacionário-suspensívoro	Epipelagico	Herbivoro	transmissão de desova
ESTUÁRIO DA BARRA DE MAMANGUAPE - PERÍODO DE CHUVA							
	DVM	Corpo	Distribuição	Alimentação	Distribuição Vertical	Nível trófico	Reprodução
Z1	sim	forma de bala	Água doce	Estacionário-suspensívoro	na	Omnivoro	na
	sim	forma de bala	Água doce	Estacionário-suspensívoro	na	Omnivoro	na
	sim	forma de bala	Água doce	Estacionário-suspensívoro	na	Omnivoro	na
Z2	sim	forma de bala	Água doce	Estacionário-suspensívoro	na	Omnivoro	na
	sim	alongado-oval	Estuarino	Emboscada-estacionário-suspensívoro	Epipelagico	Herbivoro	transmissão de desova
	sim	alongado-oval	Estuarino	Emboscada-estacionário-suspensívoro	Epipelagico-mesopelagico	Omnivoro-Herbivoro	transmissão de desova
Z3	sim	esbelto-cônico	Estuarino	Emboscada	Epipelagico	Herbivoro	ninhada de ovos
	sim	esbelto-cônico	Estuarino	Emboscada	Epipelagico	Herbivoro	ninhada de ovos
	sim	forma de charuto	Estuarino-costeiro	Emboscada-estacionário-suspensívoro	Epipelagico	Omnivoro	transmissão de desova
Z4	não	forma de bala	Estuarino-costeiro	Estacionário-suspensívoro	Epipelagico	Herbivoro	transmissão de desova
	não	forma de bala	Estuarino-costeiro	Estacionário-suspensívoro	Epipelagico	Herbivoro	transmissão de desova
	sim	forma de charuto	Estuarino-costeiro	Emboscada-estacionário-suspensívoro	Epipelagico	Herbivoro	transmissão de desova

Tabela 5– Resultados da CWM para o Estuário do Paraíba do Norte, nos períodos de seca e de chuva (No anexo I estão as referências bibliográficas analisadas na compilação dos dados desta tabela)

ESTUÁRIO PARAÍBA DO NORTE - PERÍODO DE SECA							
	DVM	Corpo	Distribuição	Alimentação	Distribuição Vertical	Nível trófico	Reprodução
Z1	sim	esbelto-cônico	Estuarino	Emboscada	Epipelágico	Herbivoro	ninhada de ovos
	sim	esbelto-cônico	Estuarino	Emboscada	Epipelágico	Herbivoro	ninhada de ovos
	sim	esbelto-cônico	Costeiro-oceânico	Emboscada	Epipelágico	Herbivoro	ninhada de ovos
Z2	sim	esbelto-cônico	Estuarino	Emboscada	Epipelágico	Herbivoro	ninhada de ovos
	não	forma de bala	Estuarino-costeiro	Estacionário-suspensívoro	Epipelágico	Herbivoro	transmissão de desova
	não	forma de bala	Estuarino-costeiro	Estacionário-suspensívoro	Epipelágico	Herbivoro	transmissão de desova
Z3	não	forma de bala	Estuarino-costeiro	Estacionário-suspensívoro	Epipelágico	Herbivoro	transmissão de desova
	não	forma de bala	Estuarino-costeiro	Estacionário-suspensívoro	Epipelágico	Herbivoro	transmissão de desova
	não	alongado-oval	Oceânico	Estacionário-suspensívoro	Epipelágico	Herbivoro	transmissão de desova
Z4	sim	alongado-oval	Estuarino	Emboscada-estacionário-suspensívoro	Epipelágico-mesopelágico	Omnívoro-herbívoro	transmissão de desova
	sim	esbelto-cônico	Estuarino	Emboscada	Epipelágico	Herbivoro	ninhada de ovos
	sim	esbelto-cônico	Estuarino	Emboscada	Epipelágico	Herbivoro	ninhada de ovos
	não	forma de bala	Estuarino-costeiro	Estacionário-suspensívoro	Epipelágico	Herbivoro	transmissão de desova
	não	forma de bala	Estuarino-costeiro	Estacionário-suspensívoro	Epipelágico	Herbivoro	transmissão de desova
	não	forma de bala	Estuarino-costeiro	Estacionário-suspensívoro	Epipelágico	Herbivoro	transmissão de desova

ESTUÁRIO PARAÍBA DO NORTE - PERÍODO DE CHUVA							
	DVM	Corpo	Distribuição	Alimentação	Distribuição Vertical	Nível trófico	Reprodução
Z1	sim	alongado-oval	Estuarino	Emboscada-estacionário-suspensívoro	Pelagic-benthopelagic	Omnivoro	transmissão de desova
	sim	alongado-oval	Estuarino	Emboscada-estacionário-suspensívoro	Pelagic-benthopelagic	Omnivoro	transmissão de desova
	sim	esbelto-cônico	Costeiro-oceânico	Emboscada	Epipelagic	Herbivoro	ninhada de ovos
Z2	sim	esbelto-cônico	Estuarino-costeiro-oceânico	Emboscada	Epipelagic	Herbivoro	ninhada de ovos
	sim	esbelto-cônico	Costeiro-oceânico	Emboscada	Epipelagic	Herbivoro	ninhada de ovos
	sim	esbelto-cônico	Estuarino	Emboscada	Epipelagic	Herbivoro	ninhada de ovos
Z3	sim	esbelto-cônico	Estuarino	Emboscada	Epipelagic	Herbivoro	ninhada de ovos
	sim	esbelto-cônico	Estuarino	Emboscada	Epipelagic	Herbivoro	ninhada de ovos
	sim	esbelto-cônico	Estuarino	Emboscada	Epipelagic	Herbivoro	ninhada de ovos
Z4	sim	esbelto-cônico	Estuarino	Emboscada	Epipelagic	Herbivoro	ninhada de ovos
	sim	esbelto-cônico	Estuarino	Emboscada	Epipelagic	Herbivoro	ninhada de ovos
	sim	esbelto-cônico	Estuarino	Emboscada	Epipelagic	Herbivoro	ninhada de ovos
	sim	esbelto-cônico	Estuarino	Emboscada	Epipelagic	Herbivoro	ninhada de ovos
	sim	esbelto-cônico	Estuarino	Emboscada	Epipelagic	Herbivoro	ninhada de ovos
	não	forma de bala	Costeiro	Estacionário-suspensívoro	Epipelagic	Herbivoro	transmissão de desova

Diversidade Funcional

Em ambos os estuários, o índice de riqueza funcional apresentou valores baixos, mas no estuário do Mamanguape esses valores foram ainda menores (Figura 9). No estuário do PB do Norte os maiores valores foram registrados durante o período de chuva nas Z2, Z3 e Z4, enquanto no estuário do Mamanguape o valor mais elevado foi registrado na Z3 no período de chuva (Figs. 9A e 9B).

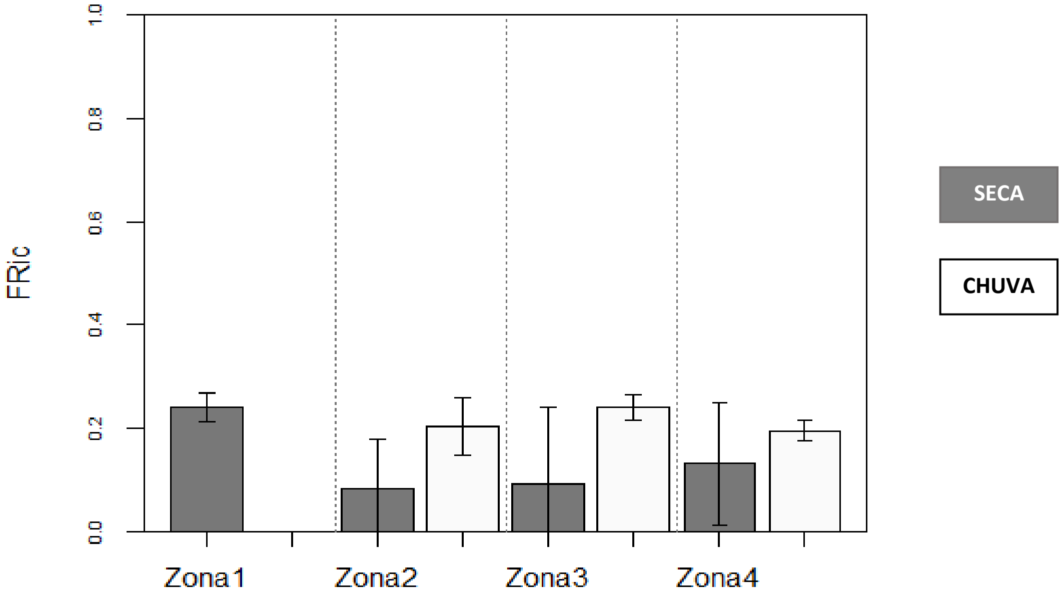

Figura 9-A: Índice de Riqueza Funcional do estuário do Paraíba do Norte.

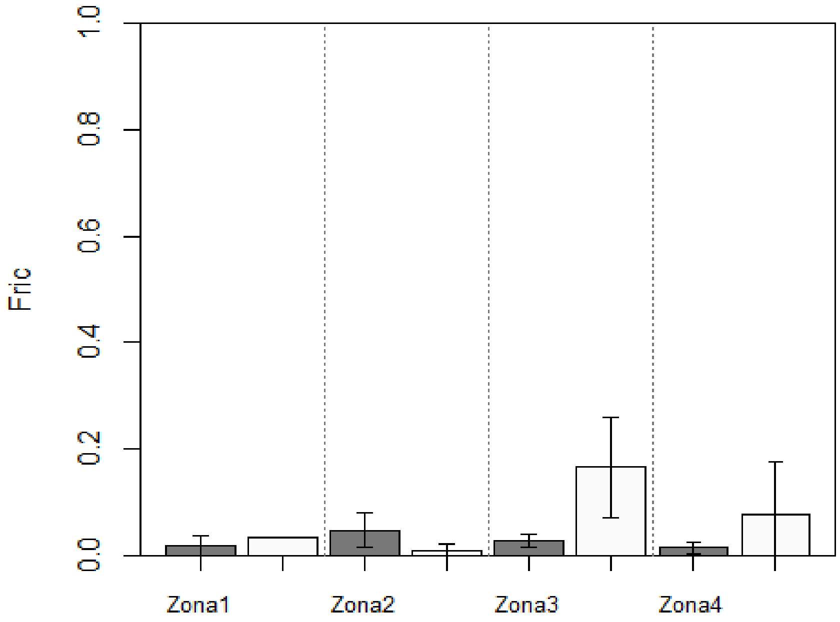

Figura 9-B: Índice de Riqueza Funcional do estuário do Mamanguape

O índice de equitabilidade funcional não apresentou grandes diferenças entre os dois estuários, concentrando o maior valor no período de chuva (Z2) do Mamanguape, e o menor valor observado na época seca do Paraíba do Norte (Z2) (Figs. 9C e 9D).

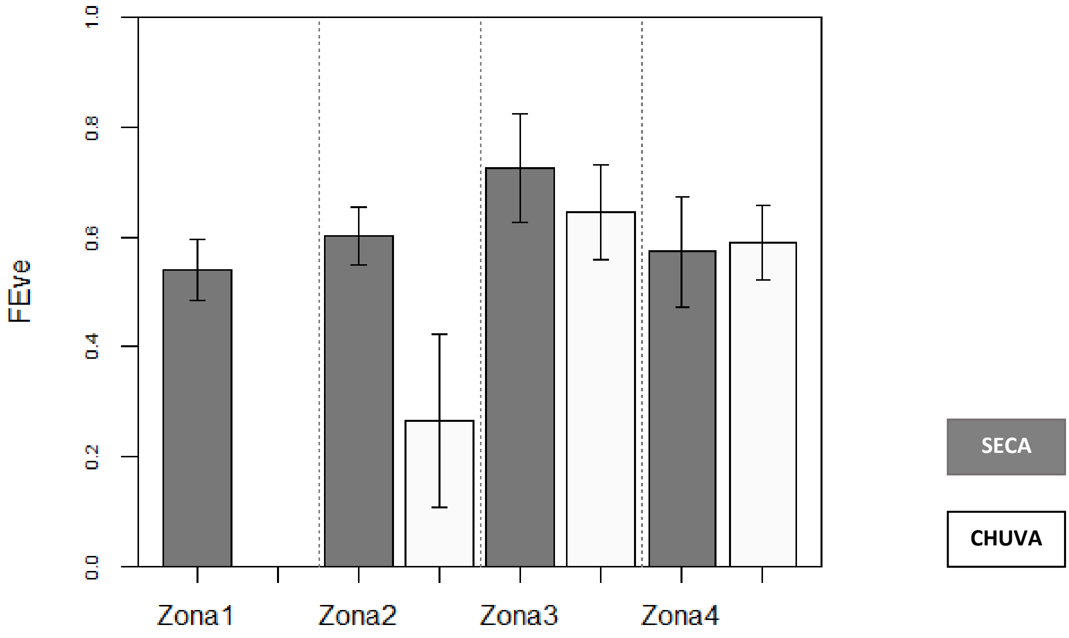

Figura 9-C: Índice de Equitabilidade Funcional do Estuário do Paraíba do Norte.

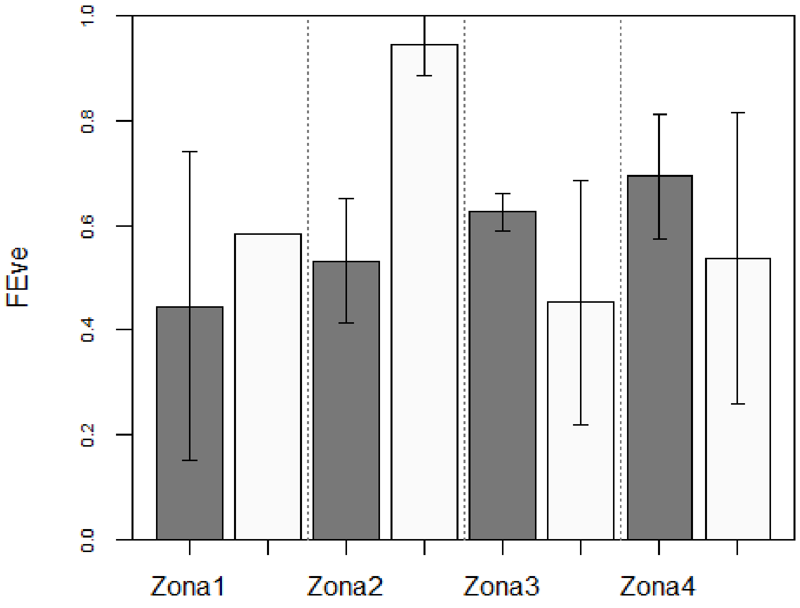

Figura 9-D Índice de Equitabilidade Funcional do Estuário do Mamanguape

Os índices de divergência funcional foram, em geral, maiores durante o período de seca em ambos os estuários, mas sem grandes variações, tendo-se registrado um valor acima na época da chuva em Z2, no estuário do Mamanguape (Figs. 9E e 9F).

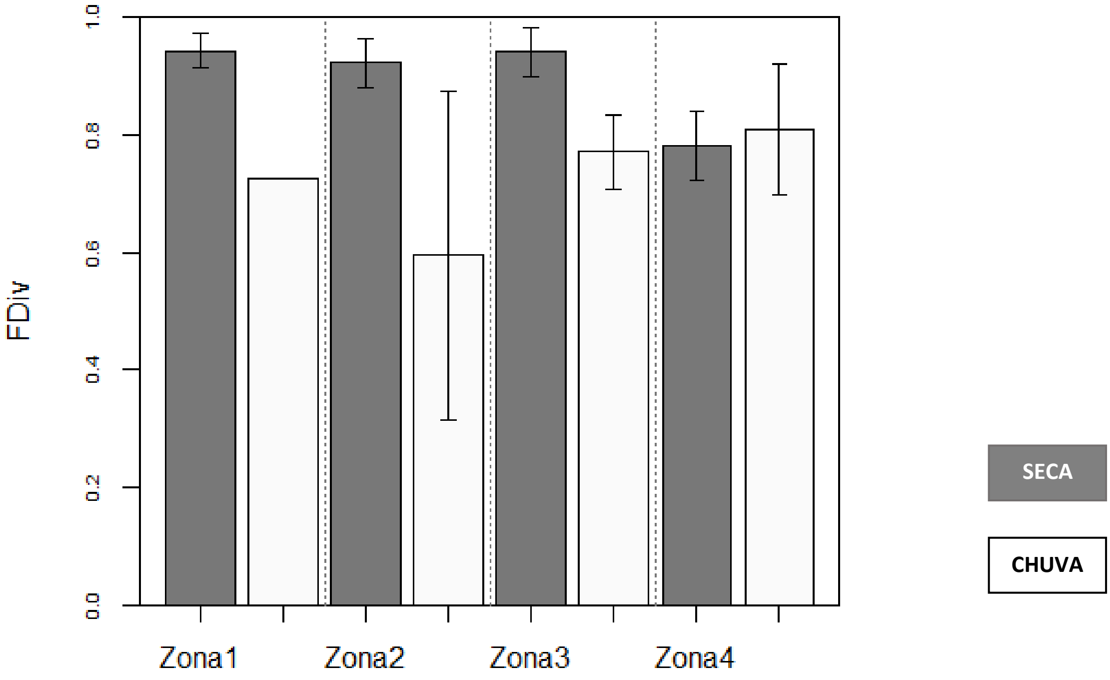

Figura 9-E: Índice de Divergência Funcional do estuário do Paraíba do Norte

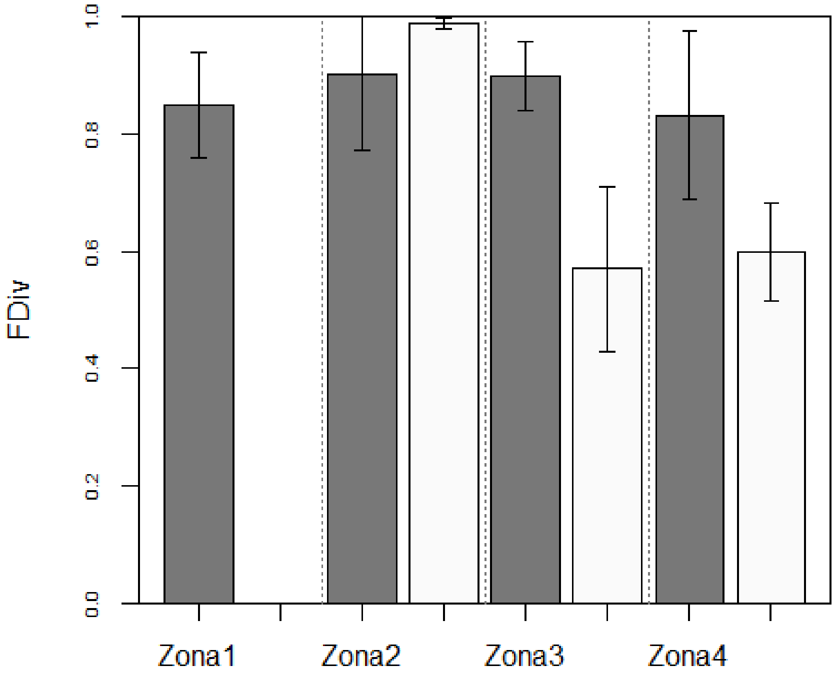

Figura 9-F: Índice de Divergência Funcional do estuário do Mamanguape

Os índices de dispersão funcional foram baixos nos dois estuários, tendo-se registrado valores mais baixos durante o período chuvoso no estuário do Paraíba do Norte do que no estuário do Mamanguape, apresentando, no entanto, menores variações neste último estuário (Figs. 9G e 9H).

