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ANDRÉA PONTES VIANA

A ICTIOFAUNA NO MONITORAMENTO DA QUALIDADE AMBIENTAL EM UM DISTRITO INDUSTRIAL DO ESTUÁRIO AMAZÔNICO

Belém

2011

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Tese apresentada ao Curso de Pós-Graduação em Ecologia Aquática e Pesca da Universidade Federal do Pará, como requisito parcial para obtenção do título de Doutora em Ecologia Aquática e Pesca.

Orientadora: Profa. Dra. Flávia Lucena Frédou

Co-Orientadora: Profa. Dra.Rossineide Martins Rocha

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"Faça o que puder, com o que tiver, onde estiver"
(Theodore Roosevelt)

"Aquilo que é o melhor para nós nem sempre é o mais fácil, mas, em última análise, é o que realmente compensa."
(Couto Nogueira)

RESUMO

Vila do Conde está localizada no município de Barcarena, Pará, Brasil. Nesta região está concentrado um importante pólo industrial de mineração, constituindo um fator de risco para a qualidade da água. Diante do exposto, este trabalho teve o objetivo de avaliar a qualidade da água no ambiente estuarino localizado no entorno de Vila do Conde utilizando a ictiofauna como bioindicador e o fígado de duas espécies de peixes como biomarcador histopatológico. As coletas do material abiótico (água) e da ictiofauna ocorreram em três áreas considerando os diferentes níveis de impacto: Zona 1, localizado no entorno do terminal portuário e industrial de Vila do Conde, considerada como alto risco de contaminação; Zona 2, localizada na ilha do Capim, na divisa dos municípios de Barcarena e Abaetetuba, , classificada com risco médio de impacto; Zona 3, localizada na ilha das Onças, município de Bracarena, classificada com risco minímo por está distante das fontes de contaminação. Para todas as áreas de estudo foram feitas amostragens tanto no ambiente de canal quanto no canal de maré ao longo de quatro coletas bimestrais -, transição chuvoso para o seco (Junho 2009), seco (Setembro 2009), transição seco para chuvoso (Janeiro 2010) e período chuvoso (Abril 2010), no período de um ano de coleta. Para a obtenção dos dados foram utilizados rede de emalhar e rede de tapagem. Como forma de abordar diferentes vertentes sobre a qualidade da água em Vila do Conde, este trabalho foi dividido em etapas. A primeira etapa consistiu do uso da ictiofauna como bioindicadora (capítulo 1). Na segunda etapa foram selecionadas duas espécies abundantes com hábitos alimentares distintos, *Plagioscion squamosissimus* e *Lithodoras dorsalis*, para avaliar a saúde do ambiente através da utilização do fígado como biomarcador histopatológico (capítulo 2). Por fim todas as famílias de descritores da comunidade estudadas nos capítulos 1 e 2 foram integralizadas através do uso de índices de integridade biológica (capítulo 3). A análise da ictiofauna como bioindicadora mostrou que, para os dois ambientes (canal e igarapé), considerando as várias famílias de descritores, foi evidente a composição diferenciada entre os locais. Das 77 espécies capturadas, apenas 23 foram encontradas na zona 1. Adicionalmente, também foi observada a diminuição de organismos de grande porte. Este

decréscimo foi considerado como uma resposta ecológica inicial as alterações antrópicas. A análise dos biomarcadores, feito através do estudo histopatológico do fígado se mostrou eficiente e demonstrou que presença antrópica naquela região está afetando a saúde da *P. squamosissimus* e *L. dorsalis*. O MAV (Mean Assessment Values), HAI (Histological Alteration Index) e o MDS (multidimensional scaling) mostraram claramente as diferenças entre as áreas estudadas. Nas áreas em que existe o contato mais próximo com o porto e as indústrias, as alterações foram mais severas e algumas consideradas irreversíveis para as duas espécies. As principais lesões encontradas nas duas espécies foram: o aumento do centro melanomacrófagos, degeneração gordurosa, inflamação nos hepatócitos, hepatite, congestão nos vasos e necrose focal. As alterações hepáticas observadas neste estudo foram mais intensas em *P. squamosissimus* que é carnívora e se alimenta na área de estudo predominantemente de camarão. Através dos índices de integridade todas as informações sobre a comunidade descritas anteriormente foram agregadas e denominadas de métricas. Para os dois ambientes (canal e igarapé), a curva de biomassa/dominância ABC mostrou que as zonas 1 e 2 apresentaram alterações, sendo estas áreas classificadas como moderadamente impactadas. Os índices BHI (Estuarine biological health index), EFCI (Estuarine fish community índex), TFCI (Transitional fish classification índex) e EBI (Estuarine biotic integrity index) foram considerados excelentes indicadores de integridade nas áreas de estudos e foram eficientes em mostrar alterações graves da comunidade de peixes na zona 1. Quanto à zona 2, já foi possível observar algum tipo de alteração no ambiente, mostrando que a contaminação não está se restringindo apenas ao entorno de Vila do Conde. As metodologias aplicadas foram capazes de detectar as interferências antrópicas na área de estudo e podem para ser replicadas em outros ambientes estuarinos. Entretanto, estudos mais detalhados e por um maior período de tempo ainda são necessários em Vila do Conde, principalmente relacionadas à bioacumulação de metais pesados nas principais espécies consumidas.

ABSTRACT

The district of Vila do Conde, in the municipality of Barcarena, is a prominent industrial hub, constituting a risk factor for water quality. Given this, the present study focused on the environmental quality of the aquatic habitats adjacent to this site, using the fish community as a bioindicator and liver two species of fish as a biomarker histopathological. The collection of abiotic data (water samples) and fish specimens was organized in three distinct zones, representing different levels of impact. Zone 1 was located in the vicinity of the Vila do Conde cargo terminal, where the risk of contamination was highest. Zone 2 was in the Capim Island, located on the border between the municipalities of Bacarena and Abaetetuba, classified as a median risk area due to its relative proximity to the port. Zone 3 was in Onças Island, which was classified as minimum risk, due to its distance from the industrial district of Vila do Conde. Two different types of environment – the main river channel and marginal tidal creek were sampled in all three zones. Samples were collected every two months, covering the region's principal climatic periods - rainy-dry transition (June 2009), dry season (September 2009), dry-rainy transition (January 2010), rainy season (April 2010), in one year collection. Data was collected using monofilament gillnets and block net. Aiming to evaluate the water quality considering different tools, this study was divided into three parts. In the the first part, the icthyofauna was used as an bioindicator of water quality (Chapter 1). In the second part, two species abundant with different feeding habits, *Plagioscion squamosissimus* and *Lithodoras dorsalis,* to assess the health of the environment through the use of liver as a biomarker histopathological (Chapter 2).

Finally, all families of descriptors (chapters 1 and 2), were combined into biological integrity indexes in chapter 3. The analysis of the icthyofauna as an bioindicator showed differences in the composition between zones and environments (main channel and tidal creek), for the different groups of descriptors. Considering the 77 species captured, only 23 species were recorded in comparison with the less impacted areas. Also, large fish were least common in zone 1. In the present study, the smaller proportion of larger-sized fish recorded in the impacted areas may reflect an ecological response to anthropogenic disturbance. Biomarker analysis, carried out through the liver histopatologic study, was efficient to detect the influence of antropogenic factors in the heatlh of *P. squamosissimus* e *L. dorsalis*. The MAV (Mean Assessment Values), HAI (Histological Alteration Index) e o MDS (multidimensional scaling) analysis all indicated clear differences between the areas surveyed. The alterations were more severe (in some cases, irreversible) in zone 1, which was closest to the port and the industrial district. The principal alterations observed in the tissue of both species included an increase in the number of melanomacrophagous centers, fatty degeneration, inflammation, congestion, hepatitis, and focal necrosis. The hepatic alterations observed in the present study were generally more intense in the carnivorous *P. squamosissimus*, which feeds mainly on shrimp in the region of the study area. Though integrity indexes, all community information were combined into metrics. In the present study, considering the ABC curve, in the main channel and creek, the zones 1 and 2 were classified as moderately disturbed, which predicts an increase in the relative abundance of opportunistic species. The BHI (Estuarine biological health index), EFCI (Estuarine fish community índex), TFCI (Transitional fish classification índex) e EBI (Estuarine biotic integrity index) were considered to be excellent indicators of the ecological integrity of the different sectors of the study area, and were especially effective for the demonstration of the critical alterations of the fish community of zone 1. It was also possible to identify alterations to the environment of zone 2. The procedures adopted in the present study were nevertheless adequate for the detection of the alterations to the environment which have occurred in the vicinity of the town's industrial district and cargo terminal and are able to be replicted in others estuarine areas. However, more reliable information on the bio-accumulation of heavy metals in these species will be necessary. This is especially important due to the prominence of both species in the diet of the local communities.

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CAPÍTULO GERAL

1. INTRODUÇÃO GERAL

Há cerca de cem anos as atividades humanas nos ambientes aquáticos começaram a ser vistas como algo que degradava o meio ambiente surgindo, a partir dessa época, iniciativas para o monitoramento biológico. Porém, estas iniciativas não ganharam destaque tornando-se secundárias e limitadas apenas a uma avaliação de contaminantes químicos e orgânicos, oriundos de fontes pontuais ou difusas, inferindo-se que estes se relacionavam diretamente com a qualidade biológica dos ecossistemas (PLANO NACIONAL DA ÁGUA, 2004, FREIRE et al., 2008).

Na década de 70, os cientistas perceberam que grandes quantidades de poluentes estavam sendo lançados no mar e que essa atividade poderia resultar em danos ao ambiente, além de prejudicar também a área estuarina, surgindo assim às primeiras reuniões visando a criação de medidas remediadoras (NRC, 2003). O planejamento hídrico e o monitoramento biológico passaram a ser discutidos como um dos instrumentos de trabalho dos gestores de recursos hídricos. Desde então meios que pudessem melhorar a qualidade foram inseridos, através do argumento de que a capacidade de sustentar uma biota balanceada é um dos melhores indicadores de "saúde", surgindo assim esforços para quantificar a integridade biótica dos corpos d'água (JARAMILLO-VILLA; CARAMASCHI, 2008).

Uma das formas de avaliar o estado de saúde do ambiente aquático é a utilização de indicadores ambientais que são definidos, de acordo com Whitfield e Elliot (2002), como medidas física, química, biológica ou socioeconômica que melhor representa os elementos chaves do ecossistema complexo. As comunidades biológicas de ecossistemas aquáticos fornecem uma medida agregada dos impactos sendo formadas por organismos que apresentam adaptações evolutivas a determinadas condições ambientais e que apresentam limites de tolerância a diferentes alterações das mesmas (JARAMILLO-VILLA; CARAMASCHI, 2008). Por esse motivo, as principais metodologias para o monitoramento ambiental abrangem o levantamento e avaliação de modificações na riqueza de espécies e índices de diversidade, abundância de organismos resistentes, perda de espécies sensíveis, medidas de produtividade primária e secundária, sensibilidade a concentrações de substâncias tóxicas (MELO; HEPP, 2008).

Clarke; Warwick (1994) citam que os efeitos biológicos causados por poluentes podem ser estudados por um conjunto variado de organismos (pelágicos e bentônicos) e que os vários componentes da biota têm suas vantagens e desvantagens quanto à sua utilização em estudos de efeitos biológicos sobre o ambiente. Nenhum índice tem se mostrado totalmente efetivo na medição dos efeitos da poluição sobre o ecossistema aquático numa ampla gama de condições (CLARKE; WARWICK, 1994, NAVAS-PEREIRA; HENRIQUE, 1996, JARAMILLO-VILLA; CARAMASCHI, 2008). Por exemplo, o plâncton possui a vantagem de se localizar em áreas bastante amplas podendo ser utilizados para mudanças globais. Porém, eles são organismos que vivem em suspensão, logo são dependentes das massas d'águas o que os tornam continuamente móveis não devendo ser utilizados para monitoramento de efeitos em uma fonte poluente local.

A principal vantagem dos organismos bentônicos está relacionada ao seu hábitat. Como são organismos que vivem no fundo, eles se alimentam dentro, sobre, e próximo aos sedimentos, onde os poluentes tendem a acumular; além disso, as comunidades bentônicas apresentam elevada diversidade biológica, o que resulta em uma maior variabilidade de respostas frente a diferentes tipos de impactos ambientais. Sua principal desvantagem é a difícil identificação taxonômica (no caso de meiobentos), o requerimento de uma grande amostragem de sedimento e, para os macrobentos, pode haver problemas devido ao recrutamento de algumas espécies que passam a fase inicial de sua vida como plâncton (SILVEIRA, 2004).

Os peixes possuem a vantagem de incluir espécies de várias categorias tróficas (onívoros, hebívoros, insentívoros, planctívoros, piscivoros) e utilizar alimentos tanto de origem aquática como terrestre. São relativamente fáceis de coletar e identificar, são consumidos por humanos, o que os torna valiosos para avaliar o risco ecológico e a saúde pública, são persistentes e se recuperam rápido dos distúrbios naturais. Porém, possuem desvantagem em relação à sua amostragem, pois pode ser seletiva se não forem usados equipamentos adequados para cada local e, dada a mobilidade sazonal de algumas espécies podem não ser bons indicadores de distúrbios localizados.

Com isto, a amostragem requer maior esforço físico (ATTRILL; DEPLEDGE, 1997, WHITFIELD; ELLIOTT, 2002, JARAMILLO-VILLA; CARAMASCHI, 2008).

Dentre os indicadores biológicos, destacam-se os bioindicadores e os biomarcadores. Os bioindicadores são baseados na avaliação do número e na distribuição de indivíduos e espécies de acordo com o estado biótico e abiótico de um meio ambiente. São utilizados, dentre estas finalidades, para indicar a biodiversidade de uma determinada região e também para medir os níveis de contaminação de uma área antes e depois de uma mudança no meio ambiente. O uso da ictiofauna como bioindicador já é tradicionalmente conhecida e se mostra eficiente quanto à avaliação da qualidade ambiental (KARYDIS; TSIRTSIS, 1996, SMITH; BARRELLA, 2000, KUPSCHUS; TREMAIN, 2001, WHITFIELD; ELLIOTT, 2002, EDDY, 2005, VIEIRA; SHIBATTA, 2007, JARAMILLO-VILLA; CARAMASCHI, 2008, MELO; HEPP, 2008).

Biomarcadores são definidos como uma variação bioquímica, celular, fisiológica ou comportamental que pode ser verificado no tecido ou em fluidos corporais em um organismo que resulta em conseqüência da exposição do animal a um agente exógeno, denominado xenobiótico (DEPLEDGE et al., 1995, WINKALER et al., 2001, DE LA TORRE et al., 2005). Os xenobióticos são substancias estranhas ao organismo as quais o ambiente aquático está exposto. Neste contexto os biomarcadores são utilizados como ferramentas na prevenção de danos para a qualidade do ambiente, pois podem ser remediados antes que ocorram danos ambientais irreversíveis o que prejudicaria o bom funcionamento da comunidade.

Estudos que utilizaram biomarcadores se mostraram eficientes para realizar diagnóstico ambiental (EVANS, 1987, HAAPARANTA et al., 1997, PLAYLE, 1998, BERNET et al., 1999, SILVA, 2002, ASUQUO et al., 2004, DURRIEU, 2005, WU et al., 2005, COSTA, 2006, MONSERRAT et al., 2007, TERRA, 2008). As principais vantagens da utilização de biomarcadores estão relacionadas à determinação da concentração, dos limites de tolerância e dos efeitos dos poluentes nos organismos. Esta metodologia permite ainda a avaliação do nível de transferência dos poluentes na cadeia trófica. As desvantagens podem ser consideradas as mesmas dos bioindicadores, por exemplo: locomoção dos organismos, dificuldade na identificação taxonômica, difícil amostragem e falta de especificidade das maiorias dos biomarcadores sobre as causas dos efeitos observados. .

Apesar dos estuários serem considerados ambientes produtivos, as atividades humanas no entorno deste ambiente acabam gerando efeitos negativos para a biota que ali vive. Como agravante muitos destes estuários ainda não possuem estudos que avaliem a sua integridade, uma vez que junto com a descarga dos rios e do transporte marinho, onde são despejados rejeitos, podem receber poluente como amônia, metais pesados, pesticidas e herbicidas (LEVINTON, 2001, LALLI; PARSONS, 1997, EDDY, 2005). Quando comparados com o ambiente marinho e de água doce, os estuários são considerados mais estáveis e resilientes quando sujeitos a estes contaminantes (NORDSTROM, 2011).

O estuário amazônico também se enquadra nesta categoria. Ele faz parte da maior bacia hidrográfica do mundo, a qual comporta um dos rios mais volumosos, o rio Amazonas. A dinâmica deste rio é resultado da sua poderosa descarga no Oceano Atlântico que, segundo Nittrouer et al. (1995), é tão forte que a água marinha raramente ultrapassa a sua desembocadura. A descarga do rio Amazonas é influenciada por quatro períodos: I) Período chuvoso, que é caracterizado pela descarga máxima de água, concentrada entre os meses de março a maio; II) Período intermediário chuvoso/seco, ocorrendo entre os meses de junho a agosto, caracterizado por ser um período de transição climática; III) Período seco, caracterizado por um clima de estiagem (pouca precipitação chuvosa) precedendo um volume mínimo de água concentrado nos meses de setembro a novembro. IV) Período intermediário seco/chuvoso, ocorrendo entre os meses de dezembro a fevereiro, caracterizado por ser um período de transição climática.

A composição da ictiofauna amazônica é marcada por variações sazonais, uma vez que alteram o teor salino e a turbidez da água variando ao longo do ano, devido à flutuação da pluma estuarina (BARTHEM, 1985). Desta forma, a fauna de peixes compensa essas mudanças na salinidade, através da substituição de espécies de água doce que dominam no período chuvoso, por espécies marinhas que dominam durante a estiagem em algumas áreas do estuário (BARTHEM, 1985, CAMARGO; ISAAC, 2001).

No estuário amazônico está localizado um dos maiores centros urbanos da região Norte, a cidade de Belém, no Estado do Pará. Esta região é influenciada pela intensiva atividade antropogênica, tanto urbana como industrial, afetando diretamente a qualidade biológica da área como demonstram alguns trabalhos já realizados na região, visando diagnosticar os efeitos da contaminação sobre a qualidade da água na bacia amazônica e no estuário, utilizando a biota (PAIVA et al., 2004, SILVA, 2006, NEVES, 2007; MONTES et al., 2010, ROCHA et al., 2010, VIANA et al., 2010).

Dentro desta região está localizado Vila Conde, no município de Barcarena, a cerca de 40 km a oeste do município de Belém. Vila do Conde foi escolhida para construção do porto por apresentar um posicionamento geográfico privilegiado, com fácil acesso marítimo, fluvial e rodoviário. Trata-se de um porto complementar ao de Belém pela sua disponibilidade para navios de maior calado (até 12,5 m). Na área adjacente ao porto está localizado o distrito industrial (com a presença de várias indústrias de mineração) fazendo este porto a movimentação dos produtos destas indústrias: bauxita, coque, alumina, alumínio primário, óleo combustível, soda cáustica, piche, fertilizantes agrícolas, manganês e caulim, para atender essas indústrias (CDP, 2010).

Desde a instalação das indústrias (em 1985), vários tipos de acidentes já foram constatados em Vila do Conde por moradores da região, entretanto, somente a partir de 2003 é que os seus efeitos sobre a qualidade da água passaram a ser diagnosticados através do Instituto Evandro Chagas, que apresentou um relatório mostrando alterações na qualidade da água. Em abril e junho de 2003 foram registrados transbordamentos de efluentes da bacia de resíduos e o derrame de soda caustica, respectivamente, provocando a mortandade de peixes nos dois casos (PORTO, 2009). Em junho de 2007 foi registrado novamente o transbordamento da bacia de resíduo de uma empresa da região causando alteração físico-química da água e colocando em risco a saúde da população local (O LIBERAL, 2007). Em setembro/2008 houve um vazamento de óleo diesel marítimo (produto derivado do petróleo), originado pelo naufrágio de um rebocador no rio Pará (DIÁRIO DO PARÁ, 2008a, 2008b). Em abril de 2009, um acidente envolvendo um vazamento de resíduo de bauxita com soda cáustica e arsênio causou mortandade de peixe, irritações e sintomas de intoxicação nos moradores da área bem como o impedimento da pesca local (LIMA et al., 2009; PEREIRA, 2009).

Diante de tantos acidentes industriais, o Instituto Evandro Chagas, através da Seção de Meio ambiente (SAMAM), criaram o "Programa de Monitoramento e Controle em saúde e Meio Ambiente nas Áreas Industriais e Portuárias dos municípios de Abaetetuba e Barcarena, Estado do Pará", cujo objetivo principal é diagnosticar e acompanhar a qualidade ambiental destes dois municípios. Apesar de este monitoramento abranger diversos aspectos ambientais e sociais não existe nenhum objetivo específico relacionado a resposta da ictiofauna diante dos diversos acidentes já ocorridos

Além da industrialização, em Barcarena também se destaca a pesca artesanal devido à elevada dependência da população em relação aos recursos pesqueiros. Em 2006, a receita bruta deste município provinda da pesca foi estimada em torno de R$ 720.000,00 e os barcos de pequeno porte foi a categoria mais rentável, com cerca de 41% da receita bruta total (PAZ, 2007). Alguns estudos realizados no entorno do porto de Vila do Conde demonstram que a ictiofauna utiliza esta área para reproduzir, crescer e se alimentar (BARBOSA, 2009, LOUREIRO et al., 2008, LUZ, 2009). Entretanto não existem informações suficientes que apontem se os impactos da área industrial de Vila do Conde estão afetando a ictiofauna.

Dentre as espécies de peixes destacam-se a pescada branca, *Plagioscion squamosissimus* (Heckel, 1840), pertencente à família Sciaenidae e o bacu, *Lithodoras dorsalis* (Valenciennes, 1840), à família Pimelodidae, que possuem importância tanto econômica como nutritiva para a comunidade que vive no município de Barcarena, além de serem abundantes ao longo de todo ano nas áreas de estudo. *P. squamosissimus,* é uma espécie carnívora, de hábito zoobentônico, com preferência alimentar para camarão e peixes (GOULDING; FERREIRA, 1984, RAIOL et al., 2006, COSTA et al., 2009). *L. dorsalis* é considerada oportunista, possuindo preferência alimentar por vegetais e sementes, sempre que disponíveis. (SANTOS et al., 2006, RAIOL et al., 2006, BARBOSA; MONTAG., 2011).

Kennish (1986) e Blaber (2000) citam que a degradação dos ecossistemas sujeitos a rejeitos de processos industriais pode reduzir a disponibilidade das populações de espécies exploráveis, resultando em problemas econômicos e sociais para as comunidades. Os processos industriais produzem rejeitos que podem liberar fluoreto, cloreto, sulfato, bicarbonato, entre outras substâncias que, em contato com a água, tendem a causar alterações significativas em sua qualidade. Além disso, o tratamento de efluentes pode aumentar os teores de alguns íons metálicos, como ferro e chumbo (RUBIO; TESSELE, 2002).

Acredita-se que algumas substâncias poluidoras lançadas nos ambientes aquáticos se acumulem nas espécies com maior nível trófico da cadeia alimentar (KASPER et al., 2007, TERRA et al., 2008). As substâncias podem ser neutralizadas, modificadas ou biotransformadas, tendo seu potencial tóxico elevado, através da biomagnificação (LOPES-POLEZA, 2004). Assim, os predadores são os mais afetados pela contaminação da água, possuindo maior probabilidade de afetar a saúde do homem, pelo consumo de pescado contaminado (LAWRENCE; HEMINGWAY, 2003).

Com base no exposto, este trabalho teve como objetivo principal avaliar a qualidade ambiental no distrito industrial no município de Barcarena, estuário do rio Pará utilizando a ictiofauna como bioindicador e o fígado das espécies *P. squamosissimus* e *L. dorsalis* como biomarcador histopatológico. Nesta pesquisa foi considerado que estudos sobre a qualidade ambiental são altamente relevantes já que a contaminação pode afetar não somente o equilíbrio ecológico do ambiente como também econômica, da região dada à dependência das comunidades ribeirinhas em relação aos recursos naturais, destacando os recursos pesqueiros. Estes tipos estudos servem para a elaboração de diagnósticos sobre o estado de saúde da biota no ambiente aquático e são eficientes como um critério para identificar o grau de impacto que um possível acidente possa ocasionar, no caso deste trabalho, no entorno de Vila do Conde.

2. HIPÓTESES

1. Os peixes são excelentes indicadores da qualidade do ambiente, pois são capazes de detectar alterações sofridas do meio aquático através da modificação da ictiofauna. Destacando que na Amazônia, a ictiofauna do canal de maré é mais afetada com a contaminação do que a ictiofauna do canal principal.

