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Campina Grande-PB
Agosto - 2015

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Dissertação de mestrado apresentada ao Programa de Pós-Graduação em Ecologia e Conservação da Universidade Estadual da Paraíba, como requisito para obtenção do título de mestre em Ecologia e Conservação.

Orientador: Dr. José Etham de Lucena Barbosa
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(Autor desconhecido)

SUMÁRIO

RESUMO

O presente estudo teve por objetivo analisar os fatores direcionadores da biomassa, diversidade funcional e ácidos graxos da comunidade zooplanctônica em dois estuários tropicais com diferentes estados tróficos (Mamanguape e Paraíba do Norte), localizados no Nordeste do Brasil. O estudo foi dividido em dois manuscritos os quais tiveram amostragens realizadas no período de seca e cheia, sendo: nov/2013 e jul/2014, para o primeiro manuscrito e dez/2014 e jul/2014 para o segundo manuscrito, respectivamente. Foram selecionados quatro zonas amostrais ao longo de cada estuário e em cada uma foram selecionados três pontos onde em cada ponto três amostras bióticas (comunidade zooplanctônica) e abióticas (variáveis ambientais) foram coletadas. Os perfis de ácidos graxos foram obtidos à partir de técnicas de cromatografia gasosa das espécies de copépodes selecionados. No primeiro estudo, foi testado se a conectividade estuarina, as relações filogenéticas e as condições ambientais locais são os principais direcionadores do padrão de distribuição da biomassa e diversidade funcional da comunidade zooplanctônica. Observou-se que esses três componentes são suficientes para explicar a variação no estuário Mamanguape que se localiza em uma área de conservação, e que diferente do que é comumente esperado, a conectividade pode apresentar uma explicabilidade maior na distribuição da comunidade, quando comparada às condições ambientais. No entanto, os três componentes utilizados não foram suficientes para explicar a variação no estuário Paraíba do Norte o qual sofre a influência de impactos antrópicos. O estudo mostra a importância da variável espacial para avaliar os fatores direcionadores da biomassa e diversidade funcional da comunidade zooplanctônica, visto que esta integra à análise os efeitos da capacidade de dispersão das espécies e das forças físicas que atuam no sistema. Além disso, uma investigação aprofundada é necessária para esclarecer os fatores que determinam e moldam as comunidades zooplanctônicas em sistemas tropicais muito impactados. No segundo estudo, foi testado se os perfis de ácidos graxos podem revelar mudanças espaciais e temporais na dieta de copépodos, e por conseguinte, possam ser usados como indicadores do estado trófico dos sistemas estuarinos. Foi possível observar que a composição de ácidos gráxos dos organismo zooplanctônicos revelaram variações sazonais e temporal na ecologia trófica dos copépodos nos dois estuários tropicais. Além disso, os perfis de ácidos gráxos foram capazes de revelar diferenças na qualidade das potenciais fontes de alimento nos dois estuários com diferentes níveis de impacto antrópico, com uma menor qualidade de fontes alimentares presente no sistema mais impactado (estuário do Paraíba do Norte). Nesse estudo os perfis de ácidos gráxos foram sensíveis à estresses naturais e antrópicos, mostrando ser uma ferramenta rápida para avaliar o estado trófico de estuários tropicais.

PALAVRAS-CHAVE: bioindicador, consensus RDA, ecologia alimentar, modelagem espacial, zooplâncton

ABSTRACT

This study aimed to analyze the drivers factors of biomass, functional diversity and fatty acids of the zooplankton community in two tropical estuaries with different trophic states (Mamanguape and Paraíba do Norte), located in Northeastern Brazil. The study was divided into two manuscripts which were sampled in dry and rainy season, as follows: Nov/2013 to Jul/2014, for the first manuscript and Dec/2014 and Jul/2014 to the second manuscript, respectively. Four sample zones were selected along each estuary and in each one were selected three sites where at each site three biotic samples (zooplankton community) and abiotic (environmental variables) were sampled. The fatty acid profiles were obtained from gas chromatography techniques from selected copepod species. In the first study, we tested whether the estuarine connectivity, the phylogenetic relationships and the local environmental conditions are the main drivers of the pattern of distribution of biomass and functional diversity of the zooplankton community. It was observed that these three components are sufficient to explain the variation in Mamanguape estuary that is located in a conservation area, and different than is commonly expected, the connectivity can provide greater explicability for the community distribution, when compared to the environmental conditions. However, the three components used were not sufficient to explain the variation in Paraíba do Norte estuary which is influenced by anthropogenic impacts. The study shows the importance of spatial variable to assess the drivers factors of biomass and functional diversity of the zooplankton community, as this part of the analysis the effects of dispersal ability of species and the physical forces acting on the system. Moreover, a thorough investigation is needed to clarify the factors that determine and shape the zooplankton communities in high impacted tropical systems. In the second study, we tested whether the profiles of fatty acids can reveal spatial and temporal changes in diet of copepods, and therefore can be used as indicators of the trophic status of estuaries. It was observed that the fatty acid composition of zooplanktonic organisms revealed seasonal and temporal variations in trophic ecology of copepods in both tropical estuaries. In addition, the profiles of fatty acids were able to reveal differences in the quality of potential food sources in the two estuaries with different levels of anthropogenic impact, with a lower quality of food sources in the most impacted system (Paraíba do Norte estuary). In this study the profiles of fatty acids were sensitive to natural and anthropogenic stresses, being a fast tool to assess the trophic status of tropical estuaries.

KEY WORDS: bioindicator, consensus RDA, feeding ecology, spatial modelling, zooplankton

1. INTRODUÇÃO GERAL

Estuários são importantes zonas de transição ou ecótonos entre rios e habitats marinhos. Eles são sistemas geomorfologicamente muito dinâmicos e transitórios, influenciados tanto por variações marítimas quanto terrestres, formando assim uma mistura complexa de diferentes habitats (MEIRE et al., 2005; CHAMPALBERT et al., 2007). Essa complexidade ecossistêmica resulta em um "*pool*" genético extremamente diverso, com a biota originada dos ambientes terrestre, marinho e de água doce (McLUSKY; ELLIOTT, 2004; TUNDISI, 1970).

Nos estuários, assim como nos demais ambientes aquáticos, a comunidade zooplanctônica é um grupo importante na teia trófica, sendo definida como o conjunto de protistas e animais (metazoários), não fotossintéticos, geralmente microscópicos, que variam desde formas unicelulares até pequenos vertebrados (GASCA et al., 1996; BONECKER et al., 2002). Estas espécies variam sazonalmente e espacialmente ao longo do estuário, podendo ser influenciada por entradas de água doce e da costa (LI et al., 2000; LI et al., 2006).

Nessa perspectiva a comunidade zooplanctônica se torna um compartimento estratégico no fluxo de energia nos ecossistemas aquáticos e na manutenção e orientação das cadeias tróficas aquáticas (ESKINAZI-SANT’ANNA et al., 2007). Seu posicionamento na cadeia alimentar, com um alto grau de conexão com os produtores primários, os torna extremamente susceptíveis a mudanças estruturais que ocorrem neste nível trófico. Em função de seus curtos ciclos de vida, as mudanças na comunidade fitoplanctônica são refletidas rapidamente pelo zooplâncton que, em seguida, pode indicar a intensidade e condições estabelecidas durante e após a consolidação destes distúrbios (KOZLOWSKY-SUZUKI; BOZELLI, 2002). Além disso, o zooplancton também apresenta forte vinculação com os níveis tróficos superiores (SORANNO el al., 1985), sendo responsável por uma importante rota de transferência de energia dos produtores primários aos peixes. Todas essas características fazem com que a comunidade zooplanctônica seja elemento-chave para o entendimento das mudanças que ocorrem nos ecossistemas aquáticos, particularmente na compreensão do potencial para a propagação destas mudanças ao longo das cadeias alimentares (SCHWAMBORN, 1997).

1.1.Padrões de distribuição da biomassa e diversidade funcional

Compreender as regras de construção das comunidades biológicas tem sido um exercício central na ecologia (PADIAL et al., 2014) e comentários recentes têm defendido a existência de relações entre os padrões de distribuição das espécies com os processos associados à sua coexistência (ACKERLY et al., 2007). Esses padrões de biodiversidade e coexistência de espécies são resultado de interações múltiplas incluindo processos evolutivos (TOFTS; SILVERTOWN, 2003; ACKERLY, 2003), variações em traços (VOGT et al., 2013), interações de espécies (ELTON, 1946, DIAMOND, 1975), padrões de dispersão (PADIAL et al., 2014), variação ambiental (PRADO-POR; LANSAC-TÔHA, 1984, LANSAC-TÔHA; LIMA, 1993) e heterogeneidade de habitats (MASSICOTTE et al., 2014).

Desde Grime (1998) o uso da biomassa para evidenciar padrões de distribuição de espécies tem sido popularizada, visto que essa medida de abundância está diretamente relacionada à quantidade de energia e recursos assimilado por uma espécie (COCHRANE; SCHULZE, 1999, HIEBER; GESSNER, 2002). No entanto, o funcionamento dos ecossistemas está diretamente ligado não só aos perfis energéticos das espécies, mas também aos traços ecológicos que estas possuem (HOOPER et al., 2005; FOLKE et al., 2004; DÍAZ et al., 2007).

A diversidade funcional é tida como uma medida robusta para esse tipo de objetivo, visto que pode ser usada tanto como (1) um indicador dos processos que direcionam a comunidade (ex. filtragem ambiental e competição; CORNWELL et al., 2006), dos impacto das perturbações (ex. alterações climáticas, pesca excessiva) e do gradiente ambiental atuante sobre a estrutura da comunidade (ex. MOUILLOT et al., 2007), quanto (2) indicador das funções do ecossistema, tais como produtividade, resiliência e ciclagem de nutrientes (ex. PETCHEY et al., 2004).

De 1999 a 2003, vários índices da diversidade funcional foram propostos (ex. WALKER et al., 1999; BOTTA-DUKAT, 2005; PETCHEY; GASTON, 2002, MANSON et al., 2003), no entanto, nenhum dos índices existentes satisfazia todos os critérios habitualmente exigidos (ex. projetado para lidar com várias traços; levar em conta a abundância, e medir todas as facetas da diversidade funcional). Diante disso, Villéger et al. (2008) propuseram três índices multidimensionais de diversidade funcional (FD) para características funcionais contínuas, cada um explorando um aspecto diferente da FD: Riqueza funcional (Fric), Uniformidade funcional (Feve), e Divergência funcional (Fdiv).

Laliberté & Legendre (2010) reformulam os índices de Villéger et al. (2008) para que as facetas da FD fossem obtidas a partir de qualquer medida de distância, número e tipo de traço, além de desenvolver um novo índice multidimensional (dispersão funcional (Fdis)). Esses índices medem diretamente a distribuição das espécies num espaço multivariado de traço funcional e são independentes um do outro e do número de espécies (Figura 1), exceto a Riqueza funcional que é correlacionada com a riqueza de espécies. Assim, através destas abordagens pode-se testar não apenas se a complementaridade de nicho melhora o funcionamento do ecossistema, mas que tipo de complementaridade melhora o funcionamento do ecossistema.

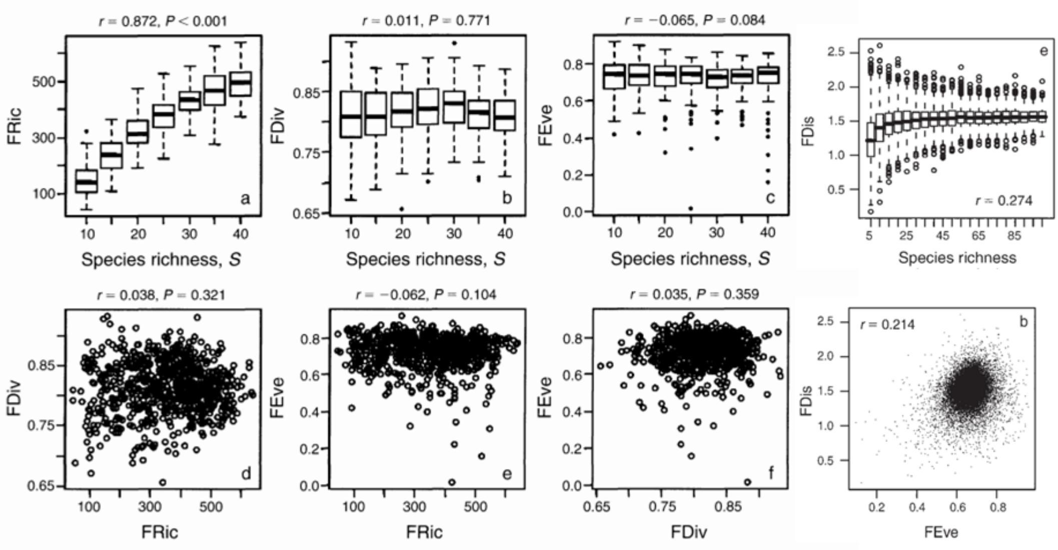

Figura 1: Propriedades dos quatro índices da diversidade funcional. Os gráficos superiores indicam as relações entre os quatro indicies e a riqueza de espécies, e os gráficos inferiores as correlações entre os índices. (Exemplo modificado de Villéger, et al. 2008 e Labireté & Legendre, 2010).

1.2. *Relações filogenéticas e distribuição espacial*

Vários trabalhos têm defendido que a variação na distribuição da biomassa e diversidade funcional da comunidade zooplanctônica em estuários é direcionada principalmente por fatores ambientais (ELLIOTT et al., 2012; LI et al., 2000; LI et al., 2006), no entanto, outros fatores como as relações filogenéticas (TOFTS; SILVERTOWN, 2003; WEBB et al., 2002; LOSOS, 2008) e habilidades de dispersão (BEISNER et al., 2006; PADIAL et al., 2014) também desempenham papel fundamental na distribuição de espécies, de modo que as condições locais não são suficientes para explicar a estrutura destas comunidades.

Segundo Webb (2002) as medidas de diversidade filogenética podem ser usadas como *proxy* das relações filogenéticas para analisar os processos ecológicos que organizam a comunidade. Essas medidas incorporam as relações filogenéticas das espécies e consequentemente diferenças entre suas histórias de vida são levados em conta em sua quantificação. A premissa é a de que espécies intimamente relacionadas dentro de uma filogenia irão partilhar traços ecológicos importantes em função da conservação evolutiva de caracteres (BLOMBERG et al., 2001). Assim, uma comunidade com espécies de abundâncias semelhantes e com parentescos próximos apresenta menor valor de diversidade em relação a outra comunidade com o mesmo número de espécies e abundâncias equivalentes, mas com parentescos distintos.

Segundo os autores que a propuseram, uma medida robusta baseada em distâncias topológicas é a Diversidade taxonômica (Δ, taxonomic diversity; WARWICK; CLARKE, 1995; CLARKE; WARWICK, 1998) e suas variações (Taxonomic Distinctness, Δ^*; Average Taxonomic Distinctness based on presence/absence of species Δ^+; Variation in Taxonomic Distinctness Λ^+ e Total Taxonomic Distinctness, $s\Delta^+$). A Diversidade Taxonômica pode ser considerada como o comprimento médio do ramo (ou braços das árvores filogenéticas) entre dois indivíduos escolhidos aleatoriamente a partir da amostra (incluindo indivíduos da mesma espécie), enquanto a Distinção Taxonômica (i.e. Taxonomic Distinctness) é o comprimento médio dos ramos entre dois indivíduos escolhidos ao acaso, à condição deles serem de diferentes espécies (ROGERS et al., 1999). A partir de dados que consistam apenas da presença ou ausência de espécies (ou seja, lista de espécies), a Distinção Taxonômica Média, uma forma mais simples da Distinção Taxonômica, pode ser pensado como o comprimento médio entre os ramos de quaisquer duas espécies escolhidas aleatoriamente presentes na amostra. O grau com que cada taxa determinado é super ou sub-representados nas amostras é outro atributo de relevância ecológica a qual reflete a Variação na Distinção Taxonômica. Finalmente, a Distinção Taxonômica Total foi proposta por Clarke & Warwick (2001) como uma medida útil de amplitude taxonômica total de uma assembleia, como uma modificação da riqueza de espécies. Segundo vários autores (ex. CLARKE; WARWICK, 1998; MAGURRAN, 2004), essas medidas são promissoras por serem robustas em relação a diferenças na amostragem.

Sabe-se que a distribuição das espécies é influenciada por gradientes ambientais (HUSTON, 1996). No entanto, segundo Lengendre & Fortin (1989) a estrutura espacial tem sido levado em consideração e ganhado força em sua utilização como preditor ou covariável em estudos que busquem entender os padrões de distribuição das comunidades (DRAY et al., 2006). Legendre (1990) propôs o uso de polinômios (3rd deg. Polynomial) de coordenadas geográficas dos pontos de amostragem para representar as relações espaciais em modelos com o objetivo de explicar a variação de espécies. No entanto, nem sempre a distância geográfica representa a verdadeira distância entre pontos amostrais, como por exemplo em estudos de comunidade aquática em sistemas que possuem curvas (Figura 2).

Figure 2: Exemplo do uso de polinômios geográficos para medir distâncias entre pontos amostras. Os pontos pretos mostram a verdadeira distância espacial para uma comunidade aquática em um rio, a qual é negligenciada pela distância geográfica (linha vermelha).

O desenvolvimento da PCNM (*Principal Coordinates of Neighbour Matrices*) forneceu uma forma nova e mais poderosa para estudar a variação espacial das comunidades (BORCARD; LEGENDRE, 2002; BORCARD et al., 2004; LEGENDRE; BORCARD, 2006). Esse modelo foi projetado para situações em que os processos físicos que geram as estruturas de resposta (por exemplo, em comunidades) não apresentam qualquer direccionalidade (ex. reservatórios, lagos). Outro modelo é o MEM (Morran's eigenfunction maps) que nada mais é que uma generalização mais recente do PCNM e possui os mesmos pressupostos (DRAY et al., 2006). No entanto, há situações em que a direccionalidade dos processos físicos influenciam a distribuição das comunidades (ex: rios, estuários), para isso Blanchet et al. (2008) desenvolveram o método de modelagem AEM (asymetric eigenfunction maps) o qual baseia-se em autofunções que utilizam a informação de direção do processo físico, coordenadas espaciais, diagrama de ligação, e pesos opcionais. Esses modelos têm sido amplamente aceitos em estudos de comunidades e metacomunidades aquáticas (FRENETTE et al., 2012; MASSICOTE et al., 2014; PADIAL et al., 2014; PEDRUSKI; ARNOTT, 2011). No entanto, nenhum destes estudos foi realizado em ambientes estuarinos onde a conectividade é mais complexa devido às suas características peculiares, como as grandes flutuações ambientais que são explicadas pelos influxos marinhos e de água doce, estes dependentes das marés e variações sazonais (ARAÚJO, 2008).

1.3 - Perfis de ácidos graxos

Diante da grande biodiversidade das comunidades aquáticas e terrestres encontradas nos estuários, as espécies da comunidade zooplanctônica podem alimentar-se de uma vasta gama de fontes alimentares (fitoplâncton, detritos, bactérias, ciliados e flagelados), dependendo da composição de espécies da comunidade e das condições ambientais (BURNS; GILBERT, 1993; KAMJUNKE, et al., 1999). Assim, existem diferenças importantes entre as guildas de zooplâncton, quanto ao seu impacto sobre os níveis tróficos inferiores, quer diretamente através de alimentação ou indiretamente por influenciar a ciclagem de nutrientes (HESSEN; LYCHE, 1991; DE MOTT, 1995).

O estudo sobre a dinâmica da cadeia alimentar pode fornecer informações importantes para entender a ecologia da linha de base dos organismos, prever as consequências à nível da comunidade de mudanças abióticas e bióticas e caracterizar as interações tróficas. Recentemente, biomarcadores lipídicos (análise de ácidos graxos) têm sido utilizados para identificar as relações específicas da teia alimentar que proporcionam informações de tempo integrado sobre a dieta de assimilação de um organismo (EL-SABAAWI et al, 2009;. VAN DEN MEERSCHE et al., 2009.; ALLAN et al., 2010; KELLY; SCHEIBLING, 2012; DALSGAARD et al., 2003). A análise de ácidos graxos (AG) especificam as fontes alimentares, além disso, a sua especificidade biológica e o fato de serem transferidos da produção primária para níveis tróficos superiores sem mudanças, fazem com que os ácidos graxos sejam ferramentas biomarcadoras adequadas (GONÇALVES et al., 2012).

Os ácidos graxos estão entre as moléculas mais importantes transferidas através da interface planta-animal em cadeias alimentares aquáticas. Classes particulares de AG, como os Ácidos Graxos Altamente Insaturados (AGAI), Ácidos Graxos Polinsaturados (AGPI), Ácidos Graxos Saturados (AGS) e Ácidos Graxos Monoinsaturados (AGMI) são importantes compostos limitantes para o crescimento somático do zooplâncton herbívoro (MÜLLER-NAVARRA, 1995; MÜLLER-NAVARRA et al., 2000; RAVET et al., 2003). Estes AGs fornecem uma contribuição substancial para a qualidade dos alimentos para invertebrados e são vitais para a manutenção somática e crescimento populacional, sobrevivência, sucesso reprodutivo (ADAMS, 1999; OLSEN, 1999; SARGENT et al., 1999), além de atuar mediando respostas imunológicas à infecções (BRETT; MÜLLER-NAVARRA, 1999).

Análises mais detalhadas da composição lipídica podem ajudar a revelar preferências alimentares das espécies investigadas. O conceito de lípidios como marcadores tróficos (Marcadores Tróficos de Ácidos Graxos - MTAG) baseia-se na premissa de que fitoplâncton, microzooplâncton e bactérias, produzem ácidos graxos taxon-específicos, que são retidos pelos seus predadores, e que podem, assim, ser utilizados para avaliar qualitativamente as posições tróficas relativas e a qualidade alimentar (HARRINGTONET et al., 1970; LEE et al., 1971; FALK-PETERSEN et al. 1990; DALSGAARD et al., 2003).

A eficácia dos MTAGs em copépodes tem sido avaliada em estudo em laboratório e campo, resultando em vários índices de omnivoria disponíveis (GRAEVE et al., 1994; STEVENS et al., 2004, GRAEVE et al., 2005). Altas proporções de 18:1n-9 e 18:1n-7 denotam carnivoria em copépodes (HAGEN et al., 2007; STEVENS et al., 2004; NYSSEN et al., 2005; SCHMIDT et al., 2006). O fato de espécies zooplanctônicas carnívoras muitas vezes terem maiores proporções de lípidos polares (ricos AGPI), do que crustáceos herbívoros, faz com que a proporção de AGPI em relação aos AGS seja também usada como um índice de carnivoria (CRIPPS; ATKINSON, 2000; STEVENS et al., 2004). Um outro índice de carnívora é a razão de ácido docosahexaenóico e ácido eicosapentaenóico (22:6n-3 / 20:5n-3, DHA / EPA) (DALSGAARD et al., 2003). DHA também é um componente importante dos lípidos polares, e é altamente conservado em cadeias alimentares marinhas (SCOTT et al., 2002; VEEFKIND, 2003). No entanto, essa relação reflete também as proporções relativas de dinoflagelados e diatomáceas nas dietas de copépodes herbívoros e onívoros visto que dinoflagelados são ricos em DHA, enquanto que as diatomáceas são ricas em EPA (VISO; MARTY, 1993).

A intensidade da luz e temperatura são provavelmente os fatores ambientais mais importantes e mais bem estudados que afetam a composição de ácidos graxos e lipídeos dos tecidos fotossintetizantes ou organismos (FARKAS, 1979; GUSCHINA; HARWOOD, 2009). Estudos recentes também evidenciam que a disponibilidade de nutrientes tem um impacto significativo e efeitos amplos sobre a composição de ácidos graxos e lipídeos em organismos aquáticos (GUSCHINA; HARWOOD, 2009).

A determinação da composição bioquímica de espécies de copépodes torna-se importante para entender suas funções fisiológicas, metabolismo e valor nutritivo que, por sua vez, são relevantes para os ecossistemas aquáticos em função dos processos de transferência de energia e produtividade secundária (VENGADESHPERUMAL et al., 2010). Diante disso, torna-se importante compreender o quanto a composição de AGs da comunidade zooplanctônica é determinada pela afiliação taxonômica e alterado pela dieta ou modificado por fatores ambientais. Por fim, é essencial analisar se o zooplâncton mantem um perfil de AGs semiconstante relativamente às suas dietas ou, alternativamente, promove bioconversão de alguns AGs em outras moléculas lipídicas (BRETT et al. 2009).