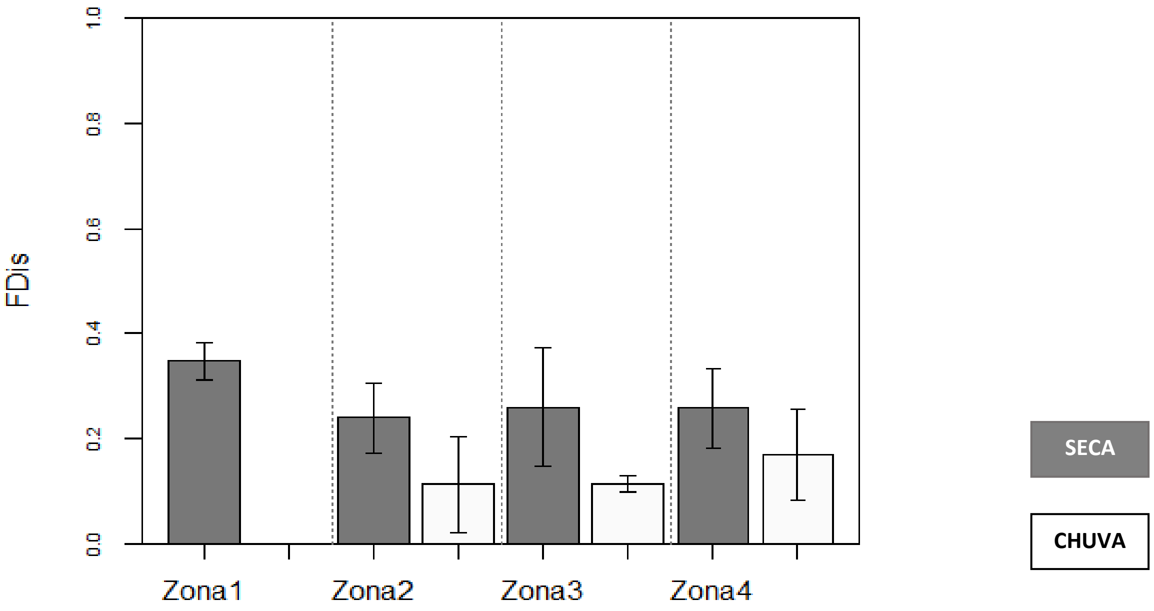

Figura 9-G: Índice de Dispersão Funcional do estuário do Paraíba do Norte

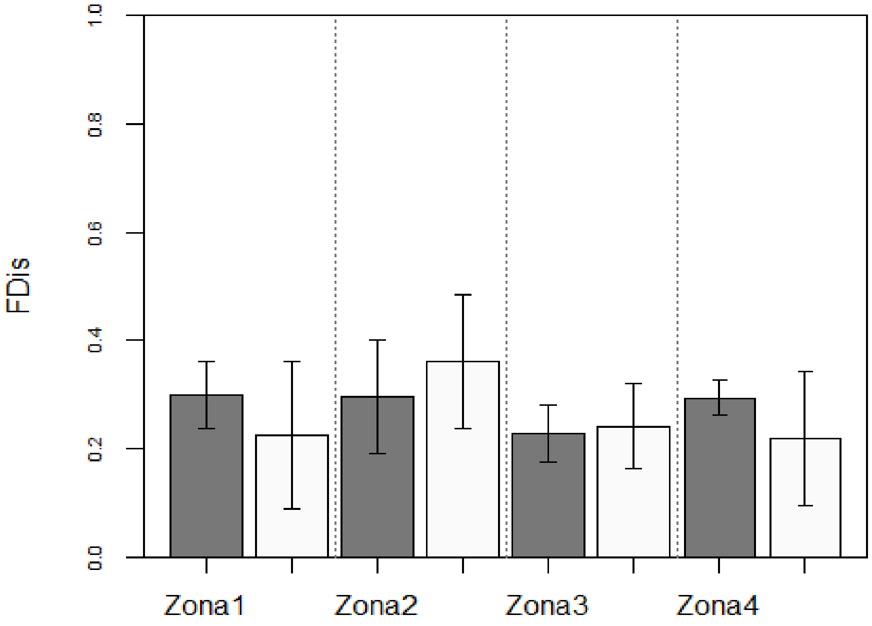

Figura 9-H: Índice de Dispersão Funcional do estuário do Mamanguape

Figura 9 – (A-B)Resultados dos índices de riqueza (FRic), (C-Dequitabilidade (FEve), (E-F) divergência (FDiv) e (G-H) dispersão funcional (FDis) para os estuários do Rio Paraíba do Norte e da Barra de Mamanguape, respectivamente. Barras brancas correspondem à época chuvosa e barras cinzentas à época de seca.

DISCUSSÃO

O baixo número de espécies dulçaquícolas apresentadas neste trabalho pode estar relacionado com períodos mais acentuados de seca extrema que se possam ter registado em anos anteriores tendo conduzido a uma redução mais acentuada das espécies de água doce. Gonçalves et al. (2012a) verificaram um efeito semelhante num estuário temperado, estuário do Mondego (Portugal), em que após uma seca extrema, a comunidade de zooplâncton marinha predominou em relação às espécies de água doce. As espécies *Notodiaptonus iheringe* e o *Microcyclops anceps*, típicas de água doce, estiveram presentes apenas na Barra de Mamanguape, entre as zonas Z1 e Z2, durante a época de chuva, onde o valor máximo de salinidade foi de 3.46 ppm. Isso possivelmente se deu pelo aumento do volume hídrico que escoa do rio durante o período de chuvas. No ciclo diário de maré, durante as marés baixas, especialmente após chuvas pesadas, espécies de água doce invadem todo o estuário (LANSAC-TÔHA & LIMA, 1993), mas uma das razões do insucesso dos organismos de água doce é a incapacidade de osmorregulação ativa necessária ao animal que vive sob condições de salinidades variáveis. A geomorfologia, o fluxo de água doce, e as marés são as variáveis dominantes que determinam a distribuição da salinidade e a circulação dentro do estuário (HANSEN& RATTRAY, 1966).

Os organismos estuarinos, apesar da sua fragilidade e vulnerabilidade diante das modificações ambientais, mantêm-se nestes sistemas bastante dinâmicos, apresentando alguma adaptação às condições existentes. Por outro lado, este equilíbrio pode ser facilmente rompido pelas atividades humanas, produzindo alterações maiores que a tolerável, ora pela adição na água de substâncias ou pela quantidade excessiva de outras, normalmente presentes (OTTMANN *et al.*, 1965)

As espécies mais representativas nos ambientes em questão foram *Oithona oswaldocruzi* e *Oithona brevicornis*. Segundo Björnberg (1981), os gêneros *Acartia* e *Oithona* estão entre os mais importantes nos estuários tropicais e subtropicais, e em conjunto com os gêneros *Parvocalanus, Euterpina, Paracalanus* e *Temora* - todos esses encontrados em ambos os estuários - compreendem os gêneros de copépodes mais abundantes. *Acartia tonsa* e *Parvocalanus campaneri* foram exclusivas do estuário da PB do Norte. *Acartia lilljeborgi*, espécie predominante em vários estuários brasileiros (DIAS, 1999), apresentou valores reduzidos de densidade e biomassa em todas as zonas de coleta e em ambos os estuários, apresentando maior valor (21ug/L) na zona Z3 do estuário do Mamanguape no período chuvoso. Santos *et al* (2009), nos estuários dos Rios Carrapicho e Botafogo, em Itamaracá – PE, observaram que as maiores densidades foram relacionadas a *A. lilljeborgi.*

Segundo Buskey (1993), esta falta de padrão é característica de muitos estuários tropicais e subtropicais, e são importantes na estruturação da comunidade. Apesar da elevada produtividade primária tornar o zooplâncton estuarino muito abundante, os fatores ambientais (como a ampla variação diária da salinidade e fatores biológicos) podem restringir a variedade das espécies quando comparado ao das áreas marinhas (KENNISH, 1990), podendo ocorrer muitas espécies no zooplâncton estuarino, mas apenas cinco ou seis constituem a maior parte da população (TUNDISI, 1970).

O estuário da Paraíba do Norte apresentou os maiores valores de fósforo total em ambos os períodos de coleta, e foi notadamente superior às mesmas informações referentes ao Mamanguape. Ainda assim o período chuvoso do Paraíba do Norte apresentou valores maiores quando comparado ao período seco. Da mesma forma, LUCAS (2006) encontrou para os estuários dos rios Botafogo e Siriji (PE) o mesmo padrão observado no presente estudo, com concentrações mais elevadas no período chuvoso, com valor máximo de 130,19 µg/L. SILVA NETO (2012), para o estuário do rio Paraíba do Norte (PB), encontrou a maior concentração de fósforo total no período chuvoso (615,0 µg/L), sendo as maiores concentrações mais expressivas nas estações a montante, ao contrário do presente estudo, que observou os maiores valores de fósforo total nas zonas intermediárias (Z2 e Z3).

Da mesma forma que o nitrogênio inorgânico dissolvido, seus valores foram significativamente mais elevados no estuário da Paraíba do Norte durante o período chuvoso. Esses dados sugerem que esse ambiente sofre mais impactos antropogênicos quando comparado ao estuário de Mamanguape e apontam um processo de eutrofização. Outra informação importante quanto aos nutrientes desses ambientes se refere à concentração de clorofila *a*, que no estuário da Paraíba do Norte apresentou valores mais elevados que no Mamanguape. A clorofila *a* atingiu valores máximos no estuário da PB durante a seca, e valores bastante reduzidos no Mamanguape. Esses dados de nutrientes revelam o quão diferentes esses ambientes se apresentam quando analisados simultaneamente, evidenciando que o Paraíba do Norte sofre mais impactos advindos das mais diversas atividades humanas que são realizadas em suas margens e entornos (navegação, cultivo de mariscos, pesca, cultivo de cana-de-açúcar), ao passo que o estuário do Mamanguape, por ser uma área de proteção ambiental, apresenta menores impactos antropogênicos.

Quanto à densidade, ocorreram diferenças importantes nos dois períodos amostrais. As maiores densidades de organismos foram registradas para as formas jovens de copépodes, nauplii e copepoditos de Cyclopoida, registro comum para esse tipo de ambiente, visto que os Copepoda são um dos grupos mais abundantes do zooplâncton. Nauplii e juvenis de copepoda são as formas mais abundantes, podendo registrar-se uma elevada abundância de uma das ordens de copepoda (Cyclopoida ou Calanoida são os mais comuns, seguidas de Harpacticoida) num determinado periodo do estudo. Além disso, as águas estuarinas são biologicamente mais produtivas do que o sistema de água doce e o oceano adjacente, devido à sua circulação hidrodinâmica característica que retem muitos nutrientes, estimulando a produção (MIRANDA *et al*., 2002), além de que a predação é menor nos estuários do que nos oceanos.

A espécie mais representativa no Mamanguape, quanto à densidade, foi *O. brevicornis* (chuva) e *O. oswaldocruzi* (seca), da mesma forma que no Paraíba do Norte, mas estas se limitaram em seus maiores valores ao período chuvoso. Leite (2004), estudando o mesozooplâncton do estuário do rio Curuçá – Pará observou que a *A. tonsa* foi a espécie de maior densidade nos locais de coleta, com valores significativamente mais elevados no período chuvoso, ao contrário do que constatamos no estuário do Rio Paraíba. Magalhães *et al* (2009), analisando a variação temporal da composição, ocorrência e distribuição de Copépodes no estuário do Taperaçu, Pará, observou que os valores médios para as densidades dos copépodes revelaram que *P. marshi* (11,7 ± 14,3 a 20.909,7 ± 50.527,1 ind.m^{-3}) foi a espécie numericamente dominante, seguida por *A. tonsa* (8 ± 11,2 a 4.604,2 ± 4.521,7 ind.m^{-3}) e *A. lilljeborgii* (0,1 ± 0,2 a 1.646,4 ± 1.237,3 ind.m^{-3}), concluindo que o estuário apresenta baixa diversidade de espécies, com os valores médios (± DP) oscilando entre 0,7 ± 0,4 (fevereiro) e 1,2 ± 0,9 (junho) bits.ind^{-1}. Santos *et al* (2009) concluiu que as diversidades médias nos estuários dos rios Carrapicho e Botafogo foram de 2,28 bits/ind e 1,91 bits/ind, respectivamente, sendo consideradas médias, quando comparadas com outros estuários de Pernambuco (NASCIMENTO, 1980; NEUMANN-LEITÃO, 1994; SILVA, 1994). Pessoa *et al* (2009), analisando a Comunidade Zooplanctônica na Baía de Suape e nos Estuários dos Rios Tatuoca e Massangana (Pernambuco), afirma que a diversidade de espécies foi média (2,827 bits/ind) devido à presença de várias espécies marinhas.

A CWM (*community-level weighted means*), composição funcional para atributos com valores contínuos, é o valor médio das características de todas as espécies presentes na comunidade, ponderada por suas abundâncias relativas (LAVOREL *et al*. 2008), revelou que seus atributos foram mais diversos no período de seca do PB do Norte e no período de chuva do Mamanguape. E, ao contrário, os traços permaneceram mais constantes nos períodos de chuva do estuário da Paraíba do Norte e de seca no estuário do Mamanguape. Essa medida mostrou ainda que durante a seca há uma invasão do estuário por espécies costeiras-oceânicas, e durante o período chuvoso predominaram espécies estuarinas, ao passo que as espécies de água doce foram registradas apenas nas primeiras zonas do estuário do Mamanguape durante o período chuvoso. Quanto aos hábitos alimentares no estuário do Paraíba do Norte, dois tipos foram predominantes no período de seca, o tipo emboscada e o tipo estacionário-suspensívoro. Este último é quando a busca pelo alimento é passiva, mas a captura pode ser ativa, enquanto o primeiro é uma busca ativa pelo alimento. O tipo emboscada também foi predominante no período chuvoso do PB do Norte, visto que a maior entrada de água doce nos estuários promove maior turbulência de suas águas, obrigando os organismos a buscarem ativamente suas fontes alimentares. Ao contrário disso, o estuário do Mamanguape apresentou o hábito alimentar do tipo emboscada mais predominante no período de seca.

Quanto à riqueza funcional, o índice de riqueza funcional mostrou que no estuário da Paraíba do Norte os valores foram mais elevados que no estuário do Mamanguape, revelando que o espaço nicho do Paraíba do Norte possui uma maior ocupação de espécies funcionalmente diferentes quando comparado ao do Mamanguape. Baixa *FRic* indica que alguns recursos (*alpha niches*) potencialmente disponíveis para a comunidade não estão sendo utilizados. A equitabilidade funcional mostrou que a distribuição das espécies em ambos os estuários não apresentou grandes variações em suas abundâncias, e o índice de divergência funcional não revelou grandes variações entre os dois estuários, mas que os maiores valores foram observados no período seco nos dois ambientes. Os índices de dispersão funcional foram baixos nos dois estuários, mas foram mais representativos durante o período de seca nos dois ambientes.

CONCLUSÃO

- Os organismos estuarinos, costeiros e/ou oceânicos são predominantes nos dois estuários estudados e apenas duas das espécies encontradas são de água doce. Algumas espécies são exclusivas de um ou de outro estuário.
- Há diferenças significativas na densidade zooplanctônica entre as zonas salinas, mas não entre os períodos sazonais, estuários e os pontos de coleta.
- O estuário do Paraíba do Norte apresenta dados relativos à nitrogênio inorgânico dissolvido, fósforo total e clorofila *a* mais elevados que o estuário de Mamanguape, confirmando que este último é menos impactado que o primeiro.
- A análise de Diversidade Funcional não demonstrou diferenças entre os ambientes analisados. O Mamanguape não apresentou maiores valores para essa análise, como era de se esperar, visto que consiste em área de proteção ambiental.

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ANEXOS

ANEXO I

Lista de Referências da Tabela 5

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ANEXO 2

Functional diversity of zooplankton communities in two tropical estuaries (NE Brazil) with different degree of human-induced disturbance.

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Abstract (150/150 max)

Functional diversity (FD) of zooplankton communities along the environmental gradient of two tropical estuaries with different levels of human impact – the environmentally protected “Mamanguape” and the urbanized “Paraiba” – was assessed. Different trait-based approaches were used to investigate the communities’ response to anthropogenic disturbance: a functional groups approach and by computing FD indices.

Results suggested that communities in each estuary share similar functions performed by few functional groups. Overall, biomass was more evenly distributed among groups, and total biomass and FD indices were slightly higher in the Mamanguape. Nevertheless, both estuaries presented similar FD levels, indicating that environmental filters seem to be the main driver of species coexistence within systems.

Despite the conservation status of the Mamanguape, anthropogenic impacts are still evident stressing the insufficiency of the measures approved to ensure good status. More effective management is essential in both estuaries to preserve biodiversity and the vital ecosystem services they support.

Keywords: Functional diversity; Biological traits; Zooplankton; Management; Tropical estuaries.

1. Introduction

Several studies are recently emerging using functional diversity and biological traits to evaluate long-standing ecological questions, yet the body of empirical evidence on the patterns of functional diversity is still small (Cadotte et al., 2011; Mason and de Bello, 2013).

Functional diversity (FD) is a key facet of biodiversity that describes the breadth of functions performed by species in a community and is measured based on traits (Tilman 2001; Maire et al., 2015). Traits (*sensu* Violle et al., 2007) are known to influence ecosystem functioning, and to reflect the mechanisms underlying species-environment relations (Diaz and Cabido, 2001; Villéger et al., 2010; Verbeck et al., 2013). Hence, analysing functional diversity patterns may provide greater insight on the responses of a community when facing anthropogenic disturbance, and assist to disentangle how ecosystems work (Mouillot et al., 2013; Gagic et al., 2015; Strong et al., 2015). FD is a multifaceted concept integrating different components, which has often been addressed through several methods (Bremner, 2008; Carmona et al., 2016), using different communities and habitats, including: benthic invertebrates (e.g. Tillin et al., 2006; Cooper et al., 2008; Bolam et al., 2016; Moretti et al., 2016; Schemera et al., 2016; Veríssimo et al., 2012; 2017; Van der Linden et al., 2016); fish (e.g. Villéger et al., 2010; Stuart-Smith et al., 2013; Wiedmann et al., 2014); plants (e.g. Pakeman et al., 2011; Schittko et al., 2014); phytoplankton (e.g. Weithoff, 2003; Edwards et al., 2013; Santos et al., 2015) and, zooplankton (e.g. Barnett and Beisner, 2007; Barnett et al., 2007, 2013; Vogt et al., 2013; Bolduc et al., 2016; Russo et al., 2016).

To date, studies addressing zooplankton functional diversity have been mostly developed for freshwater ecosystems, and a comparable lack of studies exist for marine and estuarine realms (Pomerleau et al., 2015; Helenius et al., 2016). In aquatic environments, zooplankton is at the heart of pelagic food webs, mediating energy flow to higher trophic levels and thus playing a fundamental role in many ecosystem processes (Kiørboe, 1997; Barnett et al., 2007). Further knowledge on the functioning of zooplankton communities is thus crucial, and a functional approach has been claimed as key for a comprehensive understanding of ecosystem dynamics (Russo et al., 2016). In general, the current knowledge of the relationship between functional diversity patterns and environmental variation is still poor (Dimitriadis, 2012; Mouillot et al., 2013), and is particularly scarce for the zooplankton in ecosystems with strong environmental gradients, as is the case of tropical estuaries in the Northeast Brazil. In the latter, studies have been mainly addressing zooplankton composition and structure (e.g. Almeida et al., 2009; Melo et al., 2013), focusing less on the functional aspect.