2. As espécies de peixes podem adquirir histopatologias no fígado em ambientes mais próximos a locais impactados.

3. Espécies com maior nível trófico na cadeia alimentar são mais propensas a contaminação ambiental quando comparadas às espécies de menor nível trófico.

3. METODOLOGIA

3.1. ÁREA DE ESTUDO

O porto de vila do Conde foi inaugurado pela Companhia Docas do Pará- CDP no ano de 1985. Ele está situado na margem direita do estuário do rio Pará, no distrito de Vila do Conde, município de Barcarena-PA, a cerca de 40 km a oeste de Belém, nas coordenadas 01°32'42"S 48°45'00"W (CDP, 2010). Limita-se ao norte com a Baía do Marajó e a Baía do Guajará; ao sul, com os municípios de Moju e Abaetetuba; a leste, com a Baía de Guajará e o município de Acará; a oeste, com a Baía do Marajó (CDP, 2010). Possui uma área de 1316,299 km^2 (GOVERNO DO ESTADO DO PARÁ, 1999). Esta área é uma região estuarina com grande influência de água doce, classificada de acordo com Elliott; Mclusky (2002) como um estuário de maré fluvial.

A sedimentação da área é predominantemente litoclástica arenosa variando desde afloramentos rochosos da formação barreira até areia lamosa. As correntes podem atingir até 1,24 m/s e sua hidrografia é caracterizada principalmente pela presença do rio Pará, Baía do Marajó e Golfão marajoara além dos furos e ilhas que recortam a região (Martins and Mendes, in press).

O desenho amostral deste estudo levou em consideração o grau de contaminação ambiental, decorrente da proximidade com o terminal de cargas de Vila do Conde. Nesse sentido as coletas das variáveis ambientais e da ictiofauna ocorreram em três áreas:

- Zona 1 – Localizado no estuário do rio Pará, na área de entorno do terminal portuário e do polo industrial de Vila do Conde (Figuras 1 e 2), que apresenta o maior risco de contaminação ambiental;

- Zona 2 – Localizado no estuário do rio Pará, no entorno da ilha do Capim, divisa do município de Barcarena e Abaetetuba (Figuras 1 e 3), considerada com risco mediano em relação ao grau de contaminação ambiental;

- Zona 3 – Localizado em um furo denomidado de Furo Grande, ilha das Onças, município de Barcarena (Figuras 1 e 4), com nível de impacto mínimo, dada a sua posição afastada do distrito industrial de Vila do Conde.

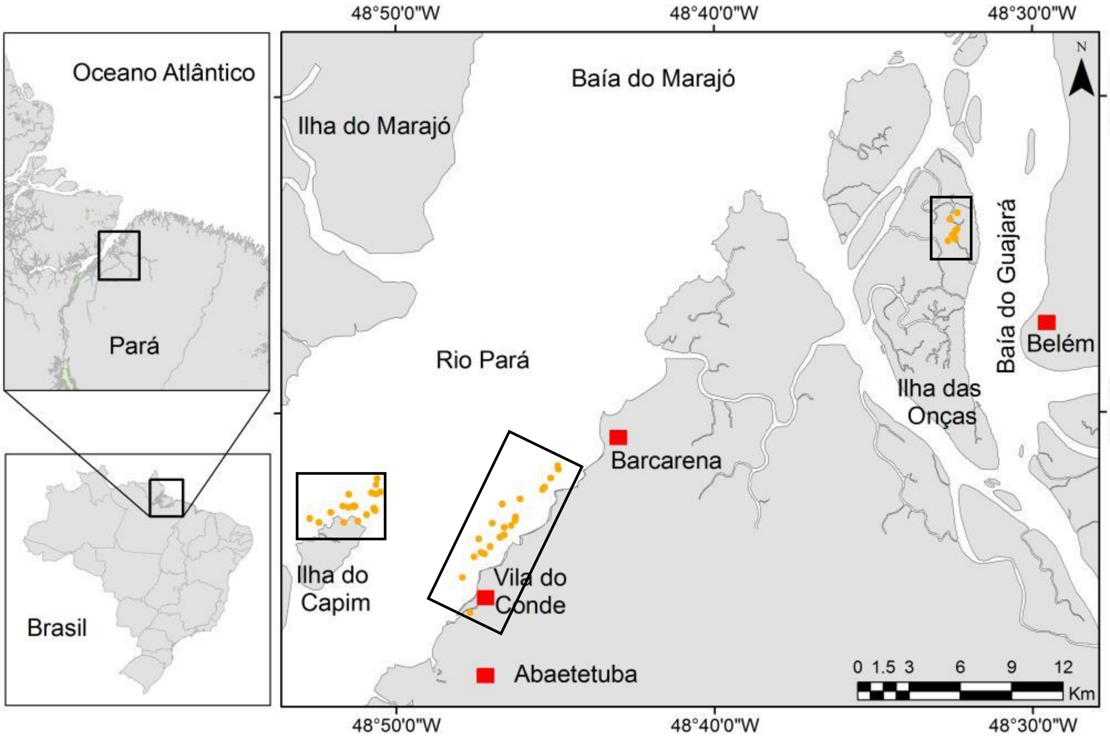

Figura 1. Mapa de localização da área de estudo. Zona 1 – Entorno do terminal portuário de Vila do Conde; Zona 2 – Ilha do Capim, divisa do município de Barcarena e Abaetetuba; Zona 3 – Ilha das Onças.

Figura 2. Entorno do terminal portuário de Vila do Conde, estuário do rio Pará, município de Barcarena-PA.

Figura 3. Ilha do Capim, estuário do rio Pará, divisa do município de Barcarena-PA e município de Abaetetuba-PA.

Figura 4. Ilha das Onças, município de Barcarena-PA.

Para todas as áreas de estudo foram feitas amostragens tanto no ambiente de canal ou curso principal do rio, quanto nos canais de maré localizados as margens do canal principal. Os pontos de amostragens foram estabelecidos de acordo as áreas de estudo e com a viabilidade de acesso para obtenção dos dados. No caso dos canais de maré, o tamanho também foi considerado. As amostragens foram padronizadas para todas as áreas de estudo e seus respectivos ambientes.

Antes da coleta efetiva de dados foi realizada uma amostragem, piloto com o objetivo de selecionar as áreas que melhor representassem cada ambiente, de acordo com as hipóteses do trabalho (Figura 5). Após estabelecido as áreas de coleta, foram feitas quatro coletas bimestrais no canal principal e no canal de maré, entre junho de 2009 a abril de 2010, considerando os diferentes períodos da região: período chuvoso (Abril/2010), transição chuvoso/seco (Junho/2009) , seco (Setembro/2009) e transição seco/chuvoso (Janeiro/2010). As coletas foram padronizadas nas luas de quadratura.

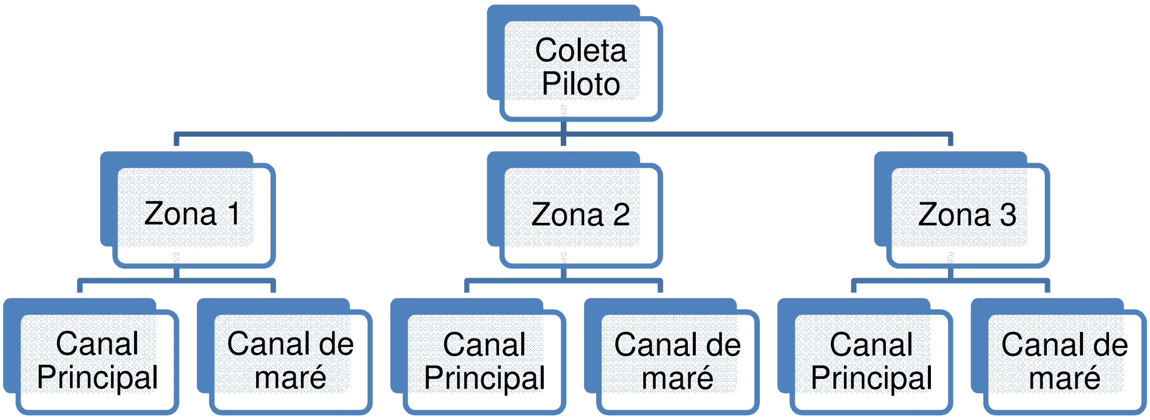

Figura 5. Fluxograma do desenho amostral para as coletas de dados mostrando a seleção das área durante a coleta piloto e a classificação da qualidade ambiental definida em diferentes níveis: Zona 1 - maior risco de contaminação ambiental; Zona 2 –risco mediano em relação ao grau de contaminação ambiental; Zona 3 – com nível de impacto mínimo.

3.2. METODOLOGIA DE CAMPO

3.2.1. Ictiofauna

Dadas as peculiaridades de cada ambiente de estudo, para o canal principal foi utilizado a rede de emalhar e para o canal de maré, a rede de tapagem. As artes de pesca foram padronizadas para todas as áreas e ambientes.

A rede de emalhar de fundo foi confeccionada com fios nylon monofilamento com diferentes tamanhos de malha – 25 mm, 35 mm, 40 mm - (medidas entre nós adjacentes). As redes foram emendadas umas as outras, apresentando desta forma comprimento total de 133,2m (Figura 6). Para cada área a rede foi lançada 3 vezes por um período de 1 hora e 30 minutos para cada lance.

Figura 6. Rede de emalhar utilizada no canal principal para todas as áreas de coleta.

A rede de tapagem foi confeccionada com rede de nylon monofilamento, com 25 mm entre nós adjacentes, 35 m de comprimento e 7 m de altura (Figura 7). Esta rede foi utilizada na saída do canal de maré, fechando-os completamente. O período de permanência da tapagem foi durante a maré vazante (em torno de 6 horas). Para cada área foi selecionado apenas um canal de maré.

Figura 7. Rede de tapagem utilizada no igarapé para todas as áreas de coleta

Todo o material pescado foi resfriado no gelo até a chegada no Laboratório de Dinâmica, Avaliação e Manejo dos Recursos Pesqueiros, na Universidade Federal do Pará, onde foram analisados.

Para as espécies *Plagioscion squamosissimus* e *Lithodoras dorsalis*, selecionadas para a avaliação da histopatologia do fígado, o processamento foi feito logo após a retirada dos indivíduos das artes de pesca. O fígado foi removido e pesado, posteriormente foi retirada uma fração do tecido e fixado com solução Bouin por um período de 48 horas. Após este período o tecido foi conservado em álcool 70% até o seu processamento.

4. ESTRUTURA DA TESE

Este trabalho foi estruturado em várias etapas seqüenciais e complementares de forma a cumprir com o objetivo proposto e responder as hipóteses formuladas:

1. A primeira etapa do trabalho consistiu na descrição da ictiofauna relacionada aos seus aspectos ecológicos. Nesse contexto foi descrita a composição e caracterização da ictiofauna, avaliando-se ainda o grau de dependência que possuem em relação aos ambientes de estudo (berçário, alimentação, reprodução). Posteriormente, as espécies da ictiofauna foram utilizadas como bioindicador na avaliação da qualidade ambiental no entorno de Vila do Conde comparando com uma área de menor influência industrial e portuária (Capítulo 1).

2. A utilização da histopatologia do fígado como ferramenta para análise da qualidade ambiental de Vila do Conde foi descrita no capítulo 2. Os biomarcadores foram avaliados nas espécies *Plagioscion squamosissimus* e *Lithodoras dorsalis*, que possuem níveis tróficos diferenciados, como forma de verificar a intensidade das patologias nestas espécies.

3. No capítulo 3 foi feita a integralização das informações biológicas através da utilização de índices de integridade. Neste capítulo, atributos e métricas foram selecionados e posteriormente integrados no calculo de diferentes índices na avaliação do estado de integridade da comunidade de peixes.

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CAPÍTULO I

ICTHYOFAUNA AS INDICATOR OF ENVIRONMENTAL QUALITY IN AN INDUSTRIAL DISTRICT OF AMAZON ESTUARY, BRAZIL

ICTIOFAUNA COMO INDICADORA DA QUALIDADE AMBIENTAL EM UM DISTRITO INDUSTRIAL NO ESTUÁRIO AMAZÔNICO, BRASIL

ICTHYOFAUNA AS INDICATOR OF ENVIRONMENTAL QUALITY IN AN INDUSTRIAL DISTRICT OF AMAZON ESTUARY, BRAZIL

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Abstract

The objective of the present study was to describe the quality environmental of the aquatic ecosystem in an industrial district (Pará, Amazon estuary), using the ichthyofauna as a bioindicator. The study area was divided into three zones: zone 1, adjacent to the industrial hub and cargo terminal, zone 2, intermediate, and zone 3, representing an area of minimum impact, isolated from industrial impacts. Impacts were assessed based on four groups of descriptors: habitat use by the different fish species, community descriptors, multivariate analysis and environmental descriptors. A total of 77 species were captured. Differences in the composition of the ichthyofauna were recorded between zones and environments (main channel and tidal channel). The ecological indices revealed clear evidence of the impact of the industrial hub and cargo terminal on the local fish communities, in comparison with the more isolated zone. In zone 1, there was a reduction in the number of thophic guilds (in main channel) and larger fish, while Shannon diversity index and Margalef's richness were also significantly lower than in the less impacted areas. The multivariate analysis separated the different zones clearly into three groups, indicating marked differences in the levels of contamination in the different parts of the study area.

Keywords: Ichthyofauna; Bioindicator; Amazon estuary; Industrial hub.

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1. Introduction

The Amazon estuary is the oceanic outlet of the World’s largest hydrographic basin, with a total discharge equivalent to approximately one sixth of that of all the rivers in the World combined, containing one fifth of all the freshwater released into the planet’s oceans (Martinelli et al*.,* 1989). The dynamics of this estuary reflect the force of this discharge into the Atlantic Ocean, which is so strong that seawater rarely passes through the mouth of the river (Nittrouer et al., 1995). The composition of the region’s ichthyofauna is influenced by seasonal fluctuations in salinity levels and the turbidity of the water, which is controlled by the estuarine plume (Barthem, 1985).

The Amazon estuary is adjacent to the municipality of Barcarena (around 84.560 inhabitants). Within this region the district of Vila do Conde is an important industrial hub, where activities include the processing of kaolin, alumina and aluminum for export. These processes produce residues which may liberate substances such as fluorides, chlorides, sulfates and bicarbonates into the aquatic environment, with significant impacts on the quality of the water. The treatment of effluents may also lead to an increase in the levels of metallic ions, such as those of iron and lead (Rubio and Tessele, 2002).

A number of studies have shown that the degradation of aquatic ecosystems exposed to discharges of industrial waste may lead to a reduction in the abundance of commercially-important species, resulting in economic and social problems for local communities (Blaber, 2000; Kennish, 1985). The residues produced by the industries of Vila do Conde constitute a risk factor for the quality of both superficial waters and underground reservoirs. The principal sources of risk include leaks from tanks and pipelines, accidental spills of toxic substances and the overflowing of residue sedimentation pools, all of which have been recorded in the study area since the establishment of the industrial installations (Carneiro et al., 2007; Lima et al., 2009).

Estuarine environments, in particular that of the Amazon, which surrounds the Vila do Conde cargo terminal, are characterized by high primary and secondary productivity and provide nurseries for numerous species of fish and other aquatic organisms, many of which are of commercial value. This study area is an estuarine environment with a considerable freshwater input, being classified as a tidal freshwater estuary according to the scheme of Elliott and McLusky (2002). This area is influenced by the Pará River, Marajó Bay and Gulf and also by several islands and channels that encompass the region. Currents in this area may reach 1.24 m/s (Martins and Mendes, in press).

Despite the intense industrial activity at Vila do Conde, the area is an important artisanal fishing ground and the local population is highly dependent on fishery resources (Paz et al., in press). In addition to its socio-economic importance, the area plays an important ecological role in the reproduction, feeding and development of many fish species (Ferreira et al., 2008; Loureiro and Frédou, 2010).

Biological monitoring is a method of assessing water quality through the responses of biological communities to changes in environmental conditions (Goulart and Callisto, 2003; Whitfield and Elliott, 2002), such as those observed in Vila do Conde. Bioindicators serve as proxies for the evaluation of contamination levels and changes in the distribution and relative abundance of bioindicator species can signal environmental deterioration (Harrison and Whitfield, 2004; Karydis and Tsirtsis, 1996). Fish can be effective bioindicators and have been used successfully for the assessment of the quality of many freshwater environments in the Amazon basin (Cleto Filho, 2003; Silva, 1995). In the case of its estuarine environments, Viana et al. (2010) used the ichthyofauna as a bioindicator for the assessment of Guajará Bay. The city of Belém, one of the largest urban centers in the Amazon region, is located in the Amazon estuary, in the northeast of the Brazilian state of Pará. This region is characterized by intense anthropogenic impacts, both urban and industrial, which have had a direct effect on the local natural systems (Aviz et al., 2006; Viana et al., 2010).

No data are available on the possible impacts of the industrial activities at Vila do Conde on the local ichtyofauna. Given this, the present study focused on the environmental quality of the aquatic habitats adjacent to this site, using the fish community as a bioindicator.

2. Material and methods

2.1. Study area and data collection

The port of Vila do Conde (01°32’42’’S, 48°45’00’’W) is located on the right bank of the estuary of Pará River in the district of Vila do Conde, part of the municipality of Barcarena in the Brazilian state of Pará. The port is approximately 40 km west of the city of Belém (CDP, 2010). The present study was designed to test the effects of the probable environmental contamination in the area adjacent to the industrial installations and cargo terminal of Vila do Conde.

For this, the collection of abiotic data and fish specimens was organized in three distinct zones, representing different levels of impact. Zone 1 was located in the estuary of Pará River, in the vicinity of the Vila do Conde cargo terminal (Fig. 1), where the risk of contamination was highest. Zone 2 was Capim Island, in the estuary Pará River, on the border between the municipalities of Bacarena and Abaetetuba (Fig. 1), classified as a median risk area due to its relative proximity to the port. Zone 3 was Onças Island in the municipality of Barcarena (Fig. 1), which was classified as minimum risk, due to its distance from the industrial district of Vila do Conde.

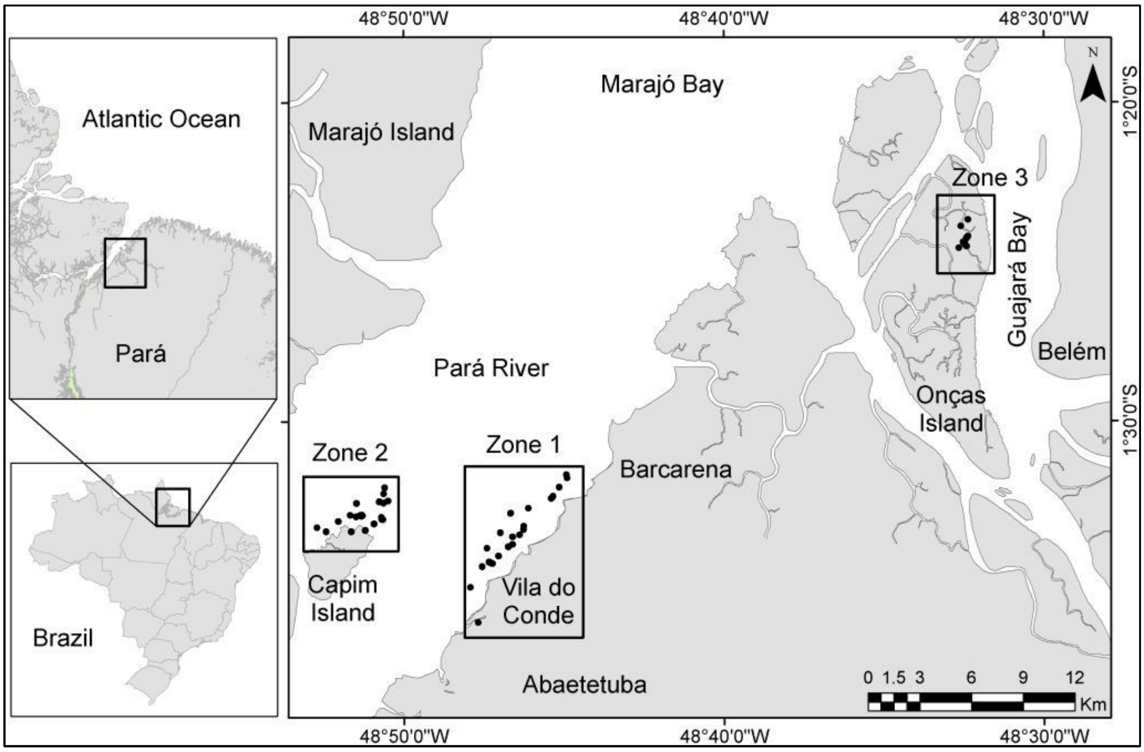

Fig. 1. Study area with the sampling points in the each zone in Amazon estuary, Brazil, from 2009 to 2010. Zone 1: located in the vicinity of the Vila do Conde cargo terminal; Zone 2: Capim Island; Zona 3: Onças Island.

Two different types of environment – the main river channel and tidal channel – were sampled in all three zones. Samples were collected every two months, between 2009 (June and September) and 2010 (January and April), covering the region’s principal climatic periods: rainy-dry transition (R-D), dry season, dry-rainy transition (D-R) and rainy season (R). All samples were collected during neap tides.

Different sampling protocols were used in the channel and tidal channel, due to their distinct dynamics. In the channel, monofilament gillnets with different stretched mesh sizes were used (25, 35 and 40 mm), stretched mesh sizes (133.2 m in total) were used. The nets were allowed to drift for an average soak time of 1 h 30 min. In the tidal channel, a block net (25 mm mesh size) was set at the mouth of the tidal channel, closing it completely. Blocking was initiated at the end of the high tide and continued throughout the entire ebb tide cycle (*c.* 6 h). The fish were caught either by being gilled in the net (smaller specimens) or collected manually in the pools remaining near the net. All specimens were stored on ice and transported to the laboratory for processing. Samples were standardized for all study areas and environments.

Water samples were collected from the surface layer. The temperature, dissolved oxygen and pH were measured in the field using a multianalyzer and by titration using the Winkler method. The analysis of nutrients – nitrate (NO_3), phosphate (PO_4) and ammonia (NH_4) – alkalinity, turbidity and total hardness was conducted on 2-liter samples of water, which were stored on ice for up to 24 hours prior to processing in a specialized laboratory. Two water samples were taken from each sampling point.

The fish specimens were identified to the lowest possible taxonomic level, based on Espirito Santo et al. (2005), FAO (1992) and Keith et al. (2000). Specimens were measured and weighed in the laboratory and had their gonads and stomach removed and weighed. Their maturation state was determined by the size and color of the gonads, based on Vazzoler (1996): stage A: immature; stage B: maturing; stage C: mature; stage D: spent.

2.2. Data analysis

For the evaluation of areas subject to different levels of contamination (zones 1, 2, and 3), the data were analyzed in the context of seasonal variation (four climatic periods) and environmental differences (main channel and tidal channel). The main channel and tidal channel habitats were analyzed separately due to the ecological differences found between them and the consequent possibility of different responses to anthropogenic impacts (Viana et al., 2010). Four descriptors or analytical approaches: (I) habitat use (evaluation of the degree of dependency of fish species on areas for reproduction and nurseries); (II) community descriptors; (III) multivariate analysis; (IV) environmental descriptors. All analyses were run in the programs Microsoft Office Excel 2007, Statistica 8.0 and Primer 6.1.6.

2.2.1. Habitat use

Based on Viana et al. (2010), individuals with mature (stage C) and spent (D) gonads were considered to be using the area as a reproduction ground. Specimens with immature gonads (stage A) indicated that the area was being used as a nursery. Possible differences in the distribution of the different stages among the study areas were evaluated using the Qui-square test.

The repletion index (RI) was calculated in order to provide a quantitative measure of the intensity of feeding. This index is provided by the formula $RI = 100\ WSWF^{-1}$, where WS = weight of the stomach contents and WF = weight of the fish. High values for this index indicate more intense feeding in the area examined (Zavala-Camin, 1996). The differences between areas and periods were tested using a one-way ANOVA. When necessary, the data were log (x+1) transformed to make the variance homogeneous. Bartlett's test was used to determine the homogeneity of the variances and differences were further explored with Tukey's *post hoc* test (Zar, 1996).

The individuals were classified by size, following Viana et al. (2010). Species with total length of less than 15 cm were classified as small, those between 15 cm and 30 cm in length as medium and fish over 30 cm in length as large. Variation in the body size of the different species among zones was analyzed using the Kruskal-Wallis test.

2.2.2. Community descriptors

The frequency of occurrence of the different fish species was evaluated based on the scheme proposed by Dajoz (1973). Species with a frequency of occurrence of ≥50% were classified as constant, those with a frequency between 25 and 50% as accessory and those with a frequency of <25% as occasional.

Feeding functional groups, based on feeding preferences and strategies, were defined for each area. For this, species were allocated to feeding guilds (based on Elliott et al., 2007 and Krumme et al., 2004): zooplanktivore (ZP); detritivore (DV); piscivore (PV); zoobenthivore (ZB); opportunist/omnivore (OP).