1.4 – Nota sobre a estrutura e objetivos do trabalho

Explorar os principais direcionadores da biomassa, diversidade funcional e ácidos graxos da comunidade zooplanctônica em estuários tropicais, tem sido um grande desafio para a ecologia de estuários, visto que facilita o entendimento dos padrões de distribuição e dinâmica trófica da comunidade no tempo e espaço. Compreender esses padrões de distribuição sob o efeito de diferentes potenciais fatores influenciadores torna-se relevante, visto que a maioria dos estudos sobre o tema utiliza unicamente as variáveis ambientais para explicar as variações na comunidade zooplanctônica.

O presente estudo possui como objetivo geral verificar quais os principais direcionadores da biomassa, diversidade funcional e ácidos graxos da comunidade zooplanctônica em dois estuários tropicais com diferentes estados tróficos. Além disso, alguns objetivos específicos foram propostos para nortear a realização do trabalho. Entre eles: (i) testar se os componentes usados são suficientes para explicar os padrões de distribuição da comunidade zooplanctônica; (ii) verificar se as comunidade existentes nos diferentes estuários amostrados sofrem influência de diferentes direcionadores; (iii) examinar as preferências alimentares das espécies de copépodes em relação as suas fontes pontenciais de alimento; e (iv) analisar os padrões sazonais e espacias dos perfis de ácidos gráxos das espécies de copépodos.

Para melhor entendimento da dissertação, esta foi dividida em dois capítulos estruturados em forma de manuscritos, que procuram discutir os objetivos propostos. As citações no texto e referências ao final do capítulo seguem as normas dos respectivos periódicos para onde serão submetidos. O primeiro manuscrito será enviado à *Oecologia*. Este trabalho versa sobre os fatores direcionadores da biomassa e diversidade funcional da comunidade zooplanctônica de dois estuários tropicais, com diferentes estados tróficos, situados no Nordeste Brasileiro. Para isso, foram modelados os efeitos da conectividade estuarina, variáveis ambientais e relações filogenéticas para explicar os padrões de distribuição das variáveis resposta em questão em função de períodos sazonais (seca e chuva) e o gradiente salino de cada estuário. O segundo manuscrito será enviado à *Ecological Indicators*. Este trabalho foi desenvolvido nos mesmos sistemas citados para o primeiro capítulo e testa se os perfis de ácidos graxos podem revelar padrões espaciais e temporais na dieta de copépodos, e se estes podem ser usados como indicadores do estados trófico dos sistemas estuarinos, que podem por sua vez refletir as variações ambientais e/ou antropogênicas. Para isso, foram investigados a composição de ácidos graxos das espécies de copépodos e suas fontes potenciais de alimento ao longo do gradiente salino e durante os períodos de seca e chuva.

2. OBJETIVO GERAL

Analisar os fatores direcionadores da biomassa, diversidade funcional e ácidos graxos da comunidade zooplanctônica em dois estuários tropicais com diferentes estados tróficos.

3. PRIMEIRO MANUSCRITO

3.1 - Pergunta: Que fatores direcionam as mudanças na biomassa e diversidade funcional da comunidade zooplanctônica em estuários tropicais?

3.2 - Hipótese: A hipótese central do estudo é que independentemente do estuário ou período sazonal, a conectividade estuarina é o componente que melhor explica a variação da biomassa e diversidades funcional da comunidade zooplanctônica.

4. SEGUNDO MANUSCRITO

4.1 - Pergunta: Perfis de ácidos graxos revelam mudanças sazonais e espaciais na dieta de copépodes em dois estuários tropicais?

4.2 - Hipótese: A hipótese central do estudo é que o perfil de ácidos gráxos de copépodes podem revelar mudanças sazonais e espaciais em sua dieta. Assim, espera-se perfis de ácidos gráxos distintos nos períodos de chuva e seca e ao longo do gradiente de salinidade de cada estuário.

Primeiro manuscrito à ser submetido à Oecologia

What factors drive changes in zooplankton biomass and functional diversity in tropical estuaries?

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GCM: analyzed the data and wrote the manuscript; JELB, AMMG and JP: conceived and designed the study and wrote the manuscript; JFV: analyzed the data; FGB: analyzed the data and provided editorial advice.

ABSTRACT

The identification of the mechanisms driving the variation within and among local assemblages is central to community ecology. Traditional ecological approaches focus on the link between community composition and environmental variables. However, considering only the local environmental conditions might not be sufficient to fully explain the structure of the zooplankton communities. We investigated the drivers of change in zooplankton biomass and functional diversity in tropical estuaries. In particular, we tested if the estuarine connectivity, the phylogenetic relationships or the local-scale environmental conditions are the main drivers of change, contributing mostly to explain the biomass patterns and functional diversity of the zooplankton community in tropical estuaries. To test our hypothesis, we carried out two sampling campaigns in the Mamanguape and Paraíba do Norte estuaries (Northeastern Brazil), during the rainy and dry seasons. Our results showed that despite having different environmental conditions, distinct zooplankton communities, with different functional traits, in both estuaries, regardless the season, estuarine connectivity was the factor that explained better the variation in biomass and functional diversity. In the Mamanguape estuary, the other two drivers in combination with connectivity also played an important role explaining the variation of biomass and functional diversity observed during the dry season. Noteworthy, however, is the unexpected finding that the local environmental variables had a poor explanatory power. To Paraíba do Norte estuary the three components are not sufficient to fully explain the variation in the biomass and functional diversity of zooplankton community. Contrarily to what is usually advocated, our results suggest that spatial processes are a driver of change in biomass and functional diversity of tropical zooplankton communities more important than local environmental variables.

KEY WORDS: connectivity, spatial modelling, taxonomic diversity, biological traits, consensus RDA.

1. INTRODUCTION

Identifying the mechanisms driving the variation within and among local assemblages is central to community ecology (Andrewartha and Birch, 1954; Ricklefs, 1987; Cornell and Lawton, 1992; Gaston, 2000; Scheiner and Willig, 2011). Traditional ecological approach focus on the processes that define the associations between community composition and environmental gradients via species sorting (Chase and Leibold, 2003). However, many other aspects have been considered when trying to understand how species in a community are structured: the influence of size or area of a focal habitat (O'Brien et al., 2004, Hoffman and Dodson, 2005), habitat connectivity (MacArthur and Wilson, 1967; Legendre and Fortin, 1989; Cottenie and De Meester, 2003), evolutionary processes (Tofts and Silvertown, 2000; Ackerly, 2003), phylogenetic relatedness (Webb et al., 2002) functional traits (McGill et al. 2006), stochastic equilibrium (Hubbel, 2001) or a combination of some of these aspects (Peres-Neto et al., 2012). To some extent, all of these factors have shown that they are useful to better understand ecological communities.

Estuaries are at the interface of continental and marine environments. As such, they present a complex mosaic of habitats and host a large number of species. Among the species often found in estuaries, zooplankton are of particular interest for a few reasons. In these ecosystems, zooplankton act as a link between primary producers and higher trophic levels, they are thus a key component of the food web (McLusky and Elliott, 2004). Zooplankton is also sensitive to environmental variations (Gonçalves et al., 2012 a, b). For these reasons, zooplankton is a valuable model group of organisms to assess the effects of environmental changes (e.g. climate changes, human impacts) and local biological processes (e.g. productivity, trophic dynamics) in estuarine ecosystems.

Local-scale environmental conditions influence the structure and dynamics of zooplankton communities (Li et al., 2000; Li et al., 2006; Elliott et al., 2012). However, because other factors such as dispersion (Besnier et al., 2006; Padial et al., 2014) and phylogenetic relatedness (Webb et al., 2002; Losos, 2008) may also play a crucial role in the distribution of species, considering the local environmental conditions might not be sufficient to fully explain the structure of the zooplankton communities.

According to Peres-Neto et al. (2012), phylogenetic relationships is a proxy for niche differentiation, which in turn can be used to analyse the ecological processes that structure a community (Webb et al., 2002). This concept relies on the idea that closely related species in a phylogeny share ecological important traits because of the evolutionary conservation of characters (Blomberg et al., 2001). Consequently, there generally is a positive relationship between the phylogenetic relatedness of two species, their histories life and ecological similarity in terms of their functional diversity (Harvey and Pagel, 1991; Silvertown et al., 1997; Clarke and Warwick, 1998). Phylogenetic topology can thus be used as a proxy of the relatedness between species and has been shown to be a robust measure that incorporates phylogenetic relatedness in community ecology studies (Webb, 2002; Ricotta, 2004; Cianciatuso, 2008).

Since the development of asymmetric eigenvector maps (AEM, Blanchet et al. 2008b), a number of ecological and ecotoxicological research have used this tool to study the dispersal of aquatic organisms assuming that the direction of flow and connectivity among samples is known (e.g. Pedruski and Arnott, 2011; Frenette et al., 2012; Massicotte et al., 2014; Padial et al., 2014). However, none of these projects have been carried out in estuaries. This gap in the literature maybe because of the complexity of these ecosystems, where high environmental fluctuations are often the result of an intricate relationship between the combined marine and freshwater influxes, and the influence of tides and seasonality. In tropical estuaries, the complexity of these relationships is increased because of the dramatic difference between the dry and rainy seasons that importantly alter the physicochemical characteristics of estuaries as well as the water level (Schwartz and Jenkins, 2000). During the rainy season, the water level and current velocity are high, whereas in the dry season, these patterns are inverted. Such important seasonal variations can strongly impact the spatial-temporal distribution of zooplankton communities (Rodriguez, 1975).

In this study, we investigated the drivers of change in zooplankton biomass and functional diversity in two tropical estuaries, Mamanguape and Paraíba do Norte. In particular, we evaluated the factors most important to explain the biomass and functional diversity of zooplankton community in tropical estuaries for the dry and rainy seasons. To achieve this goal we focused on the estuarine connectivity (used as a proxy for the dispersion ability of zooplankton), the phylogenetic relationships of zooplankton and the local-scale environmental conditions. Moreover, we hypothesised that, regardless the estuary or the season, the estuarine connectivity is the component that best explains the variation in the zooplankton biomass and functional diversity.

2. MATERIALS AND METHODS

2.1 Study area

The study was carried out in the largest and second largest estuarine system of the Paraíba state in Brazil, Paraíba do Norte and Mamanguape estuaries, respectively (Figure 1). The rainy season starts in February and ends in July, with the highest precipitation occurring from April to June. As for the dry season it span from August to January, and the driest months occur from October to December. In the study area the mean annual water temperature varies between 24 ºC and 26 ºC.

The Paraíba do Norte estuary (Figure 1A) is located mainly in an urban area (~1,000,000 inhabitants). The Cabedelo harbour, agriculture fields and aquaculture farms are located in the vicinity of the estuary. The estuary is approximately 22 km long and the river mouth 2.2 km wide, has medium flow regime, allowing the formation of small dunes, and has an average depth of three meters, except near the harbour, where the estuary is roughly 11 meters deep.

The Mamanguape estuary (Figure 1B) is located inside conservation area aimed at protecting coastal habitats and marine manatee (*Trichechus manatus* Linnaeus, 1758). The mouth of the river forms a bay about six km wide, which is nearly closed by a coastal reef line. This physical feature results in low water velocity. The estuary has well-preserved mangroves that grow around the main channel and the intertidal creeks, covering approximately 6,000 hectares, in addition to the remnants of the Atlantic rainforest (Rocha et al., 2008). Most of the area surrounding the mangroves is dedicated to sugar cane fields and in a smaller extent to crustacean aquaculture. There are ~66,000 inhabitants in the area surrounding the Mamanguape estuary.

2.2 Environmental variables

For all sites, water salinity, temperature (ºC), pH, electrical conductivity (µS/cm), turbidity (NTU) and total dissolved solids (TDS, g/L) were measured *in situ* using a multiparameter probe (Horiba/U-50). Similarly, water transparency (m) was measured with a Secchi disk. The concentrations of dissolved inorganic nitrogen (DIN, µg/L) (APHA, 2005), total phosphorous (TP, µg/L) and soluble reactive phosphorous (SRP, µg/L) (Strickland and Parsons, 1972) were measured in the laboratory. We also assessed the concentration of chlorophyll-*a* (Chl *a*, µg/L) following Lorenzen (1967).

Prior to any statistical analyses, all environmental variables were standardized (centred and divided by their standard deviation) to remove unit effects. To make sure that colinearity among environmental variables was minimal, only the variables with a variance inflation factor (VIF, Neter et al. 1996) below 2 were used.

2.3 Zooplankton sampling

We carried out two sampling campaigns in each estuary, one in the rainy season (July 2014) and the other during the dry season (November 2013). Sampling was carried out during the high tide of the full moon. In each estuary, samples were collected across four subtidal zones (Figure 1). These zones were previously chosen taking into consideration: water salinity, sediment granulometry and depth. In each subtidal zone, three sites were selected (in zone IV of the Paraíba do Norte estuary, to cover both sides of the Ilha da Restinga, we selected 6 sites) and for each site three samples were collected.

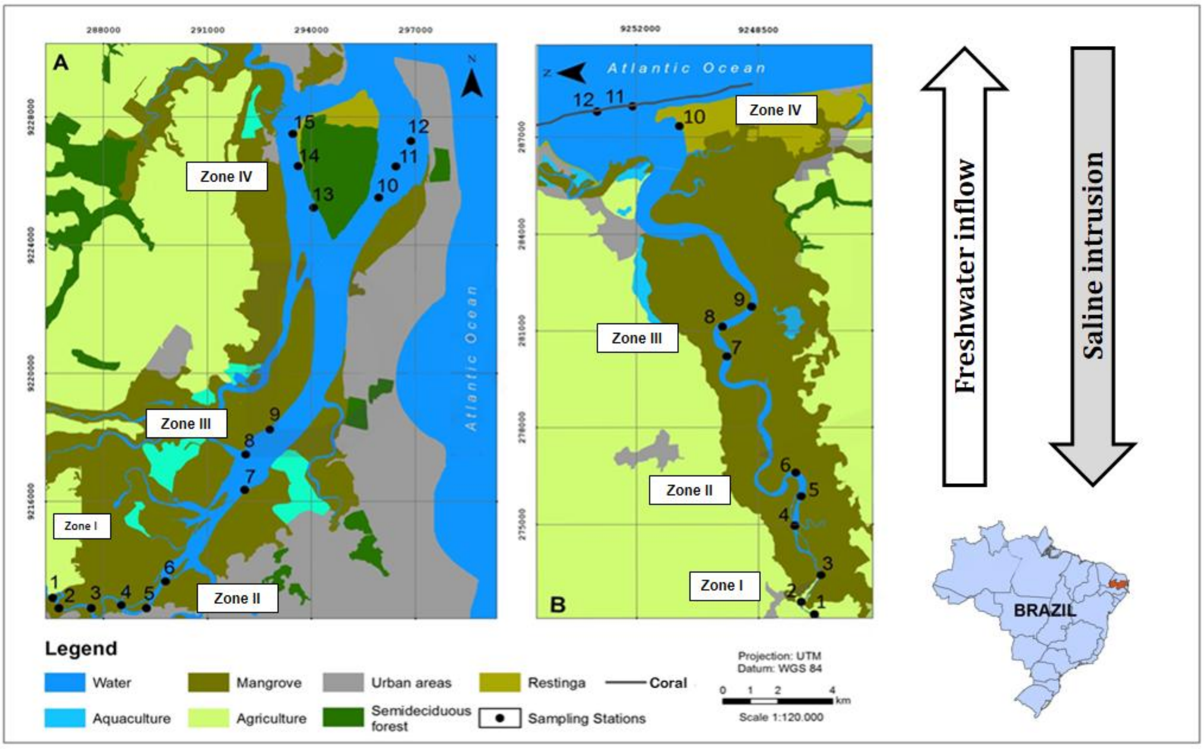


Figure 1. Study areas. A. Paraíba do Norte estuary and B. Mamanguape estuary, Brazil. The black dots (●) represent the sampling sites where 1, 2 and 3 represent the Zone I, 4, 5 and 6 the Zone II, 7, 8, 9 the Zone III, and 10, 11 e 12 the Zone IV (to Paraíba do Norte: 10, 11, 12, 13, 14 and 15 are the Zone IV). The orange area on the map of Brazil is the state of Paraíba.

Zooplankton collection at each site was carried out using a mesh size 68 µm with mouth diameter: 0.3 m. All samples were preserved in glycosylated 4% formaldehyde. The qualitative and quantitative analyses of zooplankton were carried out under a stereoscopic and inverted microscope using a Sedgewick-Rafter chamber (1 mL capacity). Organisms were identified to the lowest possible taxonomic level according to specialized literature (e.g Koste, 1978; Reid, 1985; Elmoor-Loureiro, 1997; Boltovskoy, 1999). In each sample, a minimum of 100 individuals was counted. Note that the first development stages (nauplii and copepodites) of zooplankton were not considered here because the minor taxonomic resolution could not be found. This decision did not compromise any of the analyses presented bellow because the community distribution was the same with and without these stages (Table S2).

Depending on the group of organisms, different techniques were used to estimate biomass. For copepods (harpacticoida, cyclopods and calanoids) and cladocerans a length–weight regressions was used (Dumont et al. 1975; McCauley, 1984; Ara, 2001; Azevedo et al., 2012). However, for rotifers and tintinids, the biomass was estimated by comparing body shapes with approximate geometric forms (Ruttner-Kolisko, 1977). Wet weights were estimated by assuming that $10^6 \mu m^3$ corresponds to 1 µg wet weight while dry weights were assumed to be 10% of the wet weight (Pace and Orcutt, 1981).

2.4 Functional Diversity Indices

To quantify zooplankton communities' functional diversity (FD), we focussed on five functional traits almost all defined at the species level: mean dry weight, maximum length, inshore/offshore distribution, feeding-type and trophic level (for more details see Table S1). We computed dry weights and maximum lengths directly from our zooplankton samples, while inshore/offshore distribution, feeding type and trophic level traits where derived from the literature.

Four functional diversity indices were calculated: functional richness (FRic), evenness (FEve), divergence (FDiv) (Villeger et al. 2008) and dispersion (FDis) (Laliberté and Legendre, 2010). These indices were calculated based on distances between pairs of standardized traits weighted by species relative abundance within estuary and seasons (Laliberté and Legendre, 2010). In addition, to make sure the distances had a Euclidean basis, a square root correction was applied (Lingoes, 1971; Gower and Legendre, 1986). All these calculations were carried out with the "FD" package (Laliberté et al., 2014) used within the R statistical language (R team 2015).

2.5 Taxonomic Diversity Indices

We used the hierarchical Linnean zooplankton classification as proxy for the cladograms representing the relatedness of individual species to estimate taxonomic diversity indices. Following Boxshall et al. (2014), for each site, we compiled a phylogenetic tree and considered five taxonomic levels (species, genus, family, order, class and phylum). We then calculated taxonomic diversity (Δ), taxonomic distinctness (Δ^*), average taxonomic distinctness based on presence/absence of species (Δ^+), variation in taxonomic distinctness (Λ^+) and total taxonomic distinctness ($s\Delta^+$) described by Clarke and Warwick (1998, 2001). To calculate these indices, we used the following distinctness weights: 1, for species within the same genus; 2, species of the same family but of a different genus; 3, species same order but of a different family; 4, species of the same class but of a different order; and 5, species of the same phylum but of a different class (Clarke and Warwick, 1998). Two species connected at the highest taxonomic level were given a distinctness of 100 (Clarke and Warwick, 1998, 2001). All taxonomic diversity indices were calculated using the “vegan” package (Oksanen et al. 2015) within the R statistical language. As for the environmental variables, only the taxonomic diversity indices with a VIF < 2 were used to study zooplankton biomass and functional diversity.

2.6 Estuarine connectivity

To account for connectivity among sites, we used asymmetric eigenvector maps (AEM) analysis (Blanchet et al., 2008b), which accounts for a known direction of flow in an estuarine ecosystem. However, the analysis can be used where physical forcing comes from different directions (e.g. estuarine systems) (Blanchet et al., 2011). The directional connection network in the estuaries was defined based on empirical assumptions about water flow, geographical distance between sites, seasonality and tide effects (Figure S1). The analyses weights were established following the equations 1 and 2,

$$\text{Freshwater inflow} = \left(\frac{\dfrac{\text{distance}}{\text{sd}(\text{distance})}}{\max\left(\dfrac{\text{distance}}{\text{sd}(\text{distance})}\right)} \right)^{x} + \left(\frac{1}{\exp(\text{width of stream at each site})} \right) \qquad \text{(eq. 1)}$$

$$\text{Salinity intrusion} = \left(\frac{\dfrac{\text{distance}}{\text{sd}(\text{distance})}}{\max\left(\dfrac{\text{distance}}{\text{sd}(\text{distance})}\right)} \right)^{y} + \left(\frac{1}{\exp(\text{width of stream at each site})} \right) \qquad \text{(eq. 2)}$$

where, *sd(distance)* is the standard deviation of the distance between sites, *max* is de higher value of the distance and *exp()* is Euler's number. The first part of both equations account for the geographical distance among sites using a concave up function. The second part of the equation is meant to account for the current velocity, which should be increased from upstream to downstream. Thus the width of the stream perpendicular to the current direction at the level of the site is a proxy for current velocity. The x and y define the strength of that relationship. We tested strengths ranging between 0.0 and 1.0 to x and y, resulting a total of 121 combinations of weights for each season of each estuary to make sure a good coverage of the different weights. In the rainy season, x should be larger than y because it states that the strength of the current from upstream to downstream is stronger. The inverse should be used for the dry season, that is y should be larger than x. Moran's I coefficients, which measure the degree of correlation among neighbouring observations in the network (Boots and Getis, 1988) were calculated for each eigenfunction produced by the AEM procedure. We selected only the eigenvectors with positive Moran's I autocorrelation coefficients ($I > 0$; $p < 0.05$), assuming that these eigenvectors are proxies for dispersal processes or unmeasured environmental variables that are spatially structured. We selected the AEM variables using the forward procedure (significance level: $\alpha=0.1$) proposed by Blanchet et al. (2008a). AEM analyses were performed using "AEM" package in R (Blanchet et al., 2014).

2.7 Data analyses

To evaluate if there was a difference between the two estuaries, we used a permutation-based multivariate analysis of variance (PERMANOVA, Anderson, 2001) and focused on the environmental variables and the zooplankton community composition data, independently. Similarly, using PERMANOVA with the same data, we tested if there were differences between the sampling zones and seasons for each estuary independently. Note that "subtidal zone" was nested into "season" when this analysis was performed. We assessed the significance at p-value ≤ 0.05 (after 999 permutations). The Bray-Curtis index was used for biomass data and the Euclidean Distance for environmental parameters.

Regarding the functional diversity and taxonomic diversity, we tested the differences between zones and seasons of each estuary using a two-way Analysis of Variance (two-way ANOVA, with zone and seasons as factors, with the same design as the multivariate PERMANOVA) under a significance level of $\alpha < 0.05$. The analyses was performed using the *adonis()* and *aov()* function, respectively, in the "vegan" and "stats" package (Oksanen et al., 2013)

Biomass and functional diversity of zooplankton were modelled using a set of selected environmental parameters, taxonomic diversity and estuarine connectivity variables for each season and estuaries. To identify the factors most related with changes in zooplankton biomass and functional diversity we used a consensus of canonical redundancy analysis (RDA), which makes a consensus of a group of RDA carried using different dissimilarity coefficients (Blanchet et al., 2014). Because consensus RDA averages over a group of dissimilarity coefficients it "corrects" for the mean-variance problem highlighted by Warton et al. (2012) when dealing with dissimilarity-based statistical techniques. In addition, the interpretation of the result is not constrained by the underlying dissimilarity used to carry out the RDA. The ten different asymmetrical dissimilarity coefficients compared for abundance data by Blanchet et al. (2014, see their Table 1) were used in this study. To evaluate how the different dissimilarity compared to each other for our data, we used the RV coefficient, a multivariate extension of the squared Pearson's correlation (Escoufier 1973, Robert and Escoufier 1976).