Over the last decades, most of these estuarine areas are, however, facing growing problems of environmental degradation due to increasing pressures from traditional and emerging human activities, such as tourism, agriculture, aquaculture and overfishing, together with poor management. Despite some management and conservation efforts have been made recently, from both governmental organizations and NGOs, these have proven insufficient and degradation seems to continue with potential to intensify in future (Diegues, 1999; Barletta et al., 2010).

Environmental management is committed with the safeguarding and improvement of ecosystems' environmental state through a science-based and integrated approach, in order to inform management planning effectively and regulate human pressures (Elliott, 2014). An effective management program, aiming long-term conservation and sustainable use, must thus account for an ecosystem multiple uses without compromising its ecological condition and functioning (Borja et al., 2016). In order to achieve this, an in-depth knowledge on the functioning of an ecosystem and of the responses of the biological communities to disturbances, is required. This is essential to a better understanding on how communities cope with environmental change, and such can be addressed through functional diversity.

The main goal of this manuscript was to assess zooplankton (copepods) functional diversity in two Northeast Brazilian estuaries (Paraiba and Mamanguape) experiencing distinct anthropogenic levels (mainly organic enrichment from aquaculture and agriculture). As functional diversity encompasses different "facets" (Mason et al., 2005), a combined functional approach was used to analyse the spatial and temporal response of the copepods communities along the abiotic stress gradient in each estuary: (*i*) a functional group approach: to identify and describe groups of copepods based on life-history traits, discussing their ecological relevance; and (ii), by computing complementary functional diversity indices (Functional Richness "FRic", Functional Evenness "FEve", and Functional Divergence "FDiv"). Ultimately, it is intended to increase knowledge about the functioning of tropical estuaries and to perceive the usefulness of zooplankton functional diversity analyses as tools to evaluate the effectiveness of environmental protection practices.

2. Material and Methods

2.1. Study site description

Two tropical estuaries located on the Northeast coast of Brazil, in the Paraiba state, were used as a case-study: Paraiba (6º57'30"S; 34º51'30"W) and Mamanguape (6º43'02"S; 35º67'46"W) (Fig.1). Both estuaries are classified as climatic type "As" (equatorial with a dry summer) according to the Köppen-Geiger classification. The wet season extends from February to August, with the highest precipitation occurring in June and the lowest in November. The dry season occurs mainly from August to January.

The Paraiba and the Mamanguape river basins include a hinterland region comprising the semi-arid Caatinga Biome and a narrow coastal strip originally covered by the Atlantic rainforest, with a coverage area of 20 124 km^2 and 3 522 km^2, respectively (AESA, 2015).

The Paraiba estuary (3012 ha; Fig. 1A) is the largest estuary in the state of Paraiba with about 22 km long, and is located near an urban area with approximately one million inhabitants. The Mamanguape estuary (690 ha; Fig. 1B) is the second largest estuary in the state of Paraiba and is located inside an environmental protection area (“APA”, IUCN protection category V). This estuary harbours a great variety of habitats including sandy coastal beaches bordered by field dunes, coastal reefs with dense mats of macroalgae, seagrass beds (*Halodule wrightii*), restinga forests, tableland forests and a well-preserved mangrove area with approximately 6000 ha. An important feature in this ecosystem is an 8.5 m long barrier reef perpendicular to the shoreline, which creates a protected region at the mouth of the estuary (Xavier et al., 2012; Claudino et al., 2015).

Both estuaries have mangroves that grow around the main channel and intertidal creeks, along with remnants of the Atlantic rainforest (Campos et al., 2015), and are affected by different degrees and types of human-induced disturbance. Clear signs of environmental degradation have been observed in the Paraiba estuary (highly impacted), which is located in a densely populated urban area, with sugarcane plantations along the riverbanks and intensive shrimp aquaculture activities occupying part of the remaining mangrove area. The absence of surveillance and law enforcement for specific activities contribute to a worsening of the ecological condition. In turn, the ecological importance of the coastal habitats of the Mamanguape estuary (moderately impacted), in particular for the conservation of the marine manatee (*Trichechus manatus*, Linnaeus, 1758), has been recognised by the Brazilian governmental agencies, which declared it as a conservation area in 1993. Large mangrove areas characterise this estuary, which provide several food sources and nursery areas for different species. Despite the environmental protection status, in the estuarine surrounding region there are, however, shrimp aquaculture activities and sugarcane plantations contributing to eutrophication and agrochemical pollution (Alves et al., 2005; Claudino et al., 2015).

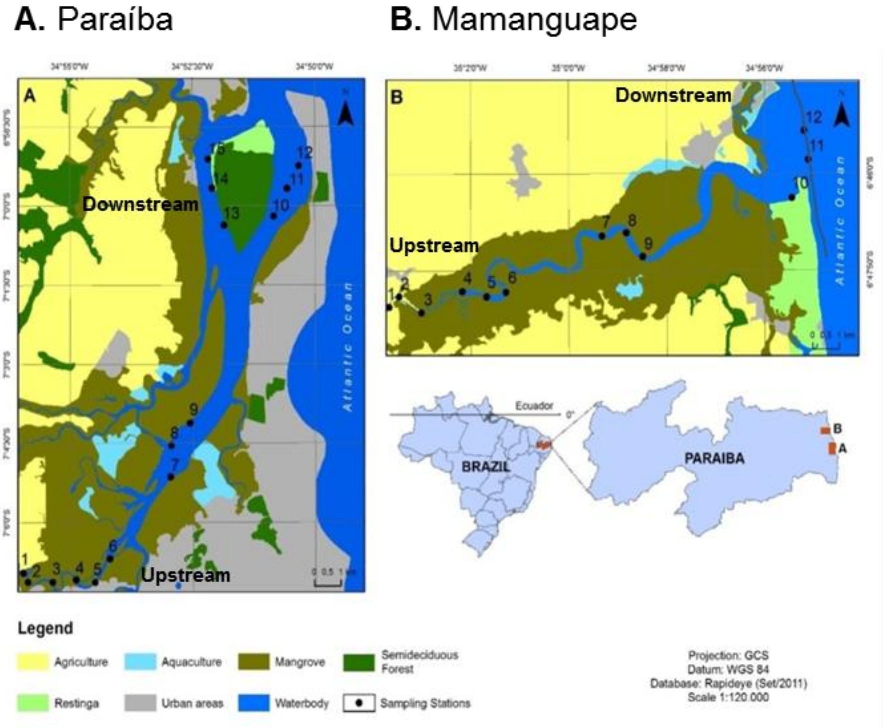


Figure 1. Geographical location of the Paraiba **(A.)** and Mamanguape **(B.)** estuaries (Northeast Brazil), sampling sites and main land uses.

2.2. Sampling and laboratory procedures

The present study focused on copepods communities which are ubiquitous and a strong contender for the most numerous metazoan class (Litchman et al., 2013), being relatively well documented in terms of distribution and biology (Razouls 2005-2015; Benedetti et al., 2015).

Two sampling campaigns were carried out in each estuary, one in the dry season (November 2013) and the other in the wet season (July 2014), during high tide. Zooplankton samples were collected using horizontal subsurface tows (bongo net: mesh size 68 µm, mouth diameter: 0.3 m) along the estuarine salinity gradient in 15 sites in the Paraiba estuary and 12 sampling sites in the Mamanguape estuary (Fig. 1). In each estuary, two estuarine sections previously defined according to biotic and abiotic variables (Alves et al., 2016; Dolbeth et al., 2016a, 2016b) can be identified: upstream (sites 1 to 6), and downstream (7 to 12/15 in Paraiba and Mamanguape estuaries, respectively). Three replicates were collected at each sampling site and samples were fixed and preserved in 4% buffered formaldehyde. In the laboratory, zooplankton samples were sorted and copepods identified preferentially to the species level under a stereoscopic microscope. Biomass was determined as µg L^{-1}. Replicates were pooled and the analyses performed at the estuarine section level.

At each sampling site several physicochemical parameters were measured *in situ*: surface water temperature (°C), dissolved oxygen (mg L^{-1}), salinity, pH and turbidity with a multiparameter probe, and transparency (m) with a Secchi disk. Additionally, water samples were collected to determine nutrients content (Ammonia (NH_4-N, µg L^{-1}), nitrite + nitrate (NO_x-N, µg L^{-1}) and total phosphorous (P, µg L^{-1})), and Chlorophyll *a* (Chl *a*, µg L^{-1}) as described in Alves et al. (2016).

2.3. Biological traits information

To build a "species by traits" data table (Table S1) for the most representative copepod species in terms of biomass and presence in the Paraiba and Mamanguape estuaries, information on 6 biological traits was gathered from different published sources including scientific papers and online data bases (e.g. "PANGEA", www.pangea.de). Traits selection, which required a compromise between information availability and its ecological relevance, was based on the choices adopted in previous zooplankton studies and included those traits that would best describe organisms' response to the environment (Barnett et al., 2007; Barton et al., 2013; Pomerleau et al., 2015; Benedetti et al., 2015). The traits chosen cover different aspects of the morphology, behaviour, life-history and ecology of the taxa, known to be functionally important: maximum body size (mm), trophic group, feeding type, reproduction mode, diel vertical migration (DVM) and vertical distribution.

2.4. Data analysis

Spatial and temporal patterns of the zooplankton functional groups, functional diversity indices and environmental variables, at the downstream and upstream sections of the Mamanguape and Paraiba estuaries, were investigated using multivariate and univariate statistical methods.

Environmental variables were explored using a Principal Component Analysis (PCA) to seek patterns between estuaries, seasons and estuarine sections. Redundant variables were removed from the analyses after inspection with Draftsman plots, ensuring that maximum variability in the data set is accounted. A resemblance matrix based on Euclidean distances was computed prior to the analysis and all variables were normalised. Those environmental variables moderately and heavily skewed in distribution were square root (turbidity and Chlorophyll *a*) and fourth root (Ammonia, Nitrite + Nitrate and Total Phosphorous) transformed.

Functional diversity assessment was carried out employing two different methods. Firstly, considering that using multiple traits may be more informative with regard to the response of assemblages to environmental disturbance than when considering isolated (Verbeck et al., 2013), zooplankton functional groups, i.e. groups of species sharing similar traits, were identified and their spatial and temporal patterns of variation were investigated. Zooplankton functional groups were identified using Multiple Correspondence Analysis (MCA) together with hierarchical agglomerative clustering (Husson et al., 2010; Legendre & Legendre, 2012). Initially, the "species by traits" matrix was ordinated using MCA, which is a method appropriate for the analysis of categorical variables, and four traits were considered in the analysis: maximum body size, trophic group, feeding type and reproduction mode. Diel vertical migration was not accounted as it exhibits high plasticity facing environmental variations and with species ontogeny (Pomerleau et al., 2015; Benedetti et al., 2015). Together with the vertical distribution, these were considered *a posteriori* in the MCA as supplementary variables, for informative purposes. After, groups of species sharing a similar set of traits (functional groups) were defined through cluster analysis based on Ward's linkage method (Ward, 1963), using Euclidean distances and the scores of taxa along the four axes of the MCA. For each functional group, a biological trait profile was created indicating for each trait the proportion of categories displayed by the group. Using the "species by samples" matrix (species biomass per sample), the groups spatial and temporal patterns of variation were evaluated.

Secondly, functional diversity was summarized based on the framework proposed by Mason et al. (2005) and Villéger et al. (2008), thus measuring its primary components through the computation of three multidimensional and complementary FD indices: Functional Richness (FRic), which represents the volume of the functional space occupied by the community; and, Functional Evenness (FEve) and Functional Divergence (FDiv), that measure the regularity and spread of the distribution of biomass in this volume, respectively. FRic is independent of species abundance/biomass, was standardised to range between zero and one, and it is expected to decrease with disturbance (Mouillot et al., 2013; Laliberté et al., 2015). Both FEve and FDiv are biomass-weighted, values are constrained between zero and one, and indices scores are expected to decrease with disturbance (Villéger et al., 2008; Mouillot et al., 2013). Indices computation included the preliminary evaluation of the quality of the functional spaces, computing all the possible spaces and selecting the most parsimonious one, in agreement with Maire et al. (2015). Detailed information on the FD indices calculation steps, R routines and functions used can be found in Mouillot et al. (2013), Villéger et al. (2013) and Maire et al. (2015), freely available at http://villeger.sebastien.free.fr/FD.html (accessed on November, 2016).

Spatial and temporal differences in the FD indices were assessed using separate PERMANOVAs based on Euclidean distance after data normalisation. A three-way experimental design was considered: “estuary” (with two-fixed levels, Paraiba and Mamanguape), “season” (with two-fixed levels, wet and dry) and “estuarine section” (with two levels - upstream and downstream – nested in “estuary” and “season”). The statistical significance of variance components was tested using 9999 permutations of residuals under a reduced model, with an *a priori* chosen significance level (*P*) of 0.05.

To infer about potential relationships between the functional diversity measures tested and estuaries environmental conditions: (*i*) Spearman’ rank correlations were used to explore the strength of association among FD indices and the environmental variables; (ii) a Distance-Based Linear Model analysis (DISTLM) was used to explore relationships between habitat characteristics and the zooplankton functional groups, with “Best” and “AICc” as model selection procedure and selection criteria, respectively (Anderson et al., 2008).

Statistical analyses were performed using: PRIMER v6 statistical package (Clarke and Gorley, 2006), together with the PERMANOVA + PRIMER add-on package (Anderson et al., 2008); Functional groups analyses (*FactoMine*R package) and FD indices computation with R software version 3.3.1 (R Development Core Team, 2016); and, Spearman correlations with Statgraphics Centurion XVI.

3. Results

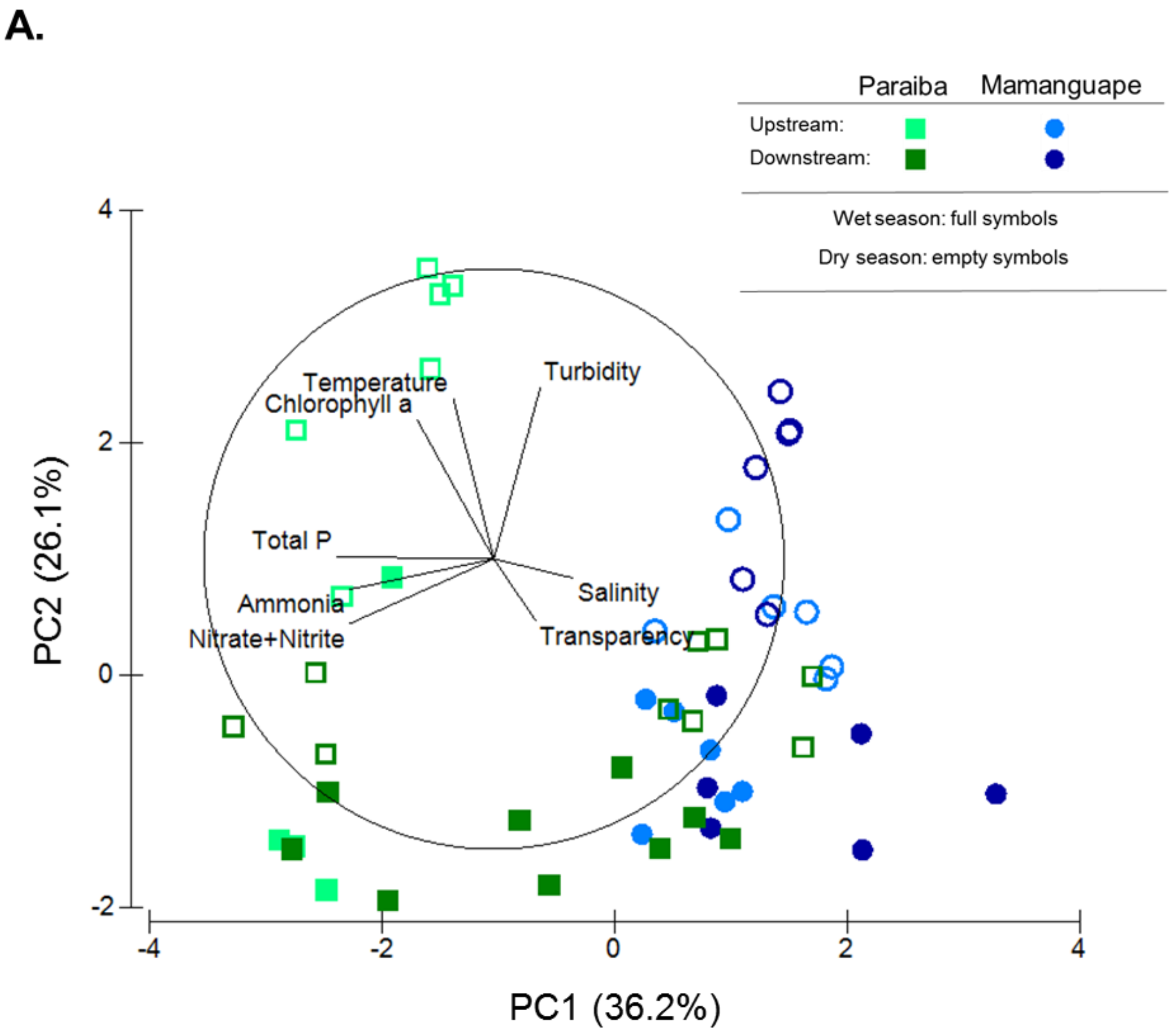
3.1. Environmental characterisation

The first two PCA axes on environmental variables explained 62.3% of the total variation clearly illustrating the relationships among samples (Fig. 2A).

A temporal distinction (seasonal) is evident, as samples from the dry and wet seasons are clearly segregated in the plot. A spatial gradient can also be identified at both estuaries, with samples from upstream section located further away from those of the downstream sector.

Characteristic of an estuarine ecosystem, a decreasing salinity gradient from the downstream to the upstream sections was observed at both estuaries (Fig. 2B). Overall, salinity was higher at the downstream sections, especially in the dry season, although in the Mamanguape estuary high levels in the upstream area have also been registered, attributable to the low freshwater input in this semi-arid region Alves et al., (2016). Higher values for turbidity and temperature were registered during the dry season, and for transparency in the wet season (Fig. 2A).

At both seasons, higher nutrient (Total P, Ammonia and Nitrate + Nitrite) and Chlorophyll *a* concentrations were observed in the Paraiba estuary, highlighting the higher degree of human-induced disturbance in this estuary (Fig. 2A). Along the estuarine gradient, nutrient levels were always higher in the Paraiba than in Mamanguape, particularly obvious for ammonia (Fig. 2B). In the Paraiba, high nutrient concentrations were registered in the wet season and mostly in the upstream section. In contrast, the less impacted estuary (Mamanguape) presented slightly higher nutrient concentrations during the dry season (Fig. 2B).