Two additional categories were included: piscivore/zoobenthivore: PV/ZB and insectivore (IS). The trophic categories found in the literature were revised according to this scheme and feeding mode functional groups were identified by combining the information available on predominant diet and feeding site. Where little information was available, trophic preferences were inferred from data gathered by the Fishbase project (Froese and Pauly, 2007). The percentage contribution of each functional category to the total species richness and individual abundance was calculated for each area. The results were compared among groups in order to assess the prevailing feeding strategies adopted by fish community.

The Catch per Unit of Effort (CPUE) was used to assess the relative abundance of fish in the main channel, while density was used for the tidal channel. The CPUE values were based on the numerical abundance or density (number of individuals, n) and biomass (total weight, b). For the main channel, CPUE = $100n$ or $b\ (AT_i)^{-1}$, where A is net length in meters, and T_i is soak time in minutes. In the case of the tidal channel, the density index was obtained by n or b/Ai, where Ai is the flooded area, which was estimated for each tidal channel at the peak of the high tide during the neap tide.

Shannon's diversity index (H'), Pielou's evenness index (J), Simpson's index (λ), total species present (S) and Margalef's index (D) were used to assess community structure. The differences in the values of these indices among the different study zones were evaluated using one-way ANOVA, followed by Tukey's *post hoc* test (Zar, 1996). For nonparametric data, the Kruskal-Wallis analysis of variance was used.

2.2.3. Multivariate analysis

A multivariate multidimensional scaling (MDS) analysis was used to evaluate the effects of spatial (Zones) and temporal variation in species composition. All groups were also tested using the analysis of similarities two-way nested ANOSIM (Clarke and Warwick, 1994). Catch per unit of effort (CPUE) was used as data entry for multivariate analyses.

2.2.4. Environmental parameters

A Principal Components Analysis (PCA) was used to evaluate the effects of the alkalinity, hardness, turbidity and the concentrations of phosphates, nitrates and ammonia in water. The data were first log-transformed (log (x+1)) for analysis. The significance of the effect of the variables on the axes was tested using the method described by Legendre and Legendre (1998), in which the coordinates of a variable in relation to a factorial axis is equal to the coefficient of correlation between this variable and the axis. A variable is considered significant when its distance (d) from the center of the plane is equal to or greater than the square root (2/m), where m is the number of variables (Valentin, 2000). The BIO-ENV routine was applied to the combined biological data (with Bray-Curtis similarity) and the reduced set of categorized environmental data (with Euclidean distance similarity) in one single analysis. All the groups formed were tested using the analysis of similarities (two-way nested ANOSIM)

3. Results

3.1. Ichthyofauna

A total of 1.708 fish specimens belonging to 77 species, 27 families and 10 orders were captured (Table 1). The Characiformes, Siluriformes and Gymnotiformes were the most abundant orders in terms of the number of families. The Cichlidae (15.2% of the total) and Loricariidae (10.1%) were the families richest in species, while *Plagioscion squamosissimus* (22.13% of the total number of specimens) and *Lithodoras dorsalis* (21.75%) were the most abundant species.

Considering both main channel and tidal channel, 23 species were captured in zone 1, 49 in zone 2, and 50 in zone 3 (Table 1). Ten species occurred in all three areas. *Plagioscion squamosissimus, L dorsalis*, *Geophagus proximus* and *Hypophthalmus marginatus* were the most abundant species (Table I). Only four species (*Brachyplatystoma rousseauxi*, *Crenicichla lugubris*, *Geophagus* sp.1 and sp.2) were exclusive to zone 1 (Table 1). A large proportion of species were exclusive to zones 2 (19) and 3 (21). In zone 2, *Cichla temensis* and *Potamotrygon orbignyi* were the most abundant species, while in zone 3, *Curimata inornata* and *Triportheus elongatus* were the most prominent.

Medium-sized fish (TL 15-30 cm) predominated in all zones, with an overall average of 65.6%. Fish of between 15 and 25 cm in length were the most abundant (Fig. 2). Large fish were least common in zone 1, whereas small fish were least common in zone 3 (Fig. 2).

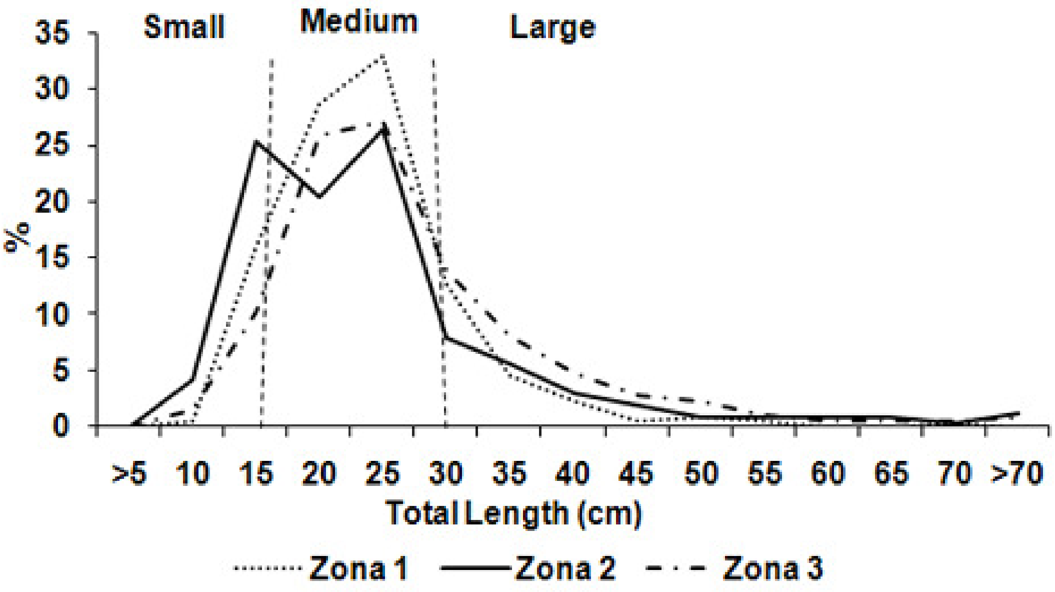

Fig. 2. Frequency of occurrence of total length by zone (Z) and body size of individuals in Vila do Conde, Amazon estuary, Brazil, from 2009 to 2010.

Table 1. Species captured in study areas in Amazon estuary, Brazil, from 2009 to 2010. Total length (TL) minimum and maximum; constancy of species: C – Constant; A – accessory; O – occasional; Mc – Main channel; Tc – Tidal Channel; 1 – Zone 1; 2 – Zone 2; 3 – Zone 3.

Ordem, Family	Species	TL (cm) min-max	Cs
CHARACIFORMES			
Anostomidae	*Leporinus fasciatus* (Bloch, 1794)	20.5-32	A(3 Mc)
	Leporinus friderici (Bloch, 1794)	23-26	A(3 Tc)
Characidae	*Astyanax fasciatus* (Cuvier, 1819)	8-12	C(2, 3 Tc)
	Specie 1	-	O(2 Tc)
	Specie 2	12.8	A(3 Tc)
	Pristobrycon calmoni (Steindachner, 1908)	7	O(3 Mc)
	Triportheus elongatus (Günther, 1864)	19-24.5	A(3 Mc)
Ctenoluciidae	*Boulengerella cuvieri* (Spix & Agassiz, 1829)	31.5-37.5	A(2 Tc)
Curimatidae	*Curimata inornata* Vari, 1989	11-16.2	C(3 Tc) A(3 Mc)
Cynodontidae	*Raphiodon vulpinus* Agassiz, 1829	34	A(3 Tc)
Erythrinidae	*Hoplias malabaricus* (Bloch, 1794)	23-25.5	A(1, 3 Tc)
Hemiodontidae	*Hemiodus unimaculatus* (Bloch, 1794)	19.5-23	O(2 Tc)
CLUPEIFORMES			

Engraulididae	*Anchoa spinifer* (Valenciennes, 1848)	7.8-16.8	A(1,2 Mc)
	Anchovia surinamensis (Bleeker, 1865)	7-11	A(3Mc; 3 Tc) O(1 Mc)
	Lycengraulis batesii (Günther, 1868)	19.4-22.5	A(3 Tc). O(3, 2 Mc)
	Pterengraulis atherinoides (Linnaeus, 1766)	17.5-21.2	A(2, 3 Tc). O(3 Mc)
Pristigasteridae	*Pellona castelnaeana* Valenciennes, 1847	20-23	O(1, 2 Mc)
	Pellona flavipinnis (Valenciennes, 1837)	17.3-52.5	C(1 Mc) O(2 Tc)
CYPRINODONTIFORMES			
Anablepidae	*Anableps anableps* (Linnaeus, 1758)	15.3-23	C(2 Tc); A(3 Tc)
GYMNOTIFORMES			
Apteronotidae	*Apteronotus albifrons* (Linnaeus, 1766)	25.5-52	A(3 Tc)
	Sternarchella terminalis (Eigenmann & Allen, 1942)	30-36	A(3 Tc)
	Sternarchorhamphus muelleri (Steindachner, 1881)	26	A(3 Tc)
Rhamphichthyidae	*Rhamphichthys marmoratus* Castelnau, 1855	36.5-78	C(3 Tc) A(1 Tc) O(3 Mc; 2 Tc)
	Rhamphichthys rostratus (Linnaeus, 1766)	50-101	C(3 Tc) A(1, 2 Tc) O(3 Mc)
Sternopygidae	*Rhabdolichops caviceps* (Fernández-Yépez, 1968)	33-41.5	A(2 Tc)
	Sternopygus macrurus (Bloch & Schneider, 1801)	30.2-58	C(1, 3 Tc) A(2 Tc) O(1, 3 Mc)
MUGILIFORMES			
Mugilidae	*Mugil incilis* Hancock, 1830	12.2-55	C(2 Tc); O(3 Mc)
PERCIFORMES			
Cichlidae	*Cichla orinocensis* Humboldt, 1821	58-69.5	A(2 Tc)

	Cichla pinima Kullander & Ferreira, 2006	25.5-39	A(2 Tc)
	Cichla pleiozona Kullander & Ferreira, 2006	15.3-63	A(2 Tc)
	Cichla temensis Humboldt, 1821	18.5-60	C(2 Cr)
	Crenicichla johanna Heckel, 1840	19.2-24.6	A(1, 3 Tc)
	Crenicichla lugubri Heckel, 1840	23.5-27	A(1 Tc)
	Crenicichla sp.	16-18	A(2 Mc)
	Geophagus proximus (Castelnau, 1855)	8.5-23.5	C(1, 2, 3 Tc) A(3 Mc)
	Geophagus sp. 1	16.5	A(1 Tc)
	Geophagus sp. 2	15	A(1 Tc)
	Geophagus surinamensis (Bloch, 1791)	10	O(2 Tc)
	Crenicichla semifasciata (Heckel, 1840)	18.5-32.5	A(3 Tc)
Sciaenidae	*Pachypops fourcroi* (Lacepède, 1802)	10.5-23.5	C(1, 2, 3 Tc) O(3 Mc)
	Plagioscion auratus (Castelnau, 1855)	15.5-31	A(2 Mc; 3 Tc) O(2 Tc)
	Plagioscion squamosissimus (Heckel, 1840)	9.5-35.5	C(1,2 Mc; 1, 2, 3 Tc) A(3 Mc)
	Plagioscion surinamensis (Bleeker, 1873)	13-27	C(3 Tc) A(1, 2 Tc; 2, 3 Mc)
PLEURONICTIFORMES			
Achiridae	*Achirus achirus* (Linnaeus,1758)	7-13	C(2 Tc)
	Apionichthys dumerili (Kaup,1858)	12-14.2	O(2 Tc)
Paralichthyidae	*Citharichthys spilopterus* Günther, 1862	-	O(2 Tc)
	Syacium papillosum (Linnaeus, 1758)	9.5-11.5	A(2 Tc)

RAJIFORMES			
Potamotrygonidae	*Potamotrygon motoro* (Müller & Henle, 1841)	26-29	C(1 Tc) A(2 Tc)
	Potamotrygon orbignyi (Castelnau, 1855)	24-38	C(2 Tc)
	Potamotrygon sp.	21.6-35.8	A(2 Tc) O(2 Tc)
SILURIFORMES			
Ariidae	*Sciades couma* (Valenciennes, 1840)	26-44	A(3 Tc) O(3 Mc)
	Sciades herzbergii (Bloch, 1794)	19-23	O(2 Mc)
Aspredinidae	*Aspredinichthys filamentosus* (Valenciennes, 1840)	25.5	A(3 Tc)
	Aspredo aspredo (Linnaeus, 1758)	19-20	A(3 Tc) O(3 Mc)
Auchenipteridae	*Ageneiosus* aff. *ucayalensis* Castelnau, 1855	9.7-27	C(3 Tc) A(2 Tc) O(2, 3 Mc)
	Ageneiosus inermis (Linnaeus, 1766)	35	A(3 Tc)
	Pseudauchenipterus nodosus (Bloch, 1794)	6.5-7.5	A(3 Tc) O(2 Tc)
	Trachelyopterus galeatus (Linnaeus, 1766)	12-21.5	C(3 Tc) A(1 Tc ; 3 Mc)
Doradidae	*Lithodoras dorsalis* (Valenciennes 1840)	10.17-21	C(1, 2 Mc; 1, 3 Tc) O(3 Mc)
	Lithodoras sp.	21-150	A(3 Tc)
Heptapteridae	*Pimelodella gr. altipinnis* (Steindachner, 1864)	13-22	C(3 Tc) A(2 Tc)
	Rhamdia quelen (Quoy & Gaimard, 1824)	20-21	A(3 Tc)
Loricariidae	*Acanthicus hystrix* Agassiz, 1829	43	O(2 Mc)
	Ancistrus sp. 1	15	A(3 Tc)
	Ancistrus sp. 2	15-19	A(3 Tc)

	Hypostomus plecostomus (Linnaeus, 1758)	14-31	A(3Mc, 3 Tc) O(2 Tc)
	Hypostomus sp.	20	O(2 Mc)
	Loricaria cf. cataphracta Linnaeus, 1758	17.5-29	A(3 Mc 2, 3 Tc)
	Peckoltia sp. 1	8.2-15	A(2, 3 Mc)
	Peckoltia sp. 2	33-45	O(2, 3 Mc)
Pimelodidae	*Brachyplatystoma rousseauxi* (Castelnau, 1855)	25.5-35	O(1 Mc)
	Brachyplatystoma vaillanti (Valenciennes, 1840)	22-37	C(3 Tc)
	Hypophthalmus marginatus Valenciennes, 1840	16.8-40	C(1, 3 Tc) O(1, 2 Mc)
	Pimelodus blochii Valenciennes, 1840	16.8-22	C(3 Tc) O(2, 3 Mc)
	Platystomatichthys sturio (Kner, 1858)	25	A(3 Tc)
	Propimelodus aff. *eigenmanni* (Van der Stigchel, 1946)	22.5	A(3 Tc)
TETRAODONTIFORMES			
Tetraodontidae	*Colomesus psittacus* (Bloch & Schneider, 1801)	10.5	O(2 Tc)

The largest proportion of species classified as constant was recorded in zone 1, in both main (33.3%) and tidal channels (44.4%). Accessory species predominated in zone 3, in both main (44%) and tidal channel (62.79%), while occasional species were common in all zones, principally the main channel (Table 1).

The CPUE values for both numerical abundance and biomass indicated a significantly higher abundance in zone 1 (ANOVA, $p < 0.05$). Seasonal differences in abundance were not recorded (ANOVA, $p > 0.05$). For tidal channel, zone 3 returned the highest density and biomass values, which were significantly different from those of the other zones (Kruskal-Wallis, $p < 0.05$).

The analysis of habitat use revealed that the species captured in the study area used main channels and tidal channels as nurseries in similar proportions (Fig. 3A). The main channel of zone 3 returned the highest mean percentage (32.5%) of use as a reproduction ground, whereas zone 1 presented the lowest values, 14.05% (Fig. 3A). In the tidal channel, the highest percentage of reproductive individuals (stages C and D) was recorded in zone 2 (29.01%), in comparison with 14% in zone 1 and 8.52% in zone 3 (Fig. 3A). The proportions of individuals using the area as a nursery were similar across seasons in all three zones.

The repletion index (RI) indicated higher rates of feeding in zones 1 and 3 (Fig. 3B). In the main channel, zone 2 presented the lowest RI values and was significantly different from zone 1 (ANOVA, $p < 0.05$). In the tidal channel, RI values for zone 3 were significantly higher (ANOVA, $p < 0.05$) than those for the other two zones, reflecting the importance of this zone as a feeding ground (Fig. 3B). No significant variation was found in this index among seasons (ANOVA, $p > 0.05$).

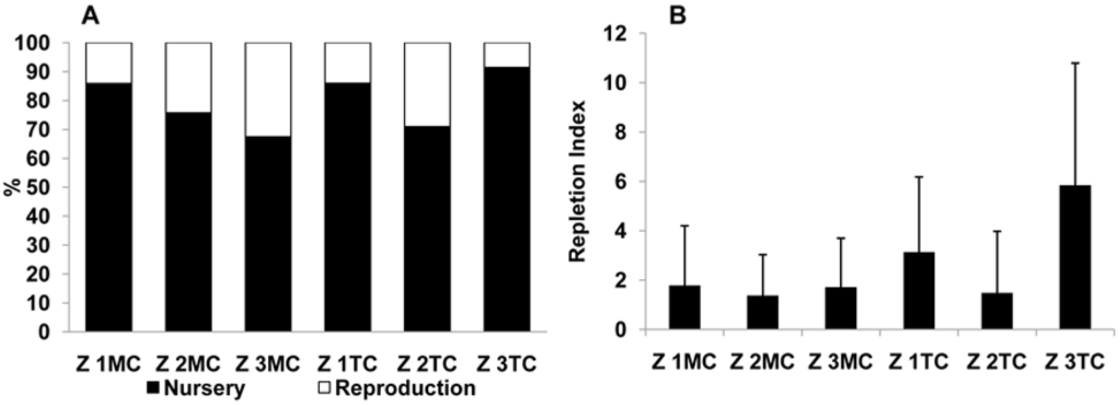

Fig. 3. Habitat use for individuals using the zones (Z) in Amazon estuary, Brazil, from 2009 to 2010 as: **A**) nursery and reproduction; **B**) Feeding Repletion index (RI). MC: Main Channel; TC: Tidal channel.

Considering the different feeding functional guilds, there was a predominance of piscivore/zoobenthivore and zoobenthivore species in all areas, in terms of the percentage of species, except in zone 1 in the main channel. Zooplanktivores were also relatively important in zone 1. Piscivores were not captured in the channel in any of the three zones and the lowest diversity of feeding guilds was recorded in zone 1 (Fig. 4A). Similar patterns are observed when individuals rather than species are considered, with a predominance of the piscivore/zoobenthivore and zoobenthivore categories, although opportunists/omnivores were prominent in both the main channel and tidal channel of zone 3 (Fig. 4B).

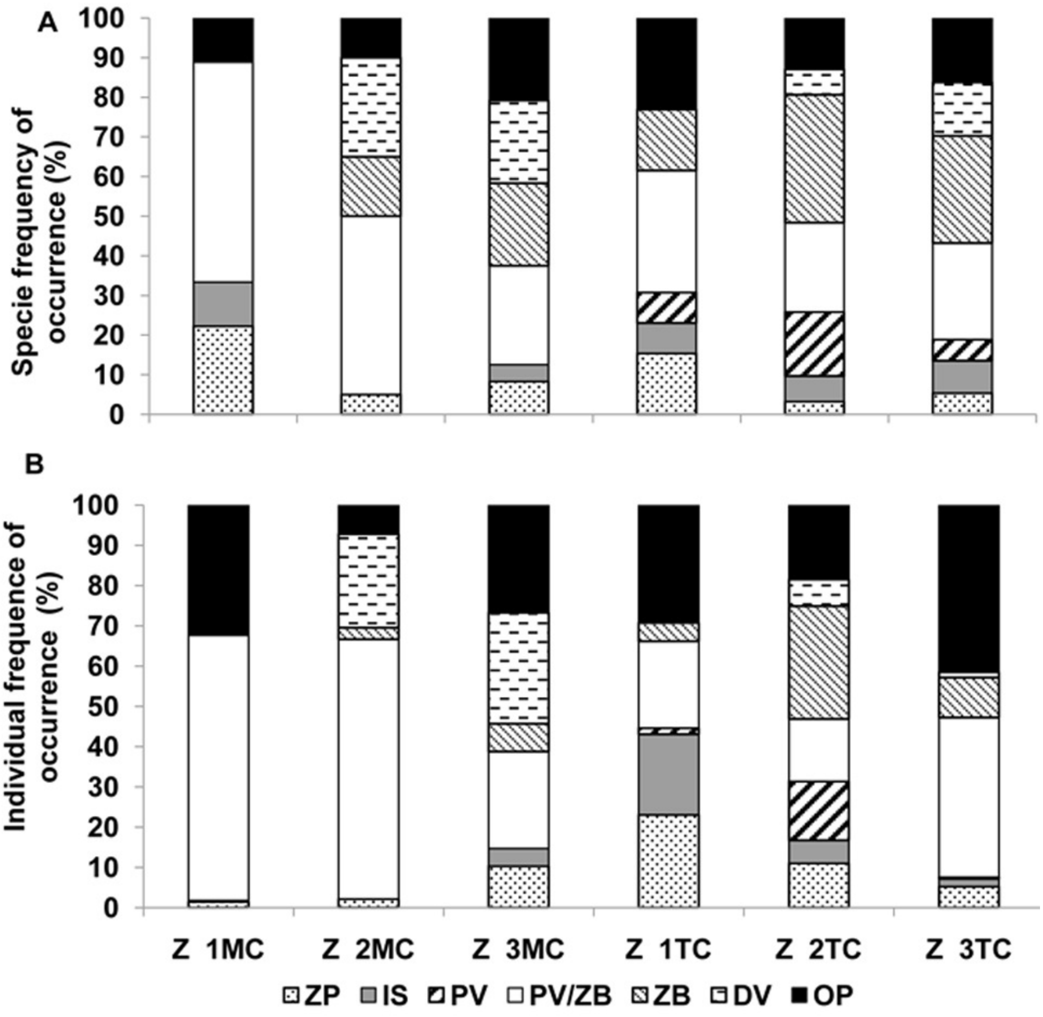

Fig. 4. Frequency of occurrence of the feeding guilds: (**A**) by number of species and (**B**) by number of individuals in Amazon estuary, Brazil, from 2009 to 2010 considering the zone (Z) and environment. MC: Main Channel; TC: Tidal channel. OP: opportunist/omnivore; DV: detritivore; ZB: zoobenthivore; PV/ZB: piscivore/zoobenthivore; PV: piscivore; IS:insectivore; ZP: zooplanktivore.

No significant seasonal variation (ANOVA, $p > 0.05$) was found in the diversity indices, although differences were found comparing the different habitats – main channel and tidal channel – in the different zones. Comparing the main channel of zone 1 with those of the other zones, all indices were significantly different (ANOVA, $p < 0.05$), except for evenness. The post test identified significantly lower values for species richness Shannon's and Margalef's index and higher values for abundance, dominance and Simpson's index (Fig. 5). In the case of the tidal channel, significant differences were found only for species richness and evenness, with lower values being recorded for the latter parameter in zone 3 (ANOVA, $p < 0.05$), but much higher species richness in comparison with zone 1 (Fig. 5).