For each estuary, we constructed ten models, one for each dissimilarity coefficient mentioned in the previous paragraph, for each of the 121 sets explanatory variables. Recall that the set of explanatory variables because of the set of AEM variables considered (see *Estuarine connectivity* section). In doing this, we were able to evaluate if any dissimilarity coefficient reacted differently from the others. In such circumstances, the dissimilarity coefficient was not further considered. With each of the remaining dissimilarity coefficient, we performed a variation partitioning analysis (Borcard et al. 1992), studying the range of R^2_a and choose the model with higher percentage of total variation explained. Following Peres-Neto et al. (2006), we used the adjusted coefficient of multiple determination (R^2_a) in the variation partitioning analysis. Variation partitioning was carried out using the ''vegan'' package (Oksanen et al., 2015). Each unique fraction of the partitioning was tested using partial RDA (9,999 random permutations).

Before the analysis described above, biomass data were transformed in "Distance between species profiles" to increases the weight given to rare species and yields little 'horseshoe effect', as described by Legendre and Gallagher (2001). All analyses were performed using the R statistical language.

3. RESULTS

3.1 Environmental characterization

In both estuaries, water temperature ranged from 27 to 29 ºC, the pH was lightly basic (6.88 to 8.35 during the rainy season) to alkaline (8.41 to 9.39 in the dry season) and the water was more turbid in the dry season (Table 1). In the Mamanguape estuary, during the dry season, the salinity ranged between 14.9 and 36.53 and, in the rainy season, between 0.4 and

34.3. The DIN ranged between 249.7 and 460.9 µg/L in the dry season and between 23.5 and 244.5 µg/L in the rainy season. The total phosphorous (TP) presented high concentrations during the dry season, where the variation was between 113.3 and 284.4 µg/L. In the rainy season the TP values ranged between 63.3 and 163.3 µg/L. In the Paraíba do Norte estuary, during the dry season, the salinity ranged between 5.73 and 34.5, and in the rainy season the variation was from 1.6 to 29.2. The dissolved inorganic nitrogen (DIN) ranged between 434.3 and 4139.59 µg/L in dry season and between 991.8 and 4037.49 µg/L during the rainy season. The TP, in the dry season varied between 153.8 and 710.189 µg/L and during the rainy season the variation was between 224.4 and 754.49 µg/L (Table I).

The environmental conditions (i.e. variables measured in the water column) were significantly different between estuaries (Pseudo-F=26.20; p=0.001). In the Mamanguape estuary, the zones had significantly different environmental conditions (Pseudo-F=2.70; p=0.006). Moreover, the environmental variables were significantly different between the two seasons (Pseudo-F=16.98; p=0.001). A similar result was found in the Paraíba do Norte estuary with significant differences between zones (Pseudo-F=4.61; p=0.01) and between seasons (Pseudo-F=3.88; p=0.04).

3.2 Structure of zooplankton groups

In total, considering both estuaries, we identified 43 zooplankton taxa (one Tintinid, 15 Rotifera, five Cladocerans, 13 Calanoida, eight Cyclopoida and one Harpacticoida). Thirty-six taxa occurred in the Mamanguape and 28 in the Paraíba do Norte estuary.

Table 1: Environmental variables (mean and standard deviation) measured *in situ* during the dry (November 2013) and rainy seasons (July 2014) in the Mamanguape and Paraíba do Norte estuaries, Northeast Brazil.

	Mamanguape estuary							
	Dry season				**Rainy season**			
	Zone I	**Zone II**	**Zone III**	**Zone IV**	**Zone I**	**Zone II**	**Zone III**	**Zone IV**
Transparency (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	0.8 ± 0.15	1 ± 0.5
Salinity	14.9 ± 1.1	20.6 ± 1.4	29.4 ± 0.6	36.5 ± 0.1	0.4 ± 0.0	3.4 ± 1.8	13.3 ± 1.2	34.3 ± 2.3
Temperature (ºC)	28.1 ± 0.0	20.6 ± 0.0	29.0 ± 0.1	28.5 ± 0.0	26.8 ± 0.4	27.2 ± 0.3	27.3 ± 0.1	27.1 ± 0.2
pH	8.4 ± 0.07	8.5 ± 0.0	8.8 ± 0.0	9.3 ± 0.1	7.7 ± 0.2	7.2 ± 0.1	7.5 ± 0.2	7.6 ± 0.4
Conductivity (mS/cm)	24.5 ± 1.6	33.0 ± 2.1	45.5 ± 0.9	55.1 ± 0.1	0.9 ± 0.04	6.3 ± 3.1	22.13± 1.8	52.0 ± 3.1
Turbidity (NTU)	55.9 ± 0.7	61.5 ± 22.4	101.7 ± 23.7	93 ± 22.4	26.8 ± 6.3	31.8 ± 6.8	32.1 ± 24.4	36.9 ± 24.4
TDS (g/L)	15.2 ± 1.0	20.2 ± 1.31	27.7 ± 0.58	33.0 ± 0.1	0.5 ± 0.0	3.9 ± 1.9	19.3 ± 5.0	31.4 ± 1.6
DIN (µg/L)	249.7 ± 70.6	263.6 ± 30.0	324.8 ± 116.2	460.9 ± 160.0	244.5 ± 231.1	136.6 ± 53.5	157.5 ± 13.0	23.5 ± 8.7
SRP (µg/L)	51.3 ± 10.0	83.5 ± 38.6	56.8 ± 6.9	43.5 ± 29.8	82.4 ± 11.7	106.8 ± 15.7	89.1 ± 3.8	63.5 ± 6.9
P total (µg/L)	284.4 ± 284.8	113.3 ± 11.5	131.1 ± 10.7	115.5 ± 27.1	131.1 33.7	163.3 ± 17.3	131.1 ± 19.2	63.3 ± 13.3
Chl *a* (µg/L)	5.2 ± 4.0	2.2 ± 1.1	2.5 ± 0.6	0.8 ± 1.5	1.4 ± 0.2	2.6 ± 1.5	1.6 ± 0.5	0.7 ± 0.6

	Paraíba do Norte estuary							
	Dry season				**Rainy season**			
	Zone I	**Zone II**	**Zone III**	**Zone IV**	**Zone I**	**Zone II**	**Zone III**	**Zone IV**
Transparency (m)	0.6 ± 0.0	0.6 ± 0.2	1.1 ± 0.1	0.8 ± 0.1	0.6 ± 0.0	0.7 ± 0.0	0.5 ± 0.0	0.9 ± 0.2
Salinity	5.7 ± 2.2	8.7 ± 4.6	21.0 ± 0.6	34.5 ± 0.0	1.6 ± 0.1	7.7 ± 0.1	18.3 ± 0.1	29.2 ± 0.2
Temperature (ºC)	29.1 ± 0.0	29.3 ± 0.2	29.1 ±0.2	28.1 ± 0.1	27.6 ± 0.0	27.2 ± 0.0	27.3 ± 0.0	27.4 ± 0.1
pH	8.4 ± 0.0	8.4 ± 0.1	8.4 ± 0.0	8.9 ± 0.0	6.8 ± 0.3	7.4 ± 0.1	7.6 ± 0.3	8.3 ± 0.1
Conductivity (mS/cm)	10.0 ± 3.6	14.9 ± 7.5	29.9 ± 7.3	52.4 ± 1.2	3.1 ± 2.0	13.4 ± 3.1	29.6 ± 2.6	45.1 ± 3.6
Turbidity (NTU)	74.7 ± 9.0	68.8 ± 12.4	24.2 ± 5.5	45.3 ± 8.4	51.9 ± 22.4	18.7 ± 3.3	16.4 ± 7.2	19.6 ± 3.47
TDS (g/L)	1.1 ± 0.6	9.26 ± 4.63	20.5 ± 0.6	31.4 ± 0.7	1.9 ± 1.2	8.3 ± 1.9	18.2 ± 1.5	27.4 ± 2.1
DIN (µg/L)	489.5 ± 170.2	1948.3 ± 1525.6	4139.5 ± 443.0	434.3 ± 186.4	2533.3 ± 1640.6	3835.6 ± 165.7	4037.4 ± 439.7	991.8 ± 819.5
SRP (µg/L)	358 ± 58.9	466.8 ± 157.4	594.6 ± 14.5	68.5 ± 26.8	405.7 ± 77.6	639.1 ± 25.4	616.8 ± 220.0	174.6 ± 76.7
P total (µg/L)	434.4 ± 270.1	668.8 ± 25.2	710 ± 18.5	153.8 ± 21.4	561.1 ±59.7	754.4 ± 94.5	650 ± 250.0	224.4 ± 65.4
Chl *a* (µg/L)	44.1 ± 18.7	18.2 ± 15.2	4.4 ± 1.1	1.2 ± 1.0	7.3 ± 3.4	2.4 ± 1.1	2.5 ± 2.5	1.7 ± 0.6

The zooplankton composition (based on biomass data) was significantly different between estuaries (Pseudo-F=6.85; p=0.001). In the Mamanguape we observed significant differences between zones (Pseudo-F=5.08; p=0.001) and seasons (Pseudo-F=3.29; p=0.005). The same result was found in the Paraíba do Norte, with significant differences in zooplankton composition between zones (Pseudo-F=3.52; p=0.001) and seasons (Pseudo-F=5.36; p=0.001).

In the Mamanguape, during the dry season, Cyclopoida was the group with the highest biomass followed by Calanoida (Figure 2). The rotifers were only represented by the species *Lecane luna* (Müller, 1776), with low biomass (0.003 µg DW/L). During the rainy season the biomass of zooplankton was higher in the intermediate zone of the estuary (Zone III), where Cyclopoida had the highest biomass (114.17 µg DW/L), followed by Calanoida (41.94 µg DW/L) (Figure 2). In the upstream zone, we observed an increase of biomass (0.38 µg DW/L) and the presence of nine others species of rotifers. Furthermore, Cladocerans were also observed in this zone with 0.16 µg DW/L together with the presence of four species (*Macrothrix mira*, *Macrothrix* sp., *Alona poppei* and *Diaphanosoma spinulosum* (Herbst, 1975). Calanoida *Notodiaptomus iheringe* (Wright, 1985) and Cyclopoida *Microcyclops anceps* (Richard, 1987) are freshwater species and appear in low biomass concentrations (1.31 and 0.119 µg DW/L, respectively).

In the Paraíba do Norte, during the dry season, the zooplankton showed the higher biomass value in the intermediate zone (Zone III), with the Cyclopoida being the group with the highest biomass (15.50 µg DW/L), followed by Calanoida (8.02 µg DW/L) (Figure 2). The rotifers occurred only in the most upstream zones (0.22 µg DW/L in Zone I and 0.12 µg DW/L in Zone II) and were represented by four species (*Asplanchna* sp., *Brachionus calyciflorus* (Pallas, 1766), *B. caudatus* (Barrois and Daday, 1894) and *B. leydigi* (Cohn, 1862). Similarly, in the rainy season, Cyclopoida were the group with the highest biomass, followed by Calanoida. The rotifers, represented by six species, occurred only in the most upstream zones, showing a decrease of biomass from Zone I (0.128 µg DW/L) to Zone II (0.002 µg DW/L).

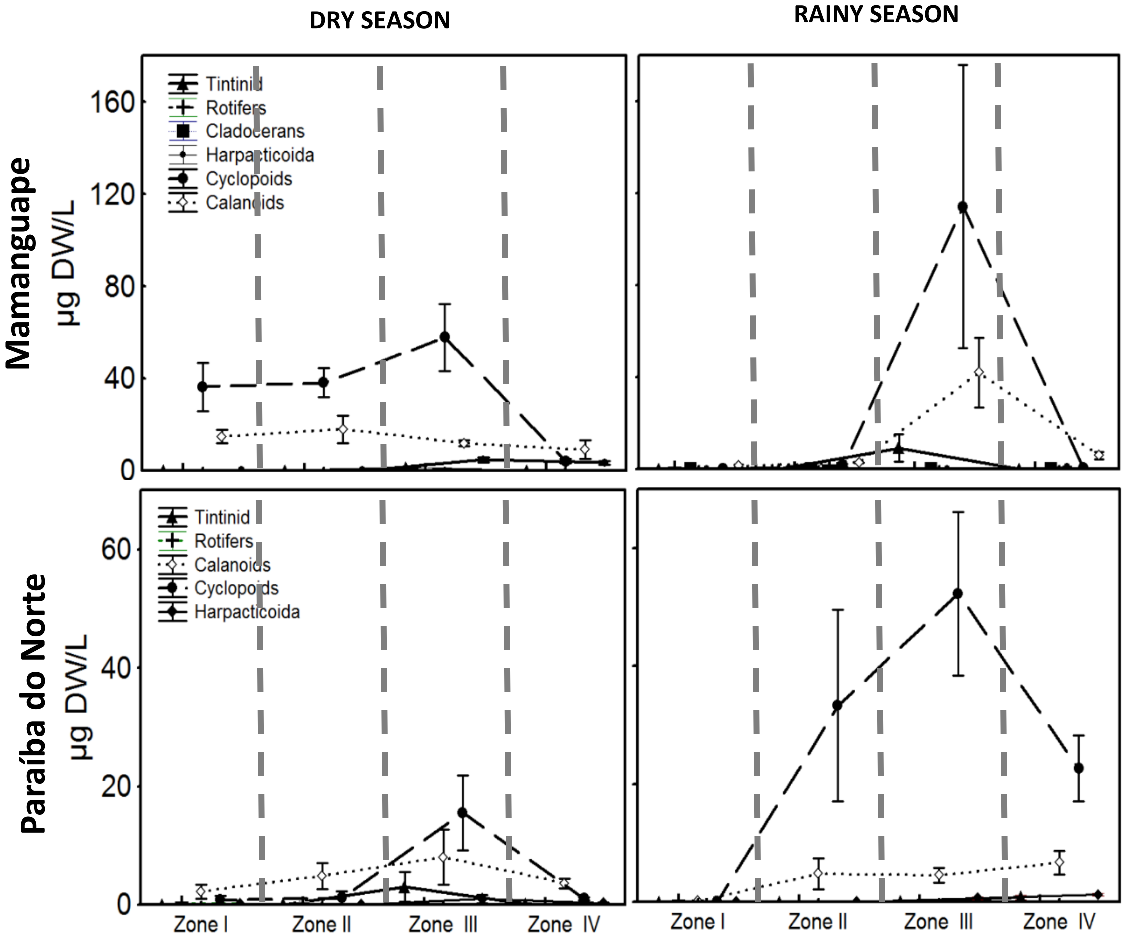
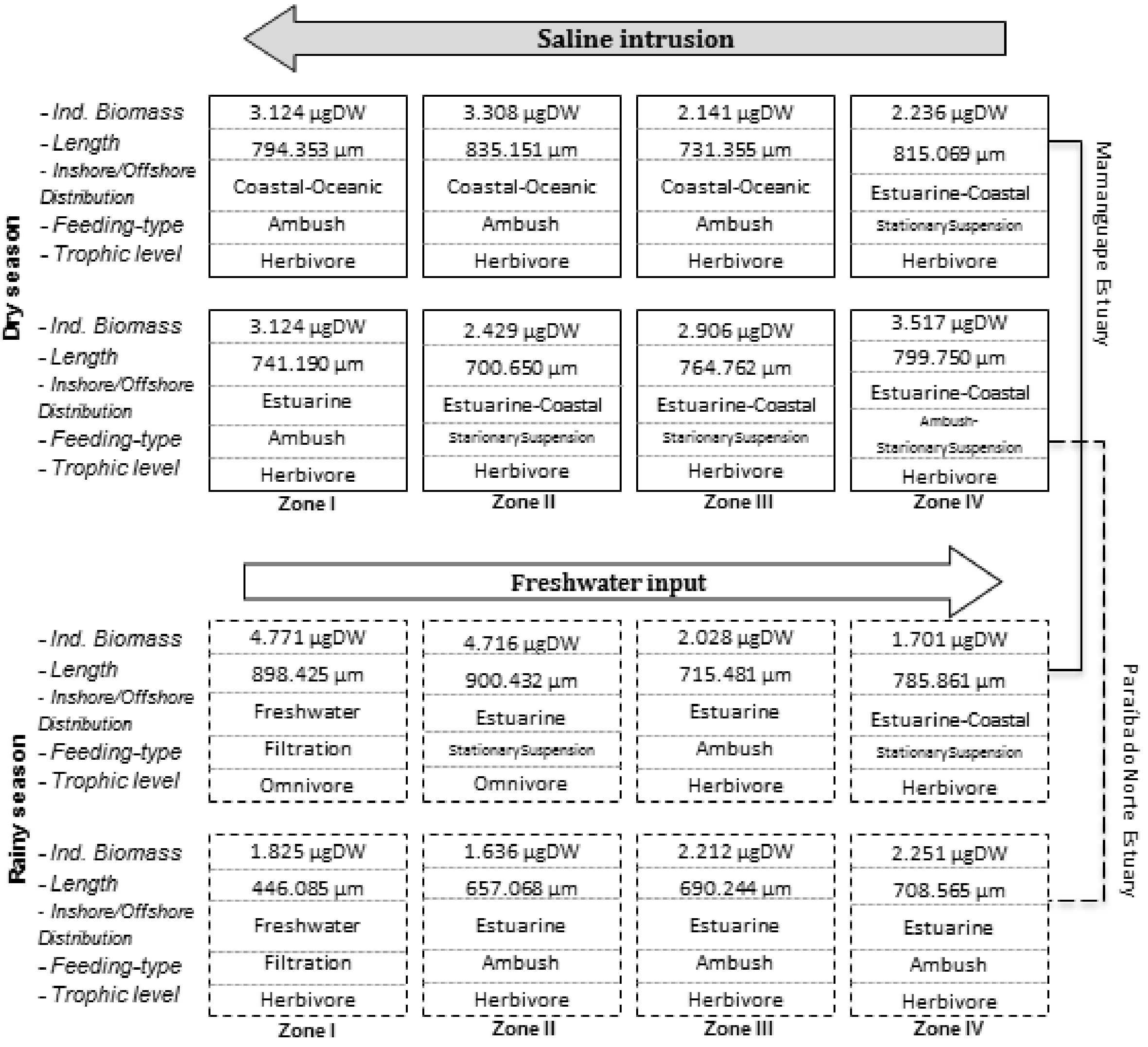


Figure 2. Spatial and seasonal variation in zooplankton biomass in the Mamanguape and Paraíba do Norte estuaries.

3.3 Functional diversity

The zooplankton communities of the Mamanguape and Paraíba do Norte estuaries are composed by species with diverse ecological traits. The biomass of individual species ranged between 0.021 and 19.55 µg DW and from 118.9 to 1587.3 µm of length for *Keratella tropica* and *Temora turbinata*, respectively (for more details see Table S1). The species presented distributions ranging from freshwater to estuarine, coastal and oceanic conditions. The feeding types included the filter feeders (and their morphotypes), surface feeders, ambushers, stationary suspension and graspers. The trophic level was considered to be herbivory or omnivory.

In general, the functional diversity showed the same pattern in both estuaries. During the dry season, the trophic level of the community was herbivory, the feeding type was mainly ambush in the upper zones and stationary suspension in the downstream zones, and the species presented estuarine, coastal and oceanic distribution (Figure 3). In the rainy season, the trophic level varied between omnivory and herbivory, without clear division between zones, and filtering species were present essentially in the upper zones. The freshwater species were, as expected, located in the upper zones and the estuarine and costal species in downstream zones; the species with oceanic distribution were completely absent (Figure 3).



	Zone I	Zone II	Zone III	Zone IV
- Ind. Biomass	3.124 µgDW	3.308 µgDW	2.141 µgDW	2.236 µgDW
- Length	794.353 µm	835.151 µm	731.355 µm	815.069 µm
- Inshore/Offshore Distribution	Coastal-Oceanic	Coastal-Oceanic	Coastal-Oceanic	Estuarine-Coastal
- Feeding-type	Ambush	Ambush	Ambush	StationarySuspension
- Trophic level	Herbivore	Herbivore	Herbivore	Herbivore

	Zone I	Zone II	Zone III	Zone IV
- Ind. Biomass	3.124 µgDW	2.429 µgDW	2.906 µgDW	3.517 µgDW
- Length	741.190 µm	700.650 µm	764.762 µm	799.750 µm
- Inshore/Offshore Distribution	Estuarine	Estuarine-Coastal	Estuarine-Coastal	Estuarine-Coastal
- Feeding-type	Ambush	StarionarySuspension	StarionarySuspension	Ambush-StarionarySuspension
- Trophic level	Herbivore	Herbivore	Herbivore	Herbivore

	Zone I	Zone II	Zone III	Zone IV
- Ind. Biomass	4.771 µgDW	4.716 µgDW	2.028 µgDW	1.701 µgDW
- Length	898.425 µm	900.432 µm	715.481 µm	785.861 µm
- Inshore/Offshore Distribution	Freshwater	Estuarine	Estuarine	Estuarine-Coastal
- Feeding-type	Filtration	StationarySuspension	Ambush	StationarySuspension
- Trophic level	Omnivore	Omnivore	Herbivore	Herbivore

	Zone I	Zone II	Zone III	Zone IV
- Ind. Biomass	1.825 µgDW	1.636 µgDW	2.212 µgDW	2.251 µgDW
- Length	446.085 µm	657.068 µm	690.244 µm	708.565 µm
- Inshore/Offshore Distribution	Freshwater	Estuarine	Estuarine	Estuarine
- Feeding-type	Filtration	Ambush	Ambush	Ambush
- Trophic level	Herbivore	Herbivore	Herbivore	Herbivore

Figure 3. Community-level weighted means (CWM) that represent the functional composition (functional identity) of zooplankton community in each zone of the Mamanguape and Paraíba do Norte estuaries, in the dry and rainy seasons.

In both estuaries, the functional richness was higher in the rainy season than in the dry season ($F_{MA} = 40.23$; $p < 0.01$, $F_{PB} = 26.13$; $p < 0.05$), but did not differ between zones (F_{MA} =

2.59; $p > 0.05$, $F_{PB} = 2.12$; $p > 0.05$) (Figure 4). In the two estuaries, the functional evenness did not differ between seasons ($F_{MA} = 1.72$; $p > 0.05$, $F_{PB} = 0.42$; $p > 0.05$) and between zones ($F_{MA} = 1.68$; $p > 0.05$, $F_{PB} = 2.47$; $p > 0.05$). In the Mamanguape, neither season ($F_{MA} = 1.35$; $p > 0.05$) nor zones ($F_{MA} = 1.39$; $p > 0.05$) had significant differences in functional divergence, whereas in the Paraíba do Norte estuary, during the dry season, we observed higher values of functional divergence ($F_{PB} = 13.65$; $p < 0.01$). In this estuary, significant differences between zones ($F_{PB} = 5.99$; $p < 0.05$) were only observed during the rainy season, with higher values in upstream zones. In both estuaries, the functional dispersion was higher in the dry season ($F_{MA} = 43.21$; $p < 0.01$, $F_{PB} = 11.72$; $p < 0.01$) but we did not observed significant differences between zones ($F_{MA} = 0.59$; $p > 0.05$, $F_{PB} = 2.44$; $p > 0.05$) (Figure 4).