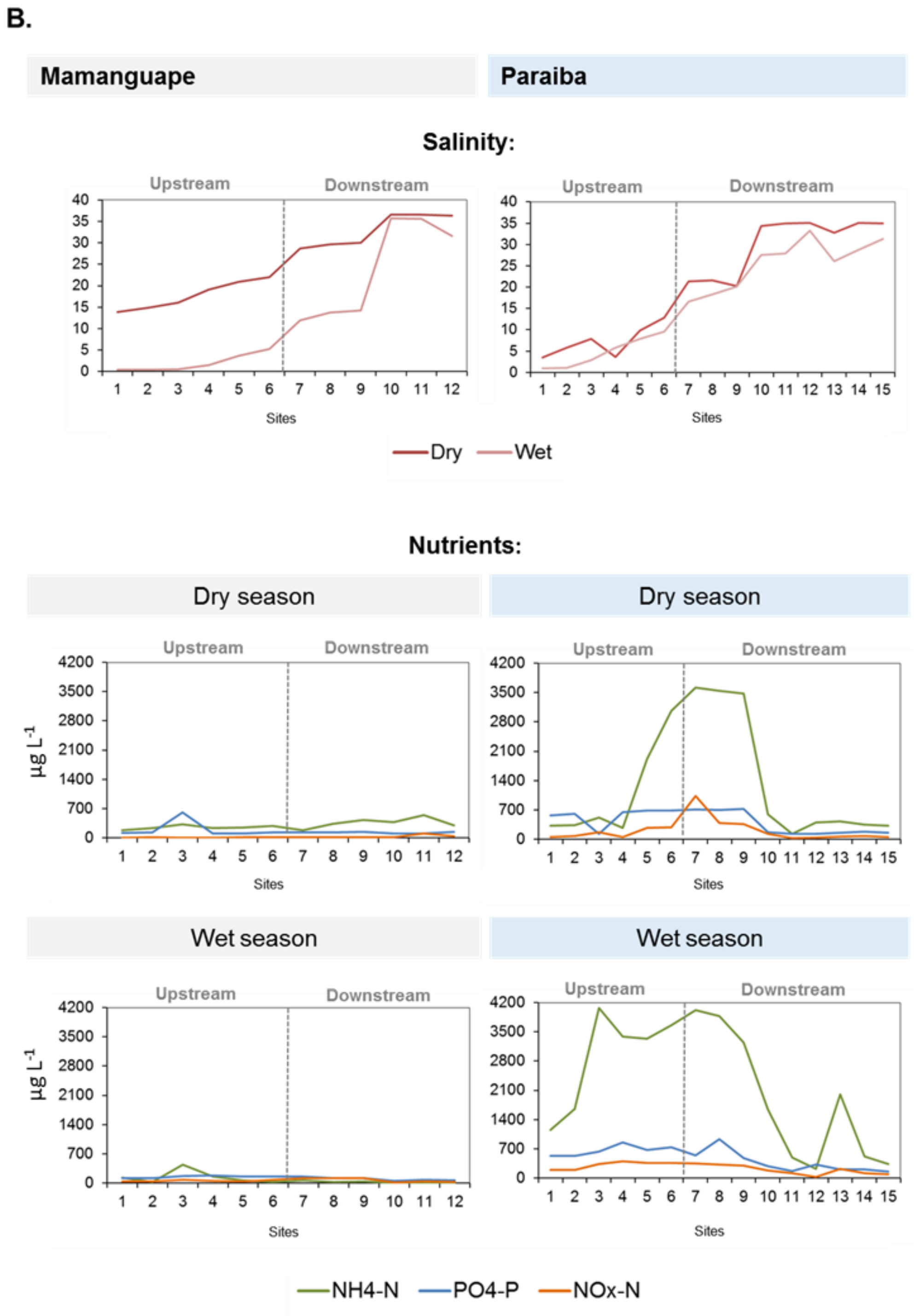


Figure 2. Environmental characterisation of the Mamanguape and Paraiba estuaries. **(A.)** Two-dimensional PCA ordination map of environmental variables in wet (full symbols) and dry (empty symbols) seasons, and upstream (light symbols) and downstream (dark symbols) sections. **(B.)** Variations in salinity and nutrient concentrations in dry and wet seasons, at the upstream and downstream sections.

3.2. Zooplankton functional groups

The four axes of the MCA performed on the traits by species matrix accounted for 90.34% of the total variance in biological traits (MCA 1: 40.23%, MCA 2: 25.59%, MCA 3: 14.02% and MCA4: 10.50%).

Overall, the first axis (MCA 1) separated small-sized (S1), egg brooders, herbivore active ambush feeders, from large-sized (S3-S4), broadcasters, omnivores and mixed/filter feeding species. The second axis (MCA 2), mainly opposed egg brood, omnivores, active ambush and mixed feeding individuals ranging several sizes, from herbivores small-sized broadcasters, filter feeders. Variances of MCA 3 and MCA 4 were explained just by a few taxa medium-sized (S3), with omnivore and herbivore feeding mode (Fig. 3B).

Trait categories contributing most to MCA axes, i.e. exhibiting higher correlation values with the different dimensions, were: (*i*) MCA 1: “very small” (S1) and “large” (S4), “omnivore” (trophic group) and “active ambush” (feeding mode); (*ii*) MCA 2: “small” (S2) and “filter” feeding; (*iii*) MCA 3 and 4, mainly “omnivore/herbivore” and “medium” size (S3).

The cluster analysis performed on the MCA output (i.e. species coordinates along the four axes; Fig. 3A), identified four distinct groups of zooplankton taxa clearly separated on the MCA space (Fig. 3B) and characterised by different sets of biological traits. Each group displayed a distinctive traits profile. Figures 3 and 4 allow to describe and characterise these groups: Group 1 (“Ambush-Herbivores”) is mainly composed of Cyclopoids of the *Oithona* genus which are mostly very small sized, active ambush-feeding herbivores and egg-brooders showing epipelagic (0 - 200 m) distribution and DVM behaviour. Group 2 (“Filter-Herbivores”) presents a larger number of species belonging to families Euterpinidae, Paracalanidae and Temoridae. Members span several size classes, are mostly herbivores and broadcast spawners, and all filter feeders. Distribution is mainly epipelagic and there is a tendency for DVM absence. Group 3 (“Mixed/ambush - Omnivores-Herbivores”) comprises two omnivore/herbivore copepods, *Mycrocyclops anceps* and *Pseudodiaptomus richardi*, with ambush and mixed feeding habits and, very-small and large body sizes, respectively. Group 4 (“Mixed-Omnivores”) includes copepods from *Acartia* and *Pseudodiaptomus* genera, with medium-large body size, all omnivore’s broadcasters and exhibiting mixed feeding strategy.

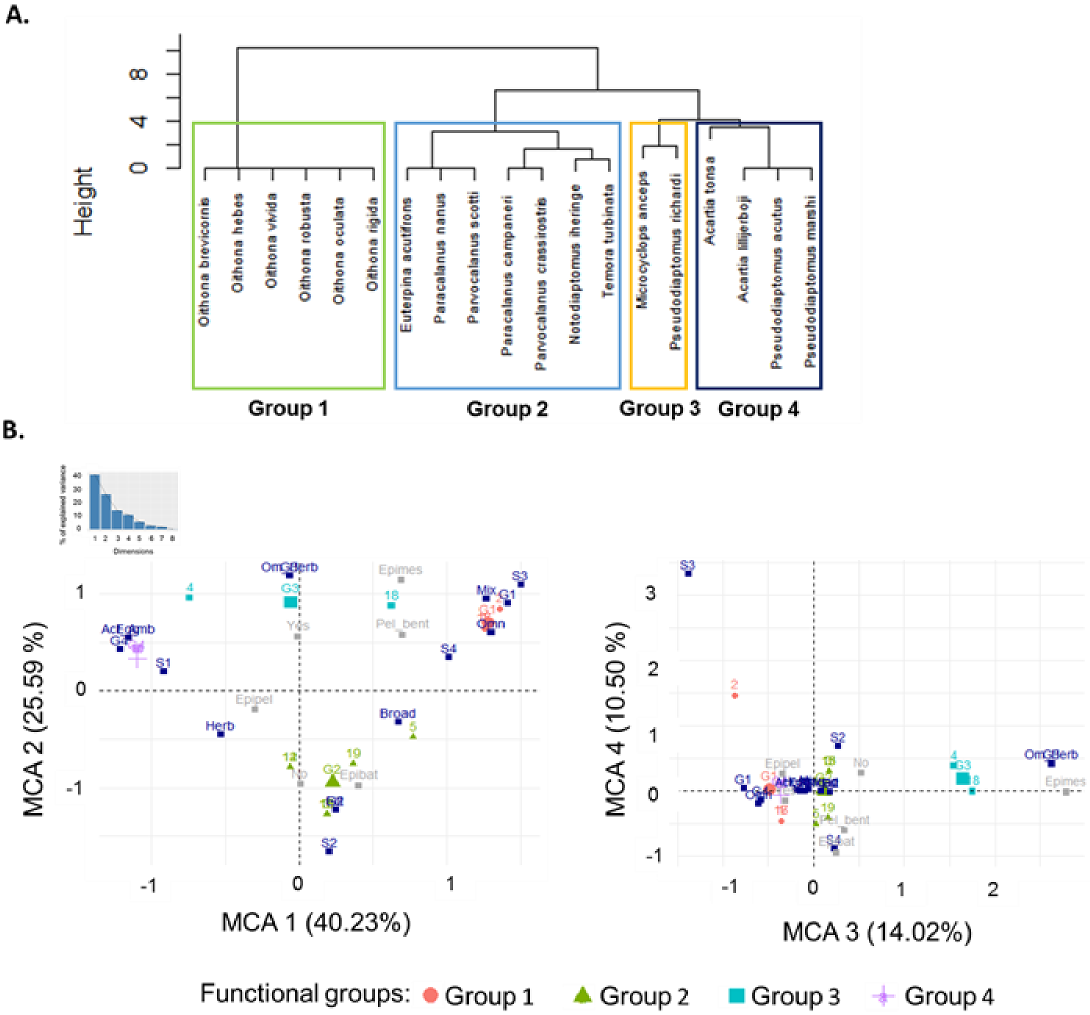


Figure 3. Classification and ordination of the zooplankton biological traits:

(A.) Dendrogram resulting from the hierarchical clustering (Ward's linkage method on Euclidean distances). Functional groups identified (1 to 4) and characterized by different sets of traits, outlined in different colour.

(B.) Histogram of eigenvalues and MCA factor maps for the axes 1 - 2 and 3 - 4. Copepod species represented by numbers; Trait categories used in the analysis shown by dark blue symbols (for categories labels please see Table S1), and superimposed supplementary variables (DVM and Vertical distribution) indicated in grey; Functional groups identified through cluster analysis (A.) represented by the symbols with different colours.

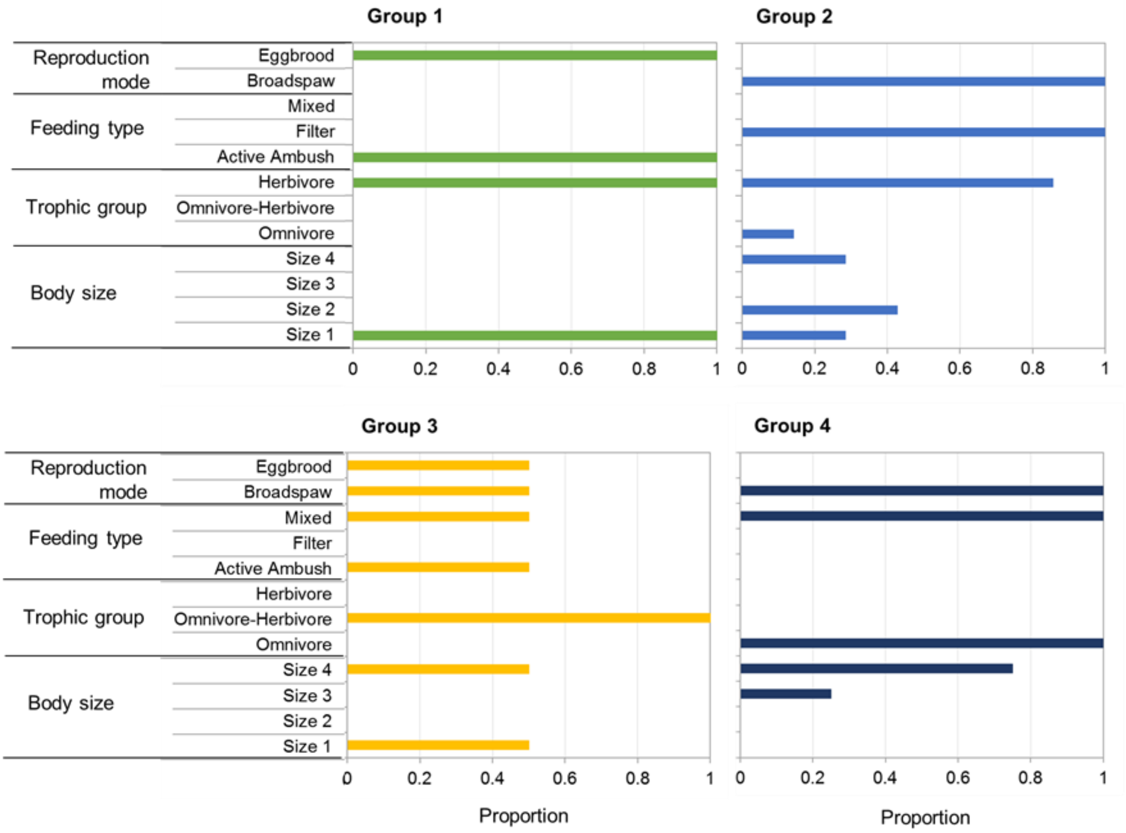


Figure 4. Biological traits profile of the zooplankton functional groups identified in the Mamanguape and Paraiba estuaries. Proportions of trait categories exhibited by the groups are shown.

Concerning the spatial and temporal variation patterns of the zooplankton functional groups, the group 4 (“Mixed-Omnivores”) was dominant in both estuaries, in particular in the saline downstream sections during the wet season, followed by groups 1 (“Ambush-Herbivores”) and 2 (“Filter-Herbivores”) (Fig. 5).

Overall, when comparing both estuaries, total biomass levels were higher in the Mamanguape than in Paraiba estuary, and the groups’ biomass appeared more evenly distributed in the former.

In the Mamanguape, an evenly biomass distribution between sections was observed during the dry season, with lower values registered at the downstream section. An inverse pattern was detected during the wet season (Fig. 5A). In the Paraiba estuary, lower total biomass values were also registered in the dry season, but this time at the upstream section (Fig. 5B).

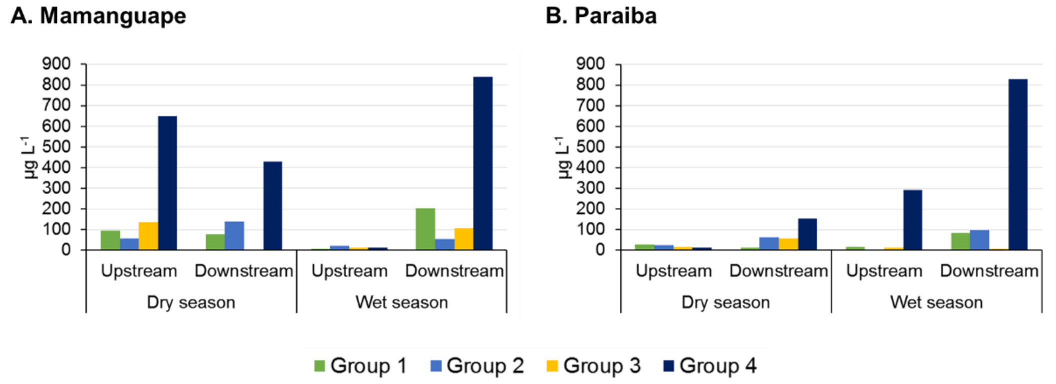


Figure 5. Total biomass of zooplankton functional groups during the dry and wet seasons, at the downstream and upstream sections of the Mamanguape (A.) and Paraiba (B.) estuaries.

Results from the DISTLM test, highlighting the relationships between environmental variables and the zooplankton groups' biomass, showed that in the Mamanguape, the most parsimonious solution (<AICc) was a 2-variable model explaining 26% of the total variability, provided by salinity and temperature. In the Paraiba estuary, 53% of the total variability was explained by four significant variables: salinity, turbidity, ammonia and chlorophyll *a*.

3.3. Functional diversity indices

The primary components of functional diversity were assessed through computation of three complementary functional diversity indices: Functional richness, evenness and divergence (FRic, FEve and FDiv, respectively). Indices behaviour was evaluated in the Paraiba and Mamanguape estuaries, spatially and temporally, i.e. at the upstream and downstream estuarine sections, during the wet and dry seasons.

Overall, when comparing both estuaries, mean FRic, FEve and FDiv were slightly higher in the Mamanguape than in Paraiba estuary (FRic: 0.38, FEve: 0.67; FDiv: 0.83; and FRic: 0.28, FEve: 0.53; FDiv: 0.80, respectively) (Fig. 6).

FRic showed spatial and temporal opposite patterns at both estuaries (Fig. 6). In the Mamanguape, it was slightly higher in the less saline upstream section during the dry season, while in the Paraiba higher mean values were registered in the downstream section and in the wet season, although these trends do not proved to be statistically significant (3-way PERMANOVA, $P>0.05$). At both estuaries, FRic generally decrease from dry to wet season in both estuarine sections, except in the downstream area of the Paraiba where it increased. This index is known to monotonically increase with species richness (Mason et al., 2013), and similar trends of variation between FRic and the number of species were observed, except in the Paraiba downstream section between the dry and wet seasons (Fig.

S1). Yet, it is important to highlight that this inconsistent result in the Paraiba may have been due to the higher number of samples containing less than three species, which prevented FRic computation for several samples and thus, may hinder our evaluation.

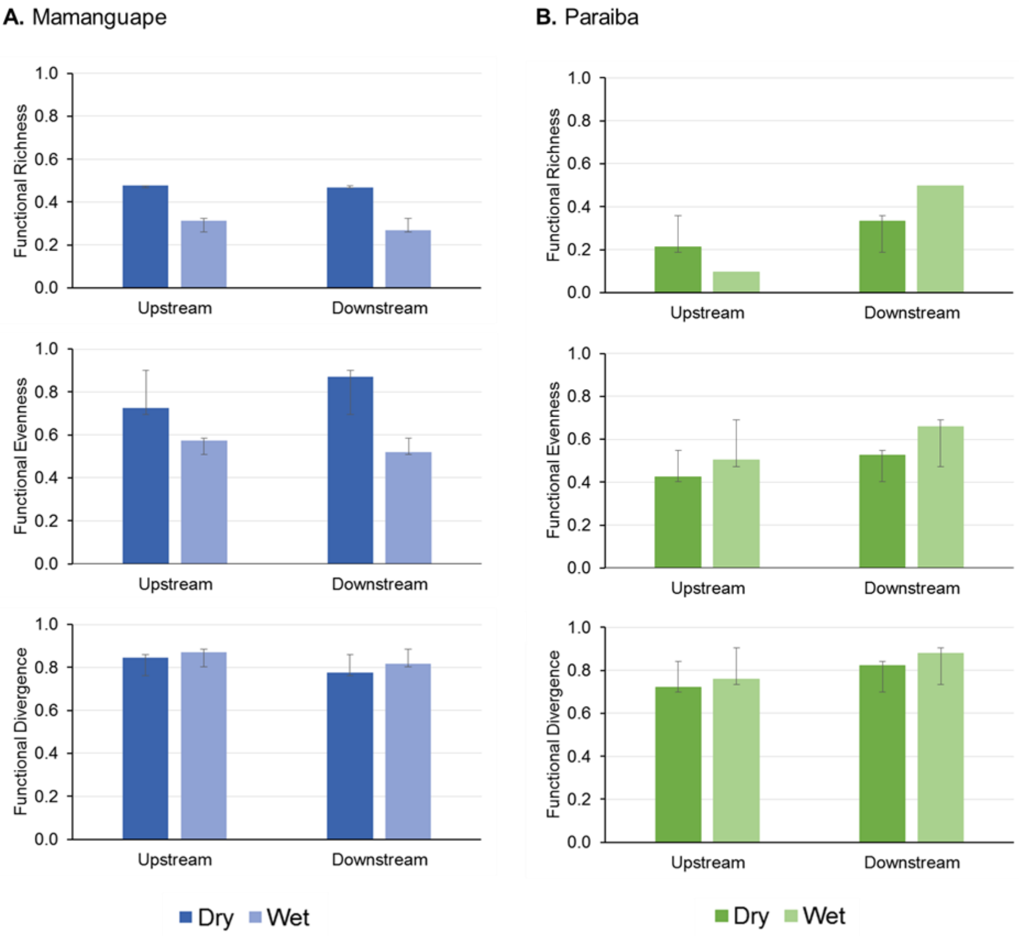


Figure 6. Variation of the functional diversity indices (Functional richness, evenness and divergence) in the downstream and upstream sections of the Mamanguape (**A.**) and Paraiba (**B.**) estuaries, during the wet and dry seasons.