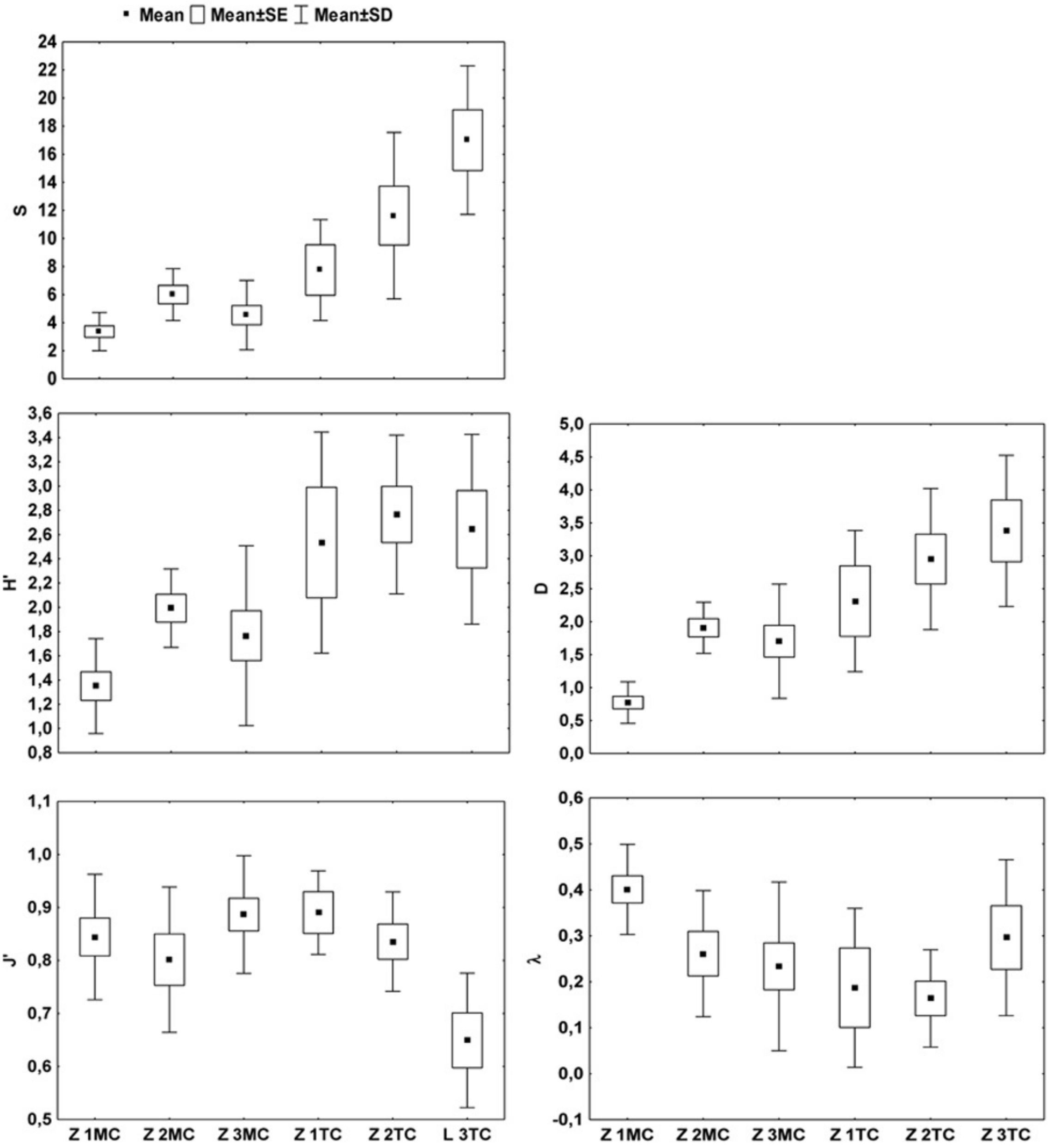

Fig. 5. Ecological indices in Amazon estuary, Brazil, from 2009 to 2010 by zone (Z) and environment. MC: Main channel; MT: Tidal channel; S: richness; J: Pielou’s evenness Index; λ: Simpson index; D: Margalef’s index; H’: Shannon’s Diversity Index.

The multivariate analysis did not reveal any significant seasonal variation for either main channels or tidal channels (ANOSIM, $p > 0.05$). However, three area significantly different groups were identified in both main channel and tidal channel (ANOSIM, $p < 0.05$) habitats (Fig. 6).

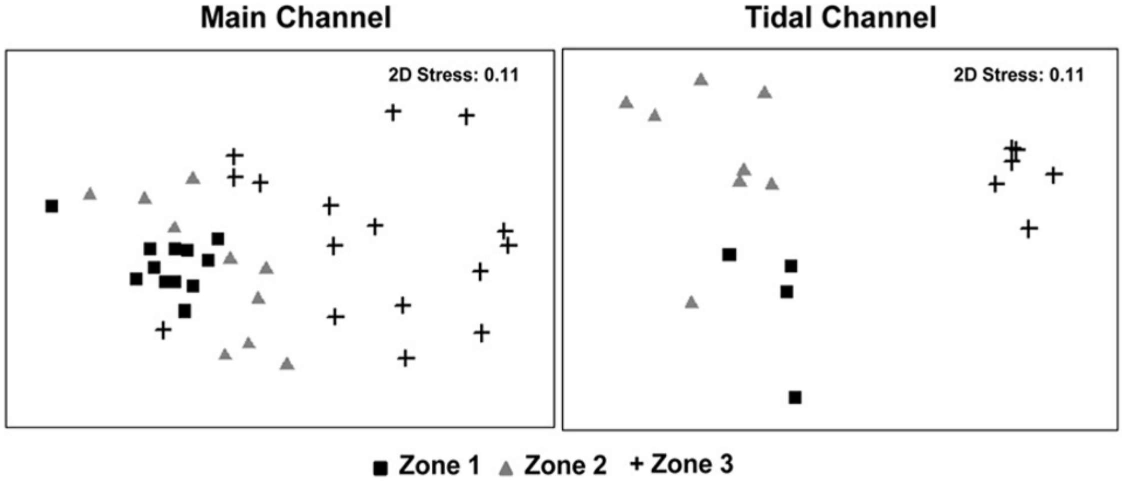

Fig. 6. Multivariate multidimensional scaling analysis in Amazon estuary, Brazil, from 2009 to 2010 by zone and environment.

3.2. Environmental parameters

Water temperatures and pH were similar among zones. Dissolved oxygen (O_2) concentrations were similar in zones 1 and 2. The O_2 concentrations in zone 3 were much lower, with values invariably below 5 mg l^{-1}. With the exception of these O_2 values in zone 3, all abiotic parameters were within the limits recommended under Brazilian legislation.

The results of the principal components analysis (PCA) indicated that 64.3% of the distribution of the data was explained by the first two axes (Table 2). Axis 1 (PC1) explained 34.8% of the variation in the samples (Table 2) and identified a significant difference (ANOSIM, $p < 0.05$) between the dry (D) and rainy (R) seasons. Alkalinity and hardness were the only environmental parameters to make a significant contribution ($d > 0.57$) to this axis (Table 2). The lowest values of these two parameters were recorded during the dry season, contrasting directly with those recorded during the rainy season (Fig. 7 A). Axis II (PC 2) accounted for 29.5% of the variation in the samples, based primarily on the differences among study areas, with zone 3 being significantly different from zones 1 and 2 (ANOSIM, $p < 0.05$). The variables involved here were ammonia and turbidity (Table 3), the concentrations of which were higher in zone 3 in comparison with the other zones (Fig. 7 B).

The BIO-ENV analysis revealed that the abiotic parameters most closely related to fish abundance were alkalinity, turbidity, hardness and the concentration of ammonia, with a maximum correlation of 0.73 for the main channel and 0.57 for the tidal channel, confirming the findings of the PCA.

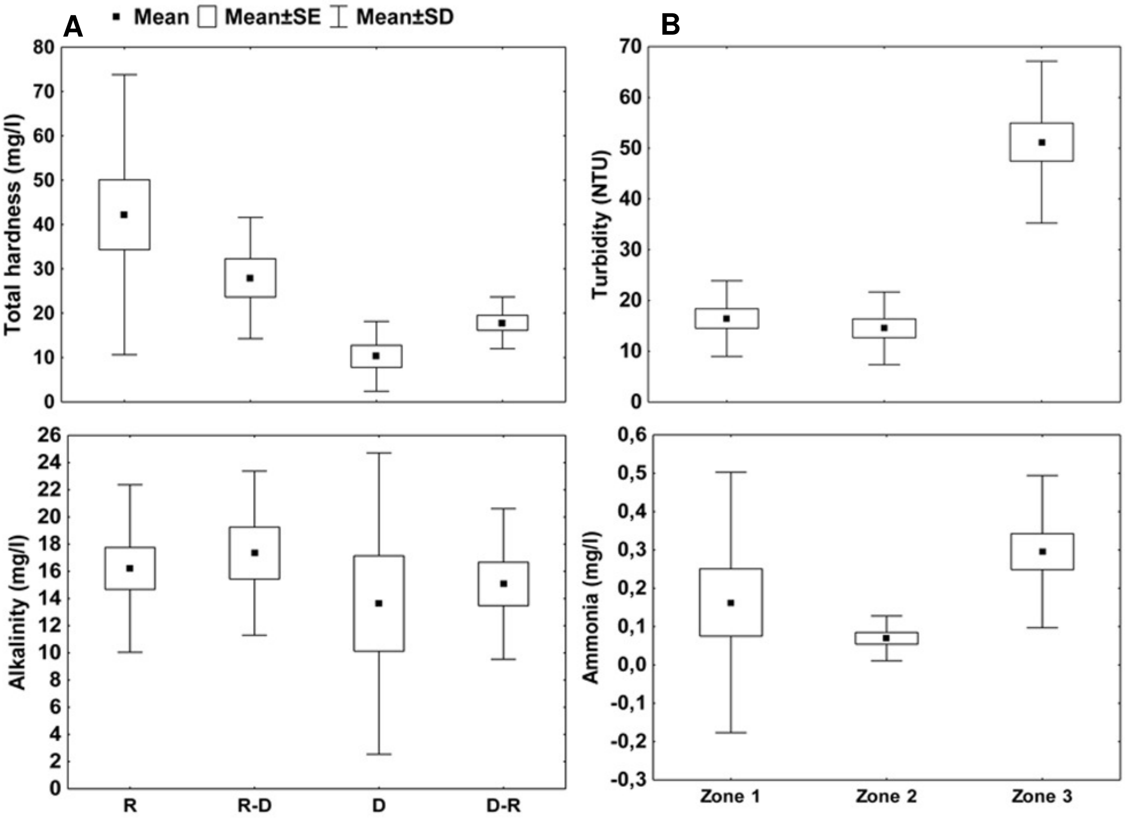

Fig. 7. Environmental parameters with a significant contribution by season (A) and zone (B) in Amazon estuary, Brazil, from 2009 to 2010. R-D: transition period between rainy and dry season; D: dry season; D-R: transition period between dry and rainy season and R: rainy season.

Table 2. Values of partial regression of the abiotic parameters. The values that showed a coefficient greater than 0.57 were considered significant (in bold).

Variable	PC1 (34.8%)	PC2 (29.05%)
Alkalinity	**0.57**	0.31
Total Hardness	**0.62**	0.16
Phosphate	-0.09	0.31
Nitrate	0.47	-0.24
Ammonia	0.20	**-0.58**
Turbidity	0.02	**-0.61**

4. Discussion

Human activities in estuarine environments tend to have negative effects on the local biota. Together with other waste, pollutants circulate extensively under the influence of the river discharge and tidal currents, often resulting in concentrations well above legally-defined limits (Eddy, 2005; Whitfield and Elliot, 2002), although in most cases, few data are available on the integrity of these environments. Vila do Conde is a case in point here and in addition to the intrinsic potential risks represented by its industries, a number of accidents have been reported since the installation of its ore-processing plants (Carneiro et al, 2007; Lima et al. 2009; Lima et al., in press).

In spite of the considerable impacts that have affected the study area since the construction of the local port by the Pará Dock Company and the subsequent installation of mineral ore-processing industries, the ichthyofauna of the estuary of the Pará River is characterized by a considerable diversity, with a total of 77 species being recorded within the study area. However, this diversity was much lower in the area closest to the port (Zone 1), where only 23 species were recorded, in comparison with the less impacted areas, which presented faunas typical of other tropical estuarine environments (Barletta et al., 2008; Barthem, 1985; Castro, 2001; Krumme et al., 2004; Montag et al., 2008; Merona, 1986/87; Paiva et al., 2008; Viana et al., 2010). This discrepancy in the number of species may be partially due to differences in sampling methods and effort between studies and to geographical particularities between the main channel of zone 1 and zone 3 (which is wider and has stronger water flow). Nevertheless, clear differences were found among zones and habitats in the composition of the ichthyofauna (main channel and tidal channel).

In general, constant species were more numerous in the tidal channel than the main channel. These species spend their whole life cycle in these habitats, which are more favorable to their development, given the relative abundance of refuges and feeding resources (Ruffino, 2004; Viana et al., 2010). However, as observed in Guajará Bay, which is adjacent to the present study area, tidal channel are also more vulnerable to contamination, resulting in a faster response from the fish species, given that it takes longer to filter out contaminants in comparison with the open channels (Viana et al., 2010).

The analysis of diversity indices is one effective way to evaluate the health of an aquatic environment (López-Rojas and Bonilla-Rivero, 2000; Whitfield and Elliot, 2002). Anthropogenic impacts are known to modify species composition through the elimination of the most sensitive taxa and the subsequent dominance of the more tolerant species (Attrill and Depledge, 1997). Biological communities provide an integral measure of modifications to the environment due to the different levels of tolerance of different species to distinct types of impact (Freire et al., 2008; Jaramillo-Villa and Caramaschi, 2008). In our study, for both types of habitats, however, while species richness was lower in zone 1, which is most vulnerable to industrial contamination, most species were constant, which appears to reflect their capacity to adapt to impacted environments. This does not necessarily means that the area is healthy given the possibility of chronic processes, such as the accumulation of heavy metals in body tissue and histological alterations of vital organs, such as the liver, kidneys and gills (Montes et al., 2010; Triebskorn et al., 2008).

Medium-sized fish (TL =15-30 cm) predominated in all three zones, although larger fish were more common in the zones further from the industrial area, presumably reflecting anthropogenic factors. As in the present study, descriptive indicators for the structure of size classes have been used by a number of authors to evaluate seasonal and spatial variation in fish communities. In Senegal, for example, a decrease in the maximum length of fish was observed after 20 years of anthropogenic impact (Ecoutin et al., 2010).

According to Yemane et al. (2008), the decline in both the mean maximum length and the number of fish species able to attain maximum length may be considered indicators of disturbances in the fish community, in this case, from overfishing. In the present study, the smaller proportion of larger-sized fish recorded in the impacted areas may reflect an ecological response to anthropogenic disturbance.

In the present study, the ecological indices indicated that the structure of the community closest to the industrial area and cargo terminal is the most impacted, based on the low values for species richness and the Shannon and Margalef indices and elevated dominance (Simpson). The most distant tidal channel (zone 3) were relatively rich (Margalef index), but equitability was low, indicating a non-uniform distribution of species. The reduced equitability was probably influenced by the dominance of *L. dorsalis* and *P. squamosissimus*.

Species representative of all different feeding modes are expected in natural estuaries, as well as a predominance of bottom-feeders (Blaber, 2000; Chaves and Umbria, 2003; Paiva et al., 2008). This pattern was observed in all parts of the study area, in terms of both the number of species and individuals, given the predominance of zoobenthivores, piscivore-zoobenthivores, opportunist-omnivores and detritivores. A reduced number of trophic categories was recorded in the main channel of zone 1. Environments that have suffered anthropogenic impacts tend to lose organisms at the top of the food chain (Browne and Lutz, 2010; Ecoutin et al., 2010), in this case, the piscivores, as well as trophic specialists. According to Garrison and Link (2000), generalist predators find prey more easily than specialized ones (such as benthophagous species) and therefore are more able to survive major disturbances. Unfortunately, when no data are available on the trophic structure of the local communities prior to current impacts, more definitive conclusions on this point are weakened (Ecoutin et al., 2010).

The abiotic parameters recorded during the present study indicated that the quality of the water was within the limits recommended by Brazilian legislation. While these values may indicate that the water is of good quality, which presumably reflects the general healthiness of the environment, these parameters may not necessarily provide an effective measure of the ecological integrity of the area (Goulart and Callisto, 2003). One possibility is that the effluents are diluted and dispersed by the system, given the hydrodynamics of lotic habitats and their capacity for the purification of contaminants. The contamination is also relatively localized in time and space, which favors the attenuation of its effects within the environment as a whole. In Guajará Bay, Gregório and Mendes (2009) and Viana et al. (2010) demonstrated that the highly dynamic mixed-energy conditions of this estuarine environment have limited the effects of the discharge of sewage and industrial effluents into the local waters.

Given this, the adoption of exclusively physical-chemical criteria for the evaluation of water quality may not necessarily provide an accurate picture of the conditions affecting local biological communities (Vieira and Chibata, 2007). In aquatic ecosystems, community composition provides an integral measure of modifications, according to the presence or absence of species with different levels of tolerance for specific types of environmental impact (Freire et al., 2008). The results of the present study indicate that the biota is an integrating element that responds systematically to alterations in the environment.

Another important consideration here is the unique intrinsic characteristics of the Amazon basin. In the study region, abiotic parameters (alkalinity and total hardness) vary seasonally in response to fluctuations in the discharge of the Amazon and Pará rivers (Nittrouer and De-Master, 1996). This variation had an important influence on some abiotic parameters, such as alkalinity and hardness, which were both lower during the dry season. The water in the area furthest from the cargo terminal was the most turbid and richest in ammonia, although the values recorded were within the legal parameters for water of good quality. The increased turbidity may reflect the reduced concentration of dissolved oxygen in this area.

Sets of indicators have been established by several authors for the monitoring of changes in the environmental quality of estuaries. However, variation in these indicators is difficult to interpret and may not fully account for the complexity of the ecosystem. In particular, some indicators are unable to identify short-term responses, demanding a much longer study period in order to demonstrate fluctuations effectively (Ecoutin et al., 2010). These variables include habitat use and the CPUE, which were evaluated in the present study.

In particular, the results of the present study demonstrated no clear pattern of habitat use, in relation to reproduction and feeding grounds or nurseries. The lack of data on the effects of contamination on reproduction grounds and nurseries in the region of the Amazon estuary and the restricted period of the present study prevent more systematic conclusions here. Courrat et al. (2009) propose that habitat loss is directly related to the level of anthropogenic pressure, which has negative effects on reproduction grounds and nurseries.

In the case of relative abundance (CPUE), the highest values were recorded in the most impacted area. This may have been related to the relative abundance of *Plagioscion squamosissimus* and *Lithodoras dorsalis*, which are the dominant species in this area. These species are not only relatively large in size, but are also resistant to anthropogenic factors.

Despite the restrictions about the use of analysis on the community structure in estuarine environments, as the case of ecological indices (Elliott and Quintino, 2007), in the studied study area is heavily influenced by the inflow of freshwater from the river Pará and Amazon.

Overall, it is clear that the presence of the cargo terminal and adjacent industries has an effect on the biological integrity of areas used by many local fish species for their reproduction and development. Many species visit the estuary of the Rio Pará during the migrations inherent to their life cycle. The juveniles tend to prefer estuaries due to the existence of favorable conditions for feeding, growth and refuge, as well their connectivity with other habitats (Barthem et al., 1991; Blaber, 2000; Courrat et al., 2009; Harrison and Whitfield, 2004; Kennish, 1985; Whitfield and Harrison, 2003). Studies have shown that the inner portion of the Amazon estuary, including Marajó and Guajará bays and the Pará estuary are used by ichtyofauna more for growth and development, rather than reproduction (Viana et al., 2010). In addition to the biological aspects of these phenomena, the local population of Vila do Conde is economically dependent on local fishery resources (Paz et al., in press).

Considering the ecological and economic importance of the estuary of the Rio Pará, the mitigation of the impacts caused by the local ore-processing installations and the cargo terminal and the systematic monitoring of the local aquatic environments should be given the highest priority. Such measures will be important to guarantee the productivity of these environments for future generations, given the importance of these resources as a source of income and subsistence for local populations.

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CAPÍTULO II

HISTOPATHOLOGY OF THE FISH LIVER AS A BIOMARKER FOR THE ANALYSIS OF THE ENVIRONMENTAL QUALITY OF THE INDUSTRIAL DISTRICT OF AMAZON ESTUARY, BRAZIL

HISTOPATOLOGIA NO FÍGADO DE PEIXE COMO UM BIOMARCADOR PARA ANÁLISE DA QUALIDADE AMBIENTAL EM UM DISTRITO INDUSTRIAL NO ESTUÁRIO AMAZÔNICO, BRASIL

HISTOPATHOLOGY OF THE FISH LIVER AS A BIOMARKER FOR THE ANALYSIS OF THE ENVIRONMENTAL QUALITY OF THE INDUSTRIAL DISTRICT OF AMAZON ESTUARY, BRAZIL

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ABSTRACT

In the present study, the occurrence, type and intensity of histological alterations of the hepatic tissue were assessed in two fish species: the South American silver croaker *Plagioscion squamossissimus* and rock-bacu *Lithodoras dorsalis*, with the objective of evaluating the quality of the water in the aquatic environments adjacent to the industrial district of Amazon estuary. None of the specimens of either species captured in zones 1 (maximum impact) and 2 (median risk) was in good health, whereas more than 60% of the specimens from zone 3 (reference area) presented healthy hepatic tissue. The principal alterations observed in the tissue of both species included an increase in the number of melanomacrophagous centers, fatty degeneration, inflammation, congestion, hepatitis and focal necrosis. The carnivorous *P. squamosissimus* presented higher levels of alteration than the herbivorous *L. dorsalis*. This study presented clear evidence of the contamination of the water of the aquatic environment adjacent to the port and industrial district of the town of Vila do Condeand of the effects of this contamination on the health of local fish populations.

Keywords: Biomonitoring, hepatic tissue, North Brazil; industrialization.

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INTRODUCTION

Ever since aquatic ecosystems have been considered to be potential sources of contamination, fish have been used as bioindicators and biomonitor organisms for the monitoring of these environments (Amado *et al.*, 2006; Schlacher *et al.*, 2007; Fernandes *et al.*, 2008; Freire *et al.*, 2008). The use of biomarkers in environmental monitoring provides information not only on the intensity, tolerance limits and effects of pollutants on the organisms, but also on the process of transfer of these substances within the trophic web (Oost *et al.*, 2003). Thus these markers serve as a "warning sign" of environmental integrity and can be used to guide the development of effective bioremediation measures before the environment has suffered irreversible damage (De La Torre *et al.*, 2005).

Histopathological analysis is widely-used in environmental monitoring research. The organs targeted in this approach respond incisively to exposure to xenobiotic chemicals or other toxic substances and these alterations can be identified relatively easily (Au, 2004; De La Torre *et al.*, 2005). In fish, the liver is considered to be an excellent organ to the study of environmental quality biomarkers, given its role in the metabolism of the individual, including the production of proteins, the oxidation, conjugation, methylation, inactivation or detoxification of substances, that is, the excretion of pollutants (Bruslé & Anadon, 1996; Bernet *et al.*, 1999; Roberts, 2000; Fernandes *et al.*, 2008; Carrola *et al.*, 2009).

The South American silver croaker *Plagioscion squamosissimus* (Heckel, 1840), Sciaenidae, and the rock-bacu *Lithodoras dorsalis* (Valenciennes, 1840), Pimelodidae, both play an important role in the local economy and the subsistence of the communities located within the area of the municipality of Barcarena, in the Brazilian state of Pará, Amazon Estuary. Both species are also relatively abundant throughout the study area. *Plagioscion squamosissimus* is a carnivorous, zoobenthic specie, which prefers to feed on shrimp and fish (Goulding & Ferreira, 1984; Raiol *et al.*, 2006; Costa *et al.*, 2009a), while *L. dorsalis* is considered to be an opportunist, feeding mainly on plant material and seeds, whenever available (Santos *et al.*, 2006; Raiol *et al.*, 2006; Barbosa & Montag, 2011).

The selection of species with different feeding habits is important for a study of this type, given that some substances may accumulate at different rates at distinct trophic levels (Kasper *et al.*, 2007; Terra *et al.*, 2008). There may also be variation in the neutralization, modification or biotransformation of different substances, depending on their potential toxicity. Given their position at the top of the food chain, carnivorous fish are more affected by the accumulation of contaminants than herbivores and are more likely to affect humans through the consumption of contaminated fish (Lawrence & Hemingway, 2003). Both the species analyzed in the present study are widely-consumed in the local communities (Espirito Santo *et al.*, 2005), which reinforces the need for the assessment of the possible risks of contamination for the human population.

The present study was based on the assessment of the occurrence, type and intensity of histological alterations of the hepatic tissue of the fish species *P. squamossissimus* and *L. dorsalis*, as an approach to the evaluation of the quality of the environment in the region of the port of Vila do Conde, in Barcarena. This locality is an important industrial hub, which includes plants for the processing of raw materials such as kaolin, alumina and aluminum. The industrial processing of these minerals produces a variety of residues, including fluorides, chlorides, sulfates and bicarbonates (Rubio & Tessele, 2002), which may provoke significant alterations in the quality of aquatic ecosystems. The treatment of these residues may also result in an increase in the levels of some metallic ions, such as iron and lead. Given these considerations, the assessment of environmental quality using biomarkers is extremely important, not only for the understanding of the effects of contamination on the ecological equilibrium of the area, but also the social and economic implications for the local riverside communities that depend on these natural resources.

MATERIALS AND METHODS

STUDY AREA AND DATA COLLECTION

The port of Vila do Conde is located on the right bank of the mouth of the Pará River (01°32’42’’S 48°45’00’’W), in the southeast of the Amazon estuary, some 40 km west of Belém, the capital of the Brazilian state of Pará (CDP, 2010). This area is subject to enormous discharges of freshwater from the Amazon and Pará rivers, and can be classified as a tidal freshwater estuary, according to the scheme of Elliott & McLusky (2002). This area is influenced by the Pará River, Marajó Bay and Gulf and also by several islands and channels that encompass the region. Currents in this area may reach 1.24 m/s (Martins & Mendes, in press).