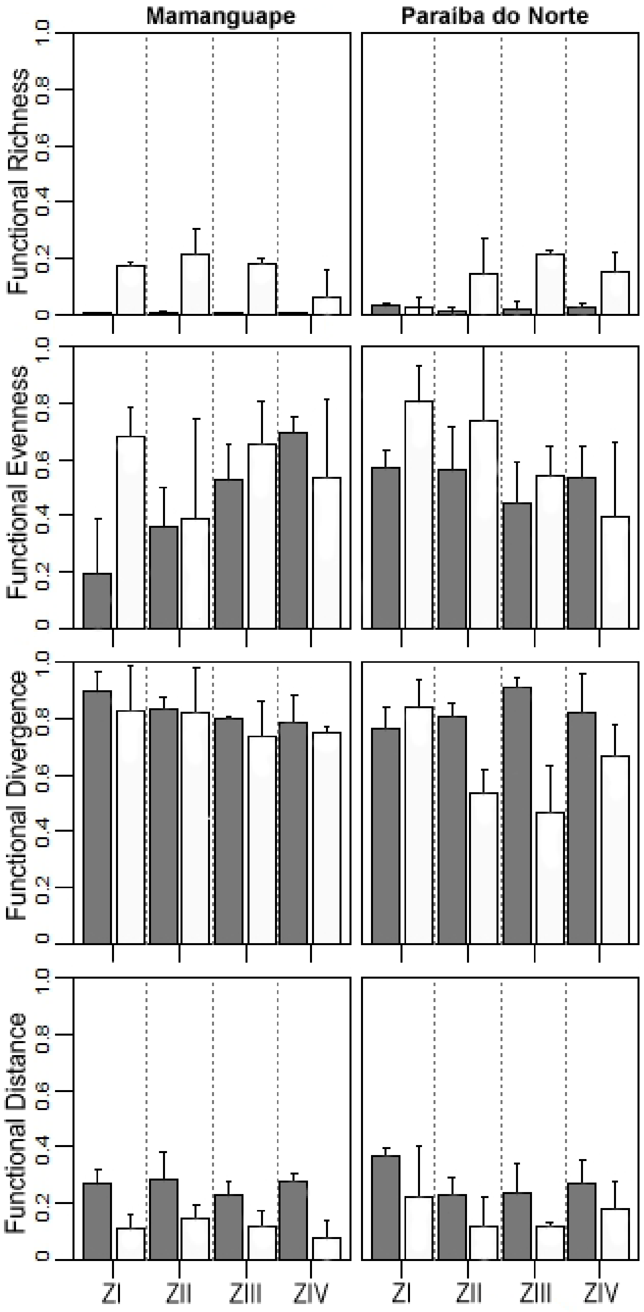


Figure 4. Functional richness, evenness, divergence and distance in different seasons (dry season = grey bars; rainy season = white bars) in the four zones of the Mamanguape and Paraíba do Norte estuaries. Data presented as mean ± SD.

3.4 Taxonomic diversity

In the Mamanguape estuary, a total of 17 species, belonging to nine genera, eight families, five orders, three classes and three phyla were found during the dry season. In the rainy season, the richness was higher, with 31 species grouped in 18 genera, 16 families, six orders, four classes and three phyla. A higher richness during the rainy season was also observed in the Paraíba do Norte estuary, with 22 species, belonging to 13 genera, 10 families, five orders, three classes and three phyla. In this estuary, during the dry season, we identified 18 species belonging to nine genera, eight families, five orders, three classes and three phyla.

In both seasons in the Mamanguape estuary we observed higher species richness in the upstream zones, where the community comprised species of different genera and families (Figure 5). Contrarily, the communities of the other two zones were composed by species of the same genus and family. This higher genetic diversity in the upstream areas favoured the increase of functional diversity once species without closely phylogenetic relatedness will not share exactly the same ecological traits due to the evolutionary conservation of characters. In the Paraíba do Norte, we observed the inverse pattern, with higher genetic diversity occurring in the more downstream zones of the estuary.

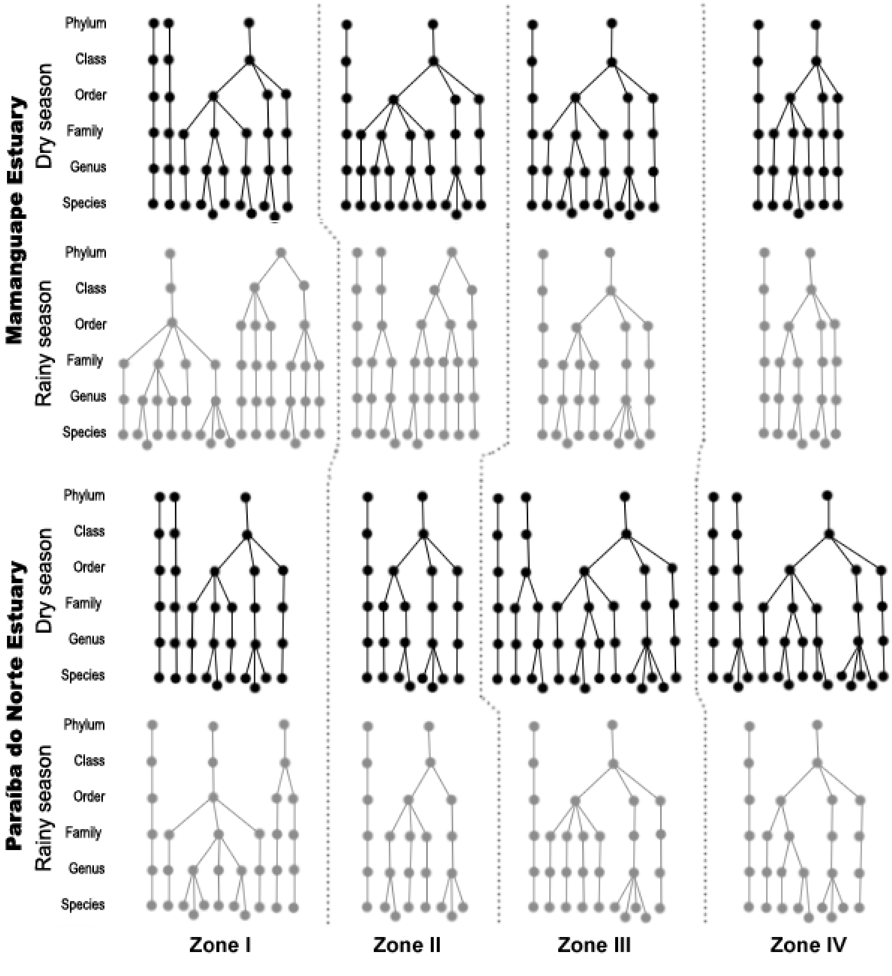


Figure 5. Hierarchical Linnean classification of the zooplankton community in time and space in the Mamanguape and Paraíba do Norte estuaries.

In the Mamanguape estuary, we did not observe differences in the Taxonomic Diversity (Δ) between season ($F_{MA} = 1.76$; $p > 0.05$) and zones ($F_{MA} = 1.25$; $p > 0.05$). However, we detected high values in the upstream zone during the rainy season due to the increase of species richness in zone I (Figure 6). In the Paraíba do Norte estuary, this index differed significantly between seasons ($F_{PB} = 23.80$; $p < 0.01$) but not between zones ($F_{PB} = 1.61$; $p > 0.05$). In the Mamanguape estuary, we only detected significant differences in Taxonomic Distinctness (Δ*) between zones during the rainy season ($F_{MA} = 4.63$; $p < 0.05$), with higher values in upstream zones, moreover the effect of seasons was not observed ($F_{MA} = 2.36$; $p > 0.05$). In the Paraiba do Norte, the Taxonomic Distinctness was not statistically different between seasons ($F_{PB} = 0.99$; $p > 0.05$) or zones ($F_{PB} = 0.92$; $p > 0.05$) (Figure 7). Regarding the Variation in Taxonomic Distinctness (Λ+), in Mamanguape estuary, the index showed significant differences between seasons ($F_{MA} = 6.16$, $p < 0.05$) and zones ($F_{MA} = 7.04$, $p < 0.01$), mainly caused by the lower values observed in the downstream zone. In the Paraíba do Norte there were no significant differences between seasons ($F_{PB} = 1.01$; $p > 0.05$) and zones ($F_{PB} = 1.16$; $p > 0.05$). When we calculated the Average Taxonomic Distinctness for presence/absence (Δ+), removing the effects of biomass in the index, in both estuaries we did not find significant differences between seasons ($F_{MA} = 0.06$; $p > 0.05$, $F_{PB} = 2.27$; $p > 0.05$) or zones ($F_{MA} = 1.62$; $p > 0.05$, $F_{PB} = 2.91$; $p > 0.05$). In both estuaries, the Total Taxonomic Distinctness (sΔ+) was significantly different between seasons ($F_{MA} = 0.12$; $p > 0.05$, $F_{PB} = 3.17$; $p > 0.05$) but differences between the zones was observed only in the Mamanguape, mainly due to the higher values of the index in the upstream zone during the rainy season ($F_{MA} = 5.88$; $p < 0.01$) (Figure 6).

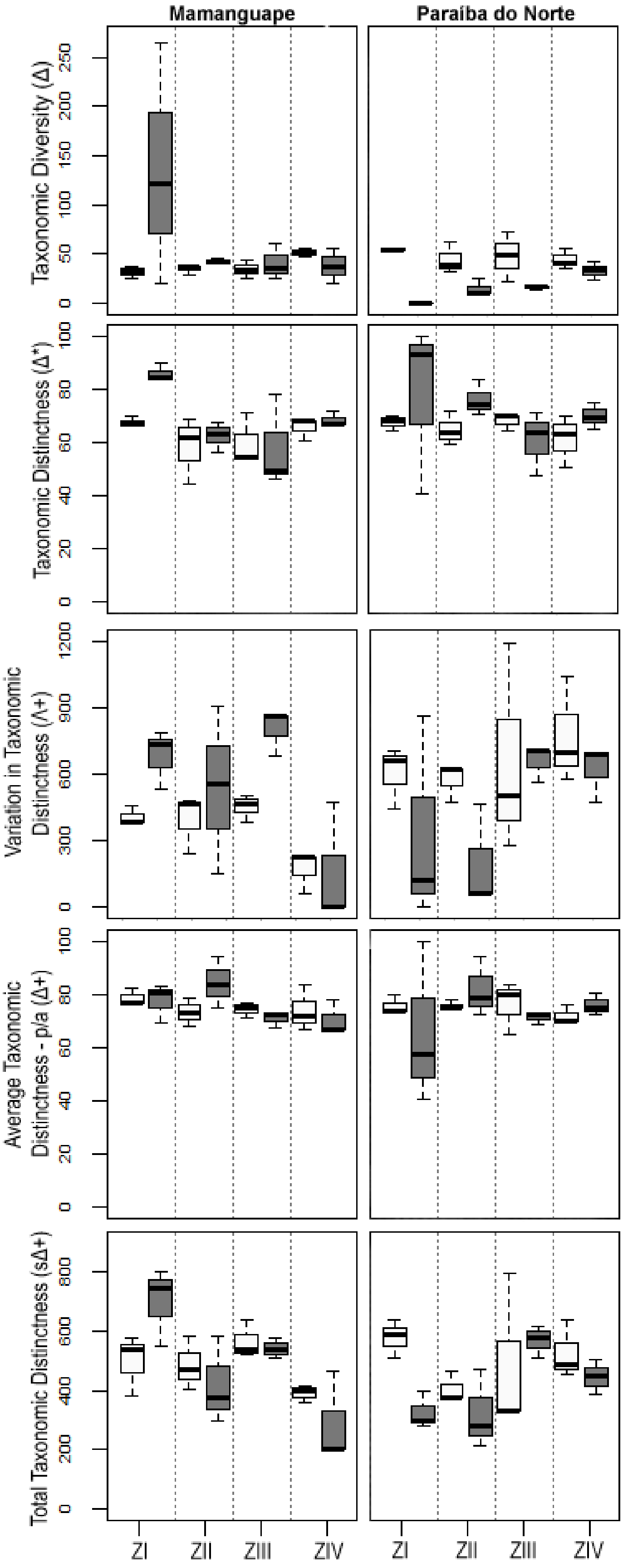


Figure 6: Temporal and spatial variations of the taxonomic diversity indices in the Mamanguape and Paraíba do Norte estuaries, per zone and season (dry season = white bars; rainy season = grey bars).

3.5 – Partitioning of Biomass variation

For the Mamanguape, during the dry season, our model was able to explain 77% of the total variation of zooplankton biomass. The greatest variation in zooplankton biomass was explained by the estuarine connectivity component and by the combination of estuarine connectivity/environment/taxonomic diversity (Figure 7A). The best estuarine connectivity model (R^2_{adj} = 0.78), with x = 0.0 and y = 0.2, retained three variables and predicted 38% (R^2_{adj}) of the zooplankton biomass distribution. Four environmental variables (Temperature, SRP, TP and Chl *a*) were retained in the RDA model explaining the biomass of zooplankton community. The above variables predicted 26% (R^2_{adj}) of the zooplankton biomass distribution. Two indices of Taxonomic Diversity were also retained (Δ and Λ^+) in the model and predicted 21% (R^2_{adj}). Only 10% was explained by the combination of connectivity/taxonomic diversity. Regarding the rainy season, our model was able to explain 94% of the total variation of the zooplankton biomass distribution. Like in the dry season, the estuarine connectivity was the most important component to explain the zooplankton biomass distribution, followed by the environmental component (Figure 7A). This means that estuarine connectivity was the most important component to be considered when explaining the zooplankton biomass distribution. The best connectivity model (R^2_{adj} = 0.94), with x = 0.4 and y = 0.1, retained three variables and predicted 46% of the variation in zooplankton biomass. The set of environmental variables retained were Temperature, Conductivity, Turbidity and TDS, explaining 36% of the variation. Three indices of Taxonomic Diversity were selected (Δ*, Δ^+, Λ^+), yielding a determination coefficient of 27%. When combined the three taxonomic Diversity components explained only 13% of the variation.

For the Paraíba do Norte, our model explained 41% and 29%, of the total variation of the zooplankton biomass distribution in the dry and rainy seasons, respectively. In this system, the components used in the model were not sufficient to explain the zooplankton biomass distribution. Nevertheless, estuarine connectivity was an important component to explain the biomass distribution in both seasons (Figure 7A). The best connectivity model (R^2_{adj} = 0.49) of the dry season, with x = 0.5 and y = 0.7, retained two variables. The best model for the rainy season (R^2_{adj} = 0.28) with x = 0.9 and y = 0.6, retained three variables.

3.5 – Partitioning of variation of Functional Diversity

In the Mamanguape, our model for the dry season was able to explain 77% of the total variation of the functional diversity distribution. The greatest variation was explained by the combination of estuarine connectivity/environment/taxonomic diversity and by the estuarine connectivity component. The best estuarine connectivity model (R^2_{adj} = 0.76), with x = 0.0 and y = 0.6, retained two variables and predicted 47% (R^2_{adj}) of the functional diversity distribution. The environmental variables predicted 18% of the functional diversity distribution and the indices of taxonomic diversity predicted 19% of the variation. Our model for the rainy season explained 64% of the total variation of the functional diversity (Figure 7B). The greatest variation in functional diversity was explained by the taxonomic diversity (78%), followed by the estuarine connectivity. The best estuarine connectivity model (R^2_{adj} = 0.64), with x = 1.0 and y = 0.3, retained three variables and predicted 29% of the variation. Only 9% of the variation was explained by the combination estuarine connectivity/environment/taxonomic diversity.

For the Paraíba do Norte, our model was able to explain 19% and 64% of the total variation of functional diversity distribution in the dry and rainy seasons, respectively. In the dry season model, the components used were not sufficient to explain the functional diversity distribution in this estuary. In the dry season, the greatest variation in functional diversity was explained by the combination estuarine connectivity/taxonomic diversity component, being able to predict 16% of the variation in functional diversity distribution. The taxonomic diversity component was the second best predictor (14%). In the rainy season model, estuarine connectivity was the component able to explain the greatest variation in functional diversity (63%), followed by the environmental parameters, with 43%.

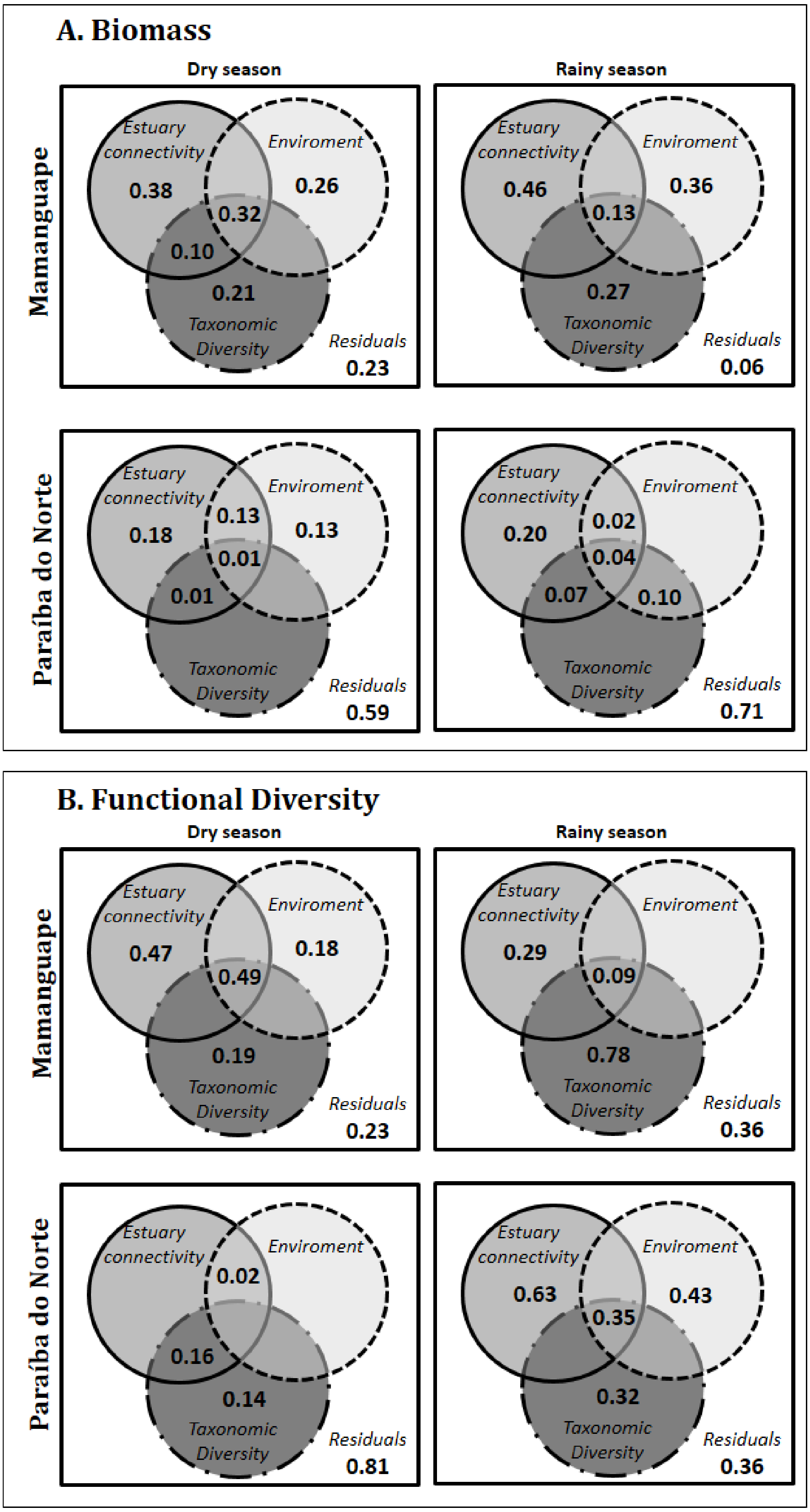


Figure 7. Partitioning of variation of (A) zooplankton biomass and (B) functional diversity distribution between “estuarine connectivity”, “local environment” and “Taxonomic Diversity” components. Values <0 not shown.

4. DISCUSSION

The identification of the mechanisms driving the variation within and among local assemblages is central to community ecology (Padial et al., 2014). Traditional ecological approaches have been focused on the link between community composition and environmental variables (e.g. Li et al., 2000; Li et al., 2006; Elliott et al., 2012) and assumed that spatial processes are not particularly important (Kneitel and Chase, 2004). However, studies showed that when included, spatial position has a higher explanatory power than water mass characteristics (e.g. Irigoien et al., 2010) and their results challenge previous interpretations of the species distribution where procedures to select and discard explanatory factors were not used and position was not included as an explanatory factor (see for example,. Albaina and Irigoien, 2007). Therefore considering only the local environmental conditions might not be sufficient to fully explain the structure of the zooplankton communities (e.g. Padial et al., 2014).

To contribute for the clarification of this topic, we investigated the drivers of change in zooplankton biomass and functional diversity in tropical estuaries. For the first time we compared the joint contribution of connectivity, phylogenetic relationship and local environmental factors in explaining the zooplankton spatial and temporal variation. To elucidate the effects of these factors, we used a complementary set of diversity indices that were reported to correlate more strongly with ecosystem properties than traditional measures of species diversity (Clarke and Warwick, 1998, 2001; Barnett and Beisner 2006). We selected two estuaries, located less than 100 km apart from each other and in the same climatic region, but with different levels and types of anthropogenic disturbance. The Mamanguape estuary is located in a protected area with less than 100,000 inhabitants, while the Paraiba do Norte in its terminal section runs mainly through an urban area, with approximately 1,000,000 inhabitants.

Our results show that, in general, despite having different environmental conditions, distinct zooplankton communities, with different functional traits, in both estuaries, regardless the season, estuarine connectivity was the factor that explained most of the variation in biomass and functional diversity. In the Mamanguape estuary, local environmental parameters, estuarine connectivity and phylogenetic relatedness are all important factors. Noteworthy, however, is the unexpected finding that the local environmental variables (e.g. temperature and salinity) had a very poor explanatory power, contrary to what is commonly expected (Kneitel and Chase, 2004; Albania and Irigoien, 2007). In both seasons, the estuarine connectivity (dispersal ability) explained better the distribution of biomass and functional diversity of zooplankton community. Our results indicate that spatial position, freshwater inflow and coastal influence are the main factors determining the zooplankton biomass and functional diversity of tropical estuarine systems. During the rainy season, the high freshwater inflow (i.e. high influence of the river basin) changes dramatically the environmental conditions (namely, the water salinity and nutrients discharge) and promotes the transport of plankton. During the wet period, the trophic level of the species varied between herbivory and onmivory and species with oceanic distribution were completely absent from the estuaries. In the dry period, the precipitation is low or almost absent and, consequently the effect of the freshwater input decreases markedly. In this season, the coastal effect is stronger, increasing the extension of the saline intrusion and promoting changes both in structure, biomass and functional identity that were expressed as communities dominated by herbivores, ambusher feeding type in the upper zones and stationary suspension feeding type in the downstream areas, with estuarine-coastal and oceanic distribution. Although several studies, where the spatial processes are not used as explanatory factors, advocate the role of a set of environmental parameters and descriptors of water quality as the main drivers of the zooplankton distribution (e.g. Vidjack et al., 2012; Mirón et al., 2014) a growing number of other studies, both in tropical and temperate coastal and estuarine systems, are in line with our results. In these studies, the variability in river discharge, geographical position, physical forces (such as wind) and dispersal ability were the principal explanatory factors of the zooplankton distribution (e.g. Montoya-Maya and Strydom, 2009; Hwang et al., 2010; Costa et al., 2011; Irigoien et al., 2011; Cloern and Jassby, 2012; Muha et al., 2012).

Noteworthy, the three selected components (i.e. connectivity, phylogenetic relatedness and local environmental parameters) in our model for the Paraíba do Norte were not sufficient to explain with confidence neither the variation of the distribution of zooplankton biomass nor the functional diversity. This is well expressed by the high values of the residuals (i.e. unexplained fraction). This estuary is highly impacted, with high concentrations of nutrients, namely with DIN concentrations varying from around 500 to 4200 $\mu g L^{-1}$ and TP ranging from 150 to 750 $\mu g L^{-1}$. Apart from this high level of nutrients, the system receives huge loads of wastewater without primary and secondary treatment, pesticides and other toxic substances from the agriculture fields and aquaculture areas (Sassi, 1991). The terminal part of the estuary is regularly dredged to maintain the navigation channels. All these activities and uses may drastically change the patterns of the zooplankton community, similarly to what was observed in temperate estuaries (e.g. Dobson, 2000; Jaworski et al., 2007; Li et al., 2012). In our model, the percentage of unexpected to variation could be attributed stochastic processes or we might not have considered the factors that are really driving the changes in zooplankton biomass and functional diversity in the Paraiba do Norte estuary, a highly human-impacted estuary. From the six drivers referenced by Cloern and Jassby (2012) as the common agents of change in the world’s estuarine-coastal systems (i.e.consumption and diversion of freshwater, modification of sediment supply, introduction of non-native species, sewage input, environmental policy, and climate shifts). The sewage input and human modification of sediment supply are probably factors that also drive the distribution of biomass and functional diversity of zooplankton community of the Paraíba do Norte estuary..

Our results clearly showed that to assess the drivers of change of zooplankton biomass and functional diversity, besides the local environmental factors we have to use spatial modelling in order to integrate the dispersion ability of the species and the effects of the physical forces acting on the system. Moreover, further investigation is urgently necessary to clarify the driving factors shaping the zooplankton communities in very impacted tropical systems. Only with the knowledge of the main driving forces shaping the target community and system the appropriate management measures can be put in place. Otherwise we risk investing time and funds monitoring the wrong variables and implementing inappropriate conservation measures.