With regard to the biomass-weighted indices (FEve and FDiv; Fig. 6), seasonally consistent trends were observed in the upstream and downstream sections of both estuaries, with higher mean values in the wet season, except for FEve in the Mamanguape, where higher values were registered in the dry season at both sections.

Overall, FEve and FDiv presented slightly higher mean values in the Mamanguape, than in Paraiba, in agreement with the lower disturbance level in this estuary. For the Paraiba estuary, higher mean FEve and FDiv values were observed in the downstream area, during the wet season. In the Mamanguape, FEve registered higher mean values downstream and in the dry season, whereas FDiv showed an inverse behaviour (higher upstream and in wet season). Statistically significant differences between upstream and downstream sections were, however, only detected for Paraiba in the wet season (3-way PERMANOVA: FEve: $F_{Sec(EsxSea)}$ = 2.84; FDiv: $F_{Sec(EsxSea)}$ = 2.97; both $P<0.05$) and Mamanguape in the dry season (3-way PERMANOVA: FEve: $F_{Sec(EsxSea)}$ = 2.29, $P<0.05$).

A few significant correlations between functional diversity indices and the environmental variables were observed (Table S2). Indices were mostly correlated with ammonia and turbidity, either in Paraiba or Mamanguape, although most of the values registered were low.

4. Discussion

The present study contributed to add knowledge on the functional diversity of the zooplankton communities of two tropical estuaries (Paraiba and Mamanguape). This type of information is scarce, in particular in the Northeast region of Brazil (Leitão et al., 2006), but crucial to a better understanding of the response of these assemblages to anthropogenic impacts, with consequent repercussions to the functioning of these ecosystems, and to inform management and conservation efforts.

Two different trait-based approaches were used to investigate the zooplankton functional diversity in the Paraiba and Mamanguape estuaries, which present dissimilar levels of human-induced disturbance: a functional group approach, and by computing complementary functional diversity indices. Both methods are known to provide different information, and therefore, to complement each other in the study of the functional diversity response to disturbance (Gagic et al., 2015). Indeed, the analyses of the spatial and temporal variation of the functional groups and indices, along the environmental gradient, shed light on zooplankton resources use and allowed us to characterize their functional trait space, and thus, to identify dissimilar areas and ways of using resources in these ecosystems. It is important to point out that zooplankton communities are highly dynamic due to its pelagic lifestyle, and the present work provides only a snapshot at a certain time and space, so that the variation patterns observed should not be interpreted strictly. Additionally, environmental condition assessment was undertaken on only two sampling occasions, and this lack of temporal replication may not be entirely translating the variability found in these estuaries. Nevertheless, Paraiba and Mamanguape estuaries have been characterised as presenting strong environmental fluctuations along their spatial gradient associated mostly to changes in salinity, temperature and turbidity (Alves et al., 2016). As one could expect, the Paraiba estuary presented higher nutrients concentration as it is most impacted due to its location in an urbanized area, near sugarcane plantations and intensive aquaculture activities. Salinity was higher in the downstream sectors of both estuaries, but in the Mamanguape higher values in the dry season were also registered upstream, indicating that the reduced freshwater runoff may promotes the influence of oceanic water further upstream, together with the lower number of river effluents discharging to this estuary.

With regard to the zooplankton functional groups, results suggested that assemblages may share similar functions at both the Mamanguape and Paraiba estuaries, translated by the low number of groups identified (four), and which seem to reflect complementarity in the use of resources within each system. This outcome could be expected if we consider that estuaries are naturally stressed and highly variable environments', therefore imposing strong habitat selection which may result in trait-convergence (de Bello et al., 2012). Habitat filtering should lessen the extent of ecological strategies, reflecting shared ecological tolerances (Cornwell et al., 2006; de Bello et al., 2012). The notion that the environment seems to filter taxa with specific characteristics allowing them to survive under determined conditions as often been highlighted (e.g. Mouillot et al. 2006; Villéger et al., 2013; Veríssimo et al., 2017), and former studies at the Paraiba and Mamanguape using fish communities have pointed in the same direction (Dolbeth et al., 2016a, 2016b).

At both the Mamanguape and Paraiba estuaries the zooplankton functional groups revealed mostly size- and feeding-structured. In fact, zooplankton communities are known to be strongly size-structured as size heavily determines its predators and prey, and influences, for instance, swimming speeds and growth rates, fecundity and metabolism (Kiørbe, 2008; Barton et al., 2011). Feeding is also known to differ in response to changing environmental conditions and prey abundance (Mariani et al., 2013; Moura et al., 2016), with the spatial and temporal distribution of feeding traits along estuarine gradients being greatly determined by trade-offs associated with food availability and presence of predators (Barton et al., 2011). Trade-offs among zooplankton traits have been often reported in the literature, in particular between size and feeding strategy, attributable to differences in metabolic requirements (e.g. Kiørbe et al., 2011; Litchman et al., 2013). For instance, Benedetti et al. (2015) studying the Mediterranean copepods found that small-sized carnivores and omnivores taxa were active ambush feeders, thus feeding passively, contrary to large carnivores which were cruise feeders and thus, needing to actively swim to search for prey, something that requires high energy expenditure. These different metabolic requirements seemed to explain size differences, as metabolic rates scale with body size (Kiørbe and Hirst, 2014). Evidence of trade-offs among zooplankton traits were also observed in the present study: very small-sized copepods were all active ambush feeders, while large-sized taxa were mostly mixed or filter feeders. Nevertheless, we agree with Benedetti et al. (2015), in that deepen knowledge of the mechanistic processes that lead to trade-offs among traits is required. Quantifying trade-offs associated with specific traits will allow predicting the behaviour, physiology and morphology that optimizes organisms fitness in particular environments and the distribution of traits along environmental gradients (Litchman et al., 2013).

The variation patterns of the zooplankton groups along the environmental gradient of both estuaries, seemed to be in line with the seasonality of environmental conditions, manifested mainly through changes in the hydrological regime, as well as with the disturbance level. Changes in the functional composition with the season were observed, reflecting the differences in the freshwater flow and thus salinity intrusion, and expressing potential different ways of coping with environmental change. Indeed, zooplankton communities have often been reported as being greatly affected by hydrological changes (Sousa et al., 2008; Melo et al., 2013; Moura et al., 2016). Overall, total zooplankton biomass was higher and appeared more evenly distributed among groups in the Mamanguape than in Paraiba estuary. Temperature and salinity were the variables most influencing groups' biomass variation in the former, whereas besides salinity, ammonia, chlorophyll *a* and turbidity, had greater contribution in the latter, reflecting the higher nutrient inputs to this estuary and it most impacted condition. Additionally, lower total fish biomass has been reported in the Mamanguape estuary as a consequence of the smaller estuarine area and the downstream reef line, which limits fish entrance from adjacent oceanic waters (Dolbeth et al., 2016a). Although no directly testing for biotic interactions and thus, predation effects, the lower predator concentrations and thus predation risk, might have been favourable for the zooplankton assemblages in this estuary, which presented higher total biomass than in Paraiba.

In the present study, functional diversity indices were used to summarise zooplankton functional diversity in each estuary, through estimation of its three “facets” (Mason et al., 2005) – richness, evenness and divergence – which enable descriptions of niche use by communities. Variable patterns in FD indices both seasonally and spatially (i.e. downstream *vs*. upstream sections), between and within each estuary, were observed. In general, slightly higher mean values for the three indices were found in the Mamanguape, although the observed difference was not pronounced neither statistically significant, which means both estuaries presenting comparable functional diversity levels.

FRic, as an indicator of the amount of potential niche space occupied by a community (Mason et al., 2005; Schleuter et al., 2010), was slightly higher downstream in the Paraiba estuary, and upstream in the Mamanguape, meaning these areas may present a broader spectrum of functions and thus, that communities are taking advantage of most of the resources available (Mason et al., 2005).

However, the results in the most saline downstream section of the Paraiba estuary should be interpreted with caution, as several samples contained less than three taxa, preventing FD indices computation, and thus, our complete evaluation. Notwithstanding, this section has been pointed as in better ecological condition (Alves et al., 2016; Dolbeth et al., 2016a) due to the lower nutrient concentrations found in this area. In turn, the lower richness found at the downstream area of the Mamanguape may be related to the longer residence time of nutrient-enriched water in this sector, as this estuary is partially dammed by a reef line in this area.

The degree to which the biomass of a community is distributed in niche space allowing an effective use of the full range of resources available is described through FEve (Mason et al., 2005). In general, a higher FEve indicates a regular distribution, and thus, that species are using the resources complementarily, whereas a lower FEve suggests that resources are being used more effectively by species with particular traits (Schleuter et al., 2010). On the other hand, FDiv translates the change in the proportion of the total biomass that is supported by the species with the most extreme traits, as well as the degree of niche differentiation. Usually, a higher FDiv value indicates a higher degree of niche differentiation, hence lower resource competition. Communities expressing high functional divergence may thus increase ecosystem function as a result of using resources more effectively (Mason et al., 2005; Mouillot et al., 2013). Complementarity of resources use among organisms coexisting at a given site may happen through the use of different resources, or by exploring the same resources but in a different way (e.g. at different times) (Diaz and Cabido, 2001).

At both the Mamanguape and Paraiba, higher mean values for FEve and FDiv were mostly observed in the downstream sections and during the wet season, with a few exceptions in the Mamanguape estuary (higher FDiv and FEve upstream and in the dry season, respectively). Overall, values were relatively high in both estuaries, consistent with regularity in the distribution and high niche differentiation within each system. Within the Paraiba and the Mamanguape estuaries, species seem to be able of using the available resources complementarily, i.e. in a different manner, in particular between sections. If higher niche complementarity within a community reflects a greater variety of potential responses to environmental conditions, and it is expected to provide a buffer against change increasing ecosystem stability and resilience (Tilman, 1996; Hewitt et al., 2008), it may be reasonable to consider the downstream sections of both estuaries to be less susceptible to disturbance than upstream areas.

In view of the great differences in human pressure resulting in greater nutrient input to the Paraiba estuary, we could expect larger differences in zooplankton functional diversity between estuaries which have, however, manifested small. These results are in line with those observed when analysing functional diversity of fish communities in these ecosystems (Dolbeth et al., 2016a). This subtle difference could be explained by the fact that the environmental protected status of the Mamanguape estuary does not imply that the system is exempt from human disturbance. Indeed, the Mamanguape estuary is also target of human activity, as verified by the enriched $\delta^{15}N$ in the biota indicating anthropogenic inputs of nitrogen (Dolbeth et al., 2016b), which probably accounts for the functional diversity levels found.

The Northeast Brazil estuarine areas have long been home of multiple and incompatible uses from increased economic and social pressures, in disagreement with a sustainable development prospect (Sassi et al., 2006; Sá et al., 2013). Human activities such as aquaculture, overfishing and agriculture (sugarcane plantation), together with an enhanced urban development, are clearly damaging these systems environmental quality (Barletta et al., 2010). Although conservation and management efforts from both governmental and non-governmental institutions have been made recently (e.g. through the creation of Ramsar and world heritage sites), environmental degradation seems to persist (Diegues, 1999).

The Mamanguape is one such example, where in spite of being a conservation unit (IUCN, category V), it is not exempt from anthropogenic pressure, presenting low nutrient levels. A management plan for this estuary was approved in 2014, with the definition of several priority conservation areas (Management plan for APA and mangrove areas - ARIE of Mamanguape River, Chico Mendes Institute – ICMBio 2014, www.icmbio.gov.br/portal/planosmanejo). Nevertheless, our results pointed towards an insufficiency of these measures to prevent future environmental quality decline. In particular, the peculiar hydro-morphology of the Mamanguape may turn it more liable to environmental damages. Its downstream reef line promotes an enhanced residence time of nutrient-enriched water, that together with its lower depth and high salinity detected upstream, in particular during the dry season, may hamper an efficient flush out from land drainage water.

Management measures promoting better regulation and surveillance of human practices, risk assessment at the watershed scale, the implementation of environment-friendly practices to decrease pollution (e.g. effluent treatment) and the involvement of local citizens seem crucial to the sustainability and conservation of both systems (e.g. Olivera and Brito, 2005; Martinelli and Filoso, 2008; Barletta et al., 2010; Sá et al., 2013). Our results reinforce the idea that efforts should be directed towards a better application and surveillance of the approved management plan, as well as, to raise awareness on the importance of adopting better public services (basic sanitation), as also previously highlighted in Dolbeth et al. (2016a).

5. Conclusions

The two selected approaches to explore the zooplankton functional diversity along the environmental gradient of the two tropical estuaries revealed useful, providing different and complementary information. When combined they are likely to provide a more robust assessment, improving knowledge about ecosystems functioning, and thus, to inform management and conservation planning more effectively. Indeed, the functional groups approach has been pointed as advantageous to help simplifying the complexity inherent to biological systems, whereas the indices approach to give insights about the ecological processes shaping community assembly (Pavoine and Bonsall, 2011).

The functional approaches used in the present study to summarize the functional diversity in each estuary, allowed us to characterise the functional space and different areas within systems, and to perceive a differential use of resources by the copepods. Our results demonstrated similar functional diversity levels across the Mamanguape and Paraiba estuaries, suggesting a certain degree of maintenance of functions between estuaries. Indeed, ‘environmental filtering’ (de Bello et al., 2012) seems to be an important driver of species coexistence in these systems, at the spatial scale analysed here, where environmental factors are thus expected to select for taxa sharing a particular set of traits that are adaptive under such conditions.

Our results provided evidence of anthropogenic impact in both systems, and despite the environmental protection status of the Mamanguape estuary, illustrating the need and reinforcing previous calls towards a more effective management and increased conservation of these ecosystems.

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Anexo lll – Artigo científico

Functional diversity of zooplankton communities in two tropical estuaries (NE Brazil) with different degree of human-induced disturbance.

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Abstract (150/150 max)

Functional diversity (FD) of zooplankton communities along the environmental gradient of two tropical estuaries with different levels of human impact – the environmentally protected "Mamanguape" and the urbanized "Paraiba" – was assessed. Different trait-based approaches were used to investigate the communities' response to anthropogenic disturbance: a functional groups approach and by computing FD indices.

Results suggested that communities in each estuary share similar functions performed by few functional groups. Overall, biomass was more evenly distributed among groups, and total biomass and FD indices were slightly higher in the Mamanguape. Nevertheless, both estuaries presented similar FD levels, indicating that environmental filters seem to be the main driver of species coexistence within systems.

Despite the conservation status of the Mamanguape, anthropogenic impacts are still evident stressing the insufficiency of the measures approved to ensure good status. More effective management is essential in both estuaries to preserve biodiversity and the vital ecosystem services they support.

Keywords: Functional diversity; Biological traits; Zooplankton; Management; Tropical estuaries.

1. Introduction

Several studies are recently emerging using functional diversity and biological traits to evaluate long-standing ecological questions, yet the body of empirical evidence on the patterns of functional diversity is still small (Cadotte et al., 2011; Mason and de Bello, 2013).

Functional diversity (FD) is a key facet of biodiversity that describes the breadth of functions performed by species in a community and is measured based on traits (Tilman 2001; Maire et al., 2015). Traits (*sensu* Violle et al., 2007) are known to influence ecosystem functioning, and to reflect the mechanisms underlying species-environment relations (Diaz and Cabido, 2001; Villéger et al., 2010; Verbeck et al., 2013). Hence, analysing functional diversity patterns may provide greater insight on the responses of a community when facing anthropogenic disturbance, and assist to disentangle how ecosystems work (Mouillot et al., 2013; Gagic et al., 2015; Strong et al., 2015). FD is a multifaceted concept integrating different components, which has often been addressed through several methods (Bremner, 2008; Carmona et al., 2016), using different communities and habitats, including: benthic invertebrates (e.g. Tillin et al., 2006; Cooper et al., 2008; Bolam et al., 2016; Moretti et al., 2016; Schemera et al., 2016; Veríssimo et al., 2012; 2017; Van der Linden et al., 2016); fish (e.g. Villéger et al., 2010; Stuart-Smith et al., 2013; Wiedmann et al., 2014); plants (e.g. Pakeman et al., 2011; Schittko et al., 2014); phytoplankton (e.g. Weithoff, 2003; Edwards et al., 2013; Santos et al., 2015) and, zooplankton (e.g. Barnett and Beisner, 2007; Barnett et al., 2007, 2013; Vogt et al., 2013; Bolduc et al., 2016; Russo et al., 2016).

To date, studies addressing zooplankton functional diversity have been mostly developed for freshwater ecosystems, and a comparable lack of studies exist for marine and estuarine realms (Pomerleau et al., 2015; Helenius et al., 2016). In aquatic environments, zooplankton is at the heart of pelagic food webs, mediating energy flow to higher trophic levels and thus playing a fundamental role in many ecosystem processes (Kiørboe, 1997; Barnett et al., 2007). Further knowledge on the functioning of zooplankton communities is thus crucial, and a functional approach has been claimed as key for a comprehensive understanding of ecosystem dynamics (Russo et al., 2016). In general, the current knowledge of the relationship between functional diversity patterns and environmental variation is still poor (Dimitriadis, 2012; Mouillot et al., 2013), and is particularly scarce for the zooplankton in ecosystems with strong environmental gradients, as is the case of tropical estuaries in the Northeast Brazil. In the latter, studies have been mainly addressing zooplankton composition and structure (e.g. Almeida et al., 2009; Melo et al., 2013), focusing less on the functional aspect.

Over the last decades, most of these estuarine areas are, however, facing growing problems of environmental degradation due to increasing pressures from traditional and emerging human activities, such as tourism, agriculture, aquaculture and overfishing, together with poor management. Despite some management and conservation efforts have been made recently, from both governmental organizations and NGOs, these have proven insufficient and degradation seems to continue with potential to intensify in future (Diegues, 1999; Barletta et al., 2010).

Environmental management is committed with the safeguarding and improvement of ecosystems' environmental state through a science-based and integrated approach, in order to inform management planning effectively and regulate human pressures (Elliott, 2014). An effective management program, aiming long-term conservation and sustainable use, must thus account for an ecosystem multiple uses without compromising its ecological condition and functioning (Borja et al., 2016). In order to achieve this, an in-depth knowledge on the functioning of an ecosystem and of the responses of the biological communities to disturbances, is required. This is essential to a better understanding on how communities cope with environmental change, and such can be addressed through functional diversity.

The main goal of this manuscript was to assess zooplankton (copepods) functional diversity in two Northeast Brazilian estuaries (Paraiba and Mamanguape) experiencing distinct anthropogenic levels (mainly organic enrichment from aquaculture and agriculture). As functional diversity encompasses different "facets" (Mason et al., 2005), a combined functional approach was used to analyse the spatial and temporal response of the copepods communities along the abiotic stress gradient in each estuary: (*i*) a functional group approach: to identify and describe groups of copepods based on life-history traits, discussing their ecological relevance; and (ii), by computing complementary functional diversity indices (Functional Richness "FRic", Functional Evenness "FEve", and Functional Divergence "FDiv"). Ultimately, it is intended to increase knowledge about the functioning of tropical estuaries and to perceive the usefulness of zooplankton functional diversity analyses as tools to evaluate the effectiveness of environmental protection practices.

2. Material and Methods

2.1. Study site description

Two tropical estuaries located on the Northeast coast of Brazil, in the Paraiba state, were used as a case-study: Paraiba (6º57’30”S; 34º51’30”W) and Mamanguape (6º43‘02”S; 35º67’46”W) (Fig.1). Both estuaries are classified as climatic type “As” (equatorial with a dry summer) according to the Köppen-Geiger classification. The wet season extends from February to August, with the highest precipitation occurring in June and the lowest in November. The dry season occurs mainly from August to January.