The experimental design of this study was based on the probable differences in the degree of environmental contamination according to the distance of the sample area from the cargo terminal at Vila do Conde. As such, the collection of abiotic data and fish specimens was conducted in three distinct areas (Fig. 1): zone 1 – was located in the estuary Pará River. The area surrounding the port of Vila do Conde, considered to be the area with the highest risk of environmental contamination; zone 2 – Capim Island, located on the border between the municipalities of Barcarena and Abaetetuba in the estuary Pará River, at an intermediate distance from the port, and thus considered to be at moderate risk of contamination; zone 3 – Onças Island in Barcarena, which is relatively isolated from Vila do Conde, and suffers minimal impact, representative of the natural conditions of the study area.

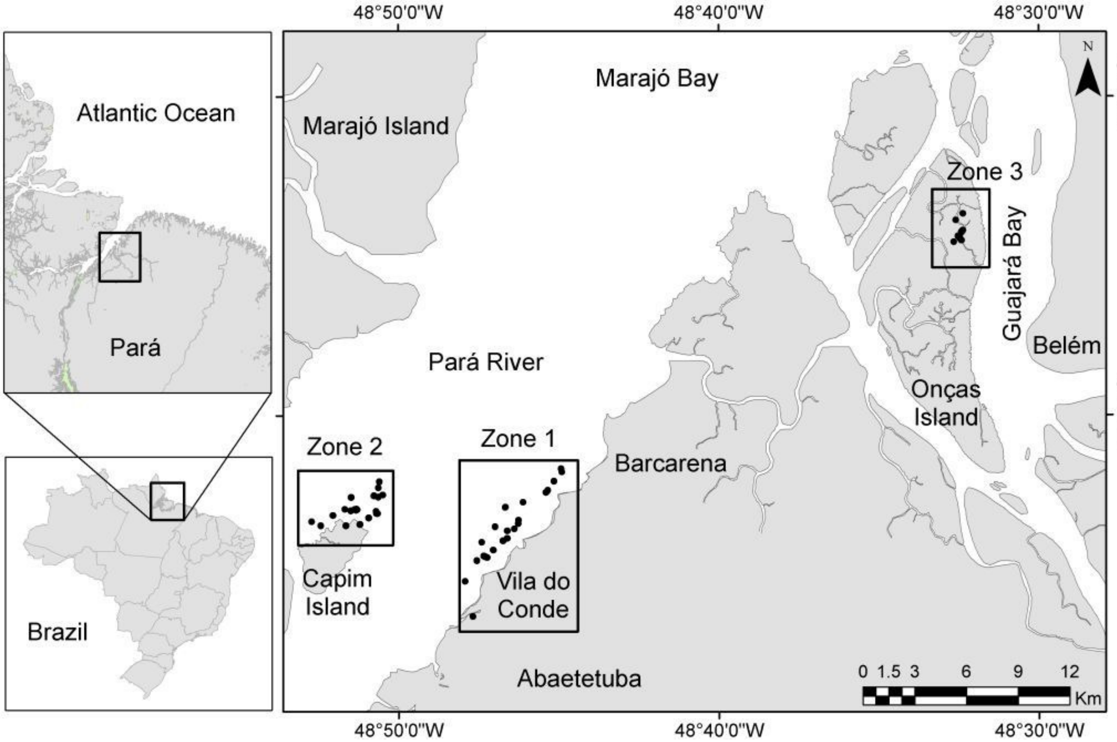

Fig. 1. Study area in Amazon estuary, Brazil, from 2009 to 2010, with the sampling points in the each area. Zone 1: adjacent to the Vila do Conde cargo terminal; Zone 2: Capim Island; Zona 3: Onças Island.

Specimens were collected during four excursions conducted between 2009 (June and September) and 2010 (January and April), in the rainy and the dry. Different sampling protocols were used in the channel and tidal channel, due to their distinct dynamics. The specimens were collected in both the main river channel and tidal channel within each of the three zones, with the aim of providing a standardized sample of the principal environments within the study area. In the main channel, fish were collected using monofilament gillnets with different stretched mesh sizes were used (25, 35 and 40 mm), stretched mesh sizes (133.2 m in total) were used. The nets were allowed to drift for an average soak time of 1h 30 min in the tidal channel, a block net (25 mm mesh size) was set at the mouth of the tidal channel, closing it completely. In the tidal channel, a block net (25 mm mesh size) was set at the mouth of the tidal channel, closing it completely. Blocking was initiated at the end of the high tide and continued throughout the entire ebb tide cycle (*c.* 6 h).

Immediately following capture, the specimens were weighed and their livers were removed and weighed. A sample of the liver was taken and fixed in

Bouin solution for 48 hours. The numbers of specimens analyzed by species, area (zones) and season (rainy and dry) are shown in Table I.

Table I. Number of *P. quamosissimus* and *L. dorsalis* specimens analyzed by zone and season in Amazon estuary, Brazil, from 2009 to 2010. Zone 1: adjacent to the Vila do Conde cargo terminal; Zone 2: Capim Island; Zona 3: Onças Island.

	P. squamosissimus			***L. dorsalis***		
Zone	**Dry**	**Rainy**	**Total**	**Dry**	**Rany**	**Total**
1	41	42	83	28	44	72
2	17	34	51	8	0	8
3	40	51	91	40	40	80
Total	99	130	225	70	77	160

During each excursion, four water samples were collected from the superficial layer of the water column, the temperature and pH were measure *in situ* using a multianalyzer. Dissolved oxygen levels were determined in the field using the Winkler method. For the analysis of nutrient concentrations – nitrates (NO_3), phosphates (PO_4) and ammonia (NH_4) – alkalinity, turbidity, conductivity and hardness, 2-liter samples of water were collected and preserved on ice for up to 24 hours prior to processing in a specialized laboratory.

ENVIRONMENTAL PARAMETERS

The mean values were calculated for the envinromental parameters. Differences among areas and seasons were tested using a one-way ANOVA. Whenever necessary, the data were log-transformed (Log (x+1)) in order to homogenize the variances. The significance of pairwise differences was assessed using Tukey's *post hoc* test (Zar, 1996). Non-normal data were examined using the Kruskal-Wallis nonparametric analysis of variance. Specific differences were examined using a ranked means multiple comparison test.

BIOLOGICAL AND HISTOLOGICAL PARAMETERS

The hepatosomatic index (HSI) was calculated to provide a quantitative measure of the metabolic activity of the liver. This index is calculated for each individual by the formula HSI = 100 liver mass (g) body mass $(g)^{-1}$. Sexually mature specimens were not included in this analysis (HSI) in order to avoid the influence of their excess weight on the results due to the modifications in their metabolism and physiology (Bruslé & Anadon, 1996).

For the histopathological analyses, the samples were embedded in paraffin, stained with HE (haematoxylin and eosin) analyzed and photographed using light microscopy (Carl Zeiss - Axiostar Plus 1169-151). Observed histopathological changes were evaluated semi-quantitatively by ranking the severity of the tissue lesions modified according to Schwaiger *et al.* (1997), which was assigned a numerical value for each animal according to a degree of change: grade 1 –mild focal changes; grade 2 - mild to moderate focal changes and grade 3 - severe and extensive pathological alterations. This ranking was used to establish an overall assessment value of the histopathological lesions of each individual fish. On the basis of these data, Mean Assessment Values (MAVs) of organ lesions were calculated for each study area.

The severity of the tissue lesions was ranked using a ranking scheme modified from Poleksic & Mitrovic-Tutundzic (1994): stage I – alterations which do not affect the normal functioning of the tissue; stage II – more severe alterations, which impair normal functioning of the tissue; stage III – severe alterations that cause irreparable damage (Table II). These data were used to estimate the Histological Alteration Index (HAI) for each animal by: HAI = $10^1\sum I+10^2\sum II+10^3\sum III$. The mean HAI values were used to assess the severity of the functional impairment of the liver and its capacity for regeneration of the each specie. Values of 0 to 10 indicate normal functioning of the organ, 11-20 represent slight damage, 21-50 moderate changes, 51-100 severe lesions, and values above 100 indicate irreversible damage to the organ.

Table II. Classification of histopathological alterations of the liver, modified from Poleksić and Mitrovic-Tutundzic (1994).

Type of alteration	Standard reaction	Stage
1. Alteration of the hepatocytes	Deformation of the cell contour	I
	Cellular hypertrophy	I
	Cellular atrophy	I
	Melanomacrophagous centers	I, II and III
	Vacuolization of the cytoplasm	I
	Degeneration of the cytoplasm	II
	Fatty degeneration	II
	Cell rupture	II
	Inflammation	II and III
	Congestion	II
2. Alteration of the blood vessels	Congestion	II
	Hepatitis	II and III
3. Necrosis	Focal necrosis	III

The differences between zones and seasons were tested using a one-way ANOVA, followed by Tukey's *post hoc* test or the Kruskal-Wallis nonparametric analysis of variances, with a ranked means multiple comparison test.

Multivariate Multidimensional Scaling (MDS) was used to compare the occurrence of histological pathologies by zone and season. This analysis was based on Euclidean distance coefficients for presence/absence data for the full set of 19 histopathological indicators for the two species. Given the reduced number of specimens collected from zone 2, for two species, it was excluded from this analysis. The significance of all the groups defined by this approach was tested through a two way nested ANOSIM. For all analysis were made according to the área and season.

RESULTS

ENVIRONMENTAL PARAMETERS

The conductivity, temperature, phosphate concentrations, alkalinity and pH of the water varied relatively little among zones or seasons (Table III). Hardness and nitrate concentrations nevertheless varied significantly between seasons (Kruskal-Wallis, d.f. = 1, $p<0.01$), with higher values being recorded during the rainy season (Table III). By contrast, ammonia, dissolved oxygen and turbidity varied significantly among zones, but not between seasons (Kruskal-Wallis, d.f. = 2, $p<0.001$). While the lowest concentrations of dissolved oxygen was recorded in zone 3, the highest values for ammonia and turbidity were recorded in this zone and were significantly different from those recorded in zones 1 and 2 (Table III).

Table III. Mean values of environmental parameters with its respective standard deviation recorded in the study area by zone and season in Amazon estuary, Brazil, from 2009 to 2010. Values in bold script represent significantly higher values than those recorded in other zones. Zone 1: adjacent to the Vila do Conde cargo terminal; Zone 2: Capim Island; Zona 3: Onças Island.

Environmental Parameter	Rainy season			Dry season		
	Zone 1	Zone 2	Zone 3	Zone 1	Zone 2	Zone 3
Alkalinity (mg L^{-1})	15.3 (± 4.5)	15.5 (± 2.3)	18.6 (± 8.5)	18.25 (± 2.1)	20 (± 2.6)	4.6 (± 5.2)
Total hardness (mg L^{-1})	30.2 (± 28.7)	29.5 (± 19.28)	47.6 (± 29.03)	17.5 (± 2.9)	18.4 (± 3.6)	7.1 (± 8)
Phosphate ((mg L^{-1})	0.04 (± 0.05)	0.02 (± 0.05)	0. 005 (± 0.005)	0.01 (± 0.03)	0	0.008 (0.006)
Nitrate (mg L^{-1})	1.06 (± 0.8)	1.07 (± 0.7)	2.5 (± 1.6)	0.46 (± 0.5)	0.54 (± 0.4)	0
Ammonia (mg L^{-1})	0.2 (± 0.4)	0.05 (± 0.06)	**0.3 (± 0.2)**	0.1 (± 0.04)	0.08 (± 0.05)	**0.19 (± 0.11)**
Dissolved oxygen (mg L^{-1})	6.6 (± 2.9)	6.5 (± 2.2)	**3.3 (± 0.4)**	6.8 (± 0.8)	7.4 (± 0.6)	**1.9 (± 0.1)**
Turbidity (NTU)	15.6 (± 8.1)	14.1 (± 7.1)	**51.9 (± 14.3)**	22.8 (± 7.1)	14.9 (± 7.6)	**50.2 (± 18.7)**
Temperature (°C)	28.6 (1.3)	28.7 (± 2)	27.28 (± 0.9)	27.9 (± 1.6)	29.87 (± 1.06)	28.10 (± 0.4)
pH	5.7 (± 9.6)	6.1 (± 8.7)	5.6 (± 0.48)	5.47 (± 0.22)	5.96 (± 0.13)	5.43 (± 0.09)
Conductivity (u S/cm)	26.8 (± 9.6)	34.6 (± 8.6)	50.1 (± 40.4)	36.5 (± 3.6)	33.1 (± 3)	45.9 (± 38.81)

BIOLOGICAL AND HISTOLOGICAL PARAMETERS

Anatomically, the livers of the two study species are typically dense and reddish-brown in color, due to their intense vascularization. The liver of *P. squamosissimus* is located posteriorly to the gills, extending along the medial region of the body towards the tail. It is divided into three lobes, with a central lobe adhered to the entrance to the stomach, pyloric ceca, gall bladder and two lateral lobes, of which the left lobe is longer than the right. In *L. dorsalis*, the liver is located directly below the cranial region, extending to the sides of the body in the direction of the pectoral fins, above the cartilaginous plates. It is composed of two lateral lobes of similar size, separated by the gall bladder and the anterior portion of the intestine. The liver of *L. dorsalis* is considerably larger, relative to body size, than that of *P. squamosissimus*.

No significant variation was found in the HIS values for *P. squamosissimus* (ANOVA, d.f. = 1, $p > 0.05$) and *L. dorsalis* (Kruskal Wallis, d.f. = 5, $p>0.05$), either between seasons or among zones.

Overall, 68.82% of the 385 specimens analyzed histologically presented some form of pathology. All individuals captured in zones 1 and 2 had pathological alterations were observed in zones 1 and 2 for either species in either season (Fig. 2). In zone 3, by contrast, approximately 60% of the *P. squamosissimus* specimens and 75% of those of *L. dorsalis* were considered healthy, based on the absence of pathological alterations of the liver (Fig. 2).

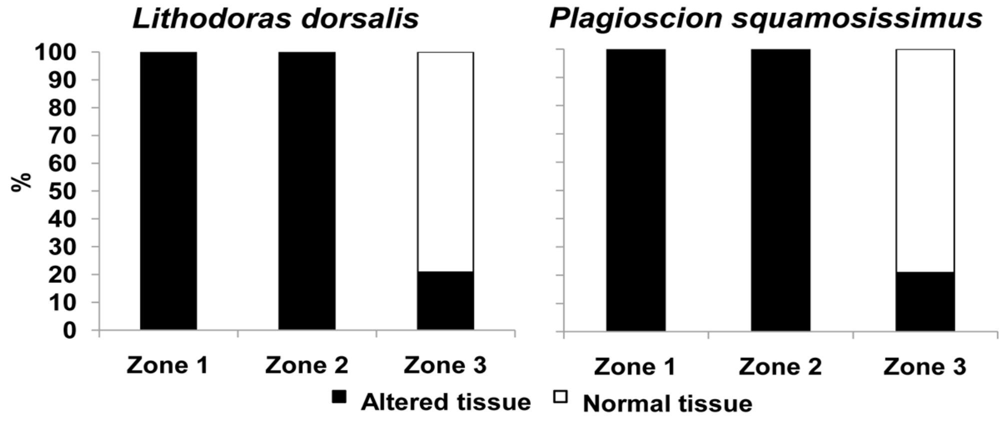

Fig. 2. Percentage of specimens with and without histopathological alterations by zone (Z) in Amazon estuary, Brazil, from 2009 to 2010.

The Mean Assessment Values (MAV) of organ lesions calculated for each species were significantly lower in zone 3 in comparison with the other zones (Kruskal-Wallis, d.f. = 2, $p<0.001$), although no seasonal effect was observed (Kruskal-Wallis, d.f. = 1, $p>0.05$, Fig. 3). No *L. dorsalis* specimens from zone 2 were examined histologically during the rainy season, which is why no MAV is presented for this period.

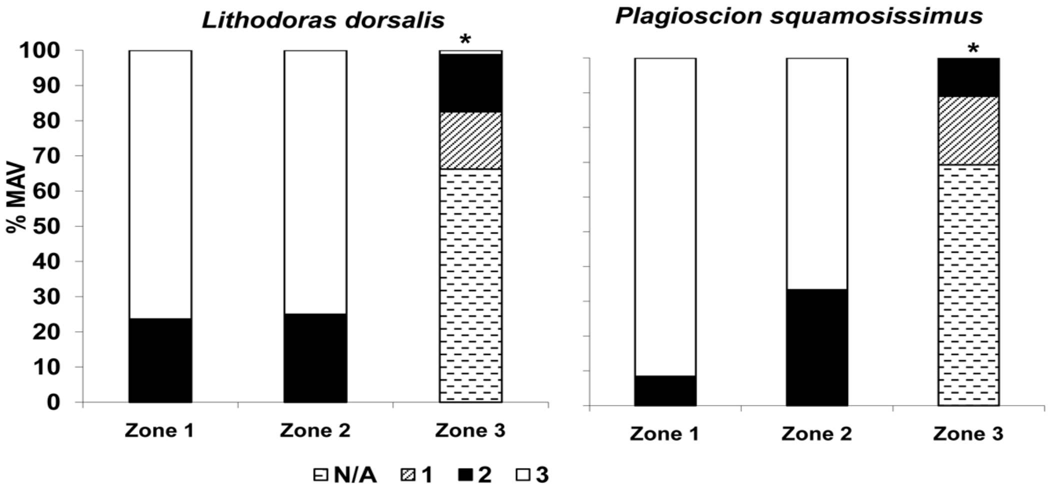

Fig. 3. Mean Assessment Values (MAV) by zone (Z) and season (Rainy and Dry) in Amazon estuary, Brazil, from 2009 to 2010. N/A = no alteration; grade 1 = mild focal changes; grade 2 = mild to moderate focal changes and grade 3 = severe and extensive pathological alterations. (*): results significant.

Histologically unaltered livers were obtained only from zone 3. These organs have a homogeneous hepatic parenchyma, with hepatocyte cords and sinusoidal capillaries surrounded by the central lobular vein (CLV), as well as hepatic arteries and portal vein (Fig. 4). Observed alterations in both species included an increase in the number of MMCs, fatty degeneration, inflammation, congestion, hepatitis and focal necrosis (Figs. 5 and 6). Other alterations are described in Table III.

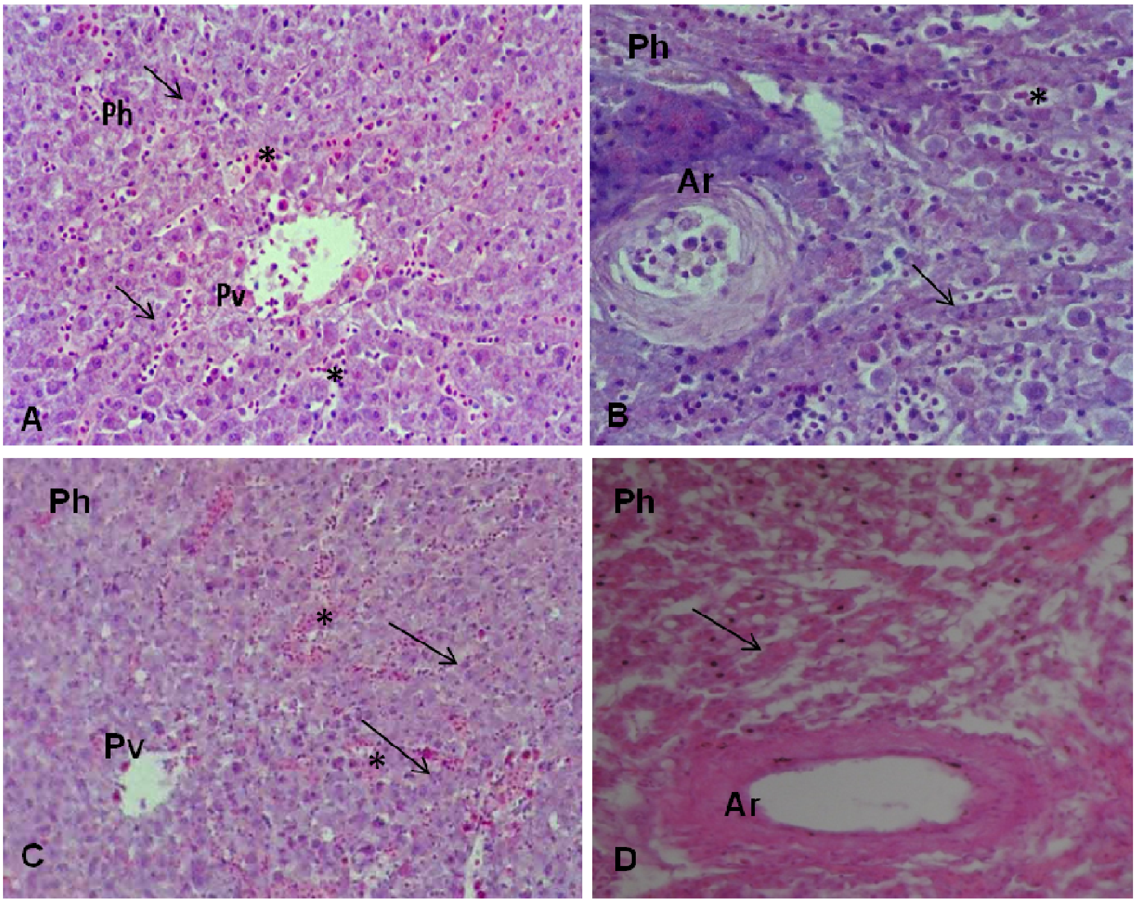

Fig. 4. Photomicrograph of normal hepatic tissue of *Plagioscion squamosissimus* (**A** and **B**) and *Lithodoras dorsalis* (**C** and **D**) captured in Amazon estuary, Brazil, from 2009 to 2010. Ph – Hepatic parenchyma with blood vessel, detail of the portal vein (Pv) and hepatic artery (Ar), hepatic cords (fine arrow) and sinusoidal capillaries (*). A and C 100x, B and D 400x. HE.

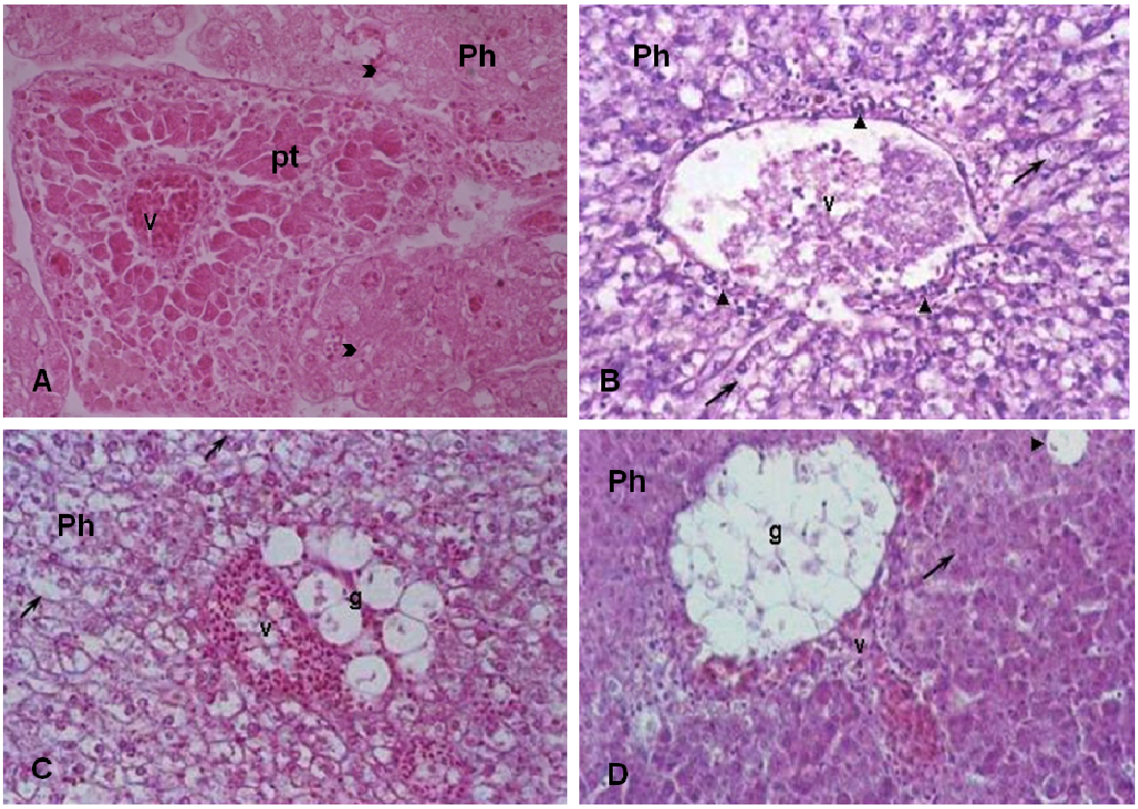

Fig. 5. Photomicrograph of hepatic tissue with pathology of *Plagioscion squamosissimus* and *Lithodoras dorsalis* captured in Amazon estuary, Brazil, from 2009 to 2010. A) hepatic tissue with congestion and presence of exocrine pancreatic tissue (pt), hepatic cords few defined (verge) 400 x; B) hepatic tissue altered by hepatitis: dilated veins (v), hypertrophied hepatocytes (fine arrow) and agglomeration of blood cells (arrow head) 400 x; C) inflamed hepatic tissue: hepatic parenchyma (ph) with spongy appearance, vacuolized hepatocyte (fine arrow) and lipid deposit (g). 100 x; D) hepatic tissue with fatty degeneration: parenchyma with isolated (arrow head) and agglomerated (g) fat globules adjacent to the blood vessel (v) and hepatocyte cords with spongy appearance (fine arrow) 100x. HE.