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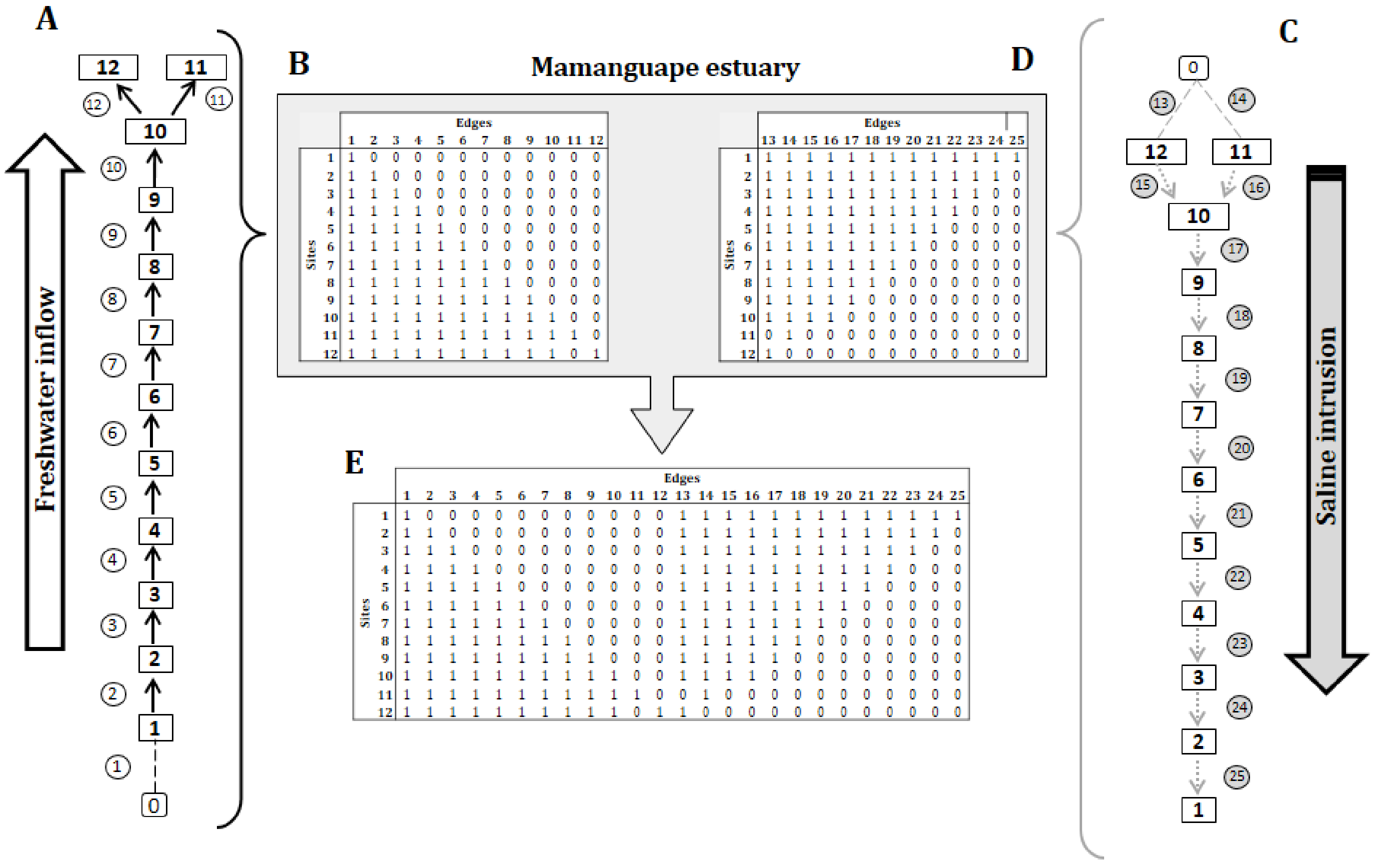
ELECTRONIC SUPPLEMENTARY MATERIAL

Table S1: Ecological traits used to compute multidimensional functional diversity (FD) indices. Sources are included in superscript numbers. * indicates traits derived at genus level. ** indicates traits derived at the family or genus level. Absence of asterisk indicates traits derived at species level, and absence of superscript number indicates traits set from personal observations. Traits derived from sampled zooplankton (†) and literature (‡).

Taxon				Ecological traits				
Taxonomic level	Family	Genus	Species	Mean dry weight† (µg)	Max length† (µm)	Inshore/Offshore Distribution‡	Feeding-type‡	Trophic level‡
Ciliophora	Ptychocylididae	*Favella*	*F.* sp.	0.114	188.19	Freshwater-Estuarine-Coastal[**8]	Filtration[**8]	Hervibore[**8]
Rotifera	Asplanchnidae	*Asplanchna*	*A.* sp.	0.834	299.36	Freshwater[5]	Filtration[**16]	Herbivore[**16]
	Brachionidae	*Brachionus*	*B. calyciflorus*	0.137	303.10	Freshwater[5]	Filtration[**16]	Herbivore[**16]
		Brachionus	*B. plicotilis*	0.085	201.75	Freshwater[5]	Filtration[**16]	Herbivore[**16]
		Brachionus	*B. quadridentatus*	0.084	156.43	Freshwater[5]	Filtration[**16]	Herbivore[**16]
		Brachionus	*B. patulus*	0.044	134.31	Freshwater[5]	Filtration[**16]	Herbivore[**16]
		Brachionus	*B. leydigi*	0.085	132.38	Freshwater[5]	Filtration[**16]	Herbivore[**16]
		Brachionus	*B. caudatus*	0.033	125.18	Freshwater[5]	Filtration[**16]	Herbivore[**16]
		Platyias	*P. quadricornis*	0.095	160.77	Freshwater[5]	Filtration[*18]	Herbivore[**16]
		Keratella	*K. tropica*	0.026	122.97	Freshwater[5]	Filtration[*16]	Herbivore[*16]
		Keratella	*K.* sp.	0.021	126.05	Freshwater[5]	Filtration[*16]	Herbivore[*16]
		Mytilina	*M. ventralis*	0.164	232.70	Freshwater[5]	Filtration[**16]	Herbivore[**16]
	Lecanidae	*Lecane*	*L. luna*	0.055	108.05	Freshwater[5]	Filtration[**16]	Herbivore[**16]
		Lecane	*L. bulla*	0.039	137.26	Freshwater[5]	Filtration[**16]	Herbivore[**16]
		Lecane	*L. leonina*	0.202	175.24	Freshwater[5]	Filtration[**16]	Herbivore[**16]
		Lecane	*L.* sp.	0.094	166.24	Freshwater[5]	Filtration[**16]	Herbivore[**16]
Cladocera	Chydoridae	*Alona*	*A. poppei*	0.870	417.80	Freshwater[5]	C-Filtration[*1]	Herbivore[*1]
		Leydigia	*L. ipojucae*	0.259	439.97	Freshwater[5]	C-Filtration[**2]	Herbivore[**2]
	Macrothricidae	*Macrothrix*	*M. mira*	0.950	498.47	Freshwater[5]	Filtration	Herbivore
		Macrothrix	*M.* sp.	0.288	275.76	Freshwater[5]	Filtration	Herbivore
	Sididae	*Diaphanosoma*	*D. spinulosum*	0.288	386.07	Freshwater[5]	S-Filtration[*2]	Herbivore[*3]
Calanoida	Acartidae	*Acartia*	*A. lillijerboji*	3.112	1178.13	Estuarine-Coastal[7]	Ambush-StationarySuspension[*6]	Omnivore[**4]
		Acartia	*A.* sp.	3.719	949.66	Estuarine-Coastal-Oceanic[*7]	Ambush-StationarySuspension[*6]	Omnivore[**4]
		Acartia	*A. tonsa*	2.024	939.08	Estuarine-Coastal-Oceanic[7]	Ambush-StationarySuspension[*6]	Omnivore[**4]
	Diaptomidae	*Notodiaptomus*	*N. iheringe*	7.226	1193.83	Freshwater[5]	StationarySuspension[**2]	Omnivore[**2]
	Paracalanidae	*Parvocalanus*	*P. crassirostris*	2.154	637.68	Coastal[7]	StationarySuspension[6]	Herbivore[9]
		Parvocalanus	*P. scotti*	3.912	794.76	Esturine-Coastal[7]	StationarySuspension[*6]	Herbivore[*9]

		Paracalanus	*P. campaneri*	2.923	791.71	Oceanic[7]	StationarySuspension[**6]	Herbivore[**9]
		Paracalanus	*P. nanus*	3.266	795.21	Oceanic[*7]	StationarySuspension[**6]	Herbivore[**9]
		Parvocalanus	*P.* sp.	3.077	749.072	Oceanic[*7]	StationarySuspension[*6]	Herbivore[**9]
	Pseudodiaptomidae	*Pseudodiaptomus*	*P. richardi*	8.651	1254.97	Estuarine[7]	Ambush-StationarySuspension	Omnivore[*15]
		Pseudodiaptomus	*P. marshi*	6.561	1147.32	Estuarine[7]	Ambush-StationarySuspension	Omnivore[*15]
		Pseudodiaptomus	*P. acutus*	8.257	1204.51	Estuarine[7]	Ambush-StationarySuspension	Omnivore[*15]
	Temoridae	*Temora*	*T. turbinata*	14.603	1329.89	Coastal-Oceanic[7]	StationarySuspension[6]	Herbivore[9]
Cyclopoida	Cyclopoidae	*Microcyclops*	*M. anceps*	1.956	658.87	Freshwater[5]	Grasping[*10]	Omnivore-Herbivore[**13]
	Oithonidae	*Oithona*	*O. brevicornis*	2.094	630.84	Estuarine[7]	Ambush[*6]	Herbivore[**9]
		Oithona	*O. robusta*	1.264	625.27	Coastal-Oceanic[7]	Ambush[*6]	Herbivore[**9]
		Oithona	*O. rigida*	1.089	583.50	Estuarine-Coastal[7]	Ambush[*6]	Herbivore[**9]
		Oithona	*O. vivida*	1.348	574.24	Coastal-Oceanic[7]	Ambush[*6]	Herbivore[**9]
		Oithona	*O. oculata*	1.882	629.63	Estuarine-Coastal[7]	Ambush[*6]	Herbivore[**9]
		Oithona	*O. hebes*	1.778	653.14	Estuarine-Coastal[7]	Ambush[*6]	Herbivore[**9]
		Oithona	*O.* sp.	1.071	591.75	Estuarine-Coastal-Oceanic[*7]	Ambush[*6]	Herbivore[**9]
Harpacticoida	Euterpinidae	*Euterpina*	*E. acutiforms*	1.227	753.20	Coastal[*7]	SurfaceFeeding[**4]	Herbivore[14]

[1] Fryer, 1968; **[2]** Barnett and Beisner, 2007; **[3]** Walseng et al., 2006; **[4]** Thorp and Covich, 2010; **[5]** Dumont et al., 1974; **[6]** Barton et al., 2013; **[7]** Boltovskoy, 1999; **[8]** Kazama et al., 2012; **[9]** Tafe and Griffiths, 1983; **[10]** Williamson and Reid, 2001; **[11]** Fryer, 1957; **[12]** Kouwenberg, 1994; **[13]** Jerling and Wooldridge, 1994; **[14]** Woods, 1993; **[15]** Stemberger, 1979; **[16]** Pourriot, 1977.



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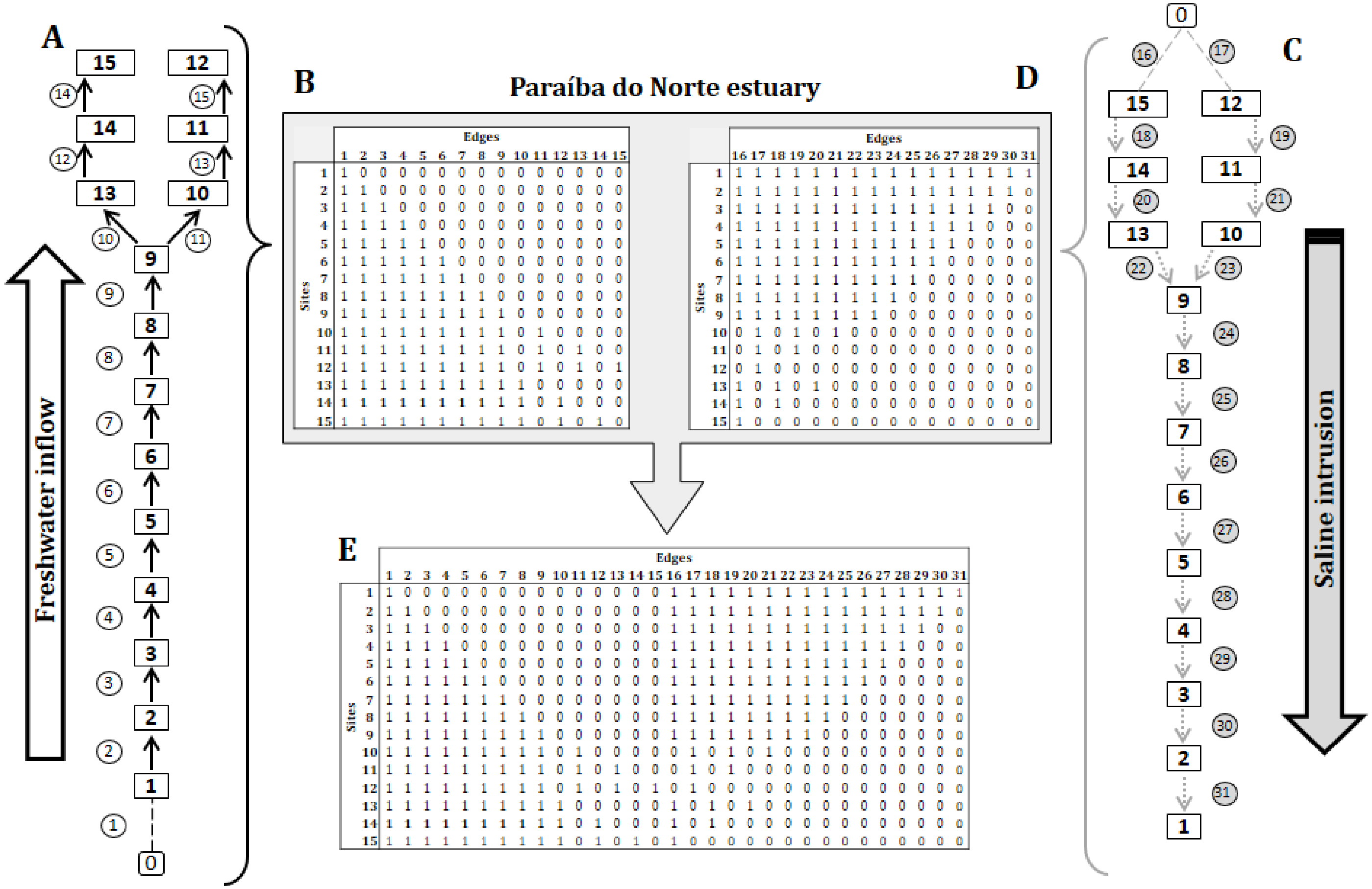

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D

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E

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Figure S1: Schematic representation describing how edges inverse to the main direction (river flow) should be handled in Mamanguape and Paraíba do Norte estuaries. (A) Sites linked by a connection diagram following the direction of the main spatial process (upstream > downstream). The resulting sites-by-edges matrix is presented in (B). The edges from downstream > upstream, inverse to the main direction of the spatial process, are considered in (C). (C) will yield the sites-by-edges matrix presented in (D). The columns of matrices (B), (D), are then bound to form matrix (E). The box represents the sites of study and the circle represents the edges.

Table S2: Influence of first / earlier stages and of dry and rainy seasons in the distribution models of rank-abundance of the zooplankton community of the estuaries of Paraíba do Norte and Mamanguape rivers. The relative abundance was performed using proportions to satisfy formatting requirements. The analysis were performed using the *radfit()* function in the "vegan" package (Oksanen et al. 2013) in R-3.1.1 Core Team (2014) graphical and statistical computing environment. The fit of all models (broken-stick, niche preemption, log-normal, Zipf and Mandelbrot-Zipf) of zooplankton relative abundance, was evaluated using Akaike's Information Criterion (AIC; Akaike 1974) where the best-fitting model is accorded to the lowest score.

	Mamanguape (dry season)				**Mamanguape (rainy season)**			
	With earlier stages		**Without earlier stages**		**With earlier stages**		**Without earlier stages**	
Model	*Deviance*	*AIC*	*Deviance*	*AIC*	*Deviance*	*AIC*	*Deviance*	*AIC*
Broken-stick	64.114	103.422	29.018	71.520	12.498	47.743	28.648	68.212
Niche Pre-emption	27.549	68.857	21.910	66.412	**4.097**	**41.343**	**6.489**	**48.053**
log-Normal	13.036	56.344	7.879	54.380	6.289	45.534	6.372	49.936
Zipf	**5.113**	**48.422**	**4.291**	**50.793**	9.977	49.222	7.795	51.359
Zipf–Mandelbrot	5.037	50.345	4.291	52.793	3.214	44.460	2.699	48.263

	Paraíba do Norte (dry season)				**Paraíba do Norte (rainy season)**			
	With earlier stages		**Without earlier stages**		**With earlier stages**		**Without earlier stages**	
Model	*Deviance*	*AIC*	*Deviance*	*AIC*	*Deviance*	*AIC*	*Deviance*	*AIC*
Broken-stick	58.3638	87.2012	65.857	109.049	42.3888	74.8317	17.1263	50.9486
Niche Preemption	19.4336	50.2711	38.504	83.696	13.6958	48.1388	2.7881	40.6103
log-Normal	9.202	42.0395	14.176	61.368	**3.6346**	**40.0775**	**4.0578**	**39.8801**
Zipf	**3.9359**	**36.7733**	**4.101**	**51.293**	7.3777	43.8206	5.8831	43.7054
Zipf–Mandelbrot	3.9359	38.7733	4.101	53.293	3.0701	41.5131	2.6767	42.499

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Segundo manuscrito à ser submetido à Ecological Indicators

Fatty acid profiles reveal seasonal and spatial shifts in the diet of copepods from two tropical estuaries

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ABSTRACT

Fatty acids are the main components of lipids and are necessary for the production and permeability of cell membranes, playing an essential role in the physiological responses of organisms. Being aware of the biochemical composition of the zooplankton is crucial to understand the processes of energy transfer and food quality in aquatic ecosystems. Zooplankton contributes to convert plant to animal food and serves as food source for higher trophic levels. For these reasons, fatty acids are often used as bioindicators of the trophic status of aquatic ecosystems. Currently, there are few studies covering this topic in tropical estuaries. Besides aiming to fill this gap of knowledge, the main aim of this study was to test if the fatty acid profiles can reveal spatial and temporal shifts in the diet of copepods and therefore can be used as indicators of the trophic status of estuarine systems. We investigated the fatty acids composition of copepod species and their possible food sources along the salinity gradient of two tropical estuaries (Paraíba do Norte and Mamanguape estuaries, Northeastern Brazil), during the rainy and dry seasons. We found clear seasonal differences regarding fatty acids composition and concentration in copepods, with maximal concentrations and diversity of total fatty acids during the rainy season. The copepods species were mainly carnivorous in the dry season and omnivorous in the rainy season and, in both estuaries, the diet of most copepods was dependent on variability of food availability. The fatty acid profiles suggest that, in general, feeding patterns of zooplankton change spatially and temporally, reflecting the shifts in their food sources abundance (i.e. dominance among diatoms and flagellates, terrestrial detritus and small animals). We observed a residual proportion of terrestrial detritus and green algae in the diets and these items were only present in the dry season. Furthermore, the food sources in the Paraiba do Norte estuary, a system with high levels of anthropogenic disturbance, showed lower feeding quality, with lower essential fatty acids concentrations. Our study showed that fatty acid profiles can be used to reveal seasonal and spatial shifts in the trophic ecology of copepods in tropical estuaries.

KEYWORDS: Zooplankton; feeding ecology; food quality; bioindicator; Brazil

HIGHLIGHTS

- The food sources in the highly impacted Paraiba estuary have lower feeding quality
- The diet of most copepods was dependent on variability of food availability
- Fatty acids presented higher abundance and diversity in the rainy season
- Fatty acids reveal seasonal and spatial shifts in the diet of copepods
- FA profiles are a good indicator to assess the trophic status of tropical estuaries

1. INTRODUCTION

Estuaries are located at the interface between the continental and marine domains and represent one of the most productive ecosystems worldwide (David et al., 2005; Isari et al., 2007). This productivity is associated with peculiar characteristics, such as the constant environmental fluctuations to which these transitional ecosystems are subjected (e.g. changes in the marine and freshwater influxes, accumulation of organic matter and nutrients that stimulate productivity, etc). Plankton (and mainly zooplankton) is known to be particularly sensitive to these variations because it is strongly influenced by climatic features and changes in hydrological conditions (Beaugrand et al., 2000; Ara, 2001). Zooplankton play a crucial role in converting plant food to animal food source, and are in turn fed upon by organisms from higher trophic levels, which gives them a key position and a considerable trophic importance. Indeed, some authors (e.g. Borja et al., 2009) have emphasized its potential as an aquatic bioindicator group of trophic status in terms of water quality. Copepods are the dominant group of zooplankton and are capable of utilizing a wide range of diets (Kleppel, 1993), playing a key role in the food web as they form a link between primary producers and secondary consumers (Richmond et al., 2007; Guschina and Harwood, 2009). The trophic flexibility of this group of organisms can act as a stabilizing force in aquatic ecosystems affecting the food chain length, and may change the quality of food available to higher consumers due to the quality of their diet (Sprules and Bowerman, 1988). Indeed, some studies in the literature reveal a direct role of phosphorous limitation on the zooplankton diet clearly demonstrating how the food quality for zooplankton affects its consumers, especially with regard to the polyunsaturated fatty acids (PUFA) (Gulati and DeMott 1997; Hartwich et al., 2013; McMeans et al., 2015). Shape and size of the food particles, food selectivity, feeding inhibition and ingestion rates, morphological defenses against digestion, nutritional inadequacy (mainly reflected in N, P and fatty acids contents) and the presence of toxins may affect zooplankton growth and reproduction and thus its consumers (Gulati and Demott 1997; Post and Takimoto, 2007; Galloway et al., 2014). Growth, health and reproduction of fish and other aquatic animals are primarily dependent upon an adequate supply of nutrients, both in terms of quantity and quality, which is closely related with the quality of food resources and the environment (Arts et al., 2001; Brett et al., 2009).

Studies on food web dynamics may provide important information to understand organisms’ baseline ecology, predict community-level consequences of abiotic and biotic changes and characterize trophic interactions (Gonçalves et al., 2012; Galloway et al., 2014). Knowledge on the biochemical composition of the zooplankton communities has become essential to understand their physiological functions, metabolism and nutritive value, as this is very relevant for the energy transfer in aquatic ecosystems (Vengadeshperumal et al., 2010; McMeans et al., 2015).

Fatty acids (FA) are one of the most important molecules transferred across the plant-animal interface in aquatic food webs (Dalsgaard et al., 2003; Allan et al., 2010;

Gonçalves et al., 2012), being claimed to be a good indicator of ecosystem health (Maazouziet al., 2008; Ramírez et al., 2013) and an indicator of stress (Sanchez-Muros et al., 2013; Neves et al., 2015). Thus, in recent decades, the interest in fatty acid composition of aquatic organisms has increased and studies *in situ* and in the laboratory have been developed (Perga et al., 2006; Bell et al., 2006; Vengadeshperumal et al., 2010; Neves et al., 2015). Many of these studies used the fatty acid trophic markers (FATMS) to identify specific food trophic relationships as they provide time-integrated information on an organism's assimilated diet (El-Sabaawi et al., 2009; Gonçalves et al., 2012; Hartwich et al., 2012). The use of FATMs is based on the premise that phytoplankton, microzooplankton, and bacteria all produce taxon-specific FA which are retained by their predators, and could be then used to qualitatively assess relative trophic positions and dietary quality (Dalsgaard et al., 2003). Indeed, ratios of FA have been used as a bioindicator to distinguish and signaling feed dietary proportions of FA within the organism and relating them with the balance required for optimal structure and/or function (Arts et al., 2009).