The Paraiba and the Mamanguape river basins include a hinterland region comprising the semi-arid Caatinga Biome and a narrow coastal strip originally covered by the Atlantic rainforest, with a coverage area of 20 124 km^2 and 3 522 km^2, respectively (AESA, 2015).

The Paraiba estuary (3012 ha; Fig. 1A) is the largest estuary in the state of Paraiba with about 22 km long, and is located near an urban area with approximately one million inhabitants. The Mamanguape estuary (690 ha; Fig. 1B) is the second largest estuary in the state of Paraiba and is located inside an environmental protection area (“APA”, IUCN protection category V). This estuary harbours a great variety of habitats including sandy coastal beaches bordered by field dunes, coastal reefs with dense mats of macroalgae, seagrass beds (*Halodule wrightii*), restinga forests, tableland forests and a well-preserved mangrove area with approximately 6000 ha. An important feature in this ecosystem is an 8.5 m long barrier reef perpendicular to the shoreline, which creates a protected region at the mouth of the estuary (Xavier et al., 2012; Claudino et al., 2015).

Both estuaries have mangroves that grow around the main channel and intertidal creeks, along with remnants of the Atlantic rainforest (Campos et al., 2015), and are affected by different degrees and types of human-induced disturbance. Clear signs of environmental degradation have been observed in the Paraiba estuary (highly impacted), which is located in a densely populated urban area, with sugarcane plantations along the riverbanks and intensive shrimp aquaculture activities occupying part of the remaining mangrove area. The absence of surveillance and law enforcement for specific activities contribute to a worsening of the ecological condition. In turn, the ecological importance of the coastal habitats of the Mamanguape estuary (moderately impacted), in particular for the conservation of the marine manatee (*Trichechus manatus*, Linnaeus, 1758), has been recognised by the Brazilian governmental agencies, which declared it as a conservation area in 1993. Large mangrove areas characterise this estuary, which provide several food sources and nursery areas for different species. Despite the environmental protection status, in the estuarine surrounding region there are, however, shrimp aquaculture activities and sugarcane plantations contributing to eutrophication and agrochemical pollution (Alves et al., 2005; Claudino et al., 2015).

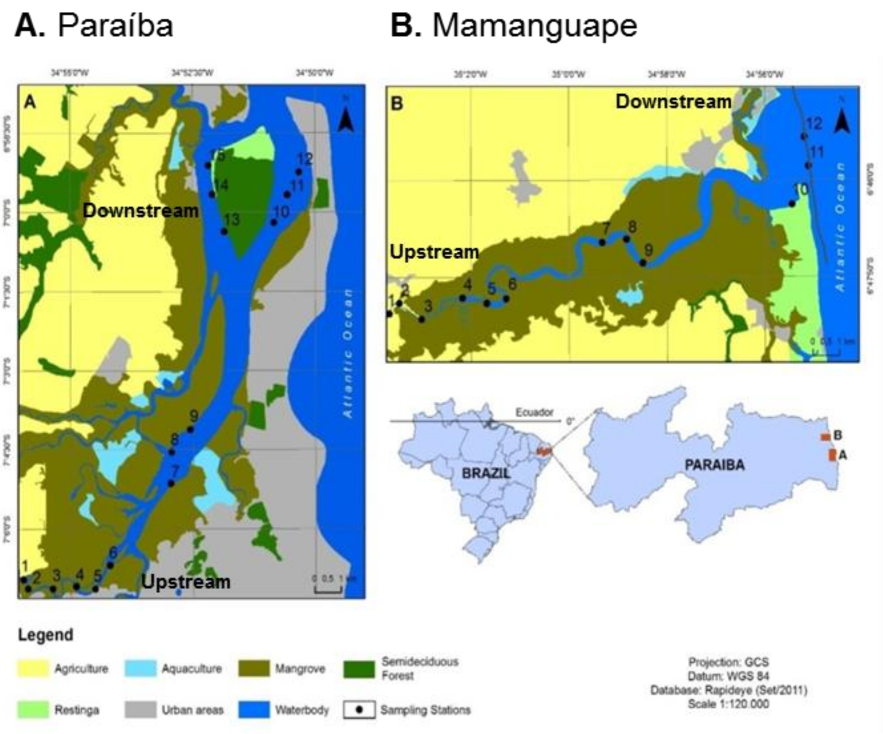


Figure 1. Geographical location of the Paraiba **(A.)** and Mamanguape **(B.)** estuaries (Northeast Brazil), sampling sites and main land uses.

2.2. Sampling and laboratory procedures

The present study focused on copepods communities which are ubiquitous and a strong contender for the most numerous metazoan class (Litchman et al., 2013), being relatively well documented in terms of distribution and biology (Razouls 2005-2015; Benedetti et al., 2015).

Two sampling campaigns were carried out in each estuary, one in the dry season (November 2013) and the other in the wet season (July 2014), during high tide. Zooplankton samples were collected using horizontal subsurface tows (bongo net: mesh size 68 µm, mouth diameter: 0.3 m) along the estuarine salinity gradient in 15 sites in the Paraiba estuary and 12 sampling sites in the Mamanguape estuary (Fig. 1). In each estuary, two estuarine sections previously defined according to biotic and abiotic variables (Alves et al., 2016; Dolbeth et al., 2016a, 2016b) can be identified: upstream (sites 1 to 6), and downstream (7 to 12/15 in Paraiba and Mamanguape estuaries, respectively). Three replicates were collected at each sampling site and samples were fixed and preserved in 4% buffered formaldehyde. In the laboratory, zooplankton samples were sorted and copepods identified preferentially to the species level under a stereoscopic microscope. Biomass was determined as µg L^{-1}. Replicates were pooled and the analyses performed at the estuarine section level.

At each sampling site several physicochemical parameters were measured *in situ*: surface water temperature (°C), dissolved oxygen (mg L^{-1}), salinity, pH and turbidity with a multiparameter probe, and transparency (m) with a Secchi disk. Additionally, water samples were collected to determine nutrients content (Ammonia (NH_4-N, µg L^{-1}), nitrite + nitrate (NO_x-N, µg L^{-1}) and total phosphorous (P, µg L^{-1})), and Chlorophyll *a* (Chl *a*, µg L^{-1}) as described in Alves et al. (2016).

2.3. Biological traits information

To build a “species by traits” data table (Table S1) for the most representative copepod species in terms of biomass and presence in the Paraiba and Mamanguape estuaries, information on 6 biological traits was gathered from different published sources including scientific papers and online data bases (e.g. “PANGEA”, www.pangea.de). Traits selection, which required a compromise between information availability and its ecological relevance, was based on the choices adopted in previous zooplankton studies and included those traits that would best describe organisms’ response to the environment (Barnett et al., 2007; Barton et al., 2013; Pomerleau et al., 2015; Benedetti et al., 2015). The traits chosen cover different aspects of the morphology, behaviour, life-history and ecology of the taxa, known to be functionally important: maximum body size (mm), trophic group, feeding type, reproduction mode, diel vertical migration (DVM) and vertical distribution.

2.4. Data analysis

Spatial and temporal patterns of the zooplankton functional groups, functional diversity indices and environmental variables, at the downstream and upstream sections of the Mamanguape and Paraiba estuaries, were investigated using multivariate and univariate statistical methods.

Environmental variables were explored using a Principal Component Analysis (PCA) to seek patterns between estuaries, seasons and estuarine sections. Redundant variables were removed from the analyses after inspection with Draftsman plots, ensuring that maximum variability in the data set is accounted. A resemblance matrix based on Euclidean distances was computed prior to the analysis and all variables were normalised. Those environmental variables moderately and heavily skewed in distribution were square root (turbidity and Chlorophyll *a*) and fourth root (Ammonia, Nitrite + Nitrate and Total Phosphorous) transformed.

Functional diversity assessment was carried out employing two different methods. Firstly, considering that using multiple traits may be more informative with regard to the response of assemblages to environmental disturbance than when considering isolated (Verbeck et al., 2013), zooplankton functional groups, i.e. groups of species sharing similar traits, were identified and their spatial and temporal patterns of variation were investigated. Zooplankton functional groups were identified using Multiple Correspondence Analysis (MCA) together with hierarchical agglomerative clustering (Husson et al., 2010; Legendre & Legendre, 2012). Initially, the “species by traits” matrix was ordinated using MCA, which is a method appropriate for the analysis of categorical variables, and four traits were considered in the analysis: maximum body size, trophic group, feeding type and reproduction mode. Diel vertical migration was not accounted as it exhibits high plasticity facing environmental variations and with species ontogeny (Pomerleau et al., 2015; Benedetti et al., 2015). Together with the vertical distribution, these were considered *a posteriori* in the MCA as supplementary variables, for informative purposes. After, groups of species sharing a similar set of traits (functional groups) were defined through cluster analysis based on Ward’s linkage method (Ward, 1963), using Euclidean distances and the scores of taxa along the four axes of the MCA. For each functional group, a biological trait profile was created indicating for each trait the proportion of categories displayed by the group. Using the “species by samples” matrix (species biomass per sample), the groups spatial and temporal patterns of variation were evaluated.

Secondly, functional diversity was summarized based on the framework proposed by Mason et al. (2005) and Villéger et al. (2008), thus measuring its primary components through the computation of three multidimensional and complementary FD indices: Functional Richness (FRic), which represents the volume of the functional space occupied by the community; and, Functional Evenness (FEve) and Functional Divergence (FDiv), that measure the regularity and spread of the distribution of biomass in this volume, respectively. FRic is independent of species abundance/biomass, was standardised to range between zero and one, and it is expected to decrease with disturbance (Mouillot et al., 2013; Laliberté et al., 2015). Both FEve and FDiv are biomass-weighted, values are constrained between zero and one, and indices scores are expected to decrease with disturbance (Villéger et al., 2008; Mouillot et al., 2013). Indices computation included the preliminary evaluation of the quality of the functional spaces, computing all the possible spaces and selecting the most parsimonious one, in agreement with Maire et al. (2015). Detailed information on the FD indices calculation steps, R routines and functions used can be found in Mouillot et al. (2013), Villéger et al. (2013) and Maire et al. (2015), freely available at http://villeger.sebastien.free.fr/FD.html (accessed on November, 2016).

Spatial and temporal differences in the FD indices were assessed using separate PERMANOVAs based on Euclidean distance after data normalisation. A three-way experimental design was considered: "estuary" (with two-fixed levels, Paraiba and Mamanguape), "season" (with two-fixed levels, wet and dry) and "estuarine section" (with two levels - upstream and downstream – nested in "estuary" and "season"). The statistical significance of variance components was tested using 9999 permutations of residuals under a reduced model, with an *a priori* chosen significance level (*P*) of 0.05.

To infer about potential relationships between the functional diversity measures tested and estuaries environmental conditions: (*i*) Spearman' rank correlations were used to explore the strength of association among FD indices and the environmental variables; (ii) a Distance-Based Linear Model analysis (DISTLM) was used to explore relationships between habitat characteristics and the zooplankton functional groups, with "Best" and "AICc" as model selection procedure and selection criteria, respectively (Anderson et al., 2008).

Statistical analyses were performed using: PRIMER v6 statistical package (Clarke and Gorley, 2006), together with the PERMANOVA + PRIMER add-on package (Anderson et al., 2008); Functional groups analyses (*FactoMine*R package) and FD indices computation with R software version 3.3.1 (R Development Core Team, 2016); and, Spearman correlations with Statgraphics Centurion XVI.

3. Results

3.1. Environmental characterisation

The first two PCA axes on environmental variables explained 62.3% of the total variation clearly illustrating the relationships among samples (Fig. 2A).

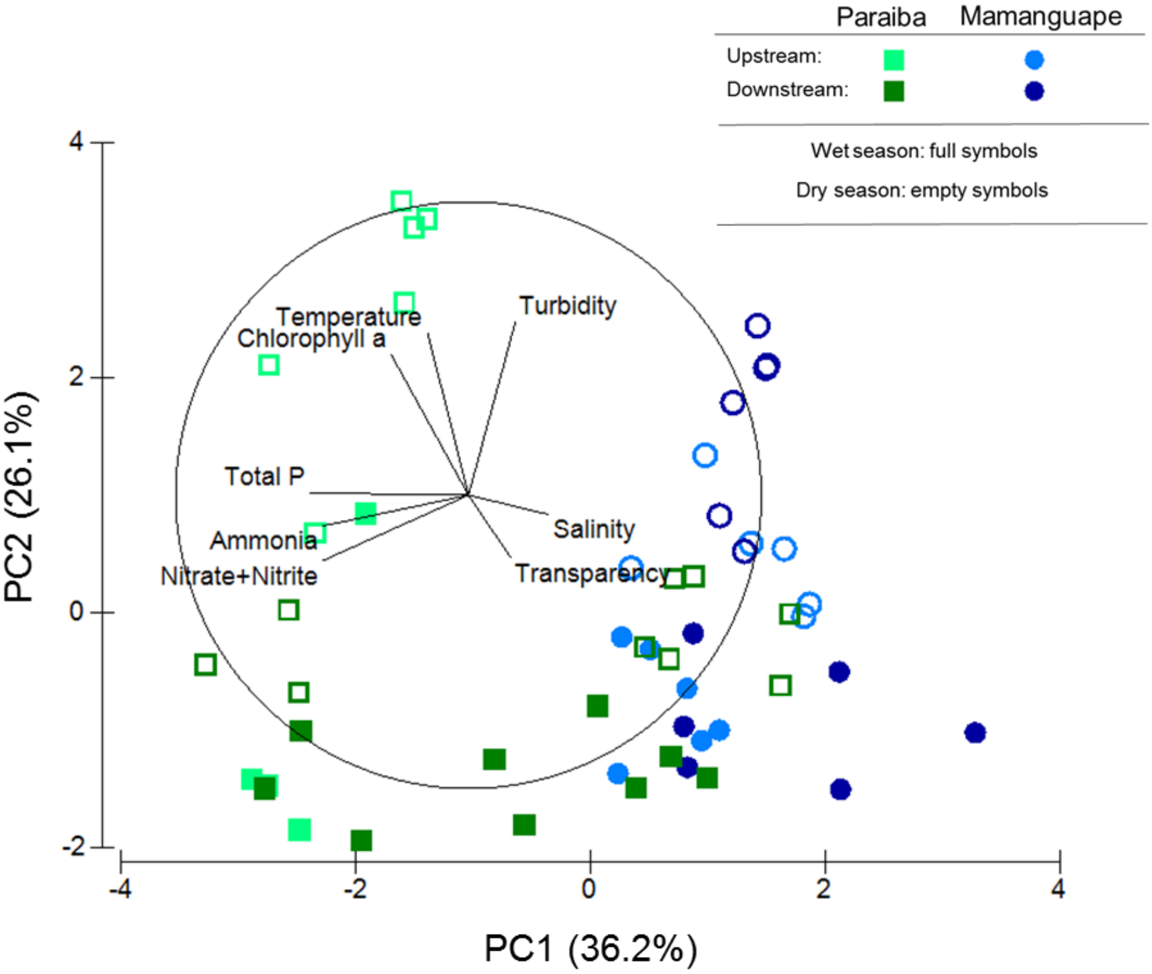
A temporal distinction (seasonal) is evident, as samples from the dry and wet seasons are clearly segregated in the plot. A spatial gradient can also be identified at both estuaries, with samples from upstream section located further away from those of the downstream sector.

Characteristic of an estuarine ecosystem, a decreasing salinity gradient from the downstream to the upstream sections was observed at both estuaries (Fig. 2B).

Overall, salinity was higher at the downstream sections, especially in the dry season, although in the Mamanguape estuary high levels in the upstream area have also been registered, attributable to the low freshwater input in this semi-arid region Alves et al., (2016). Higher values for turbidity and temperature were registered during the dry season, and for transparency in the wet season (Fig. 2A).

At both seasons, higher nutrient (Total P, Ammonia and Nitrate + Nitrite) and Chlorophyll *a* concentrations were observed in the Paraiba estuary, highlighting the higher degree of human-induced disturbance in this estuary (Fig. 2A). Along the estuarine gradient, nutrient levels were always higher in the Paraiba than in Mamanguape, particularly obvious for ammonia (Fig. 2B). In the Paraiba, high nutrient concentrations were registered in the wet season and mostly in the upstream section. In contrast, the less impacted estuary (Mamanguape) presented slightly higher nutrient concentrations during the dry season (Fig. 2B).

A.


Paraiba
Mamanguape
Upstream:
Downstream:
Wet season: full symbols
Dry season: empty symbols
Temperature
Chlorophyll a
Turbidity
Total P
Ammonia
Nitrate+Nitrite
Salinity
Transparency
4
2
0
-2
-4
-2
0
2
4
PC2 (26.1%)
PC1 (36.2%)

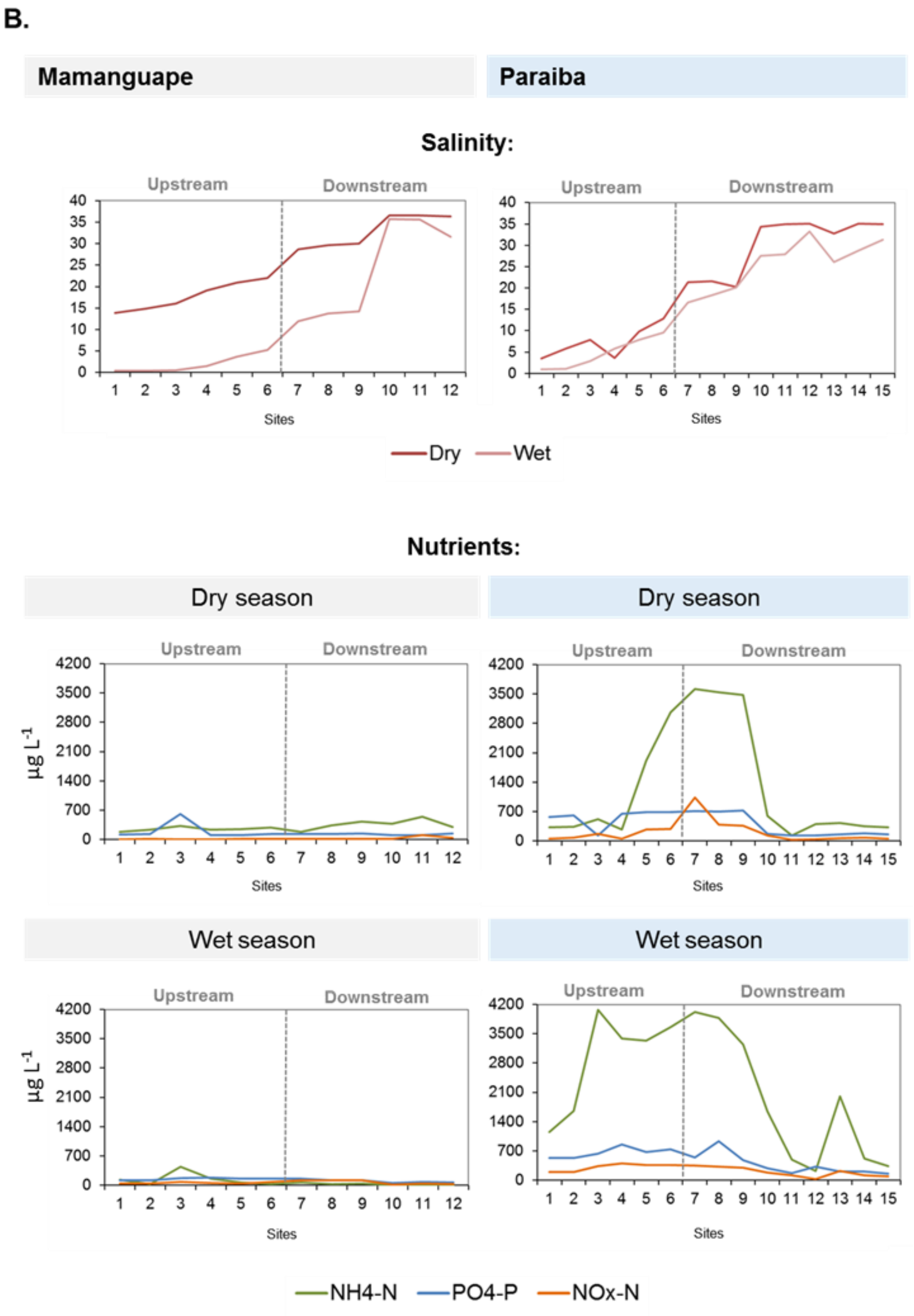


Figure 2. Environmental characterisation of the Mamanguape and Paraiba estuaries. **(A.)** Two-dimensional PCA ordination map of environmental variables in wet (full symbols) and dry (empty symbols) seasons, and upstream (light symbols) and downstream (dark symbols) sections. **(B.)** Variations in salinity and nutrient concentrations in dry and wet seasons, at the upstream and downstream sections.