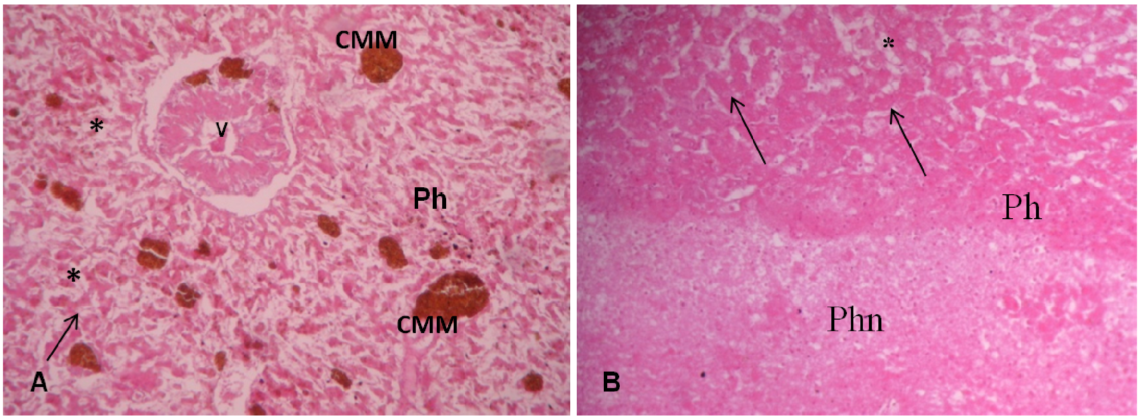

Fig. 6. Photomicrograph of hepatic tissue with pathology of *Plagioscion squamosissimus* and *Lithodoras dorsalis* captured in Amazon estuary, Brazil, from 2009 to 2010. A) hepatic tissue with concentration of melanomacrophagous centers (MMCs) and congested vein (v), sinusoidal capillaries (*) and hepatocyte cords (fine arrow), 100x; B) necrotic tissue with intense cell death (Phn), 100x. HE.

The specimens from zones 1 and 2 also presented higher frequencies of alterations at all stages of severity in comparison with zone 3, where no stage II alterations were recorded. While the two species presented similar patterns of alteration, those observed in *P. squamosissimus* were generally more severe than those recorded in *L. dorsalis*.

The Histological Alteration Index (HAI) varied significantly among zones for both *P. squamosissimus* (Kruskal-Wallis, d.f. = 2, $p<0.001$) and *L. dorsalis* (Kruskal-Wallis, d.f. = 2, $p<0.001$). The results for zone 3 indicate that the specimens had relatively healthy livers, with HAI values of less than 10 (Fig. 7), with a mean values of 2.52 (S.D. ±5.1) for *P. squamosissimus* and 2.70 (S.D. ±5.33) for *L. dorsalis*. In zones 1 and 2, by contrast, all the livers analyzed presented some form of function-impairing alteration, with HAI values above 100 in both areas (Fig. 7). The HAI values for *P.squamosissimus* were also significantly higher than those recorded for *L. dorsalis* (Kruskal-Wallis, d.f. = 1, $p<0.05$), with an mean value of 115.84 and 90.25, respectively. No significant difference was recorded between seasons Rainy and Dry (Kruskal-Wallis, d.f. = 1, $p>0.05$).

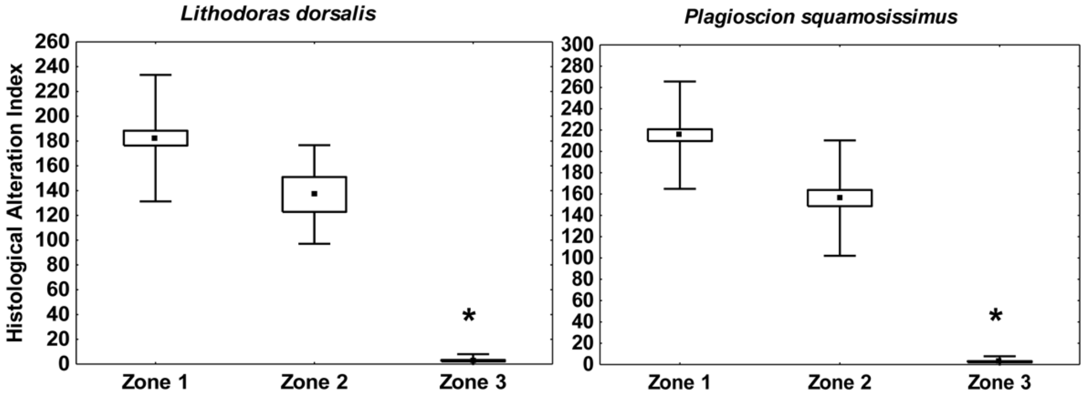

Fig. 7. The Histological Alteration Index (HAI), with mean values of the individuals captured in Amazon estuary, Brazil, from 2009 to 2010 by area. (*): results significant.

The multivariate analysis of the set of hispathological indicators revealed significant differences among zones for both species (two-way ANOSIM, d.f. = 3, 19, R = 0.95, p<0.01) (Fig. 8). The MDS analysis indicated a clear separation between zones 1 and 3, which was due primarily to the much lower frequency of histpathological alterations in zone 3.

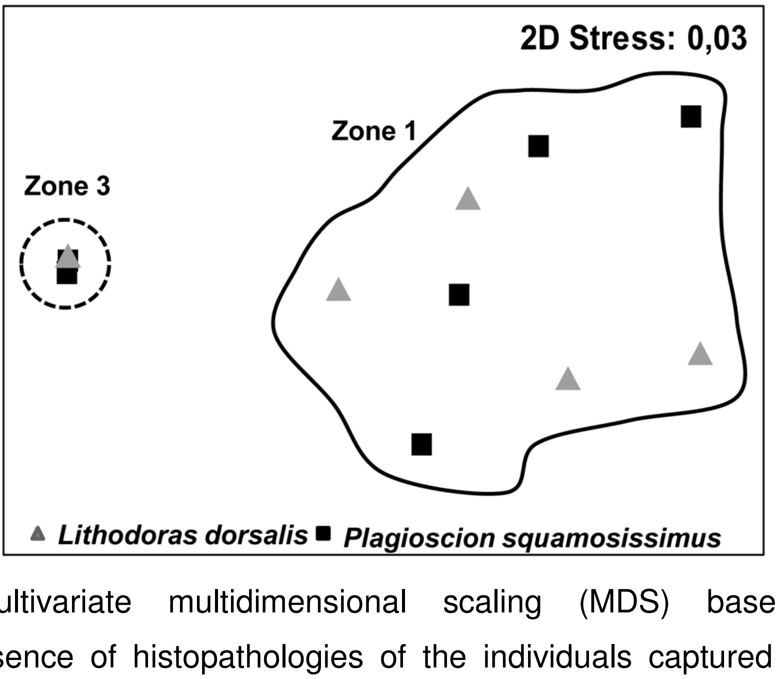

Fig. 8. Multivariate multidimensional scaling (MDS) based on the presence/absence of histopathologies of the individuals captured in Amazon estuary, Brazil, from 2009 to 2010. Zone 1: adjacent to the Vila do Conde cargo terminal; Zona 3: Onças Island.

DISCUSSION

The use of histopathological biomarkers is increasingly considered to be one of the best procedures for the evaluation of the effects of pollutants on aquatic ecosystems. The liver is often used for such studies and produces reliable results for the evaluation of environmental quality (Schwaiger, 2001; Freire *et al.*, 2008; Ozmen *et al.*, 2008; Costa *et al.*, 2009b; Adams *et al.*, 2010; Rocha *et al.* 2010).

In the present study, the biological condition of the specimens was assessed through the hepatosomatic index (HSI) result in modifications of the metabolism and physiology of the fish (Klein *et al.*, 2005; Pyle *et al.*, 2005; Yang & Bauman, 2006; Greco *et al.*, 2010). In healthy bony fish, HSI values range between 1 and 2 (Bruslé & Anadon, 1996). In the present study, however, the mean values recorded for *P. squamosissimus* were below 1 in all three zones, whereas they were between 1 and 2 – i.e. normal levels – for *L. dorsalis*. Values outside this range do not necessarily reflect anthropogenic impacts, however, given that other factors, such as feeding behavior, climate, sex, age and breeding patterns may also affect this index (Bruslé & Anadon, 1996; Yang & Bauman, 2006; Solé *et al.*, 2009).

The physical-chemical variables analyzed in the present study were within the limits considered tolerable by Brazilian legislation, indicating that the quality of the environment of the study area was satisfactory. While these parameters are good indicators of water quality, these results may reflect the system's capacity to filter out pollutants and purify the water before it is sampled (Goulart & Callisto, 2003), especially considering the limited time scale of the sampling. Gregório & Mendes (2009) and Viana *et al.* (2010) have shown that the highly dynamic and mixed-energy environment of Guajará Bay, adjacent to the study area, results in the rapid dissipation of both sewage and industrial effluents. In Vila do Conde itself, Berrêdo *et al.* (2001) also found that the physical-chemical properties of the water were generally within accepted limits and it seems likely that high levels of contamination are restricted to the areas adjacent to the effluent outlets (Lima *et al.* , 2007).

A number of studies have shown that the concentrations of some metals – such as lead (Pb), copper (Cu), chrome (Cr) and nickel (Ni) – in the water at

Vila do Conde exceed the levels permitted by Brazilian legislation (Berrêdo *et al.*, 2001; Carneiro *et al.*, 2007). This situation appears to be related primarily to the local processing of bauxite, in addition to the industrial accidents that have occurred within the study area. High levels of heavy metals can be extremely damaging to the aquatic biota, due to their accumulation in both sediments and in the trophic web through transference. This appears to be confirmed by the hepatic alterations observed in the fish specimens analyzed in the present study.

The liver participates in processes such as the transformation and excretion of toxic substances from the body, and may have its structure altered in response to external factors (Bruslé & Anadon, 1996; Dyk *et al.*, 2007). In the present study, the histopathological analyses supported emphatically the conclusion that local anthropogenic impacts are affecting the health of both fish species: *P. squamosissimus* and *L. dorsalis*. The MAV and HAI values and the MDS analysis all indicated clear differences between the areas surveyed. The alterations were more severe (in some cases, irreversible) in zone 1, which was closest to the port and the industrial district. In their experimental study, Schwaiger *et al.* (1997) recorded high MAVs for the livers of *Salmo trutta f. fario* and *Barbatula barbatula* exposed to contaminants, in comparison with control specimens, which reinforces the effectiveness of histopathological alteration in this organ a biomarker of environmental quality.

In zones 1 and 2 the alterations considered most significant in both species were the increase in the number of melanomacrophagous centers (MMCs), fatty degeneration, inflammation, hepatitis, congestion of the blood vessels and focal necrosis. Similar results have been obtained for contaminated natural environments with adequate water quality in studies involving *Pleuronectes vetulus* (Stehr *et al.*, 2003), some cyprinids species (Gül *et al.*, 2004), *Salmo trutta f. fario* (Carrola *et al.*, 2009) and *Solea senegalensis* (Costa *et al.*, 2009b).

In this study, the presence of melanomacrophagous centers (MMCs) was evident in the hepatic parenchyma of both species. These structures represent the first line of defense of the organism and will also store foreign matter through the capture and processing of antigens and the exogenous products of cell degradation (Agius & Roberts, 2000; Borucinska *et al.*, 2009). As a result there is increase in size and number of MMCs mainly in tissues degenerate. Fish that live in environments impacted by the degradation may reflect the change in the immune system (Agius & Roberts, 2000; Bucke *et al.*, 1992).

Fatty degeneration was confirmed in both species through the observation of isolated or grouped fatty globules in the vicinity of the hepatic vein. In their analysis of histopathological alterations in *Clarias gariepinus* from polluted natural aquatic environments, Marchand *et al.* (2008) observed both MMCs and fatty degeneration, which were associated with contamination by metals. A similar situation is likely in the present study area given the evidence of contamination by heavy metals (Berrêdo *et al.*, 2001; Carneiro *et al.*, 2007).

Hepatitis is a degenerative lesion of the liver, associated with a process of cellular inflammation, while congestion is an accumulation of blood in the sinusoids, which was observed in both species analyzed in the present study. Rocha *et al.* (2010) recorded both hepatitis and congestion in their study of the dourado catfish, *Brachyplatystoma rousseauxii*, in environments affected by urban pollution.

Focal necrosis is associated with inflammatory processes. Nero *et al.* (2006) found a strong correlation between petroleum concentrations and focal necrosis in the livers of *Perca flavescens* and *Carassius auratus*. While this condition is irreversible, it does not disable the liver completely, given that the associated release of chemical signals induces the proliferation of cells that substitute the necrotic material, thus maintaining the structure and functional condition of the liver (Melo *et al.*, 2008). Fernandes *et al.* (2008) also associated focal necrosis with inflammatory processes in their study of *Liza saliens* in a contaminated natural environment.

Many metallic pollutants are found in the environment at sub-lethal levels, and are overlooked when they do not cause immediate mortality in fish populations. This type of contamination is known as chronic exposure (Dyk *et al.*, 2007). These authors showed that chronic exposure or exposure to high concentrations of pollutants such as chemical and metallic products, may cause severe alterations to the liver, given its role in the detoxification of the organism and that the characteristics of these alterations will depend on exposure time and the concentration of the contaminant. Additionally, the consumption of contaminated organisms by heavy metals by humans can cause serious health problems, especially for those populations with a diet restricted mainly fish.

Studies of the sub-lethal effects of metal and hydrocarbon contaminants include those of Liu *et al.* (2010), who focused on copper in *Synechogobius hasta*, Simonato *et al.* (2008) who analyzed the effects of diesel oil on *Prochilodus lineatus*, and Riba *et al.* (2005) who studied the effects of a number of different heavy metals in a variety of fish species. All these studies recorded alterations to the hepatic tissue, including swelling and the accumulation of fat, necrosis, congestion of the blood vessels, presence of MMCs, hypertrophy, hyperplasia, and stagnation of the ducts. These alterations were also observed in the present study and constitute clear evidence of the contamination of the water or sediment in the study area.

The hepatic alterations observed in the present study were generally more intense in the carnivorous *P. squamosissimus*, which feeds mainly on shrimp in the region of the study area (Ferreira *et al.*, 2008). Carnivorous fish tend to ingest and accumulate more toxins than organisms occupying lower trophic levels (Terra *et al.*, 2008), given that they normally feed on organisms that are already contaminated. This is especially true when the prey species are benthic organisms (Asuquo *et al.*, 2004; Durrieu *et al.* 2005; Terra *et al.*, 2008). Shrimp are bottom-feeders that tend to accumulate more heavy metals than other organisms (Çoğun *et al.*, 2005).

This study presented clear evidence of the contamination of the water of the aquatic environment adjacent to the port and industrial district of Vila do Conde and of the effects of this contamination on the health of local fish populations. This was the first study to histological alteration in the liver as a biomarkerfor the evaluation of environmental quality in this part of the Amazon basin. The data on the histological pathology of *P. squamosissimus* and *L. dorsalis* may provide environmental managers with important insights into monitoring needs and the potential for remediation of the impacted area. However, more reliable information on the bio-accumulation of heavy metals in these species will be necessary in order to reinforce the conclusion that these toxins are being transferred through the trophic web. This is especially important due to the prominence of both species in the diet of the local communities.

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CAPÍTULO III

MEASURING THE ECOLOGICAL INTEGRITY OF AN INDUSTRIAL DISTRICT LOCATED IN THE AMAZON ESTUARY, BRAZIL

AVALIAÇÃO DA INTEGRIDADE ECOLÓGICA EM UM DISTRITO INDUSTRIAL LOCALIZADO NO ESTUÁRIO AMAZÔNICO, BRASIL

MEASURING THE ECOLOGICAL INTEGRITY OF AN INDUSTRIAL DISTRICT LOCATED IN THE AMAZON ESTUARY, BRAZIL

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Abstract

The aim of the present study is to provide information on the ecological integrity of an industrial district (Pará, Amazon estuary), by applying a selection of fish based multimetric indices of ecosystem integrity: Abundance Biomass Comparation (ABC); Biological Health Indexes; Estuarine Fish Community, Transitional Fish Classification and Estuarine Biotic Integrity Indexes. To evaluate the impacts of the industrial area and cargo terminal, three areas were considered: Zone 1, (maximum impact), Zone 2 (median risk) and Zone 3 (reference area). The procedures adopted were nevertheless adequate for the detection of the alterations to the environment. All the biological indices used were considered to be excellent indicators of the ecological integrity of the different sectors of the study area and were especially effective for the demonstration of the critical alterations of the fish community of zone 1 and zone 2.

Keywords: Integrity index, Ichthyofauna, Amazon estuary; Industrial hub.

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1. Introduction

Estuarine systems have been coming increasingly to the attention of environmental institutions due to explosive population growth and increasingly intense anthropogenic impacts, which affect both water quality and aquatic biodiversity through the discharge of effluents and pollutants, such as ammonia, heavy metals, pesticides and herbicides (Asuquo et al., 2004; Eddy, 2005).

Biological indicators now constitute an integral component of water quality monitoring programs throughout the world (Dolph et al., 2010; Qadir and Malik, 2009). As they are formed by a variety of organisms endowed with different adaptive responses to given environmental conditions and varying degrees of tolerance to alterations in these conditions, the analysis of the biological communities of aquatic ecosystems can provide a useful aggregate measure of impacts (Goulart and Callisto, 2003). Given this, the principal approaches to the monitoring of environments involve the recording and evaluation of modifications in specific variables, such as species richness, diversity indices, the abundance of tolerant organisms and the loss of sensitive species, measures of primary and secondary productivity and ecotoxicological assays (Harrison and Whitfield, 2004; Jaramillo-Villa and Caramaschi, 2008; Karydis and Tsirtsis, 1996).

Procedures that integrate data on the community in order to generate a diagnosis of the quality of the environment are known as integrity indices. These indices were initially developed by Karr (1981) for freshwater environments and were subsequently adapted to other systems, such as estuaries and marine environments (Coates et al., 2007; Deegan et al., 1997; Harrison and Whithield, 2004). While these indices have certain limitations, they are still considered to be highly effective for the monitoring of water quality and have even been integrated into the environmental legislation of most European countries and US states (Ferreira and Casatti, 2006; Jaramillo-Villa and Caramaschi, 2008; Tejerina-Garro et al., 2005). In addition to their effectiveness, these indices are relatively simple to produce and are easily understandable to lay people, politicians and the general public.

In Brazil, this approach has been widely-used in studies of streams, rivers and reservoirs (Araújo et al., 2003; Casatti et al., 2009; Costa and Schulz, 2010; Ferreira and Casatti, 2006). In estuarine environments, however, few studies have involved the formulation and application of indices of biotic integrity. In the Amazon region, up to now, only two recent studies have used indices of this type for the monitoring of water quality, both of which focused on estuarine environments (Lemos and Frédou, 2010; Neves and Frédou, 2007).

The estuary of the Amazon River is adjacent to the municipality of Barcarena (around 84.560 inhabitants). Within this region the district of Vila do Conde is an important industrial hub, responsible for the processing and exportation of products such as kaolin, alumina and aluminum. The industrial processing of these minerals produces residues which may liberate substances such as fluorides, chlorides, sulfates and bicarbonates into the water, causing significant modifications to its quality. The treatment of effluents may also result in an increase in the levels of some metallic ions, such as those of iron and lead (Rubio and Tessele, 2002). A number of accidents have been recorded since the installation of the industrial zone (Carneiro et al, 2007; Lima et al., 2009), which indicates that local industrial activities constitute a key risk factor for the quality of the aquatic environments in Vila do Conde, including both surface and subterranean water resources and biota.

The aim of the present study is to provide detailed insights into the ecological integrity of the aquatic ecosystems of an industrial district (Pará, Amazon estuary), based on multimetric indices derived from the analysis of the local fish community. In addition to their intrinsic value for the monitoring of the study area, it is hoped that the results of this analysis will provide an important baseline for the development of effective procedures for the evaluation of similar aquatic systems.

2. Study area and data collection

The port of Vila do Conde (01°32’42”S, 48°45’00”W) is located on the right bank of the estuary Pará River in the district of Vila do Conde, part of the municipality of Barcarena in the Brazilian state of Pará, Amazon. The port is approximately 40 km west of the city of Belém (CDP, 2010). This study area is an estuarine environment with a considerable freshwater input, being classified as a tidal freshwater estuary according to the scheme of Elliott and McLusky (2002).

The present study was designed to test the effects of the probable environmental contamination in the area adjacent to the industrial installations and cargo terminal of Vila do Conde. For this, the collection fish specimens were organized in three distinct zones, representing different levels of impact: Zone 1 was located in the estuary of Pará River, in the vicinity of the Vila do Conde cargo terminal (Fig. 1), where the risk of contamination was highest. Zone 2 was Capim Island, in the estuary of Pará River, on the border between the municipalities of Bacarena and Abaetetuba (Fig. 1), classified as a median risk area due to its relative proximity to the port. Zone 3 was Onças Island in the municipality of Barcarena (Fig. 1), which was classified as minimum risk, due to its distance from the industrial district of Vila do Conde.

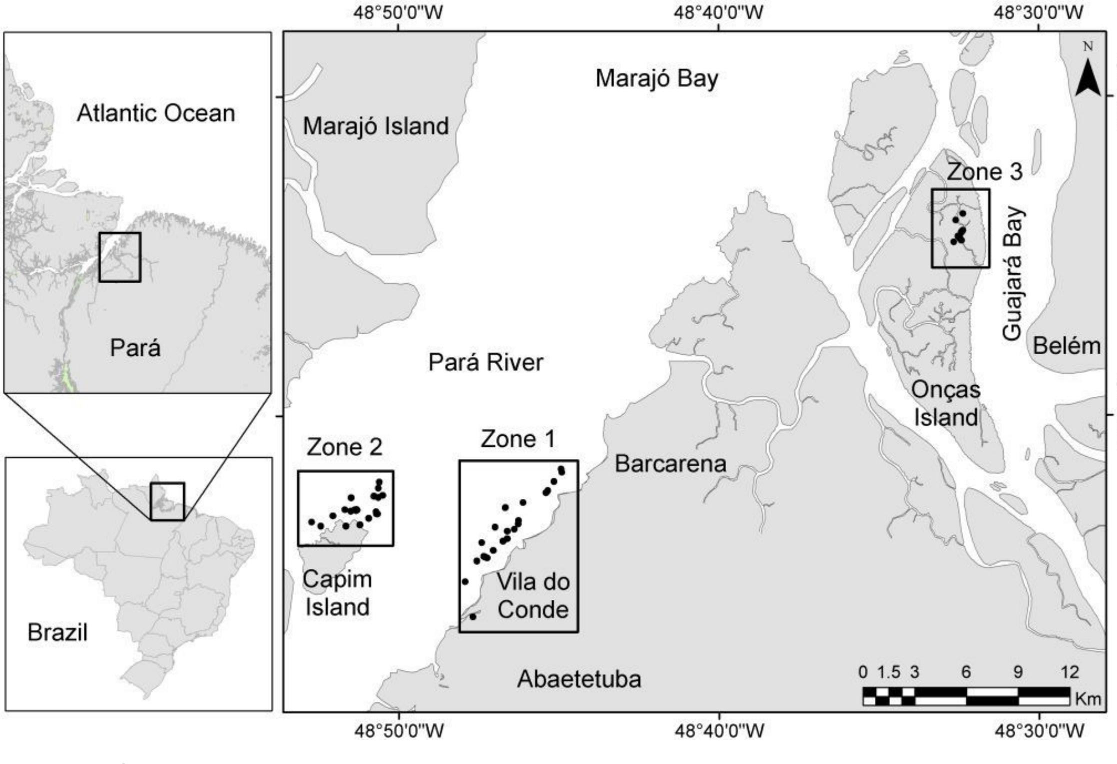

Fig. 1. Study area in Amazon estuary, Brazil, from 2009 to 2010, with the sampling points within the each zone. Zone 1: located in the vicinity of the Vila do Conde cargo terminal; Zone 2: Capim Island; Zona 3: Onças Island.