Currently, the studies reported in the literature refer mainly to temperate systems, being scarce the knowledge of trophic dynamics of the zooplankton community in tropical estuaries, particularly regarding the biochemical processes (e.g. fatty acid profiles) and seasonal variations *in situ*. Up-to-date tracing techniques such as fatty acid profiling can contribute to answer questions related with global changes, such as, for example, how structural changes in species composition are linked to functional changes or to species' response to environmental changes. Thus, the use of biomarkers to assess the effects of different stressors (natural and/or anthropogenic) on biochemical processes that govern organismal health and fitness in complex ecosystems might provide much more relevant information than other indirect measurements alone (Fleeger et al., 2003; Neves et al., 2015). Environmental stressors interfere with sub-organismal constituents such as cells and tissues, therefore, biochemical levels are sensitive and quick-responding indicators to stressors (Adams & Greeley, 2000). Therefore, a better knowledge of the ecosystem functioning, particularly regarding the trophic dynamic, is crucial to predict the potential impact and threats of future environmental changes in aquatic communities.

Besides aiming to fill the gap of knowledge regarding the zooplankton trophic dynamics in tropical systems, the main aim of this study was to test if the FA profiles of copepods can reveal spatial and temporal shifts in the diet of copepods and therefore can be used as indicators of the trophic status of estuarine systems, which may in turn reflect environmental and/or anthropogenic variations. We investigated the FA composition of copepod species and their possible food sources along the salinity gradient of two tropical estuaries (Paraíba do Norte and Mamanguape estuaries, Northeastern Brazil), during the rainy and dry seasons. Zooplankton species were characterized in terms of FA in order to 1) examine the dietary preferences of copepods species in relation to potential food sources and 2) to analyze spatial and seasonal patterns in the FA profiles. The central hypothesis of our study is that the FA profile of copepods can reveal seasonal and spatial shifts in their diet. We expect distinct FA profiles in the rainy and dry periods and along the salinity gradient of each estuary.

2. MATERIALS AND METHODS

2.1 Study site

The study was developed in two transitional systems located in the Northeast of Brazil - Paraíba do Norte and Mamanguape estuaries (Figure 1). The climate of the region is AS’ type according to Köppen, i.e. hot and wet. Precipitation varies from 2000 mm/season to less than 30 mm/season. The rainy season starts in February and ends in July, with higher precipitation from April to June, while the dry season is from August to January, with minimum precipitation from October to December. The mean annual of water temperature is between 24 ºC and 26 ºC.

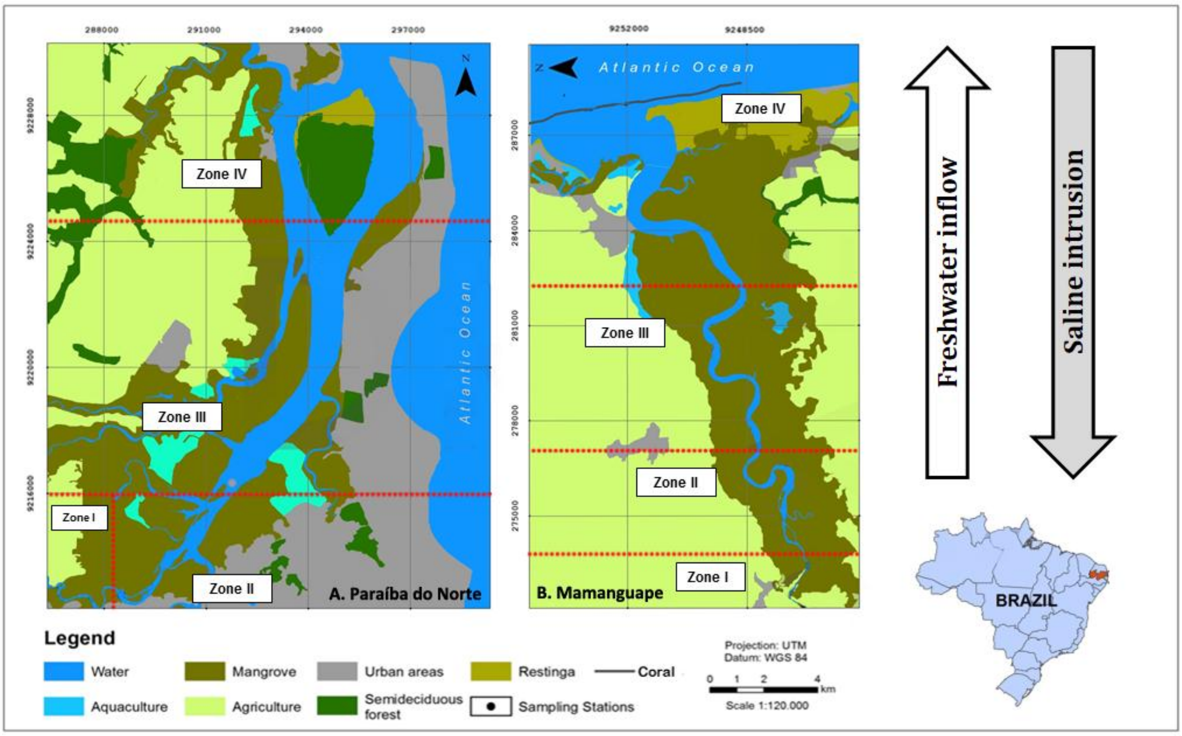

Figure 1. Study areas. A. Paraíba do Norte estuary and B. Mamanguape estuary. The red lines represent the sampling zones (Author: S. Vital).

The Paraíba do Norte estuary (Figure 1A) is located mainly in an urban area, with approximately 1,000,000 inhabitants. In the vicinity of the estuary there are also agriculture fields, aquaculture areas and the Cabedelo harbor. The estuary is approximately 22 km long and the river mouth is 2.2 km wide. The estuary reveals typical features of a river subjected to a regime of medium flow, allowing the formation of small dunes and with a mean depth of three meters, except near the harbor, where it has 11 meters.

The Mamanguape estuary (Figure 1B), after the Paraíba do Norte estuary, is the second largest estuary in the state of Paraíba. The estuary is located inside an environmental protection area (“APA”), whose main goal is to protect the coastal habitats and the marine manatee *Trichechus manatus* Linnaeus, 1758. The mouth of the river forms a bay six km wide that is nearly closed by a coastal reef line, which results in calm and quiet waters. The estuary has well-preserved mangroves composed mainly by *Rhizophora mangle, Avicennia schaueriana, Avicennia germinans, Laguncularia racemosa,* and *Conocarpus erectus* that grow around the main channel and the intertidal creeks, comprising approximately 6,000 hectares, in addition to the remnants of the Atlantic rainforest (Rocha et al., 2008). On the edge of the mangrove area there are extensive sugar cane fields and aquaculture areas dedicated to crustacean production. The riverside community is about of 66,000 inhabitants.

2.2 Sample collection and laboratory analyses

To test our study hypothesis, in each estuary, we carried out two sampling campaigns: one in the rainy season (July 2014) and the other during the dry season (December 2014). In each estuary, samples were collected in four subtidal zones along the estuarine gradient (Figure 1), during the high tide of the full moon. These zones were previously defined taking into consideration: water salinity, sediment granulometry and depth. In each subtidal zone, three replicates were collected.

Samples were collected by horizontal subsurface tows (mesh size 68 µm, mouth diameter: 0.3 m). Zooplankton samples were brought to the laboratory, sorted alive at species level, concentrated on GF/F Whatman filters (25 mm diameter) and stored in nitrogen liquid in eppendorfs. For each species, three replicates containing a minimum of 150 individuals each were prepared. To quantify potential food sources present in the water column, in each season and zones we collected water samples (1L) and filtered them on GF/F Whatman filters (25 mm diameter).

As proxy of the zooplankton community, to perform the FA analysis, we selected the copepod species that represented more than 30% of the abundance value in each sampling zones. In the Paraíba do Norte estuary we selected the species: *Paracalanus campaneri* (Giesbrecht, 1889), *Acartia lilljeborgi* (Björnberg, 1982), *Pseudodiaptomus richardi* (Dahl F., 1894), *Oithona* sp., *Oithona brevicornis* (Giesbrecht, 1891), and *Euterpina acutifrons* (Dana, 1847) and in the Mamanguape estuary we selected the species: *Pseudodiaptomus marshi* (Wright, 1936), *A. lilljeborgi*, *Paracalanus crassirostris* (Dahl, 1894), *Parvocalanus scotti* (Früchtl, 1923), *Oithona* sp., *O. brevicornis* (Giesbrecht, 1891) and *E. acutifrons* (Dana, 1847).

The extraction of total lipids of food sources, copepods and methylation to fatty acid methyl esters (FAMEs) was achieved by a modified one step derivatisation method, after Gonçalves et al. (2012). The boron trifluoride-methanol reagent was replaced by a 2.5% H_2SO_4-methanol solution since BF_3-methanol can cause artefacts or loss of PUFAs (Eder, 1995). The fatty acid Methylnonadecanoate C19:0 was added as an internal standard for the quantification (Fluka 74208). Samples were then centrifuged in a Thermo Scientific Heraeus Megafuge 16R, stored and frozen in new vials. The FAMEs obtained were separated and quantified using gas chromatography (GC) Thermo Scientific Trace 1300 coupled with a flame ionization detector (FID). All samples were run in splitless mode, with a 5µL injection per run, using a biodiesel for FAME column (60 m × 0.250 mm × 0.20 µm) with He flow rate of 0.6 ml/min. The column temperature was set at 120ºC and then programmed to increase up to 240ºC at a ratio of 4ºC/min. The detector and injector were set at 250ºC.

FAMEs were identified by comparison with the retention times and mass spectra of authentic standards and available ion spectra in WILEY mass spectral libraries. Quantification of individual FAMEs was accomplished by the use of external standards (Supelco™ 37 Component FAME Mix, Supelco # 47885, SigmaAldrich Inc., USA). The quantification function of each FAME was obtained by linear regression applied to the chromatographic peak areas and corresponding known concentrations of the standards (ranging from 5 to 250 $mg.ml^{-1}$).

2.3 Fatty acid trophic markers (FATMS)

Fatty acid ratios were calculated and used as biomarkers based on El-Sabaawi et al. (2009) and Dalsgaard et al. (2003) to inspect whether animal, bacteria or algae class ratios were maintained in the lipid extracts of copepods species thus reflecting their trophic position and dietary quality. The trophic and dietary tracers used in this study are summarized in Table 1 (for further details see Table 1 in El-Sabaawi et al., 2009).

Table 1. Trophic and dietary fatty acid markers used in this study.

Marker	**Formula**	**Source**	**Reference**
DHA/EPA	22:6(n-3)/20:5(n-3)	Dinoflagellates/diatoms, carnivory	Budge and Parrish, 1998
18:2(n-6)	-	Terrestrial detritus or green algae	Dalsgaard et al., 2003
PUFA/SFA	(sum of all polyunsaturated fatty acids)/ (sum of all saturated fatty acids)	Carnivory	Stevens et al., 2004
D/F	(16PUFA + 16:1(n-7) + 20:5(n-3) / (18PUFA + 18:2(n-6) + 22:6(n-3)[a]	Diatoms/flagellates	Adapted from Dalsgaard et al., 2003

[a] 16PUFA includes all PUFA containing 16 carbon atoms, and 18PUFA includes all PUFA containing 18 carbon atoms.

Typically, carnivorous zooplankton shows higher quantities of polar lipids (rich in PUFA) than herbivorous crustaceans. Thus, the ratio PUFA/SFA (saturated fatty acids) denote carnivory in copepods (Cripps and Atkinson, 2000). Another index used to determine the degree of carnivory is the ratio DHA/EPA (eicosapentaenoic acid, 20:5(n-3)) (Dalsgaard et al., 2003). DHA (docosahexaenoic acid, 22:6(n-3)) is highly conserved in food webs, as it is an important component of polar lipids (Scott et al., 2002). Thus, the ratio DHA/EPA should increase towards higher trophic levels. This ratio may also reflect the proportion of dinoflagellates and diatoms in the diets of omnivorous and herbivorous organisms, as DHA is often dominant in dinoflagellates, whereas EPA is mainly found in diatoms (Dalsgaard et al., 2003). The proportion of all diatom markers (D=16PUFA+16:1n7+20:5n3) to all flagellate markers (F=18PUFA+18:2n6+22:6n3), D/F, is used to distinguish between diatom and dinoflagellate-based diet (El-Sabaawi et al., 2009). High proportions of C18:2n-6 denote the presence of terrestrial detritus or green algae in the zooplankton diet (Dalsgaard et al., 2003).

2.4 Data analysis

The FA profiles of copepods were reported over a spatio-temporal scale, by determining total (mg.ind^{-1}) or relative (%) fatty acid concentrations. To examine the variation in FA composition among zones and seasons in each estuary the data of food source and copepods species were transformed using log(x+1) and converted into similarity triangular matrices using the Bray-Curtis resemblance measure and we run a two-way analysis of similarity (ANOSIM - two-way: “season” and “zone”). The distance between samples was represented using non-metric multidimensional (n-MDS) plots and the stress values of each representation are shown. The contribution of individual FAs to similarities and dissimilarities within and between sample groups (zone × season) were tested using the similarity percentage analysis routine (SIMPER). These multivariate analyses were done using the PRIMER 6 software package (Clarke and Gorley, 2006).

To highlight any seasonal, spatial or interspecific pattern of the copepods diet we ran a Principal Component Analysis (PCA) on fatty acid trophic markers (FATMs), using the “*vegan”* package (Oksanem et al., 2015) of R Core Team (2014).

3. RESULTS

3.1 Food sources present in the water column

In both estuaries and seasons, the FA profile of the food sources present in the water column samples were mainly composed by PUFA (polyunsaturated fatty acids) and HUFA (highly unsaturated fatty acids) (Figure 2; Table S1). Moreover, the samples of the Mamanguape estuary showed higher concentration and diversity of PUFA and HUFA than the ones of the Paraíba do Norte estuary. In both estuaries, SFA was the group with the lowest relative concentration and the lowest diversity of FA. During the dry season, C24:1(n-9), C20:2(cis-11,14), C22:2(cis-13,16), EPA, DHA and ARA (eicosatetraenoic acid, 20:4(n-6)) were abundant in the food sources collected in the water column of the Paraíba do Norte. In the Mamanguape estuary, during the dry season, ARA was the FA with the highest concentration, followed by C24:1(n-9). In the same estuary, during the rainy season, DHA (C22:6(n-3)) was the most abundant FA, followed by C20:2(cis-11,14). In the Paraíba do Norte, during the rainy season, besides the two FA just mentioned (DHA and C20:2(cis-11,14)), the C24:1(n-9) and EPA showed also high relative concentrations in the food sources present in the water column.

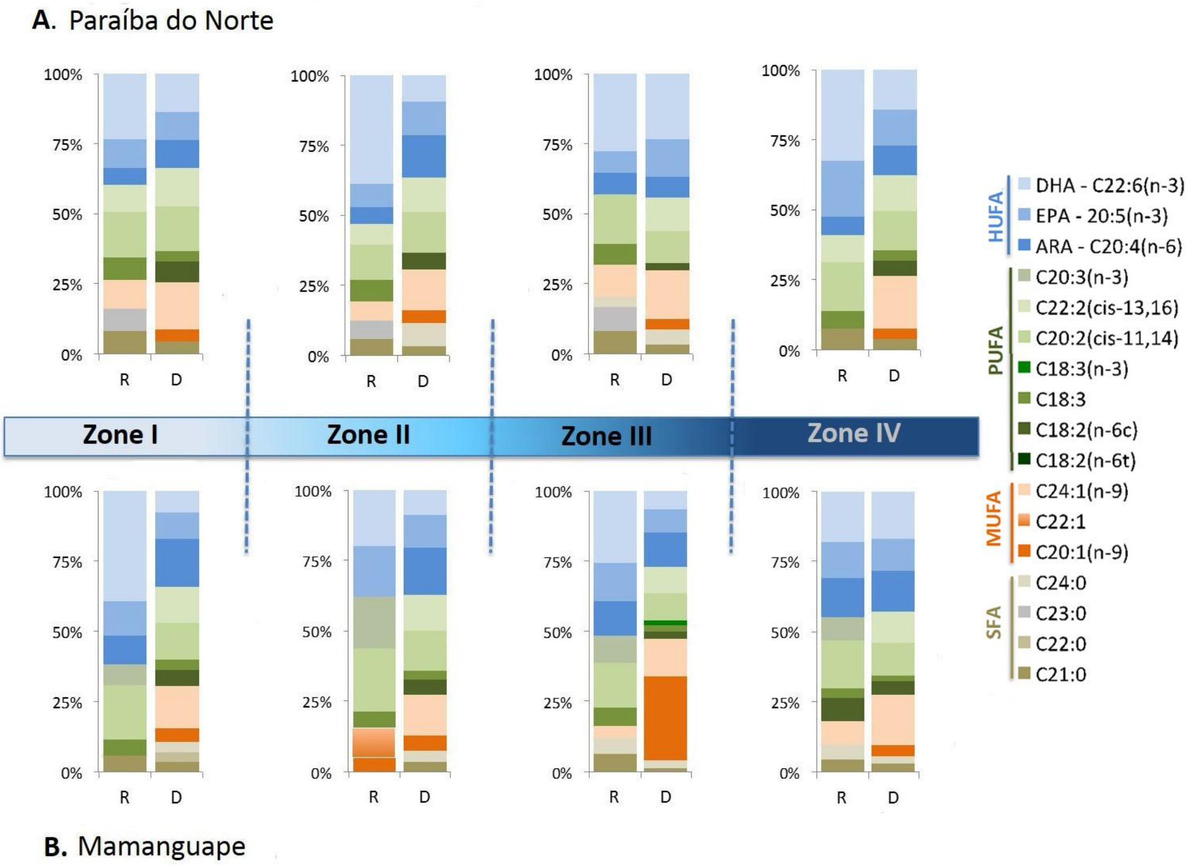

Figure 2.Relative fatty acid concentration (%) of the food sources present in the water column of each zone in (A) Paraíba do Norte (B) Mamanguape estuaries, in both seasons (R = rainy season; D = dry season).

The FA composition of the food sources present in the water column was significantly different between seasons (Group I – rainy season and Group II – dry season, Figure 3) in the Paraíba do Norte (R Global$_{PB}$ = 0.781, p = 0.029) and Mamanguape estuaries (R Global$_{MA}$ = 0.667, p = 0.029). There were significant differences regarding the FA composition of the food sources only between the zones I / II and between zones I / IV of the Mamanguape estuary (R Global$_{ZI/ZII}$ = 0.218, p = 0.004; R Global$_{ZI/ZIV}$ = 0.242, p = 0.008), but not in the Paraíba do Norte estuary (R Global$_{PB}$= 0.024, p = 0.299). In the Paraíba do Norte estuary, twelve FA explained 94% of the dissimilarity between Groups I and II, where C24:1(n-9), DHA, C24:0 and C22:2(cis-13,16) contributed to 50% of this value. The same pattern was observed in the Mamanguape estuary where 92% of the dissimilarity was explained by twelve FA, where C24:1(n-9), C24:0, DHA and C22:2(cis-13,16) contributed to 55% of the differences (for more details, please see Table S1 in the Online Supplementary Material).

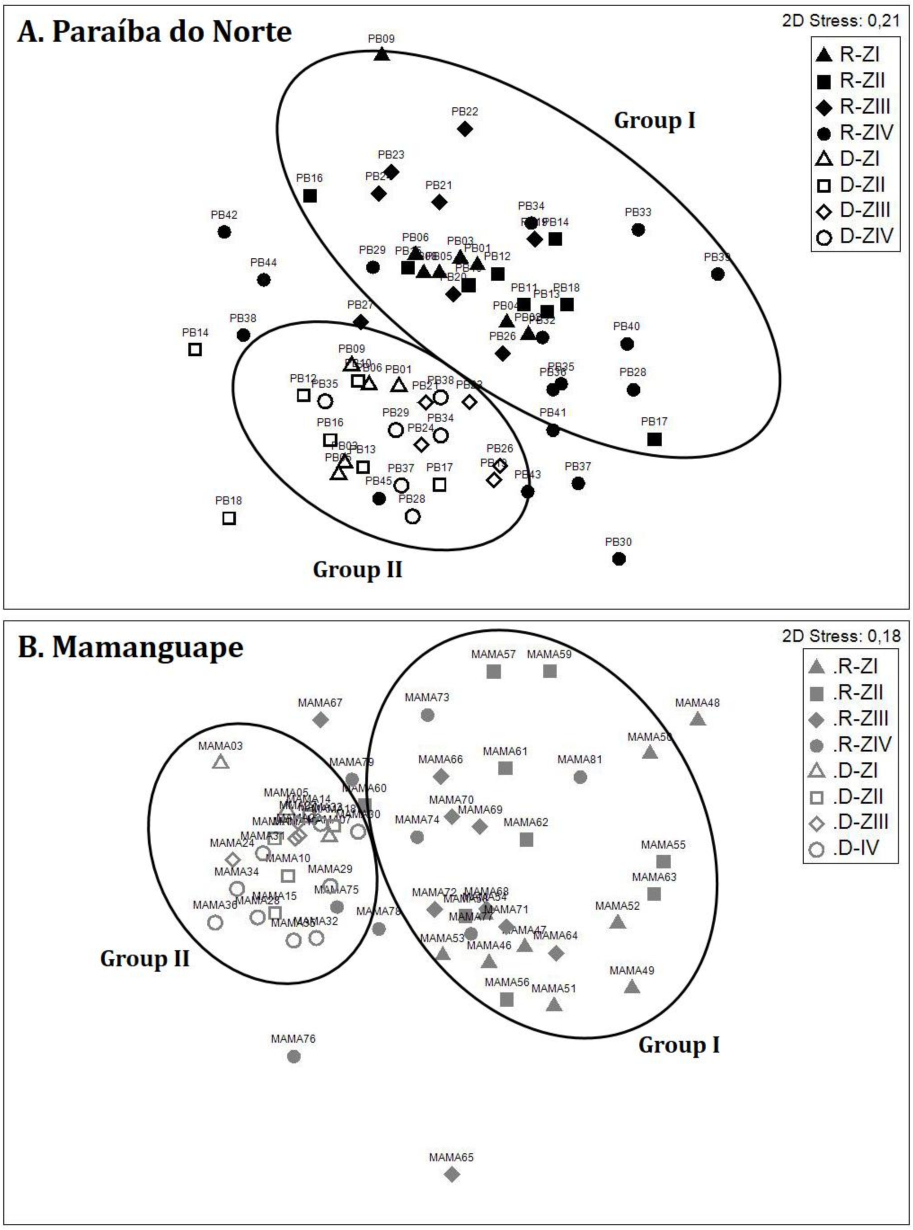

Figure 3. Two-dimensional n-MDS ordination plots of the fatty acid composition of the food sources present in the water samples from Paraíba do Norte (A) and Mamanguape (B) estuaries. R = rainy season; D = dry season. Sampling zones: ZI to ZIV.

3.2 Fatty acid composition of copepods

Similar to the food sources' profiles (Figure 2), the rainy season of both estuaries was characterized by copepod species with higher relative concentrations and diversity types of PUFA and HUFA, whereas in the dry season the pattern was not clear. (Figure 4; Tables S3 and S4). The relative concentration of the other FAs groups (SFA and MUFA, monounsaturated fatty acids) did not change clearly between seasons.

In the Paraíba do Norte, the PUFA were mainly represented by C20:2(cis-11,14) and C20:3(n-3), whereas in the Mamanguape the PUFA was mainly constituted by C20:2(cis-11,14), C22:2(cis-13,16) and C18:2(n-6cis). The FA C18:2(n-6cis) was observed in all species only during the dry season. HUFA was a group present in all species from both estuaries and represented mainly by high concentrations of DHA (C22:6(n-3)) followed by EPA (C20:5(n-3)) and ARA (C20:4(n-6)). The selected copepod species in the Paraíba do Norte had higher relative concentration of SFA and more diversity of FA from this group when compared with the species from the Mamanguape estuary, with FA ranging from C16:0 to C24:0, mainly constituted by longer chains of C (C23:0 and C24:0). C24:1(n-9) was the most abundant MUFA in the two seasons, in both estuaries.