3.2. Zooplankton functional groups

The four axes of the MCA performed on the traits by species matrix accounted for 90.34% of the total variance in biological traits (MCA 1: 40.23%, MCA 2: 25.59%, MCA 3: 14.02% and MCA4: 10.50%).

Overall, the first axis (MCA 1) separated small-sized (S1), egg brooders, herbivore active ambush feeders, from large-sized (S3-S4), broadcasters, omnivores and mixed/filter feeding species. The second axis (MCA 2), mainly opposed egg brood, omnivores, active ambush and mixed feeding individuals ranging several sizes, from herbivores small-sized broadcasters, filter feeders. Variances of MCA 3 and MCA 4 were explained just by a few taxa medium-sized (S3), with omnivore and herbivore feeding mode (Fig. 3B).

Trait categories contributing most to MCA axes, i.e. exhibiting higher correlation values with the different dimensions, were: (*i*) MCA 1: “very small” (S1) and “large” (S4), “omnivore” (trophic group) and “active ambush” (feeding mode); (*ii*) MCA 2: “small” (S2) and “filter” feeding; (*iii*) MCA 3 and 4, mainly “omnivore/herbivore” and “medium” size (S3).

The cluster analysis performed on the MCA output (i.e. species coordinates along the four axes; Fig. 3A), identified four distinct groups of zooplankton taxa clearly separated on the MCA space (Fig. 3B) and characterised by different sets of biological traits. Each group displayed a distinctive traits profile. Figures 3 and 4 allow to describe and characterise these groups: Group 1 (“Ambush-Herbivores”) is mainly composed of Cyclopoids of the *Oithona* genus which are mostly very small sized, active ambush-feeding herbivores and egg-brooders showing epipelagic (0 - 200 m) distribution and DVM behaviour. Group 2 (“Filter-Herbivores”) presents a larger number of species belonging to families Euterpinidae, Paracalanidae and Temoridae. Members span several size classes, are mostly herbivores and broadcast spawners, and all filter feeders. Distribution is mainly epipelagic and there is a tendency for DVM absence. Group 3 (“Mixed/ambush - Omnivores-Herbivores”) comprises two omnivore/herbivore copepods, *Mycrocyclops anceps* and *Pseudodiaptomus richardi*, with ambush and mixed feeding habits and, very-small and large body sizes, respectively. Group 4 (“Mixed-Omnivores”) includes copepods from *Acartia* and *Pseudodiaptomus* genera, with medium-large body size, all omnivore’s broadcasters and exhibiting mixed feeding strategy.

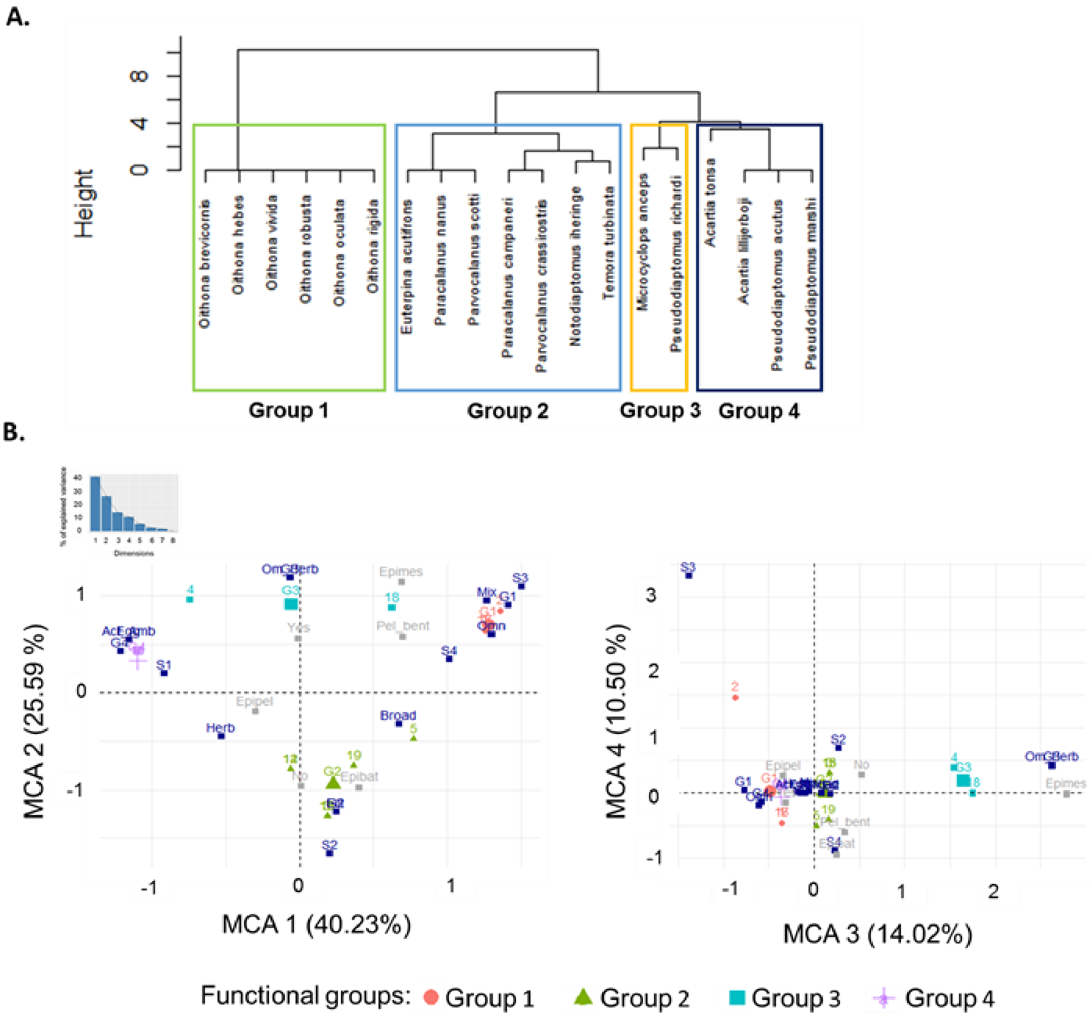


Figure 3. Classification and ordination of the zooplankton biological traits:

(A.) Dendrogram resulting from the hierarchical clustering (Ward's linkage method on Euclidean distances). Functional groups identified (1 to 4) and characterized by different sets of traits, outlined in different colour.

(B.) Histogram of eigenvalues and MCA factor maps for the axes 1 - 2 and 3 - 4. Copepod species represented by numbers; Trait categories used in the analysis shown by dark blue symbols (for categories labels please see Table S1), and superimposed supplementary variables (DVM and Vertical distribution) indicated in grey; Functional groups identified through cluster analysis (A.) represented by the symbols with different colours.

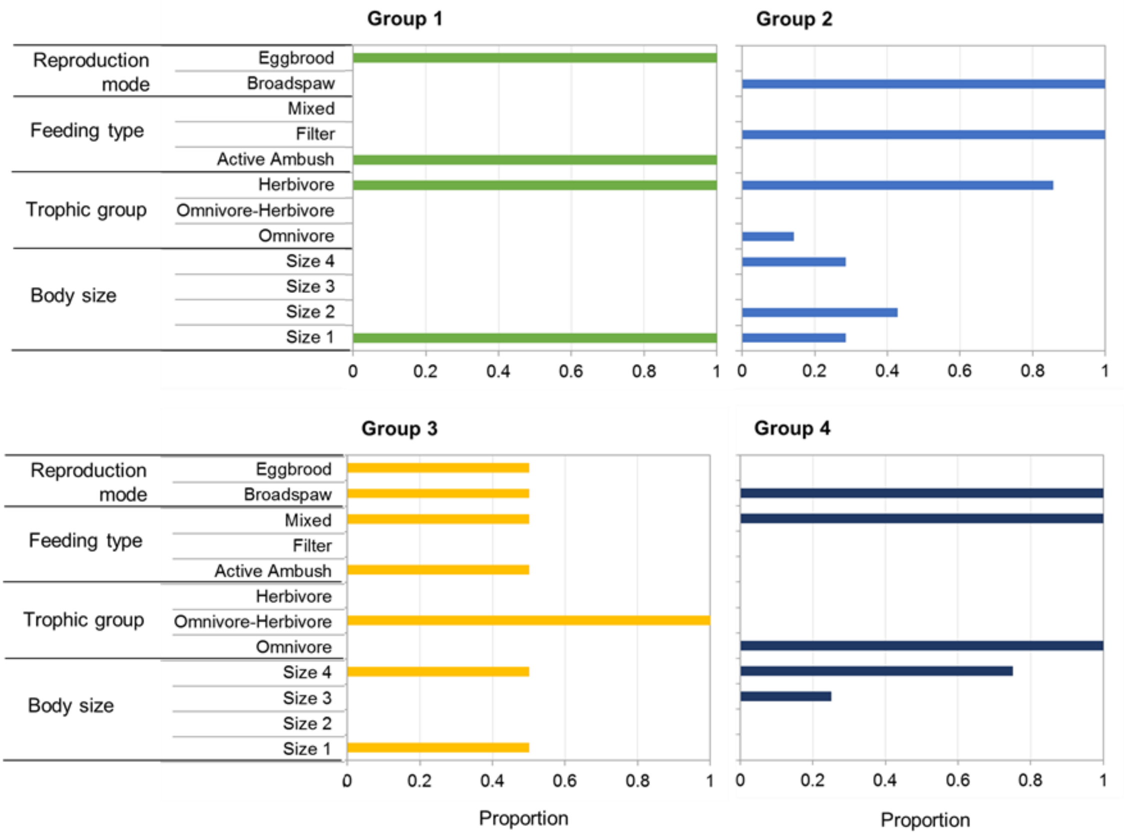


Figure 4. Biological traits profile of the zooplankton functional groups identified in the Mamanguape and Paraiba estuaries. Proportions of trait categories exhibited by the groups are shown.

Concerning the spatial and temporal variation patterns of the zooplankton functional groups, the group 4 (“Mixed-Omnivores”) was dominant in both estuaries, in particular in the saline downstream sections during the wet season, followed by groups 1 (“Ambush-Herbivores”) and 2 (“Filter-Herbivores”) (Fig. 5).

Overall, when comparing both estuaries, total biomass levels were higher in the Mamanguape than in Paraiba estuary, and the groups’ biomass appeared more evenly distributed in the former.

In the Mamanguape, an evenly biomass distribution between sections was observed during the dry season, with lower values registered at the downstream section. An inverse pattern was detected during the wet season (Fig. 5A). In the Paraiba estuary, lower total biomass values were also registered in the dry season, but this time at the upstream section (Fig. 5B).

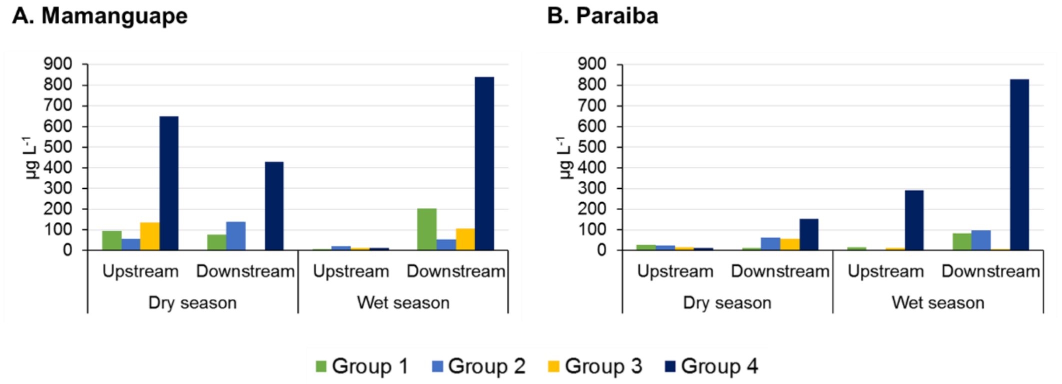


Figure 5. Total biomass of zooplankton functional groups during the dry and wet seasons, at the downstream and upstream sections of the Mamanguape (A.) and Paraiba (B.) estuaries.

Results from the DISTLM test, highlighting the relationships between environmental variables and the zooplankton groups' biomass, showed that in the Mamanguape, the most parsimonious solution (<AICc) was a 2-variable model explaining 26% of the total variability, provided by salinity and temperature. In the Paraiba estuary, 53% of the total variability was explained by four significant variables: salinity, turbidity, ammonia and chlorophyll *a*.

3.3. Functional diversity indices

The primary components of functional diversity were assessed through computation of three complementary functional diversity indices: Functional richness, evenness and divergence (FRic, FEve and FDiv, respectively). Indices behaviour was evaluated in the Paraiba and Mamanguape estuaries, spatially and temporally, i.e. at the upstream and downstream estuarine sections, during the wet and dry seasons.

Overall, when comparing both estuaries, mean FRic, FEve and FDiv were slightly higher in the Mamanguape than in Paraiba estuary (FRic: 0.38, FEve: 0.67; FDiv: 0.83; and FRic: 0.28, FEve: 0.53; FDiv: 0.80, respectively) (Fig. 6).

FRic showed spatial and temporal opposite patterns at both estuaries (Fig. 6). In the Mamanguape, it was slightly higher in the less saline upstream section during the dry season, while in the Paraiba higher mean values were registered in the downstream section and in the wet season, although these trends do not proved to be statistically significant (3-way PERMANOVA, *P*>0.05). At both estuaries, FRic generally decrease from dry to wet season in both estuarine sections, except in the downstream area of the Paraiba where it increased. This index is known to monotonically increase with species richness (Mason et al., 2013), and similar trends of variation between FRic and the number of species were observed, except in the Paraiba downstream section between the dry and wet seasons (Fig. S1). Yet, it is important to highlight that this inconsistent result in the Paraiba may have been due to the higher number of samples containing less than three species, which prevented FRic computation for several samples and thus, may hinder our evaluation.

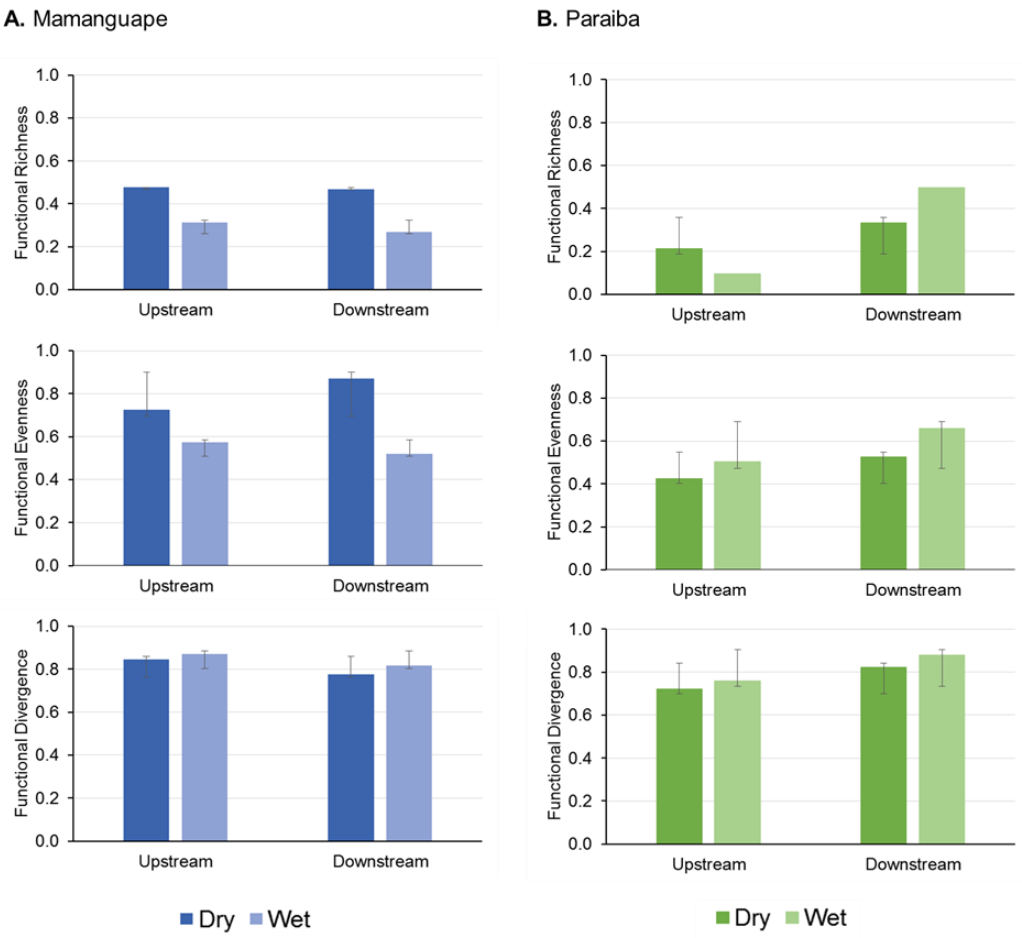


Figure 6. Variation of the functional diversity indices (Functional richness, evenness and divergence) in the downstream and upstream sections of the Mamanguape (**A.**) and Paraiba (**B.**) estuaries, during the wet and dry seasons.

With regard to the biomass-weighted indices (FEve and FDiv; Fig. 6), seasonally consistent trends were observed in the upstream and downstream sections of both estuaries, with higher mean values in the wet season, except for FEve in the Mamanguape, where higher values were registered in the dry season at both sections.

Overall, FEve and FDiv presented slightly higher mean values in the Mamanguape, than in Paraiba, in agreement with the lower disturbance level in this estuary. For the Paraiba estuary, higher mean FEve and FDiv values were observed in the downstream area, during the wet season. In the Mamanguape, FEve registered higher mean values downstream and in the dry season, whereas FDiv showed an inverse behaviour (higher upstream and in wet season). Statistically significant differences between upstream and downstream sections were, however, only detected for Paraiba in the wet season (3-way PERMANOVA: FEve: $F_{Sec(EsxSea)} = 2.84$; FDiv: $F_{Sec(EsxSea)} = 2.97$; both $P<0.05$) and Mamanguape in the dry season (3-way PERMANOVA: FEve: $F_{Sec(EsxSea)} = 2.29$, $P<0.05$).

A few significant correlations between functional diversity indices and the environmental variables were observed (Table S2). Indices were mostly correlated with ammonia and turbidity, either in Paraiba or Mamanguape, although most of the values registered were low.

4. Discussion

The present study contributed to add knowledge on the functional diversity of the zooplankton communities of two tropical estuaries (Paraiba and Mamanguape). This type of information is scarce, in particular in the Northeast region of Brazil (Leitão et al., 2006), but crucial to a better understanding of the response of these assemblages to anthropogenic impacts, with consequent repercussions to the functioning of these ecosystems, and to inform management and conservation efforts.