Two different types of environment – the main river channel and tidal channel – were sampled in all three zones. Samples were collected every two months between June, 2009 (June and September) and 2010 (January and April), All samples were collected during the neap tides. As the main channels and tidal channel show distinct dynamics (Viana et al., 2010), different sampling protocols were used. In the channel monofilament gillnets with different stretched mesh sizes were used (25, 35 and 40 mm), stretched mesh sizes (133.2 m in total) were used. In the tidal channel, a block net was set at the mouth of the tidal channel, closing it completely. Samples were standardized for all study areas and their environments.

All the fish specimens were identified to the lowest possible taxonomic level, based on Espirito Santo et al. (2005), FAO (1992) and Keith et al. (2000) Specimens were measured and weighed in the laboratory and had their gonads and stomach removed and weighed. Their maturation state was determined by the size and color of the gonads, based on Vazzoler (1996): stage A: immature; stage B: maturing; stage C: mature; stage D: spent.

The liver of species *Plagioscion squamosissimus* and *Lithodoras dorsalis* were selected as biomarker for the evaluation of histopathological alterations for each environment. Both species are relatively abundant within the study area and are important as resources for both subsistence and commercial fisheries based in communities within the municipality of Barcarena. The two species also represent distinct trophic levels and thus different levels of vulnerability to contamination. Immediately after being caught the fish were weighed and had their livers removed and weighed. Part of the liver sample was fixed in Bouin solution over a period of 48 hours and then conserved in 70% ethanol. In the laboratory, the organs were embedded in paraffin, sectioned (5 µm) and the slides were stained with hematoxylin and eosin (HE). These sections were examined by optical microscopy using Schwaiger et al. (1997) as a basic reference.

3. Data analysis

The three distinct areas (zones 1, 2, and 3), represented different levels of contamination, with a main channel environment and a tidal channel being chosen within each zone. As it was furthest from the source of the pollution, zone 3 was considered to be the reference. For the evaluation of the impacts caused by the Vila do Conde industrial district, three distinct groups of integrity indices were used – (I) Abundance-Biomass dominance curves (ABC); (II) Biological Health Index; (III) Estuarine fish community, transitional fish classification and estuarine biotic integrity indices.

3.1. Abundance-Biomass dominance curves (ABC)

The K-dominance curves for number of individuals and biomass were plotted together and the curves compared for main channel and for tidal channel. The direction and degree of separation of the curves is expressed by Clarke's W statistic (Clarke, 1990). Complete dominance of biomass and an even distribution of abundance are represented by a W value of +1, whereas the opposite situation scores -1. Negative values indicate disturbed environments (Yemane et al., 2005).

3.2. The Biological Health Index (BHI) of the estuary

The BHI developed by Cooper et al. (1994) is a measure of the degree of similarity between the potential community that would have been found originally within the study area and the actual community observed in that area (Whitfiled and Elliot, 2002). The basic assumption is that the difference between the two communities is due to habitat degradation. This index is calculated by: $BHI=10(J)[\ln P \ln P_{max}]^{-1}$, where J is the number of species in the area divided by the number of species in the reference area, P (potential species) is the number of species in the reference area and $Pmax$ (maximum potential species) is the total number of species from all communities. The value of the index varies from 0 to 10, with 10 representing excellent quality (Table 5).

3.4. Fish community, transitional classification and indices of biological integrity

The Estuarine Fish Community Index (EFCI) was created by Harrison and Whitfield (2004) and modified by Coates et al. (2007) into the Transitional Fish Classification Index (TFCI). The Estuarine Biotic Integrity Index (EBI) was adapted by Deegan et al. (1997) and Breine et al. (2007) from the Integrity biotic Index (IBI). These index are differentiated by the number and the nature of the metric used. The three integrity indices were calculated based on the respective reference environment (zone 3) in order to evaluate the degree of impact. The metrics and attributes adapted for the present study area and each integrity index are presented in Table 1.

Selection and adaptation of metrics

Some of the metrics used to calculate the indices were used in their original form, whereas others were adapted according to the conditions found in the present study. These modifications were necessary due to the environmental differences between the study area and the regions in which the indices were originally developed. A total of 25 metrics were selected and arranged in five attributes (Table 1): I - Species Diversity and Composition (SDC), II - Species Abundance (SA), III - Nursery Function (NF), IV - Trophic Integrity (TI), and V - Species health (SH). The values used to calculate these metrics were obtained from both the data collected during the present study and those available in the literature.

For the Species Diversity and Composition (SDC) attribute, four metrics were estimated: (1) total number of taxa; (2) number of individuals; (3) species composition, based on the presence/absence of species and estimated by Sorensen's coefficient of similarity and (4) Shannon's diversity index.

For Species Abundance (SA), only two metrics were adopted: relative abundance (5), which was based on the mean relative abundance of each species, used to calculate the Bray-Curtis similarity index and number of species contributing to 90% of the recorded abundance (6).

The attribute Nursery Function (NF) was represented by eight metrics: number of resident estuarine taxa (7), or the "estuarine species" guild, according to the classification of Elliot et al. (2007) functional guild composition the relative abundance of this guild (8). The species that use the study area as a nursery or for spawning were classified according to the scheme of Viana et al. (2010), which considered the presence of individuals with mature (stage C) and spent (stage D) gonads as evidence of the use of the area as a spawning ground and that of individuals with immature gonads (stage A) as evidence of its use as a nursery. The total number of species that use the area as a nursery and spawning ground represent metrics 9 and 10, respectively, while metrics 11 and 12 are the same parameters for estuarine species. All species were also classified according to their position in the water column – pelagic and demersal (13) or benthopelagic and benthic (14). These characteristics were inferred from the data available on the Fishbase database (Froese and Pauly, 2007). Species that are found in the study area during only part of their life cycle were classified as migratory (15).

The TI (Trophic Integrity) attribute also consisted of eight metrics: Metric 16 was based on functional guilds (marine stragglers, marine migrants, estuarine species, freshwater migrants, and freshwater stragglers) and metric 17 on feeding guilds (zooplanktivore, herbivore, piscivore, zoobenthivore, piscivore/zoobenthivore, and miscellaneous/opportunist). These classifications were based on the preferential habitats (16) and the feeding preferences and strategies (17) of the species, based on the approach of Elliot et al. (2007) and Krumme et al. (2004), adapted to the conditions of the present study. Trophic categories were adapted from those available in the literature and the feeding mode functional groups were identified by combining the data on predominant diet and feeding location. Where insufficient information was available, trophic and habitat preferences were inferred from the database of the Fishbase project (Frose and Pauly, 2007). Metrics 18 through 23 were determined by the number and abundance of bentic invertebrate (zoobenthivorous taxa), piscivorous and piscivororous/zoobenthivorous taxa.

Species Health (SH) was based on the analyses of two species, *Plagioscion squamosissimus* (metric 24) and *Lithodoras dorsalis* (metric 25), based on their Histological Alteration Indices (HAI). Following an approach adapted from Poleksic and Mitrovic-Tutundzic (1994), the severity of tissue lesions was ranked and the alterations observed in each organ were classified in three progressive stages of tissue damage: stage I (alterations which do not affect the normal functioning of the tissue), stage II (alterations which impair normal tissue function) and stage III (severe alterations which cause irreparable damage). The HAI was calculated for each animal based on: HAI = $10^1\sum I+10^2\sum II+10^3\sum III$, where I, II, III represent the stages outlined above. The mean values of HAI were used to relate environmental impact with the degree of functional impairment of the liver and its capacity for regeneration.

Table 1. Attributes and metrics adapted for the integrity indices and response of the community and/or individual to anthropogenic impacts. Species Diversity and Composition (SDC); Species Abundance (SA); Nursery Function (NF); Trophic Integrity (TI) and Species Health (SH). ↓ - decreases; ↑ - increases; ↑↓ - extremely low or high values indicate low quality, due to the absence or dominance of the species in the community.

Attribute	Metrics	Siglas das métricas	Index	Response to stress
SDC	1- Total number of taxa	TNT	EFCI, TFCI, EBI	↓
SDC	2 - Number of individuals	NI	EBI	↓
SDC	3 - Species composition (% Sorensen similarity)	SC	EFCI, TFCI	↓
SDC	4 - Shannon's diversity index	SDI	EFCI, TFCI, EBI	↓
SA	5 - Relative abundance (% Bray-curtis similarity)	RA	EFCI, TFCI	↓
SA	6 - Number of species that make up 90% of abundance	NS90%	EFCI, TFCI, EBI	↓
NF	7 - Number of estuarine resident taxa	NER	EFCI, TFCI	↓
NF	8 - Relative abundance of estuarine resident taxa	RAER	EFCI	↓↑
NF	9 - Number of nursery species (immature gonads - stage A)	NNS	EBI	↓
NF	10 - Number of spawners species (mature and spent gonads - stages C and D, respectively)	NSS	EBI	↓
NF	11 - Number of estuarine nursery species	NENS	EBI	↓
NF	12 - Number of estuarine spawners species	NESS	EBI	↓
NF	13 - Number of species demersal + Pelagic	NSDP	EBI	↓
NF	14 - Number species benthopelagic + benthonic	NSBB	EBI	↓
NF	15 - Number of migratory species	NMS	EBI	↓
TI	16 - Functional guild composition (number)	FUGC	TFCI	↓
TI	17 - Feeding Guild composition (number)	FEGC	TFCI	↓
TI	18 - Number of bentic invertebrate feeding taxa (zoobenthivores)	NZB	EFCI, TFCI, EBI	↓
TI	19 - Number of piscivorous taxa	NPV	EFCI, TFCI, EBI	↓
TI	20 - Number of piscivorous/zoobenthivorous taxa	NPV/ZB	EFCI, TFCI, EBI	↓

TI	21 - Relative abundance of bentic invertebrate feeding taxa	RAZB	EFCI	↓
TI	22 - Relative abundance of piscivorous taxa	RAPV	EFCI	↓
TI	23 - Relative abundance of piscivorous/zoobenthivorous taxa	RAPV/ZB	EFCI	↓
SH	24 - Histological Alteration Index of *Lithodora dorsalis*	HAILD	EBI	↑
SH	25 - Histological Alteration Index of *Plagioscion squamosissimus*	HAIPS	EBI	↑

Reference condition

Two separate reference conditions were calculated for each integrity index, representing the main channel and tidal channel environments (Tables 2-4). The reference limits were determined based on the values recorded in the area of least impact (zone 3).

Metrics 11, 12, 20, and 21 were not determined for the main channels due to the lack of an adequate number of species for the calculation of the parameter. Some metrics were not determined in comparison with the reference area (zone 3), but rather in relation to the community as a whole. This was the case for species composition (metric 3) and relative abundance (metric 5). Metric 3 was based on the presence or absence of species in each zone, with Sorensen's coefficient of similarity being calculated based on these data. The results of this analysis were compared with those from zone 3 in order to classify the quality of the metric. For relative abundance, the most abundant species were first identified, corresponding to the species with a total relative abundance of at least 1%. Species below this level were excluded from the analysis. The reference community (all areas) was based on the 14 most abundant taxa recorded in the main channel and the 18 most abundant taxa registered in the tidal channel. The Bray-Curtis similarity index was then calculated and the results were compared with those from zone 3, once again, in order to evaluate the quality of the metric.

The reference limits for the feeding guild composition metric were based on the relative contribution of each guild to total abundance. For the definition of an undisturbed environment, the references limits were adapted: 1% for piscivores, 5% for zoobenthivores and 10% for piscivores/zoobenthivores. In the case of histopathological alterations in *P. squamosissimus* and *L. dorsalis*, healthy fish were defined as those with mean HAI values lower than 10.

Table 2. Estuarine Fish Community Index (EFCI) scores for the main channel and tidal channel in Amazon estuary, Brazil, from 2009 to 2010. The scores based on the reference zone were classified as follows: 1 (<50%); 3 ([50%-90%[); 5 (≥ 90%).

Métricas EFCI	**1**	**3**	**5**	**Ambiente**
Total number of taxa	< 12	[12-23[	≥ 23	Main channel
	< 22	[22-39[	≥ 39	Tidal channel
Species composition (% Sorensen similarity)	< 50%	[50%-80%[	≥ 80%	Main channel and Tidal channel
Shannon's diversity index	< 0.88	[0.88-1.59[	≥ 1.59	Main channel
	< 1.32	[1.32-2.37[	≥ 1.59	Tidal channel
Relative abundance (% Bray-curtis similarity)	< 40%	[40%-60%[	≥ 60%	Main channel and Tidal channel
Number of species that make up 90% of abundance	< 9	[9-16[	≥ 16	Main channel
	< 6	[6-12[	≥ 12	Tidal channel
Number of estuarine resident taxa	< 1	[1-2[	≥ 2	Main channel
	< 2	[2-4[	≥ 4	Tidal channel
Relative abundance of estuarine resident taxa	<54 e ≥486	[54-135[e [405-486[	[135-405[	Main channel
	<117 e ≥1049	[117-292[e [874-1049[	[292-874[	Tidal channel
Number of bentic invertebrate feeding taxa (zoobenthivores)	< 3	[3-5[	≥ 5	Main channel
	< 5	[5-9[	≥ 9	Tidal channel
Number of piscivorous taxa	-	-	-	Main channel
	< 1	[1-2[	≥ 2	Tidal channel
Number of piscivorous/poobenthivorous taxa	< 3	[3-6[	≥ 6	Main channel
	< 5	[5-8[	≥ 8	Tidal channel
Relative abundance of bentic invertebrate feeding taxa	< 2.5%	[2.5%-5%[	≥ 5%	Main channel and Tidal channel
Relative abundance of piscivorous taxa	< 0.5%	[0.5%-1%[	≥ 1%	Main channel and Tidal channel
Relative abundance of piscivorous/zoobenthivorous taxa	< 5%	[5%-10%[	≥ 10%	Main channel and Tidal channel

Table 3. Transitional Fish Classification Index (TFCI) scores for the main channel and tidal channel in Amazon estuary, Brazil, from 2009 to 2010. The scores based on the reference zone were classified as follows: 1 (<20%); 2 ([20%-40%[); 3 ([40%-60%[); 4 ([60%-80%[) 5 (≥80%).

Métricas TFCI	**1**	**2**	**3**	**4**	**5**	Ambiente
Total number of taxa	<5	[5-10[	[10-15[	[15-20[	≥ 20	Main channel
	<9	[9-18[	[18-26[	[26-35[	≥ 35	Tidal channel
Species composition (% Sorensen similarity)	<20%	[20%-40%[	[40%-60%[	[60%-80%[	≥ 80%	Main channel and Tidal channel
Shannon's diversity index	<0.35	[0.35-0.71[	[0.71-1.06[	[1.06-1.41[	≥ 1.41	Main channel
	<0.53	[0.53-1.06[	[1.06-1.59[	[1.59-2.12[	≥ 2.12	Tidal channel
Relative abundance (% Bray-curtis similarity)	<20%	[20%-40%[	[40%-60%[	[60%-80%[	≥ 80%	Main channel and Tidal channel
Number of species that make up 90% of abundance	<5	[4-7[	[7-10[	[10-14[	≥ 14	Main channel
	<3	[3-5[	[5-8[	[8-11[	≥ 11	Tidal channel
Number of estuarine resident taxa	<0.4	[0.4-0.8[	[0.8-1.2[	[1.2-1.6[	≥ 1.6	Main channel
	<1	[1-2[	[2-3[	[3-4[	≥ 4	Tidal channel
Number of bentic invertebrate feeding taxa (zoobenthivores)	<1	[1-2[	[2-3[	[3-4[	≥ 4	Main channel
	<2	[2-4[	[4-6[	[6-8[	≥ 8	Tidal channel
	-	-	-	-	-	Main channel
Number of piscivorous taxa	<0.4	[0.4-0.8[	[0.8-1.2[	[1.2-1.6[	≥ 1.6	Tidal channel
Number of piscivorous/poobenthivorous taxa	<2	[2-3[	[3-4[	[4-5[	≥ 5	Main channel
	<1	[1-2[	[2-3[	[3-4[	≥ 4	Tidal channel
Functional guild composition (number)	<1	[1-2[	[2-3[	[3-4[	≥ 4	Main channel
	0	[0-1[	[1-2[	[2-3[	≥ 3	Tidal channel
Feeding guild composition (number)	0	1	2	3	≥ 4	Main channel and Tidal channel

Table 4. Values of the Estuarine Biotic Integrity Index (EBI) for the main channel and tidal channel in Amazon estuary, Brazil, from 2009 to 2010. The scores based on the reference zone were classified as follows: 1 (<33%); 3 ([33%-66%[); 5 (≥66%).

Métricas EBI	1	2	3	Environment
Total number of taxa	<9	[9-17[	≥17	Main channel
	<15	[15-29[	≥29	Tidal channel
Number of individuals	<39	[39-78[	≥78	Main channel
	<246	[246-491[	≥491	Tidal channel
Shannon's diversity index	<0.58	[0.58-1.17[	≥1.17	Main channel
	<0.87	[0.87-1.74[	≥1.74	Tidal channel
Species composition (% Sorensen similarity)	<6	[6-12[	≥12	Main channel
	<5	[5-9[	≥9	Tidal channel
Number of nursery species (immature gonads - stage A)	<5	[5-10[	≥10	Main channel
	<11	[11-22[	≥22	Tidal channel
Number of spawners species (mature and spent gonads - stages C and D, respectively)	<5	[5-10[	≥10	Main channel
	<6	[6-11[	≥11	Tidal channel
Number of estuarine nursery species	-	-	-	Main channel
	<1	[1-2[	≥2	Tidal channel
Number of estuarine spawners species	-	-	-	Main channel
	<1	[1-2[	≥2	Tidal channel
Number of species demersal + Pelagic	<5	[5-10[	≥10	Main channel
	<6	[6-12[	≥12	Tidal channel
Number species benthopelagic + benthonic	<4	[4-8[	≥8	Main channel
	<7	[7-14[	≥14	Tidal channel
Number of migratory species	<3	[3-6[	≥6	Main channel
	<8	[8-15[	≥15	Tidal channel
Number of bentic invertebrate feeding taxa (zoobenthivores)	<2	[2-4[	≥4	Main channel

	<4	[4-7[	≥7	Tidal channel
Number of piscivorous taxa	-	-	-	Main channel
	<1	[1-2[	≥2	Tidal channel
Number of piscivorous/zoobenthivorous taxa	<2	[2-4[	≥4	Main channel
	<3	[3-6[	≥6	Tidal channel
Histological Alteration Index of *Lithodora dorsalis*	<51	[11-50]	≤ 10	Main channel and Tidal channel
Histological Alteration Index of *Plagioscion squamosissimus*	<51	[11-50]	≤ 10	Main channel and Tidal channel

Score system

The TFCI values are proportions, varying from 0 to 1 (“relative score”) and were calculated by dividing the sum of the 10 metrics by the highest possible score (55 for main channels and 60 for tidal channel). The EFCI and BHI scores used in the present study were those used by Henriques et al. (2008), which the authors adapted by classifying the values in five categories (quintiles): (a) bad, (b) poor, (c) moderate, (d) good, and (e) excellent environmental quality (Table 5). The EBI scores were based on the original approach of Karr (1981).

Table 5. Ecological scale in Amazon estuary, Brazil, from 2009 to 2010 adapted from Henriques et al. (2008) for the different indices. Descriptors adapted from Roth et al. (2000). Estuarine Biological Health Index (BHI); Estuarine Biotic Integrity Index (EBI); Estuarine Fish Community Index (EFCI); Transitional Fish Classification Index (TFCI).

Rating	Scores				Descriptions
	BHI	EBI	EFCI	TFCI	
Bad	[0-2[	≤ 23	[16-20[	[0.2-0.36[	Strong deviation from reference conditions, with many aspects of biological integrity endangered, indicating severe degradation
Poor	[2-4[	[28-35[	[22-38[	[0.36-0.52[	Significant deviation from reference conditions, with many aspects of biological integrity not resembling the quality of minimally impacted area.
Moderate	[4-6[	[39-44[	[40-44[	[0.52-0.68[	Comparable with reference area, but with some aspects of biological integrity compromised
Good	[6-8[	[48-52[	[46-62[	[0.68-0.84[	Comparable with reference area and regarded as minimally affected.
Excellent	[8-10]	[57-60]	[64-68]	[0.84-1]	Comparable with reference area.

4. Results

The ABC dominance curves indicated that the main channel in zones 1 and 2 are suffering a moderate degree of disturbance. The abundance and dominance curves for these two zones were very close to one another, often crossing, with values of W close to 0 (Fig. 2). In the case of the tidal channel, the curves indicated moderate disturbance in zone 1, while in zones 2 and 3, the biomass curve extended above the abundance curve, with values of W greater than 0 (W = 0.142 and 0.103, respectively), indicating the predominance of K-selected species (Fig. 2).

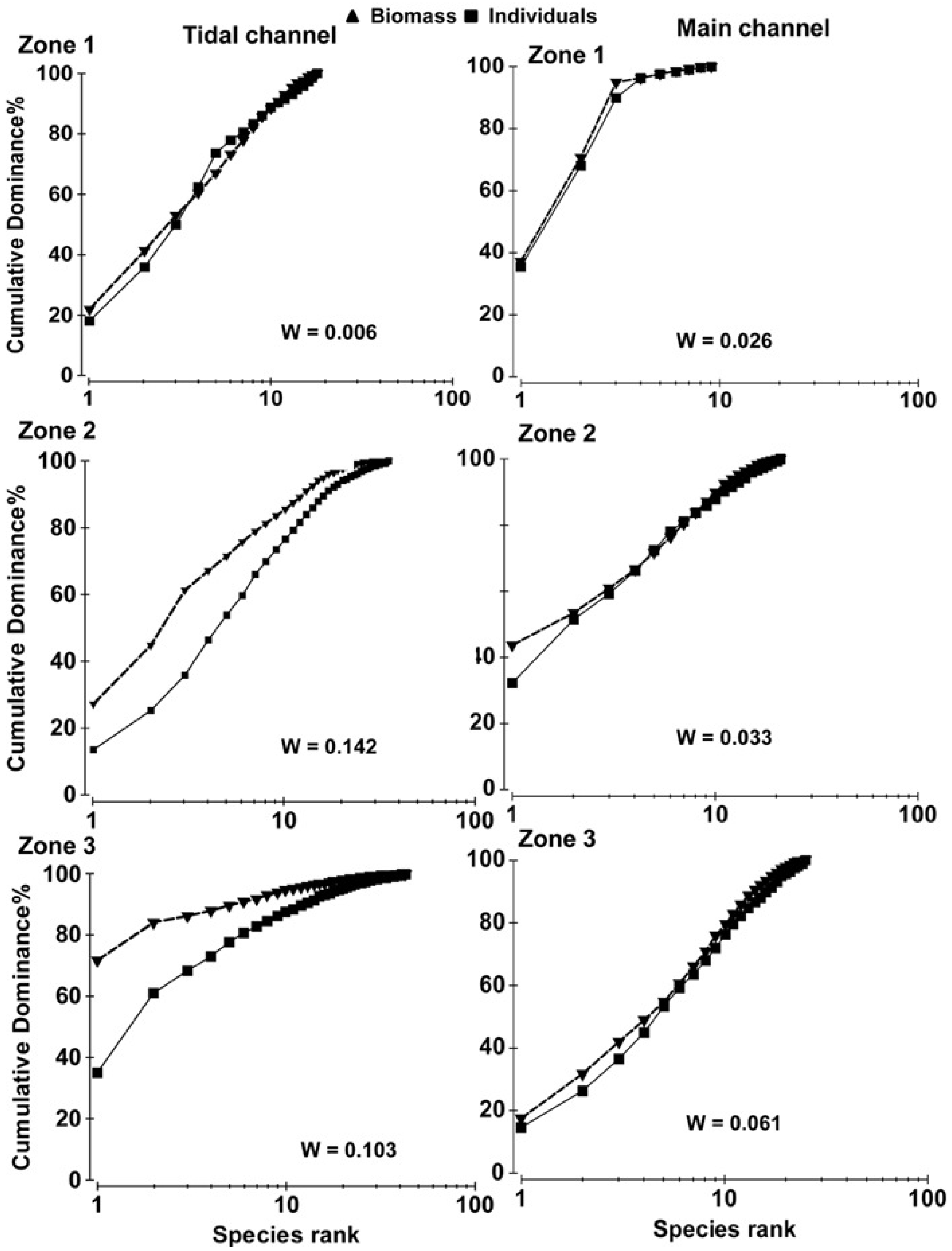

Fig. 2. ABC dominance curves by zone and environment (main channel and tidal channel) in Amazon estuary, Brazil, from 2009 to 2010. Zone 1: located in the vicinity of the Vila do Conde cargo terminal; Zone 2: Capim Island; Zona 3: Onças Island.