In the Paraíba do Norte, during the rainy season, *P. campaneri* and *A. lillijeborgi* were the copepod species with the highest diversity in FAs, whereas in the Mamanguape estuary, *P. scotti* and *O. robusta* were the copepod species with the richest FA profile (Figure 4).

In both estuaries, we observed a clear seasonal pattern of the sampled species based on their FA composition and concentration (Figure 5). Group I is formed by the samples of the dry season of both estuaries. Group II is formed by the Paraíba do Norte estuary samples of the rainy season, and Groups IIIa and IIIb are constituted by samples from the rainy season of the Mamanguape estuary (R Global = 0.698; p = 0.001). However, we did not observe significant differences between the overall.

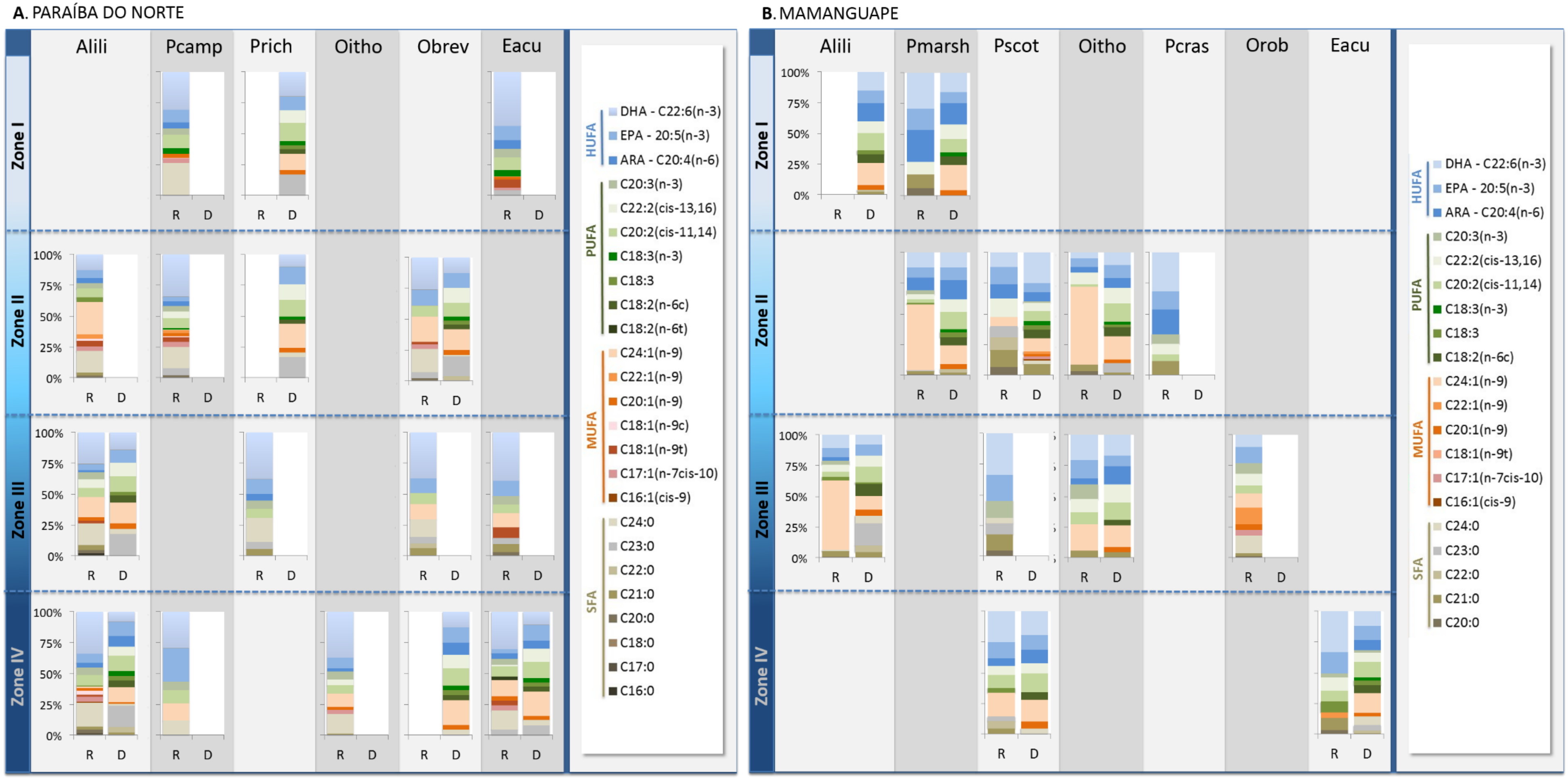

Figure 4.Relative fatty acid concentration (%) in the selected copepods species by zone and season (R = rainy season; D = dry season) in (A) Paraíba do Norte and (B) Mamanguape estuaries.

FA composition (i.e. considering the different set of species collected in each zone) of the zones within each estuary (R $Global_{PB}$ = 0.072, p = 0.121; R $Global_{MA}$ = -0.006, p = 0.491).

Interestingly, however, analyzing the FA profiles of each species individually (see Figure 4) we observed some very clear temporal and spatial differences occurring in some of the species. For instance, analyzing those species that were collected in both seasons in at least two zones of the same estuary (e.g. *A. lilljeborgi* in the Paraiba do Norte; *P. marshi and Oithona* sp. in the Mamanguape estuaries) we observed major changes in the FA profile of the species during the rainy season and minor changes in the FA profile during the dry season. Moreover, during the rainy season we observed marked changes in the FA profiles among zones of the same estuary (e.g. *A. lilljeborgi*, *P. campaneri* and *E. acutifrons* in Paraíba do Norte and *P. marshi*, *P. scotti*, *Oithona* sp. in Mamanguape estuaries) (Figure 4).

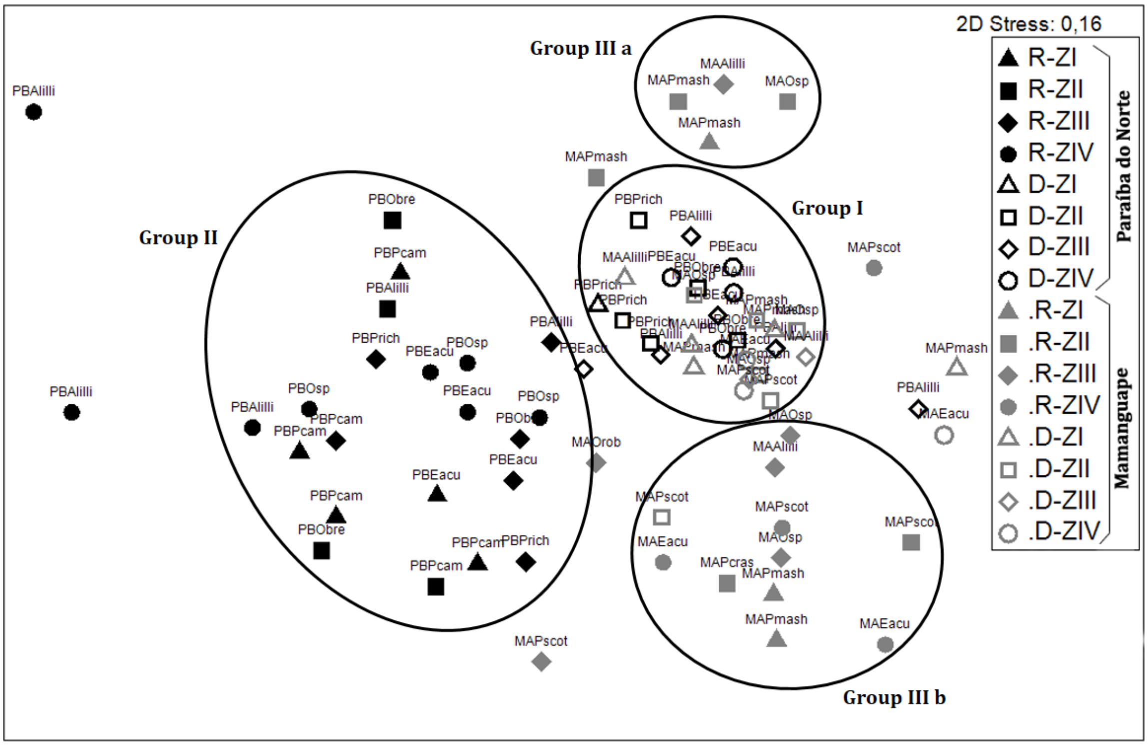

Figure 5. Two-dimensional n-MDS ordination plot of the fatty acid composition of copepods at Paraíba do Norte (black symbols) and Mamanguape (grey symbols) estuaries. R = rainy season; D = dry season. Sampling Zones: ZI to ZIV.

The FA composition and concentration of the species collected was significantly different between the seasons in the two estuaries (0.50≤R Global≤0.99, p=0.029, Table 2), with high R values indicating a good segregation of groups, as observed in the n-MDS plots. We did not observe significant differences regarding the FA composition of the species collected in each estuary during the dry season (Group I).

Table 2. ANOSIM pairwise test results comparing the fatty acid profile of selected copepod species of the Paraíba do Norte (PB) and Mamanguape (Mama) estuaries in both seasons (Rainy and Dry). In bold, p>0.05.

Groups	**R**	***p***
Rainy PB vs. Rainy Mama	0.969	0.029
Rainy PB vs. Dry PB	0.99	0.029
Rainy PB vs. Dry Mama	0.99	0.029
Dry PB vs. Rainy Mama	0.76	0.029
Rainy Mama vs. Dry Mama	0.50	0.029
Dry PB vs. Dry Mama	**0.396**	**0.089**

Ten FA explained 90% of the Group I (Dry season of Mamanguape and Dry season of Paraíba do Norte estuaries) similarity, with C24:1(n-9), DHA and C20:2(cis-11,14) together contributed with 48% to this similarity (Table S5). Nine FA explained 91% of the similarity within Group II, where DHA and C24:0 contributed to 50% of it. Finally, in the Group IIIa / IIIb eight FA contributed 94% to the within group similarity, where DHA, EPA and C22:2(cis-13,16) were responsible for 58% of the similarity (Table S5).

3.3 Dietary fatty acid biomarkers

According to several studies (Dalsgaard et al., 2003; Arts et al., 2009; Gonçalves et al., 2012), the FA content in copepods resembles the fatty acid signature of its preys and their nutritional value is subject to the type of food consumed. In our study, in general, the majority of copepod species were omnivorous, feeding on small animals and consuming diatoms which is evidenced by the increase of DHA/EPA and the decline of D/F, respectively (Table 3). The omnivory was more intensive in the Paraíba do Norte than in the Mamanguape estuary. Indeed, the FAs profile of the copepods collected during the dry season in the Paraíba do Norte estuary suggest a higher affinity of these species for carnivory (PUFA/SFA) and a lower herbivory index (D/F) (Figure 6; Table 3).

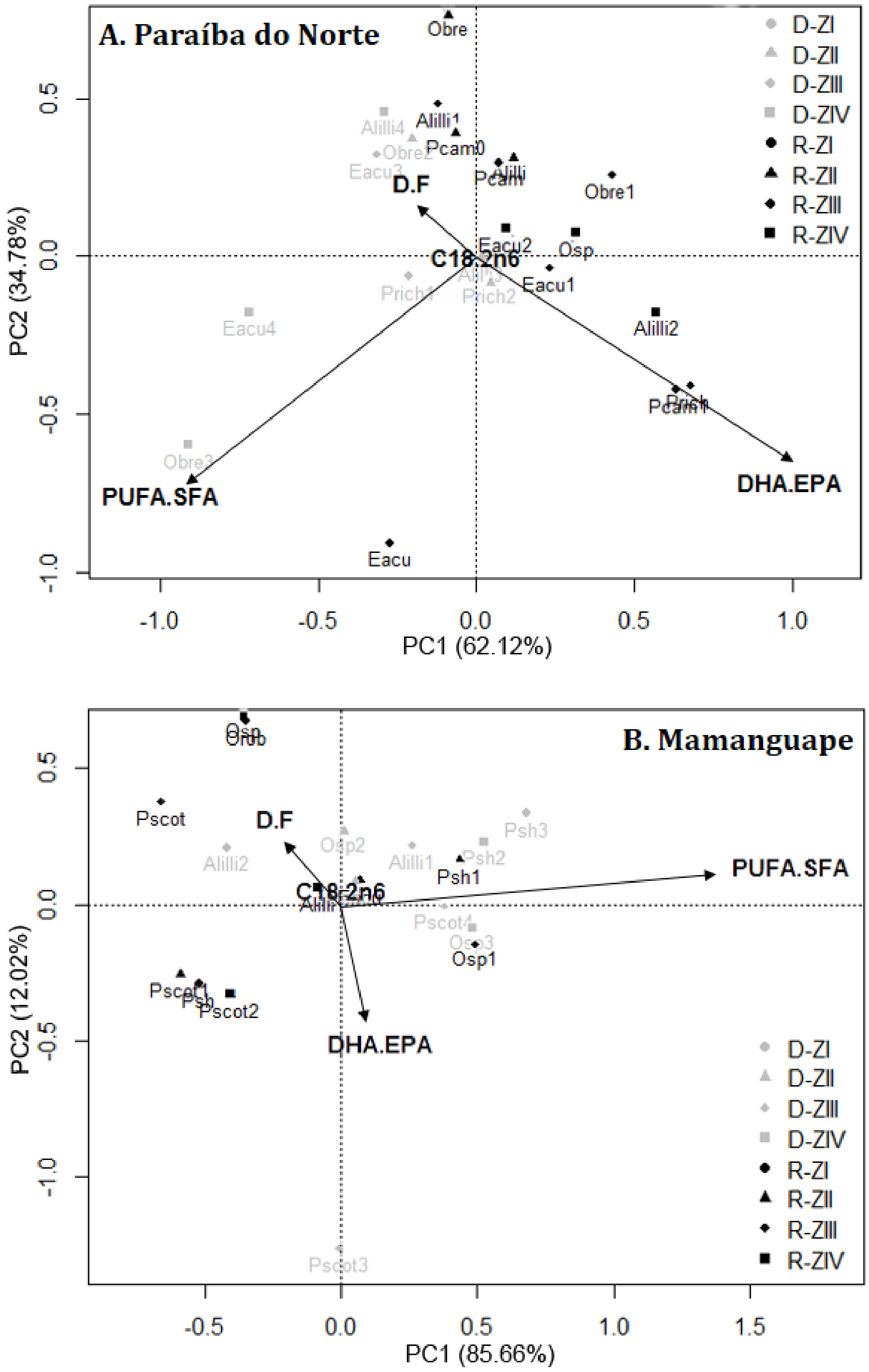

Figure 6. Biplot of Principal Component Analysis (PCA) of fatty acid trophic markers (FATMS) for copepods collected in the four zones (ZI: Zone I; ZII: Zone II; ZIII: Zone III; ZIV; Zone IV) of the Paraíba do Norte (A) and Mamanguape (B) estuaries, during the dry (D) and rainy (R) seasons. Pcam = *Paracalanus campaneri*; Eacu = *Euterpina acutifrons*; Alilli = *Acartia lilljeborgi*; Obre = *Oithona brevicornis*; Orob = *Oithona robusta*; Osp = *Oithona* sp.; Prich = *Pseudodiaptomus richardi*; Psh = *Pseudodiaptomus mashi*; Pscot = *Parvocalanus scotti*.

Furthermore, during the rainy season in the zone II of the Mamaguape estuary, *P. scotti*, *Oithona* sp. and *O. robusta* showed a higher preference for flagellates than for diatoms (Figure 6B; Table 3). A similar trend was observed in *O. brevicornis,* in the Paraíba do Norte estuary. All the other species, no matter the estuarine zone or season, showed the opposite behavior, i.e. higher consumption of diatoms relatively to flagellates.

In both estuaries, the selected copepods species presented a low affinity with the biomarker for terrestrial detritus or green algae (C18:2(n-6)). In fact, this FA was only observed in the copepods collected during the dry season and in low concentrations (Figure 6).

The dietary quality varied between the two seasons, suggesting that the copepods diet quality is mainly dependent of the food availability that varies seasonally. For example, in the Paraíba do Norte, during the rainy season, the majority of the larger copepods (*P. richardi, A. lilljeborgi* and *P. campaneri*) were omnivorous, feeding in small animals and in flagellates, fact that is visible by the increase of DHA/EPA and the decrease of D/F. During the dry season, these copepods, mainly *P. richardi*, fed mostly on small animals, observation supported by the increase of PUFA/SFA, low presence of terrestrial detritus and green algae and the presence of C18:2(n-6), respectively. The same behavior was observed in *O. brevicornis* that showed a higher carnivory affinity (high PUFA/SFA) and a lower herbivory affinity (low D/F), being is diet composed largely by flagellates, during the dry season, whereas in the rainy season the species maintained the consumption of small animals but feeding also on diatoms at zone II and on flagellates on zone III (Table 3). The calanoids and cyclopoids collected in the Mamanguape estuary during the dry season feed mainly on small animals (high PUFA/SFA), however, during the rainy season this consumption reduced significantly unless to *P. mashii* (Zone II), *Oithona* sp. (Zone III) and *E. acutifrons* (Zone IV).

Table 3. Seasonal interspecific differences in fatty acid trophic markers (FATMS) in selected copepods from A. Paraíba do Norte and B. Mamanguape estuaries

A. PARAÍBA DO NORTE

	P. richardi			*P. campaneri*			*A. lilljerborgi*					*O. brevicornis*				*Oithona* sp.	*E. acutifrons*				
	Rainy	Dry		Rainy			Rainy			Dry		Rainy		Dry		Rainy	Rainy			Dry	
	ZIII	ZI	ZII	ZI	ZII	ZIII	ZII	ZIII	ZIV	ZIII	ZIV	ZII	ZIII	ZII	ZIV	ZIV	ZI	ZIII	ZIV	ZIII	ZIV
DHA/EPA	7.69	1.78	2.59	1.92	11.40	7.51	1.91	1.17	5.59	2.33	0.77	0.87	3.19	1.03	1.07	3.37	3.86	3.27	21.73	0.90	0.85
18:2n6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PUFA/SFA	0.69	2.14	1.44	0.81	0.86	0.76	0.71	0.91	0.56	1.38	1.28	0.50	0.30	1.23	8.39	0.68	5.29	0.97	1.02	1.59	4.64
D/F	0.13	0.37	0.28	0.55	0.08	0.13	0.41	0.85	0.18	0.30	0.57	1.15	0.31	0.54	0.48	0.37	0.23	0.31	0.05	0.50	0.54

B. MAMANGUAPE

	P. mashii				*A. lilljerborgi*			*P. scotti*					*Oithona* sp.				*O. robusta*	*E. acutifrons*	
	Rainy		Dry		Rainy	Dry		Rainy			Dry		Rainy		Dry		Rainy	Rainy	Dry
	ZI	ZII	ZI	ZII	ZIII	ZI	ZIII	ZII	ZIII	ZIV	ZII	ZIV	ZII	ZIII	ZII	ZIII	ZIII	ZIV	ZIV
DHA/EPA	1.67	1.54	1.26	1.17	1.27	1.19	0.86	0.87	1.58	1.79	4.21	1.65	0.72	2.04	0.97	1.85	0.73	1.37	1.24
18:2n6	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PUFA/SFA	0.63	7.28	8.48	11.32	2.40	5.14	0.96	0.38	0.44	0.94	2.50	6.36	1.35	7.85	3.01	7.68	1.39	3.43	3.26
D/F	0.60	0.65	0.36	0.33	0.50	0.44	0.47	1.15	0.63	0.56	0.22	0.46	1.39	0.49	0.43	0.43	1.36	0.57	0.39

4. DISCUSSION

4.1 Fatty acid profiling and anthropogenic impact

Fatty acid profiling revealed changes in the quality of the potential food sources of two tropical estuaries with different levels of anthropogenic impact. In both estuaries, regardless the season, the FA profiles of the food sources present in the water column were mainly composed by PUFA and HUFA, with higher concentration and diversity of these FA in the samples of the Mamanguape estuary than those of the Paraíba do Norte estuary. PUFA and HUFA are FA groups mainly composed by essential fatty acids (EFA). These EFA play a key role on the health and function of animals at all trophic levels, potentially limiting growth, reproduction, immune responses and adaptation to environmental changes of zooplankton in the pelagic food web (Evjemo et al., 2008; Hartwick et al. 2013; Taipale et al., 2014). In general, these FA cannot be synthesized *de novo*, or at least not in sufficient amount for somatic growth, reproduction and survival by consumers, being obtained mainly by feeding (Bell and Tocher, 2009; Perumal et al., 2010). Moreover, they are known to have beneficial effects on plasma lipids and lipoproteins (Harris 1997a,b), cardiovascular diseases (Kris-Etherton et al., 2002), cancer (Shahidi and Miraliakbari, 2004), inflammatory and autoimmune diseases (Simopoulos, 2002), brain development and function (Ruxton et al., 2004), and adipose tissue hypertrophy (Parrish et al., 1990).

The lower concentrations of these FA groups in the Paraíba do Norte estuary, when compared the Mamanguape (located inside an environmental protection area), is most probably related with the high levels of anthropogenic activity that are impacting this estuary. The Paraiba do Norte estuary has high concentration of nutrients, receives huge loads of wastewater without sanitary treatments, and has high levels of pesticides and other toxic substances coming from agriculture fields and aquaculture areas (Sassi, 1991). Moreover, the terminal part of the estuary is regularly dredged to maintain the navigation channels. All these activities and uses have the potential to decrease drastically the quality of food available to higher trophic levels, with severe repercussions in the flow of energy and matter within the system.

4.2 Fatty acid profiling and the seasonality

Fatty acid profiling revealed clear temporal shifts in the trophic ecology of zooplankton species in the studied tropical estuaries. Our results showed clear seasonal differences regarding FA composition and concentration in copepods and their potential food sources, with maximal concentrations and diversity of total FA during the rainy season. In this wet period, PUFA and HUFA where the groups with higher concentration, while in the dry season the dominance of these FA was not so clear. The seasonal variations in PUFA accumulation in copepods species may be related with different physiological demands, which are probably linked to species-specific life history and life cycle strategies (Heckmann et al., 2008; Schlotzet al., 2012). Moreover, the higher concentration of n-3 HUFA in copepods may be explained by their ability to adjust their n-3HUFA content to temperature variations (Farkas, 1979; Martin-Creuzburget al., 2012). The presence of high concentrations of DHA is often linked with the role that this FA plays in the development of the nervous system (Persson and Vrede, 2006). Thus, according to Gapasin and Duray (2001), copepods with higher content of DHA tend to have a higher nutritional value for higher trophic levels. Several other studies report the same pattern of high concentrations of DHA in copepods (Persson and Vrede, 2006; Smyntek et al., 2008; Burns et al., 2011; Mariash et al., 2011), and the seasonal variations in fatty acid profile are widely discussed (Kainz et al., 2008; Lau et al., 2012). Our results also showed that the selected copepods species of each estuary presented a low affinity with the biomarker for terrestrial detritus or green algae (C18:2(n-6)) and that this FA was only part of the profiles in the dry season. The profiles of food sources suggest that the copepods only have access to food sources with this FA during the dry season, so it is natural assimilate them only in this season. This fact suggest that in the dry season, without the amount of freshwater input, the system is more dependent on local food sources, being the presence of food source with this FA, a result of drainage of intertidal creeks and mangrove that grow around the main channel.

The seasonal differences regarding FA composition and concentration in copepods and their potential food sources in the Paraiba do Norte and Mamanguape estuaries are probably linked with marked changes in the rainfall pattern. These changes, in turn, produce variations in the river flow and induce fluctuations in salinity, nutrients concentrations and availability of potential food sources (Li et al., 2006; Hartwich et al., 2012). Being the FA profile a reflection of the food consumed (“you are what you eat” principle); our results suggest that, in general, in the two estuaries, the feeding patterns of zooplankton changed reflecting the shifts in the abundance of their food sources (i.e. dominance among diatoms and flagellates, terrestrial detritus and small animals). During the rainy season, the copepods were omnivorous and, in the dry period, the species were mainly carnivorous. Therefore, our results corroborated the perception of other authors that some species have the ability to change their feeding strategy, showing food preferences, catching their food particles based on size, nutritive value (e.g Tackx et al., 1989; Adrian and Schneider-Olt, 1999) and physiological requirements (e.g. Hartwich et al. 2013).