Two different trait-based approaches were used to investigate the zooplankton functional diversity in the Paraiba and Mamanguape estuaries, which present dissimilar levels of human-induced disturbance: a functional group approach, and by computing complementary functional diversity indices. Both methods are known to provide different information, and therefore, to complement each other in the study of the functional diversity response to disturbance (Gagic et al., 2015). Indeed, the analyses of the spatial and temporal variation of the functional groups and indices, along the environmental gradient, shed light on zooplankton resources use and allowed us to characterize their functional trait space, and thus, to identify dissimilar areas and ways of using resources in these ecosystems. It is important to point out that zooplankton communities are highly dynamic due to its pelagic lifestyle, and the present work provides only a snapshot at a certain time and space, so that the variation patterns observed should not be interpreted strictly. Additionally, environmental condition assessment was undertaken on only two sampling occasions, and this lack of temporal replication may not be entirely translating the variability found in these estuaries. Nevertheless, Paraiba and Mamanguape estuaries have been characterised as presenting strong environmental fluctuations along their spatial gradient associated mostly to changes in salinity, temperature and turbidity (Alves et al., 2016). As one could expect, the Paraiba estuary presented higher nutrients concentration as it is most impacted due to its location in an urbanized area, near sugarcane plantations and intensive aquaculture activities. Salinity was higher in the downstream sectors of both estuaries, but in the Mamanguape higher values in the dry season were also registered upstream, indicating that the reduced freshwater runoff may promotes the influence of oceanic water further upstream, together with the lower number of river effluents discharging to this estuary.

With regard to the zooplankton functional groups, results suggested that assemblages may share similar functions at both the Mamanguape and Paraiba estuaries, translated by the low number of groups identified (four), and which seem to reflect complementarity in the use of resources within each system. This outcome could be expected if we consider that estuaries are naturally stressed and highly variable environments’, therefore imposing strong habitat selection which may result in trait-convergence (de Bello et al., 2012). Habitat filtering should lessen the extent of ecological strategies, reflecting shared ecological tolerances (Cornwell et al., 2006; de Bello et al., 2012). The notion that the environment seems to filter taxa with specific characteristics allowing them to survive under determined conditions as often been highlighted (e.g. Mouillot et al. 2006; Villéger et al., 2013; Veríssimo et al., 2017), and former studies at the Paraiba and Mamanguape using fish communities have pointed in the same direction (Dolbeth et al., 2016a, 2016b).

At both the Mamanguape and Paraiba estuaries the zooplankton functional groups revealed mostly size- and feeding-structured. In fact, zooplankton communities are known to be strongly size-structured as size heavily determines its predators and prey, and influences, for instance, swimming speeds and growth rates, fecundity and metabolism (Kiørbe, 2008; Barton et al., 2011). Feeding is also known to differ in response to changing environmental conditions and prey abundance (Mariani et al., 2013; Moura et al., 2016), with the spatial and temporal distribution of feeding traits along estuarine gradients being greatly determined by trade-offs associated with food availability and presence of predators (Barton et al., 2011). Trade-offs among zooplankton traits have been often reported in the literature, in particular between size and feeding strategy, attributable to differences in metabolic requirements (e.g. Kiørbe et al., 2011; Litchman et al., 2013). For instance, Benedetti et al. (2015) studying the Mediterranean copepods found that small-sized carnivores and omnivores taxa were active ambush feeders, thus feeding passively, contrary to large carnivores which were cruise feeders and thus, needing to actively swim to search for prey, something that requires high energy expenditure. These different metabolic requirements seemed to explain size differences, as metabolic rates scale with body size (Kiørbe and Hirst, 2014). Evidence of trade-offs among zooplankton traits were also observed in the present study: very small-sized copepods were all active ambush feeders, while large-sized taxa were mostly mixed or filter feeders. Nevertheless, we agree with Benedetti et al. (2015), in that deepen knowledge of the mechanistic processes that lead to trade-offs among traits is required. Quantifying trade-offs associated with specific traits will allow predicting the behaviour, physiology and morphology that optimizes organisms fitness in particular environments and the distribution of traits along environmental gradients (Litchman et al., 2013).

The variation patterns of the zooplankton groups along the environmental gradient of both estuaries, seemed to be in line with the seasonality of environmental conditions, manifested mainly through changes in the hydrological regime, as well as with the disturbance level. Changes in the functional composition with the season were observed, reflecting the differences in the freshwater flow and thus salinity intrusion, and expressing potential different ways of coping with environmental change. Indeed, zooplankton communities have often been reported as being greatly affected by hydrological changes (Sousa et al., 2008; Melo et al., 2013; Moura et al., 2016). Overall, total zooplankton biomass was higher and appeared more evenly distributed among groups in the Mamanguape than in Paraiba estuary. Temperature and salinity were the variables most influencing groups' biomass variation in the former, whereas besides salinity, ammonia, chlorophyll *a* and turbidity, had greater contribution in the latter, reflecting the higher nutrient inputs to this estuary and it most impacted condition. Additionally, lower total fish biomass has been reported in the Mamanguape estuary as a consequence of the smaller estuarine area and the downstream reef line, which limits fish entrance from adjacent oceanic waters (Dolbeth et al., 2016a). Although no directly testing for biotic interactions and thus, predation effects, the lower predator concentrations and thus predation risk, might have been favourable for the zooplankton assemblages in this estuary, which presented higher total biomass than in Paraiba.

In the present study, functional diversity indices were used to summarise zooplankton functional diversity in each estuary, through estimation of its three “facets” (Mason et al., 2005) – richness, evenness and divergence – which enable descriptions of niche use by communities. Variable patterns in FD indices both seasonally and spatially (i.e. downstream *vs*. upstream sections), between and within each estuary, were observed. In general, slightly higher mean values for the three indices were found in the Mamanguape, although the observed difference was not pronounced neither statistically significant, which means both estuaries presenting comparable functional diversity levels.

FRic, as an indicator of the amount of potential niche space occupied by a community (Mason et al., 2005; Schleuter et al., 2010), was slightly higher downstream in the Paraiba estuary, and upstream in the Mamanguape, meaning these areas may present a broader spectrum of functions and thus, that communities are taking advantage of most of the resources available (Mason et al., 2005).

However, the results in the most saline downstream section of the Paraiba estuary should be interpreted with caution, as several samples contained less than three taxa, preventing FD indices computation, and thus, our complete evaluation. Notwithstanding, this section has been pointed as in better ecological condition (Alves et al., 2016; Dolbeth et al., 2016a) due to the lower nutrient concentrations found in this area. In turn, the lower richness found at the downstream area of the Mamanguape may be related to the longer residence time of nutrient-enriched water in this sector, as this estuary is partially dammed by a reef line in this area.

The degree to which the biomass of a community is distributed in niche space allowing an effective use of the full range of resources available is described through FEve (Mason et al., 2005). In general, a higher FEve indicates a regular distribution, and thus, that species are using the resources complementarily, whereas a lower FEve suggests that resources are being used more effectively by species with particular traits (Schleuter et al., 2010). On the other hand, FDiv translates the change in the proportion of the total biomass that is supported by the species with the most extreme traits, as well as the degree of niche differentiation. Usually, a higher FDiv value indicates a higher degree of niche differentiation, hence lower resource competition. Communities expressing high functional divergence may thus increase ecosystem function as a result of using resources more effectively (Mason et al., 2005; Mouillot et al., 2013). Complementarity of resources use among organisms coexisting at a given site may happen through the use of different resources, or by exploring the same resources but in a different way (e.g. at different times) (Diaz and Cabido, 2001).

At both the Mamanguape and Paraiba, higher mean values for FEve and FDiv were mostly observed in the downstream sections and during the wet season, with a few exceptions in the Mamanguape estuary (higher FDiv and FEve upstream and in the dry season, respectively). Overall, values were relatively high in both estuaries, consistent with regularity in the distribution and high niche differentiation within each system. Within the Paraiba and the Mamanguape estuaries, species seem to be able of using the available resources complementarily, i.e. in a different manner, in particular between sections. If higher niche complementarity within a community reflects a greater variety of potential responses to environmental conditions, and it is expected to provide a buffer against change increasing ecosystem stability and resilience (Tilman, 1996; Hewitt et al., 2008), it may be reasonable to consider the downstream sections of both estuaries to be less susceptible to disturbance than upstream areas.

In view of the great differences in human pressure resulting in greater nutrient input to the Paraiba estuary, we could expect larger differences in zooplankton functional diversity between estuaries which have, however, manifested small. These results are in line with those observed when analysing functional diversity of fish communities in these ecosystems (Dolbeth et al., 2016a). This subtle difference could be explained by the fact that the environmental protected status of the Mamanguape estuary does not imply that the system is exempt from human disturbance. Indeed, the Mamanguape estuary is also target of human activity, as verified by the enriched $\delta^{15}N$ in the biota indicating anthropogenic inputs of nitrogen (Dolbeth et al., 2016b), which probably accounts for the functional diversity levels found.

The Northeast Brazil estuarine areas have long been home of multiple and incompatible uses from increased economic and social pressures, in disagreement with a sustainable development prospect (Sassi et al., 2006; Sá et al., 2013). Human activities such as aquaculture, overfishing and agriculture (sugarcane plantation), together with an enhanced urban development, are clearly damaging these systems environmental quality (Barletta et al., 2010). Although conservation and management efforts from both governmental and non-governmental institutions have been made recently (e.g. through the creation of Ramsar and world heritage sites), environmental degradation seems to persist (Diegues, 1999).

The Mamanguape is one such example, where in spite of being a conservation unit (IUCN, category V), it is not exempt from anthropogenic pressure, presenting low nutrient levels. A management plan for this estuary was approved in 2014, with the definition of several priority conservation areas (Management plan for APA and mangrove areas - ARIE of Mamanguape River, Chico Mendes Institute – ICMBio 2014, www.icmbio.gov.br/portal/planosmanejo). Nevertheless, our results pointed towards an insufficiency of these measures to prevent future environmental quality decline. In particular, the peculiar hydro-morphology of the Mamanguape may turn it more liable to environmental damages. Its downstream reef line promotes an enhanced residence time of nutrient-enriched water, that together with its lower depth and high salinity detected upstream, in particular during the dry season, may hamper an efficient flush out from land drainage water.

Management measures promoting better regulation and surveillance of human practices, risk assessment at the watershed scale, the implementation of environment-friendly practices to decrease pollution (e.g. effluent treatment) and the involvement of local citizens seem crucial to the sustainability and conservation of both systems (e.g. Olivera and Brito, 2005; Martinelli and Filoso, 2008; Barletta et al., 2010; Sá et al., 2013). Our results reinforce the idea that efforts should be directed towards a better application and surveillance of the approved management plan, as well as, to raise awareness on the importance of adopting better public services (basic sanitation), as also previously highlighted in Dolbeth et al. (2016a).

5. Conclusions

The two selected approaches to explore the zooplankton functional diversity along the environmental gradient of the two tropical estuaries revealed useful, providing different and complementary information. When combined they are likely to provide a more robust assessment, improving knowledge about ecosystems functioning, and thus, to inform management and conservation planning more effectively. Indeed, the functional groups approach has been pointed as advantageous to help simplifying the complexity inherent to biological systems, whereas the indices approach to give insights about the ecological processes shaping community assembly (Pavoine and Bonsall, 2011).

The functional approaches used in the present study to summarize the functional diversity in each estuary, allowed us to characterise the functional space and different areas within systems, and to perceive a differential use of resources by the copepods. Our results demonstrated similar functional diversity levels across the Mamanguape and Paraiba estuaries, suggesting a certain degree of maintenance of functions between estuaries. Indeed, ‘environmental filtering’ (de Bello et al., 2012) seems to be an important driver of species coexistence in these systems, at the spatial scale analysed here, where environmental factors are thus expected to select for taxa sharing a particular set of traits that are adaptive under such conditions.

Our results provided evidence of anthropogenic impact in both systems, and despite the environmental protection status of the Mamanguape estuary, illustrating the need and reinforcing previous calls towards a more effective management and increased conservation of these ecosystems.

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Supplementary material

Functional diversity of zooplankton communities in two tropical estuaries (NE Brazil) with different degree of human-induced disturbance

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Table S1. Biological traits and respective categories for the copepod species in the Paraiba and Mamanguape estuaries.

Traits	Categories	Codes
Maximum body size (mm)	Size 1 (0.50-0.70)	S1
	Size 2 (0.70-0.90)	S2
	Size 3 (0.90-0.1.10)	S3
	Size 4 (>1.10)	S4
Trophic group	Herbivore	Herb
	Herbivore-Omnivore	Om-Herb
	Omnivore	Omn
Feeding type	Active ambush	Act_Amb
	Filter	Filt
	Mixed	Mix
Reproduction mode	Broadcast spawning	Broad
	Egg-brooding sac	Egg
Vertical distribution	Epipelagic	Epipel
	Epimesopelagic	Epimes
	Epibathypelagic	Epibat
	Pelagic-benthopelagic	Pel_bent
Diel Vertical Migration (DVM)	Yes	Yes
	No	No

Table S1. (*cont.*)

Estuary	Species	Maximum body size (mm)	Trophic group	Feeding type	Reproduction mode	Vertical distribution	Diel Vertical Migration
Ma/PB	*Acartia lillijerboji*	1.18	Omnivore [8]	Mixed [6]	Broadcast spawning [13, 14]	Epipelagic [9]	yes [1]
PB	*Acartia tonsa*	0.94	Omnivore [8]	Mixed [6]	Broadcast spawning [13, 16]	Epipelagic [9]	yes [1]
Ma	*Euterpina acutifrons*	0.75	Herbivore [12]	Filter [18]	Broadcast spawning [15]	Epipelagic [3]	no [1]
Ma/PB	*Microcyclops anceps*	0.66	Omnivore-Herbivore [11]	Active Ambush	Egg-brooding sac [17]	Pelagic-benthopelagic [17]	yes[19]
Ma/PB	*Notodiaptomus iheringi*	1.19	Omnivore [7]	Filter [7]	Broadcast spawning [17]	Pelagic-benthopelagic [17]	yes [2]
PB	*Oithona brevicornis*	0.63	Herbivore [9]	Active Ambush [5]	Egg-brooding sac [16]	Epipelagic [5]	yes [4]
Ma/PB	*Oithona hebes*	0.65	Herbivore [9]	Active Ambush [5]	Egg-brooding sac [16]	Epipelagic [5]	yes [4]
Ma/PB	*Oithona oculata*	0.63	Herbivore [9]	Active Ambush [5]	Egg-brooding sac [16]	Epipelagic [5]	yes [4]
Ma/PB	*Oithona rigida*	0.58	Herbivore [9]	Active Ambush [5]	Egg-brooding sac [16]	Epipelagic [5]	yes [4]
Ma	*Oithona robusta*	0.63	Herbivore [9]	Active Ambush [5]	Egg-brooding sac [16]	Epipelagic [5]	yes [4]
Ma/PB	*Oithona vivida*	0.57	Herbivore [9]	Active Ambush [5]	Egg-brooding sac [16]	Epipelagic [5]	yes [4]
Ma	*Paracalanus campaneri*	0.79	Herbivore [9]	Filter [17]	Broadcast spawning [3]	Epipelagic [3]	no[1]
Ma/PB	*Paracalanus nanus*	0.80	Herbivore [9]	Filter [17]	Broadcast spawning [3]	Epipelagic [3]	no[1]
Ma/PB	*Parvocalanus crassirostris*	0.64	Herbivore [9]	Filter [6]	Broadcast spawning [3]	Epipelagic [3]	no [1]
Ma/PB	*Parvocalanus scotti*	0.79	Herbivore [9]	Filter [6]	Broadcast spawning [3]	Epipelagic [3]	no[1]
Ma/PB	*Pseudodiaptomus acutus*	1.20	Omnivore [10]	Mixed	Broadcast spawning [15]	Pelagic-benthopelagic [11]	yes [3]
Ma	*Pseudodiaptomus marshi*	1.15	Omnivore [10]	Mixed	Broadcast spawning [15]	Pelagic-benthopelagic [11]	yes [3]
Ma	*Pseudodiaptomus richardi*	1.25	Omnivore-Herbivore [3]	Mixed	Broadcast spawning [15]	Epimesopelagic [3]	yes [3]
Ma/PB	*Temora turbinata*	1.33	Herbivore [11]	Filter [6]	Broadcast spawning [15]	Epibathypelagic [11]	no [1]

Table S1 labels: Ma (Mamanguape estuary); PB (Paraiba estuary).

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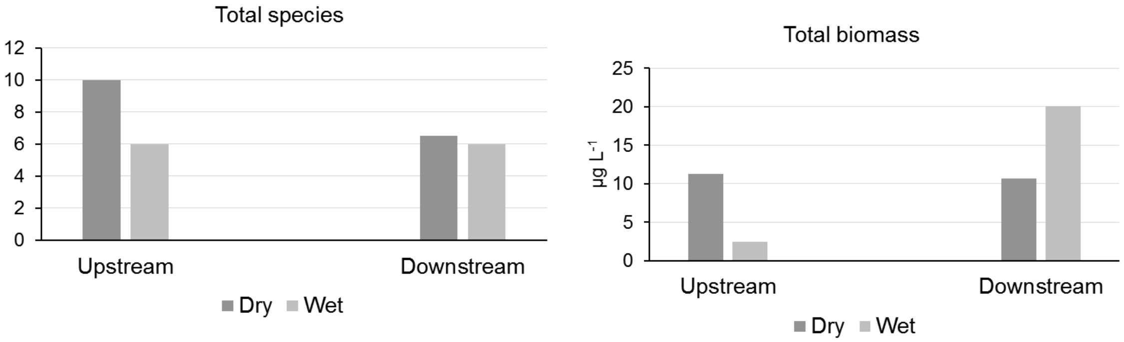
A. Mamaguape



B. Paraiba

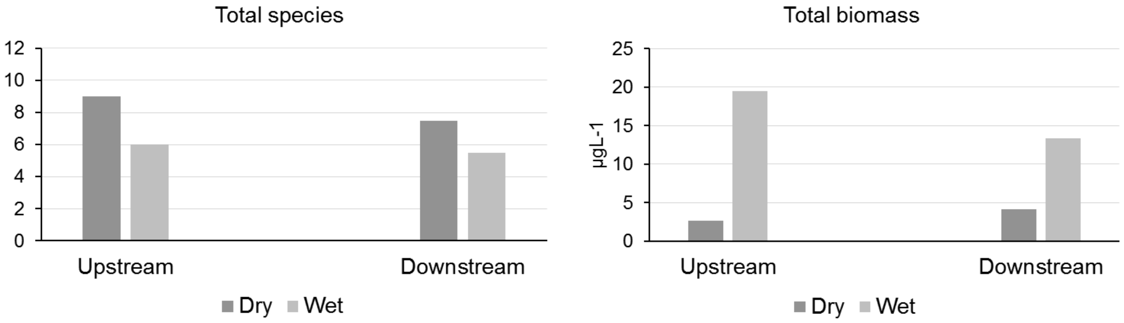


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Figure S1. Total number of species and total biomass during the dry and wet seasons, in the upstream and downstream sections, of the Mamanguape (A.) and Paraiba (B.) estuaries.

Table S2. Spearman rank correlations between functional diversity indices and environmental variables in the Mamanguape and Paraiba estuaries. Only correlation values with statistically significant *P*-values (<0.05) are presented.

	Mamanguape			**Paraiba**		
	FRic	**FEve**	**FDiv**	**FRic**	**FEve**	**FDiv**
Ammonia	0.58	0.71	No significant correlations found		0.40	0.38
Salinity		0.48				
Temperature		0.69				
Turbidity		0.53		-0.46	-0.53	-0.58