4.3 Fatty acid profiling and the spatial gradient

Fatty acid profiling revealed spatial changes in the trophic ecology of zooplankton species in the two tropical estuaries. Analyzing the FA profiles of each species individually we observed marked changes in the FA profiles among zones of the same estuary during the rainy season. Probably during the wet period the species are transported along the estuary with the freshwater inflow, limiting their dispersal ability and making difficult to select the grazing grounds locals (Irigoien et al. 2011). Thus, the feeding behavior of species is limited by the food sources that are available in the area and not strictly by the species physiological requirements (Hartwich et al. 2013) or by the nutritive value of food (Adrian and Schneider-Olt, 1999). Nevertheless, our results also showed that in both estuaries and seasons, if we consider together the FA composition of all species collected in a certain zone, there were no significant variations regarding the FA composition within the zones of the same estuary. This fact suggests that the differences in FA profiles found among species collected in the same zone are greater than the differences between the "overall FA composition" among the zones along the salinity gradient.

5. CONCLUSIONS

In this study the FA composition of zooplankton organisms revealed temporal and seasonal shifts in the trophic ecology of copepods in two tropical estuaries under different levels of anthropogenic impacts and environmental conditions. Besides, FA profiling was able to reveal changes in the quality of the potential food sources of two tropical estuaries with different levels of anthropogenic impact, with lower-quality food sources present in the most impacted system. Our study showed that FA profiles were sensitive to natural and anthropogenic stress, proving to be a fast and powerful tool to assess the ecological trophic status of tropical estuaries.

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ONLINE SUPPLEMENTARY MATERIAL

Table S1. Relative fatty acid concentration (%) in the potential food sources present in the water column of each zone in Paraíba do Norte and Mamanguape estuaries, in both seasons (dry and rainy).

Table S2. Results of SIMPER showing the average dissimilarity of the fatty acids composition of the food sources between the two groups in each estuary. The two groups were defined based on the ANOSIM / n-MDS results.

Table S3. Relative fatty acid concentration (%) in the most abundant species of copepods per zone and season in Mamanguape estuary.

Table S4. Relative fatty acid concentration (%) in the most abundant species of copepods per zone and season in Paraíba do Norte estuary.

Table S5. Results of SIMPER showing the average similarity of the fatty acids composition of copepods within of the groups in each estuary. The groups were defined based on the ANOSIM / n-MDS results.

Table S1. Relative fatty acid concentration (%) in the food sources present in the water column of each zone in Paraíba do Norte and Mamanguape estuaries, in both seasons (dry and rainy).

		Mamanguape estuary								**Paraíba do Norte estuary**							
		Dry season				**Rainy season**				**Dry season**				**Rainy season**			
	Fatty acids	Zone I	Zone II	Zone III	Zone IV	Zone I	Zone II	Zone III	Zone IV	Zone I	Zone II	Zone III	Zone IV	Zone I	Zone II	Zone III	Zone IV
SFA	C21:0	3.3	3.3	1.5	2.9	5.9	0.0	6.3	4.6	4.5	3.5	3.4	3.7	8.3	5.6	8.0	7.6
	C22:0	3.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	C23:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.8	6.7	8.7	0.0
	C24:0	3.9	4.5	4.0	2.9	0.0	0.0	5.8	5.3	0.0	7.9	5.5	0.0	0.0	0.0	3.9	0.0
	Total SFA	10.9	7.8	5.5	5.9	5.9	0.0	12.1	10.0	4.5	11.4	8.8	3.7	16.1	12.3	20.6	7.6
MUFA	C20:1(n-9)	4.3	5.0	41	3.8	0.0	5.0	0.0	0.0	4.3	4.9	3.5	3.9	0.0	0.0	0.0	0.0
	C22:1	0.0	0.0	0.0	0.0	0.0	10.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0,0	0.0
	C24:1(n-9)	15.4	14.7	18.1	17.9	0.0	0.0	4.4	8.4	16.8	14.2	17.3	18.7	10.3	7.1	11.1	0.0
	Total MUFA	19.7	19.7	22.2	21.7	0.0	15.5	4.4	8.4	21.1	19.1	20.9	22.6	10.3	7.1	11.1	0.0
PUFA	C18:2(n-6t)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	C18:2(n-6c)	5.8	5.2	4.0	4.8	0.0	0.0	0.0	7.8	7.2	6.3	2.8	5.3	0.0	0.0	0.0	0.0
	C18:3	3.3	3.0	2.7	1.9	5.7	5.9	6.3	3.6	4.1	0.0	0.0	3.8	8.0	7.6	7.2	6.4
	C18:3(n-3)	0.0	0.0	2.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	C20:2(cis-11,14)	13.4	14.4	14.0	11.7	19.2	22.4	16.0	17.3	15.5	14.4	11.4	14.0	16.4	12.3	17.9	17.3
	C22:2(cis-13,16)	12.7	12.7	12.4	11.3	0.0	0.0	0.0	0.0	13.9	12.2	11.8	12.8	9.7	7.4	0.0	9.8
	C20:3(n-3)	0.0	0.0	0.0	0.0	7.7	18.2	9.7	8.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Total PUFA	35.2	35.3	35.2	29.8	32.6	46.6	32.1	36.9	40.7	32.9	26.0	36.0	34.1	27.3	25.1	33.5
HUFA	ARA - C20:4(n-6)	16.9	17.0	16.7	14.3	10.0	0.0	12.2	13.9	9.7	15.0	7.4	10.5	5.6	6.4	7.9	6.4
	EPA - 20:5(n-3)	9.4	11.5	11.3	11.5	12.4	17.8	13.4	12.8	10.2	12.0	13.4	12.7	10.7	7.9	7.6	19.9
	DHA - C22:6(n-3)	7.9	8.7	9.1	16.7	39.2	20.0	25.8	18.1	13.7	9.5	23.5	14.5	23.3	38.9	27.7	32.7
	Total HUFA	34.2	37.1	37.1	42.6	61.5	37.9	51.4	44.7	33.7	36.6	44.4	37.7	39.6	53.2	43.2	58.9
	N	12	11	12	11	7	8	9	12	10	10	10	10	9	9	9	7

Table S2. Results of SIMPER showing the average dissimilarity of the fatty acids composition of the food sources between the two groups in each estuary. The two groups were defined based on the ANOSIM / n-MDS results.

	Dissimilarity	**Fatty acids**	**Av. Abund (Group I)**	**Av. Abund (Group II)**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Group I & II (Paraíba do Norte estuary)	43.43	C24:1(n-9)	0.02	0.04	6.86	1.55	15.78	15.78
		DHA	0.05	0.03	6.69	1.32	15.4	31.19
		C20:4	0.01	0.03	4.23	1.31	9.73	40.92
		C22:2(cis-13,16)	0.01	0.03	3.91	1.47	9	49.92
		EPA	0.02	0.03	3.55	1.39	8.18	58.1
		C18:2(n-6c)	0	0.01	2.61	2.02	6.01	64.11
		C20:2(cis-11,14)	0.03	0.03	2.58	1.26	5.93	70.04
		C23:0	0.01	0	2.3	1.37	5.29	75.33
		C24:0	0	0.01	2.23	1.25	5.14	80.46
		C18:3	0.01	0	2.17	1.4	5	85.47
		C20:1(n-9)	0	0.01	1.86	1.94	4.29	89.75
		C21:0	0.01	0.01	1.84	1.27	4.24	93.99
Group I & II (Mamanguape estuary)	43.71	C24:1(n-9)	0.01	0.05	8.67	2.88	19.83	19.83
		C20:4	0.02	0.05	6.54	1.78	14.97	34.8
		DHA	0.04	0.04	4.88	1.2	11.18	45.97
		C22:2(cis-13,16)	0.02	0.04	4.31	1.57	9.86	55.83
		EPA	0.02	0.03	3.06	1.52	6.99	62.83
		C18:2(n-6c)	0	0.01	2.37	1.96	5.41	68.24
		C20:2(cis-11,14)	0.03	0.04	2.34	1.51	5.35	73.59
		C24:0	0.01	0.01	2.27	1.13	5.2	78.79
		C20:1(n-9)	0	0.01	2.02	1.99	4.62	83.41
		C21:0	0.01	0.01	1.46	1.35	3.34	86.75
		C18:3	0.01	0.01	1.23	1.38	2.82	89.57
		C22:1	0.01	0	1.18	0.52	2.69	92.26

Table S3.Relative fatty acid concentration (%) in the most abundant species per zone and season in Mamanguape estuary.

		P. marshi				*A. lilijeborgi*			*P. crassirostis*	*P. scotti*					*Oithona* sp.				*O. robusta*	*E. acutifrons*	
		Rainy		Dry		Rainy	Dry		Rainy	Rainy			Dry		Rainy		Dry		Rainy	Rainy	Dry
	Fatty acids	I	II	I	II	III	I	III	II	II	III	IV	II	IV	II	III	II	III	III	IV	IV
SFA	C20:0	6.0	0.9	0.0	0.0	0.8	0.0	0.0	0.0	6.3	4.5	0.0	0.0	0.0	3.4	0.0	0.0	0.0	1.1	3.5	0.0
	C21:0	10.6	2.3	0.0	2.0	4.5	2.3	4.6	11.4	14.4	13.3	4.5	9.0	0.0	5.2	5.8	1.9	4.4	2.6	9.8	0.9
	C22:0	0.0	0.0	0.0	3.2	0.0	1.7	5.1	0.0	9.9	0.0	6.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0
	C23:0	0.0	1.2	0.0	0.0	0.0	0.0	18.4	0.0	9.2	9.0	3.5	0.0	0.0	0.0	0.0	8.0	0.0	0.0	0.0	4.3
	C24:0	0.0	0.0	0.0	0.0	1.2	0.0	6.2	0.0	0.0	4.5	0.0	2.9	4.7	0.0	0.0	0.0	0.0	14.2	0.0	7.0
	Total SFA	16.7	4.3	0.0	5.2	6.6	4.1	34.3	11.4	39.8	31.3	14.3	11.9	4.7	8.6	5.8	9.9	4.4	17.9	13.3	14.2
MUFA	C16:1(cis-9)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	C17:1n-7(cis-10)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	0.0	0.0	0.0	0.0	0.0	4.6	0.0	0.0
	C18:1(n-9t)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	C20:1(n-9)	0.0	0.0	4.3	3.9	0.0	3.8	4.7	0.0	0.0	0.0	0.0	2.1	5.5	0.0	0.0	2.8	4.0	4.7	0.0	3.1
	C22:1(n-9)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.2	0.0	0.0	0.0	0.0	0.0	13.6	4.5	0.0
	C24:1(n-9)	0.0	53.2	20.9	15.2	56.4	18.1	11.3	0.0	7.4	0.0	19.4	11.0	17.7	63.4	21.6	19.0	17.8	11.4	0.0	16.1
	Total MUFA	0.0	53.2	25.3	19.1	56.4	21.9	16.0	0.0	7.4	0.0	19.4	18.5	23.2	63.4	21.6	21.9	21.9	34.4	4.5	19.2
PUFA	C18:2(n-6c)	0.0	0.0	6.9	6.3	0.0	6.9	9.8	0.0	0.0	0.0	0.0	6.6	6.3	0.0	0.0	7.0	4.4	0.0	0.0	6.2
	C18:3	0.0	1.2	0.0	4.6	2.8	3.4	1.5	0.0	0.0	0.0	4.1	3.7	0.0	0.0	0.0	2.2	0.0	0.0	8.8	4.3
	C18:3(n-3)	0.0	0.0	2.7	2.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.4	0.0	0.0	0.0	2.9	0.0	0.0	0.0	2.2
	C20:2(cis-11,14)	0.0	3.2	11.1	14.0	4.6	14.1	12.6	5.4	0.0	0.0	10.1	8.2	15.0	2.4	9.9	14.7	14.4	6.9	8.8	12.8
	C22:2(cis-13,16)	10.5	3.8	11.9	10.5	5.6	9.6	9.1	8.7	15.1	0.0	7.4	6.7	8.3	9.3	10.1	12.6	14.9	9.2	10.8	7.2
	C20:3(n-3)	0.0	3.7	0.0	0.0	3.1	0.0	0.0	7.9	0.0	13.9	0.0	1.4	0.0	0.0	12.4	0.0	0.0	8.8	3.0	2.2
	Total PUFA	10.5	11.9	32.6	37.8	16.1	34.0	33.0	22.0	15.1	13.9	21.5	30.0	29.6	11.7	32.4	39.5	33.8	24.9	31.5	34.9
HUFA	C20:4(n-6) (ARA)	26.3	10.0	17.6	15.5	2.7	14.7	0.0	20.2	12.0	0.0	6.5	7.0	11.4	4.4	4.9	7.9	14.4	0.0	0.0	8.6
	C20:5(n-3) (EPA)	17.2	8.0	9.1	10.0	7.7	10.4	9.0	14.9	13.8	21.2	13.2	7.5	11.7	7.0	14.7	10.8	9.0	13.2	17.5	11.3
	C22:6(n-3) (DHA)	29.3	12.6	15.4	12.4	10.5	14.9	7.7	31.4	12.0	33.6	25.0	25.1	19.4	5.0	20.6	10.1	16.6	9.7	33.3	11.8
	Total HUFA	72.8	30.5	42.1	37.9	20.9	40.0	16.7	66.5	37.7	54.8	44.8	39.6	42.5	16.3	40.2	28.8	40.0	22.8	50.8	31.7
	N	6	11	9	12	11	11	12	7	9	10	14	16	9	12	12	12	9	16	13	15

Table S4. Relative fatty acid concentration (%) in the most abundant species per zone and season in Paraíba do Norte estuary.

		P. campaneri			*A. lilijerborgi*					*P. richardi*			*Oithona* sp.	*O. brevicornis*				*E. acutifrons*			
		Rainy			Rainy			Dry		Rainy	Dry		Rainy	Rainy		Dry		Rainy			Dry
	Fatty acids	I	II	IV	II	III	IV	III	IV	III	I	II	IV	II	III	II	IV	I	III	IV	IV
SFA	C16:0	0.0	0.0	0.0	0.0	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	C17:0	0.0	0.0	0.0	0.0	0.0	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	C18:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	C20:0	0.0	2.1	0.0	1.6	2.7	3.0	0.0	0.0	0.0	0.0	0.0	0.0	2.2	0.0	0.0	0.0	0.0	2.7	0.0	0.0
	C21:0	0.3	0.0	0.0	2.6	3.8	2.8	0.0	2.2	5.5	0.0	0.0	1.2	0.0	6.2	0.0	0.0	0.0	6.7	0.0	0.0
	C22:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.4	0.0	0.0	0.0	0.0	0.0	3.5	3.6	0.0	0.0	0.0	0.0	0.0
	C23:0	0.0	5.3	0.0	0.0	0.0	0.0	17.4	17.2	5.5	16.6	16.7	0.0	5.0	5.5	16.0	0.0	4.2	5.0	4.6	7.6
	C24:0	25.9	17.2	11.8	17.5	17.6	18.7	4.4	1.3	19.7	0.0	4.0	15.9	18.3	14.4	1.5	4.4	0.0	0.0	15.5	4.8
	Total SFA	27.0	24.6	11.8	21.7	25.9	25.9	25.4	25.2	30.7	16.6	21.1	20.0	26.0	29.5	21.1	4.4	4.2	14.4	20.1	15.4
MUFA	C16:1(cis-9)	0.0	0.0	0.0	0.0	0.0	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	C17:1(n-7cis-10)	3.8	4.7	0.0	3.5	0.0	4.3	0.0	0.0	0.0	0.0	0.0	3.4	4.1	0.0	0.0	0.0	2.2	0.0	4.1	0.0
	C18:1(n-9t)	0.0	3.8	0.0	4.7	2.5	1.5	0.0	0.0	0.0	0.0	0.0	0.0	2.1	0.0	0.0	0.0	6.5	8.8	3.8	0.0
	C18:1(n-9c)	0.3	0.5	0.0	1.6	0.0	2.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	C20:1(n-9)	3.1	2.3	0.0	0.0	2.7	2.5	4.4	1.6	0.0	3.8	3.7	2.7	0.0	0.0	3.8	3.6	2.1	0.0	3.7	3.3
	C22:1(n-9)	0.0	2.7	0.0	3.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0
	C24:1(n-9)	0.0	0.6	14.0	26.3	16.5	1.1	16.5	12.2	0.0	13.1	19.4	10.4	20.2	12.5	17.0	20.1	0.0	11.1	13.0	19.3
	Total MUFA	16.1	14.6	14.0	39.9	21.7	13.4	20.9	13.8	0.0	16.9	22.7	13.1	26.4	12.5	20.8	23.7	11.2	20.0	24.7	21.4
PUFA	C18:2(n-6t)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.1	0.0
	C18:2(n-6c)	0.0	0.0	0.0	0.0	0.0	0.0	6.1	5.4	0.0	4.0	3.6	0.0	0.0	0.0	3.5	4.6	0.0	0.0	0.0	4.5
	C18:3	0.0	0.0	0.0	3.5	0.0	1.0	2.5	3.6	0.0	3.2	0.0	0.0	0.0	0.0	3.3	3.7	0.0	0.0	0.0	3.1
	C18:3(n-3)	4.7	1.3	0.0	0.0	0.0	0.0	0.0	4.0	0.0	2.9	2.3	0.0	0.0	0.0	3.3	4.1	4.9	0.0	0.0	3.7
	C20:2(cis-11,14)	11.2	8.0	10.5	7.5	7.3	8.5	13.0	12.4	7.4	15.1	13.6	7.0	8.6	8.8	11.0	13.5	10.7	7.5	8.2	13.2
	C22:2(cis-13,16)	0.0	5.1	0.0	0.0	7.0	0.0	11.3	7.3	0.0	10.3	13.0	4.3	0.0	0.0	12.6	11.2	0.0	0.0	0.9	10.4
	C20:3(n-3)	4.9	5.0	7.0	4.5	5.8	6.0	0.0	0.0	6.6	0.0	0.0	6.5	0.6	0.0	0.0	0.0	6.8	6.5	5.1	0.0
	Total PUFA	20.1	19.4	17.5	15.5	20.1	15.5	30.5	32.7	14.0	35.5	30.5	20.3	13.0	8.8	33.8	37.1	22.4	14.0	17.3	33.2
HUFA	C20:4(n-6) (ARA)	5.3	3.1	0.0	3.7	1.4	3.9	0.0	8.5	5.6	0.0	0.0	2.8	0.0	0.0	0.0	9.8	6.9	0.0	4.7	6.8
	C20:5(n-3) (EPA)	9.8	4.0	27.4	6.6	5.4	7.2	10.3	11.9	11.7	11.2	13.7	8.6	12.9	11.7	11.3	12.1	11.4	12.1	3.0	12.7
	C22:6(n-3) (DHA)	30.8	34.3	29.4	12.6	25.5	34.0	14.1	7.9	38.0	19.9	10.1	37.2	25.9	37.4	12.9	12.9	43.9	39.6	30.3	10.5
	Total HUFA	47.2	41.4	56.8	22.9	32.4	45.2	24.4	28.3	55.3	31.0	27.8	43.1	38.8	49.2	24.3	34.7	62.2	51.7	38.0	41.7
	N	11	16	6	14	13	16	10	14	8	13	10	11	10	8	12	11	11	9	13	12

Table S5. Results of SIMPER showing the average similarity of the fatty acids composition of copepods within of the groups in each estuary. The groups were defined based on the ANOSIM / n-MDS results.

MDS Groups	Similarity	Fatty acids	Av. % abun.	Av. sim.	Sim/SD	Contrib. %	Cum. %
Group I (Dry Mamanguape / Dry Paraíba do Norte)	69.79	C24:1(n-9)	0.01	13.05	4.45	18.69	18.69
		C22:6(n-3)(DHA)	0.01	11.04	3.59	15.82	34.51
		C20:2(cis-11,14)	0.01	9.80	3.60	14.05	48.56
		C20:5(n-3)(EPA)	0.01	8.67	6.44	12.43	60.99
		C22:2(cis-13,16)	0.01	7.32	2.25	10.49	71.48
		C18:2(n-6c)	0.00	4.59	3.38	6.58	78.06
		C20:1(n-9)	0.00	3.47	7.04	4.97	83.03
		C20:4(n-6)	0.00	3.14	0.62	4.50	87.53
		C23:0	0.00	2.82	0.50	4.04	91.57
Group II (Rainy Paraíba do Norte)	55.46	C22:6(n-3)(DHA)	0.04	18.58	1.84	33.49	33.49
		C24:0	0.02	9.32	1.32	16.8	50.29
		C20:2(cis-11,14)	0.01	6.33	3.82	11.42	61.71
		C20:5(n-3)(EPA)	0.01	5.12	1.88	9.23	70.95
		C20:3(n-3)	0.01	4.02	2	7.25	78.2
		C24:1(n-9)	0.01	2.46	0.57	4.44	82.63
		C21:0	0.00	1.9	1.03	3.43	86.07
		C18:1(n-9t)	0.00	1.49	0.66	2.69	88.76
		C17:1(n-7cis-10)	0	1.37	0.83	2.47	91.23
Group III a / III b (Rainy Mamanguape)	45.98	C22:6(n-3)(DHA)	0.01	11.75	2.1	25.55	25.55
		C20:5(n-3) EPA)	0.01	9.4	3.09	20.43	45.99
		C22:2(cis-13,16)	0	5.73	1.44	12.47	58.45
		C24:1(n-9)	0.02	4.57	0.35	9.95	68.4
		C21:0	0	4.36	1.54	9.48	77.88
		C20:4(n-6)	0	3.38	0.81	7.35	85.23
		C20:3(n-3)	0	2.13	0.6	4.64	89.87
		C20:2(cis-11,14)	0	2.11	0.72	4.58	94.45

6. CONCLUSÃO GERAL

Vários estudos têm evidenciado que os variáveis ambientais são suficientes para explicar os padrões de distribuição da comunidade zooplanctônica em sistemas estuarinos. No entanto, desde que outros fatores como à conectividade estuarina e as relações filogenéticas também desempenham papel fundamental para entender esses padrões, usar apenas os variáveis ambientais para identificar a variação estrutural na comunidade zooplanctônica pode não ser suficiente. Através dos fatores amostrados no Capítulo I (variáveis ambientais, conectividade e relação filogenética), observou-se que esses três componentes são suficientes para explicar a variação na comunidade zooplanctônica no estuário Mamanguape que se localiza em uma área de conservação, e que diferente do que é comumente esperado, a conectividade pode apresentar uma explicabilidade maior na distribuição da comunidade, quando comparada às variáveis ambientais. No entanto, os três componentes utilizados não foram suficientes para essa variação no estuário Paraíba do Norte o qual sofre a influência de fatores antrópicos.

O presente estudo mostra claramente que para avaliar os fatores direcionadores da mudança de biomassa e diversidade funcional da comunidade zooplanctônica, além dos fatores ambientais locais, temos que usar a modelagem espacial, a fim de integrar a capacidade de dispersão das espécies e dos efeitos das forças físicas que atuam no sistema. Além disso, uma investigação mais aprofundada é urgentemente necessária para esclarecer os fatores que determinam e que moldam as comunidades zooplanctônicas em sistemas tropicais muito impactados. Somente com o conhecimento das principais forças que dão forma a comunidade alvo e sistema de medidas de gestão apropriadas podem ser postas em prática. Caso contrário, corremos o risco de investir tempo e fundos em monitoramentos de variáveis erradas e implementação de medidas inadequadas de conservação.

Quando avaliei os perfis de ácidos gráxos para conhecer a dinâmica tróficas das espécies de copépodos nos dois estuários tropicais amostrados, concluí que a composição de ácidos gráxos dos organismo zooplanctônicos revelaram variações sazonais e temporal na ecologia trófica dos copepodos nos dois estuários tropicais. Além disso, os perfis de ácidos gráxos foram capaz de revelar diferenças na qualidade das potenciais fontes de alimente nos dois estuários com diferentes níveis de impacto antrópico, com uma menor qualidade de fontes alimentares presente no sistemas mais impactado (estuário do Paraíba do Norte). Nesse estudo os perfis de ácidos gráxos foram sensíveis à estresse naturais e antrópicos, mostrando ser uma ferramenta rápida para avaliar o estado trófico de estuários tropicais.

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