The lowest BHI values were recorded in zone 1, in both the main channel (2.19) and the tidal channel (2.90). The highest values were recorded in zone 3, for both the main channel (8.91) and the tidal channel (9.01), reflecting the good quality of the environment in these two areas (Fig. 3).

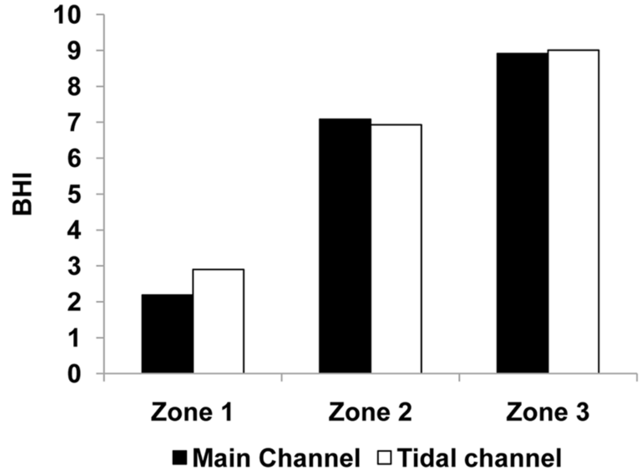

Fig. 3. Estuarine biological health index (BHI) by zone and environmet (main channel and tidal channel) in Amazon estuary, Brazil, from 2009 to 2010. Zone 1: located in the vicinity of the Vila do Conde cargo terminal; Zone 2: Capim Island; Zona 3: Onças Island.

Considerable differences in the integrity indices and almost all their respective metrics were observed among zones. In both main channel and tidal channel habitats, the communities recorded in zone 1 were the most impaired in comparison with the reference, zone 3 (Figs. 4 and 5).

An overview of the scores by zone and habitat (Table 6, Figs. 4 and 5) reveals integrity indices ranging from bad to excellent. While the ecological status of the different areas varied considerably according to the index used, the sum of the evidence indicated clearly that the integrity of zone 1 – with scores varying from bad to moderate – has been damaged, whereas the environmental quality of zone 2 has been largely maintained, despite suffering some anthropogenic impact, except for the values of EFCI, which indicate reduced integrity (Table 6, Fig 5). The results also indicated that, in zone 1, the fish community in the main channel has been more heavily impacted than that of the tidal channel.

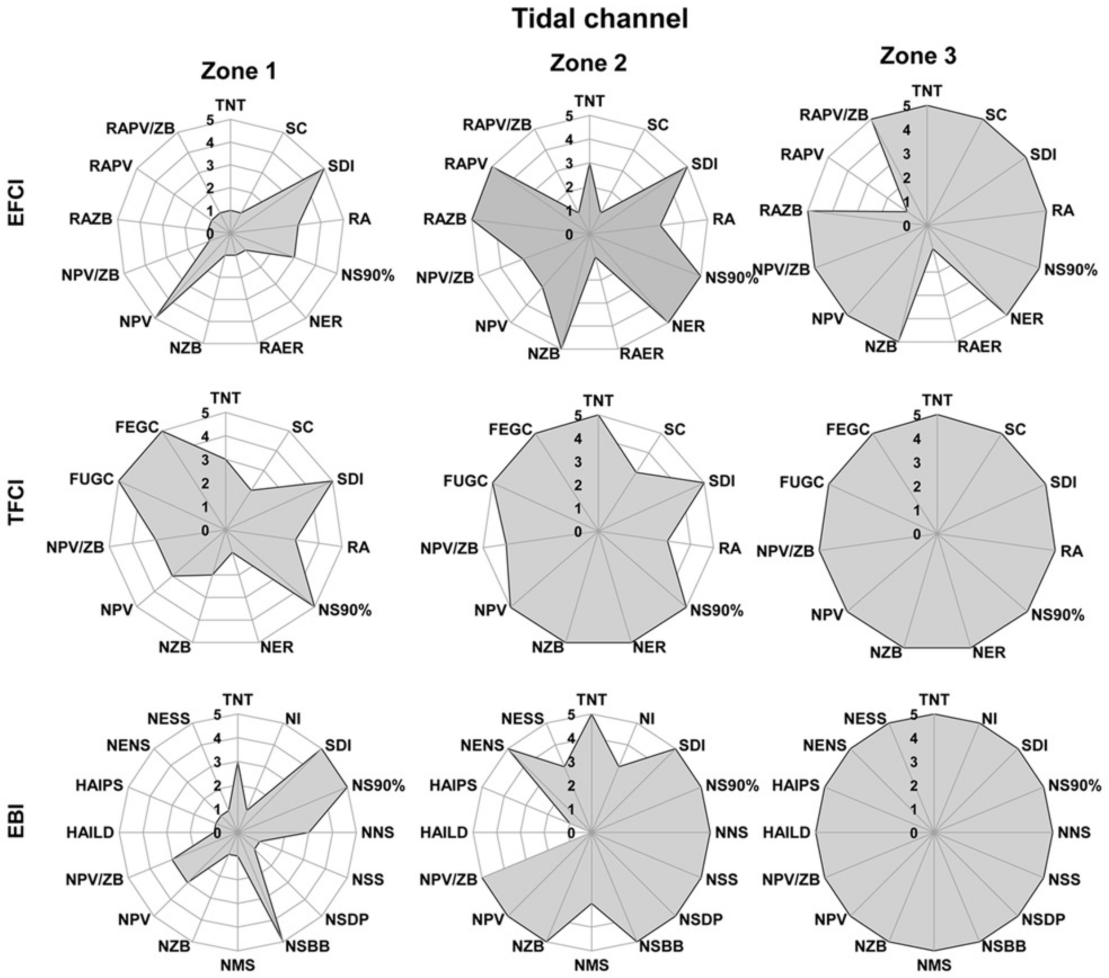

Fig. 4. Quality of the each metric recorded in the tidal channel of zone 1 (located in the vicinity of the Vila do Conde cargo terminal), zone 2 (Capim Island) and zone 3 (Onças Island) in Amazon estuary, Brazil, from 2009 to 2010, by integrity index. Scale for EFCI and EBI: 1 – bad; 3 – moderate; 5 - excellent. Scale for TFCI: 1 - bad; 2- poor; 3 – moderate; 4 – good; 5 – excellent. The meaning of the symbol representing each metric is provided in Table 1.

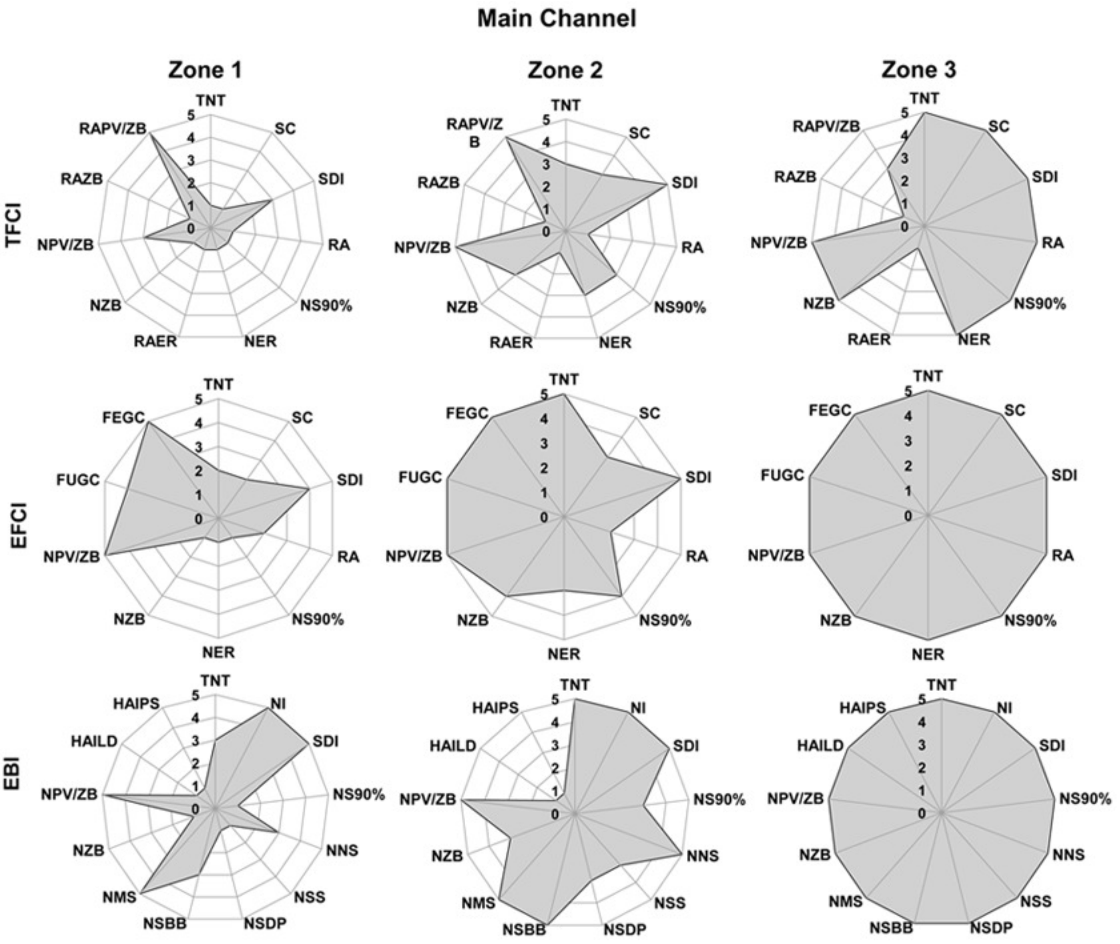

Fig. 5. Quality of the each metric recorded in the main channel of zone 1 (located in the vicinity of the Vila do Conde cargo terminal), zone 2 (Capim Island) and zone 3 (Onças Island) in Amazon estuary, Brazil, from 2009 to 2010**,** by integrity index. . Scale for EFCI and EBI: 1 – bad; 3 – moderate; 5 - excellent. Scale for TFCI: 1 - bad; 2- poor; 3 – moderate; 4 – good; 5 – excellent. The meaning of the symbol representing each metric is provided in Table 1.

Table 6. Ecological status obtained for each index by zone and habitat in Amazon estuary, Brazil, from 2009 to 2010.

	Index	**Zone 1**	**Zone 2**	**Zone 3**
Tidal channel	EFCI	Poor (25)	Good/moderate (45)	Good (57)
	TFCI	Moderate (0.62)	Good (0.83)	Excellent (0.92)
	EBI	Moderate/Poor (36)	Excellent (65)	Excellent (80)
	BHI	Poor (2.90)	Good (6.93)	Excellent (9.01)
Mean Channel	EFCI	Bad (19)	Poor (33)	Good/moderate (45)
	TFCI	Poor (0.49)	Good (0.75)	Excellent (0.91)
	EBI	Moderate/Poor (35)	Good/moderate (49)	Excellent (65)
	BHI	Poor (2.19)	Good (7.08)	Excellent (8.91)

5. Discussion

The biota can be considered to be one of the best indicators for the evaluation of the health of an aquatic ecosystem (Breine et al., 2010; Delpech et al., 2010). However, when contamination originates a negative effect on the biota of an area, the environment is typically already at an advanced stage of degradation (Norris and Thoms, 1999). Indices of integrity are considered to be effective tools for the evaluation of environmental (Breine et al., 2010; Qadir and Malik, 2009). The important feature of these indices is their inclusion of attributes that represent the biological structure and function of the community, based on metrics that assess parameters such as reproductive and trophic levels, species health, diversity and abundance (Ferreira and Casatti, 2006; Roset et al., 2007). These indices of biological integrity may be more effective than the adoption of exclusively physical-chemical criteria for the evaluation of water quality, which may not necessarily provide an accurate depiction of the conditions faced by local communities (Vieira and Chibata, 2007). While these criteria may provide a reliable assessment of water quality *per se*, they may not necessarily offer an effective measure of the ecological integrity of the area (Goulart and Callisto, 2003), given that they merely provide a “snapshot” of environmental conditions at a given point in time. By contrast, biological indicators offer an integrated overview of the accumulated effects of pollution on the biota.

In the present study, a variety of different indices were used to provide information on the ecological integrity of the aquatic ecosystem adjacent to Vila do Conde. This town has become an important industrial hub, with installations for the processing of mineral ores and production of chemicals. The residues produced by these industries constitute a risk factor for the quality of both surface and underground water resources. Despite the intense industrial activity, this area is an important artisanal fishing ground and the local population is highly dependent on its fishery resources (Paz et al., in press). The application of different integrity indices provided useful complementary insights into the ecological quality of the different environments. Overall, the results indicated very clear differences among the three zones.

The ABC curve represents the relationship between r- and K-strategist species. With their larger body size and longer life cycle, K-strategists tend to be less abundant, but dominant in terms of biomass. When exogenous factors disturb a community, these species tend to be the most impacted, while more opportunistic species – generally r-selected taxa characterized by relatively small body size and short life cycles – become numerically dominant (Casatti et al., 2006; Clarke and Warwick, 1994). In the present study, the main channel in zones 1 and 2 and the tidal channel in zone 1 were classified as moderately disturbed, which predicts an increase in the relative abundance of opportunistic species. Dias and Tejerina-Garro (2010) found that the presence or absence of organisms of relatively large size has a marked effect on the ABC biomass curve, often causing it to rise above the abundance curve. A reduction in the abundance of large-bodied individuals was observed in zone 1 in comparison with zones 2 and 3 (Viana and Frédou, unpublished results).

The BHI scores were relatively effective in the context of the present study, revealing alterations in the integrity of the impacted areas, in comparison with the reference. This index has received some criticism due to the use of simple presence/absence data and its dependence on sample size (Henriques et al., 2008; Whitfield and Elliott, 2002). These authors also concluded that, in isolation, BHI is not a good indicator of environment quality, but in association with other approaches, such as those applied in the present study, it may be a useful tool for the characterization of fish communities.

Some of the integrity indices used in the present study, such as TFCI, EFCI and EBI - which was derived from Karr (1981) - Index of Biological Integrity), include a number of different metrics which, when combined, provide insights into each biological parameter and thus into the health of the aquatic environment as a whole (Breine et al., 2010; Deegan et al., 1997). The metrics were calculated based on the reference area (zone 3) and characterized by the distance from the industrial district (zones 1 and 2). Henriques et al. (2008) concluded that, while the reference limits are not real or exact values, they can be effectively compared with values for impacted areas, as long as the sampling procedures were both adequate and standardized and the same calculating principles were applied, as was the case in the present study. The results of the present study showed clear differences in these indicators between the reference area and the impacted zones.

Not all the metrics used for the calculation of different indices responded to anthropogenic impacts in the same way. These dissimilarities are related to the differences in the approach to the classification of the quality of an environment. The TFCI and EFCI scores provide a more rigorous system for the classification of environments of good quality (score = 5) when compared with the EBI.

Anthropogenic impacts are known to modify species composition through the elimination of the most sensitive taxa (species richness declines) and the subsequent dominance of the more tolerant species (Casatti et al., 2009; Delpech et al., 2010; Dias and Tejerina-Garro, 2010; Magurram, 2005). In the present study, the ecological indices indicated that the community closest to the industrial district is the most impacted, based on the reduced values recorded for the species diversity and composition attribute. Despite the restrictions about the use of analysis on the community structure in estuarine environments, as the case of ecological indices (Elliott and Quintino, 2007), in the studied study area is heavily influenced by the inflow of freshwater from the river Pará and Amazon.

The metrics of the nursery function attribute indicated a reduction in the quality of zone 1 in comparison with zones 2 and 3. Many of these metrics, which are related to habitat use, are influenced by the migratory species, which utilize estuarine environments as staging posts during specific periods of their life cycle, such as development, feeding or spawning (Loureiro and Frédou, 2010; Raiol et al., 2006; Viana et al., 2010). The metrics included in this category evaluate the capacity of the environment to provide feeding resources and refuges over the short term. Migratory species are especially sensitive to habitat degradation and exposure to toxic substances, making them particularly useful as indicators for the evaluation of the integrity of an environment (Breine et al., 2010; Breine et al., 2007; Courrat et al., 2009; Delpech et al., 2010).

The structure of a community's trophic guilds has been considered to be a good indicator of trophic integrity (Deegan et al., 1997; Elliot et al., 2007; Henriques et al., 2008). A reduced number of trophic categories were recorded in the main channel of zone 1, in particular, the absence of piscivores, zoobenthivores, and detritivores. Browne and Lutz (2010) and Ecoutin et al. (2010) also found that anthropogenic disturbances may result in the loss of organisms at the top of the trophic web, in addition to the more specialist taxa. Generalist predators may be able to find prey more easily than specialized ones, such as benthophagous species (Garrison and Link, 2000) and are therefore better prepared to survive major disturbances. The values of the metrics related to trophic guilds are consistent with the results of the ABC curves. Clarke and Warwick (1994) concluded that the presence of predator guilds tends to push the biomass curve above the abundance curve. However, when no data are available on the trophic structure of the local communities prior to the observed impacts, it is much more difficult to understand their effects on community structure (Ecoutin et al., 2010).

Henriques et al. (2008) concluded that, when the sample is dominated by a few species, this will be reflected in all the metrics that include abundance parameters. In the present study, the metrics Relative Abundance of Estuarine Resident Taxa (RAER), Relative Abundance of Piscivorous/Zoobenthivorous Taxa (RAPV/ZB), Relative Abundance of Bentic Invertebrate Feeding Taxa (RAZB) and Relative Abundance of Piscivorous Taxa (RAPV) all indicated reduced quality in the reference area (zone 3). The abundance of the two dominant species (*P. squamosissimus* and *L. dorsalis*) affected these results. Both species are relatively common in the Amazon basin and are considered to be relatively tolerant of contamination, given that studies in other parts of the estuary have found that their abundance is not affected by anthropogenic disturbances (Viana et al., 2010). In future studies, it would be useful to eliminate the effects of the dominance of these species, in order to increase the reliability of the indices that include abundance data.

The values of HAI for both *P. squamosissimus* and *L. dorsalis* revealed clear evidence of histological alterations in the specimens captured in the most impacted area (zone 1). This approach is considered to be an excellent tool for environmental monitoring, given that the liver responds rapidly to exposure to pollutants, especially chemical products and metals, given its role in the detoxification of the organism (Dyk et al., 2007; Stehr et al., 2003). When the integrity of this metric is modified, as in zone 1, severe and irreversible alterations of the liver have occurred.

Despite certain differences, all the biological indices used in the present study were considered to be excellent indicators of the ecological integrity of the different sectors of the study area and were especially effective for the demonstration of the critical alterations of the fish community of zone 1. It was also possible to identify alterations to the environment of zone 2. The pattern of disturbance points clearly to the influence of the industrial district and cargo terminal of Vila do Conde and in particular the kaolin and bauxite processing. A number of products, including alumina and primary aluminum, caustic soda, fuel oil and manganese, are also shipped out of the port (CDP, 2010).

Previous studies of the sediments of zone 1 have recorded levels of heavy metals (Cadmium, Iron, Zinc, Aluminum, and Sodium) above the limits permitted by Brazilian legislation (Bêrredo et al., 2001; Carneiro et al., 2007; Lima et al., 2007). This scenario may cause disease and mortality in local fish (Casatti et al., 2009), as observed in the accidents recorded previously in Vila do Conde (Lima et al., 2007), as well as affecting the ecological functions of the fish community and causing a reduction in the trophic integrity of the environment, as shown in the present study.

The results of the present study will provide an important database for the future and clearly very necessary ecological monitoring of the aquatic environments adjacent to Vila do Conde. Given the lack of historical data for the study area, however, a number of adjustments will be required in order to adapt the metrics to the region’s unique conditions. The procedures adopted in the present study were nevertheless adequate for the detection of the alterations to the environment which has occurred in the vicinity of the town’s industrial district and cargo terminal.

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CONSIDERAÇÕES FINAIS

1. CONSIDERAÇÕES FINAIS

O diagnóstico da qualidade da água na área industrial de Barcarena, em Vila do Conde utilizando a ictiofauna como indicador, foi a proposta deste trabalho. A decisão de estudar a comunidade biológica partiu do principio, abordado por vários autores (CLARKE; WARWICK, 1994, NAVAS-PEREIRA; HENRIQUE, 1996, SMITH; BARRELLA, 2000, SILVEIRA, 2004, JARAMILLO-VILLA; CARAMASCHI, 2008) de que a biota, em situações adversas, é capaz de se adaptar e responder a diferentes níveis de degradação ambiental. No caso dos peixes especificamente, além dos aspectos ecológicos, os mesmos são consumidos pela população o que gera também um apelo econômico e social, no tocante à necessidade da preservação da qualidade do ambiente e saúde do animal.

Na região amazônica, a pesca é uma atividade complexa, ligada culturalmente a tradições pesqueiras (BRAGA, 2001). Esta atividade vem sendo realizada em áreas com grande influência antrópica. Dentro do estuário Amazônico, a Vila do Conde, localizada na cidade de Barcarena, é conhecida por abrigar diversas indústrias de beneficiamento de minérios e por possuir um terminal portuário pertencente a “Companhia Docas do Pará” (CDP), que faz o escoamento da produção. Adicionalmente, este porto manuseia produtos químicos necessários para o beneficiamento da bauxita, além de outros produtos como transporte de gado vivo, coque e piche (CDP, 2010). Considerando estas características, o ambiente aquático no entorno desta região portuária e industrial acabou se tornando suscetível a eventuais acidentes, como já noticiado diversas vezes pela mídia (BERREDO et al., 2001; CARNEIRO et al., 2007; LIMA et al., 2009; PEREIRA, 2009; PORTO, 2009; LIMA et al., in press;).

Como forma de abordar diferentes vertentes sobre a qualidade da água em Vila do Conde, este trabalho foi dividido em etapas. A primeira etapa consistiu no uso da ictiofauna como bioindicador (**capítulo 1**). Na segunda etapa foram selecionadas duas espécies abundantes com hábitos alimentares distintos, pescada branca (*Plagioscion squamosissimus*) e bacu (*Lithodoras dorsalis*), para avaliar a saúde do ambiente através da utilização do fígado como biomarcador histopatológico (**capítulo 2**). Por fim, todas as famílias de descritores da comunidade estudada nos capítulos 1 e 2 foram integradas, através do uso de índices de integridade biológica (**capítulo 3**). Estes três capítulos permitiram testar metodologias baseadas em pressupostos distintos, identificando as mais eficientes no monitoramento e aquelas cujas adaptações se fazem necessárias ao ambiente estuarino amazônico.

A análise da ictiofauna como bioindicador (capítulo 1) mostrou que, para os dois ambientes (canal principal e canal de maré), as várias famílias de descritores, foram eficientes em detectar a composição diferenciada entre os locais. Dentre os descritores, a riqueza, porte das espécies, constância, índices de diversidade de Shannon, riqueza observada e de Margalef, índice de dominância de Simpson, uso dos habitat, guilda funcional e análise multivariada são metodologias indicadas para o monitoramento ambiental.

Apesar das restrições existentes quanto ao uso de análises de comunidade em ambiente estuarinos, como é o caso dos índices ecológicos (ELLIOTT; QUINTINO, 2007), na área de estudo esta restrição não prejudicou os resultados, pois esta zona do ambiente amazônico apresenta grande influência de água doce proveniente dos rios Pará e Amazonas, podendo ser classificada como um estuário de maré fluvia (ELLIOTT; MCLUSKY, 2002).

Em contraste, a equitabilidade e a CPUE (índice de abundância) não se mostraram bons indicadores. Descritores relacionados com a abundância relativa, na qual há presença de espécies dominantes, influenciam no resultado e já foi alvo de críticas para este tipo de abordagem (HENRIQUES et al. 2008). Quando a amostra é dominada por poucas espécies a elevada abundância é um reflexo das espécies com grande distribuição e resistência à poluição. Este foi o caso do presente estudo, onde a maior abundância esteve relacionada a uma grande quantidade de indivíduos das espécies *Plagioscion squamosissimus* e *Lithodoras dorsalis* que dominaram nesta área, apresentam maior porte e são resistentes a fatores antrópicos.

A análise dos biomarcadores histopatológicos do fígado se mostrou eficiente e demonstrou que a presença antrópica naquela região está afetando a saúde da *P. squamosissimus* e *L. dorsalis*. Nas áreas em que existe o contato mais próximo com o porto e as indústrias, as alterações foram mais severas e algumas consideradas irreversíveis para as duas espécies, mostrando o grau de comprometimento da qualidade ambiental em Vila do

Conde. A análise da intensidade das alterações histopatológicas, considerando os diferentes níveis tróficos das duas espécies, indicou alterações mais severas para *P. squamosissimus,* de hábito carnívoro/zoobentívoro.

Uma vez que o fígado participa de processos como transformação e excreção de substâncias tóxicas do corpo, podendo ter sua estrutura alterada em resposta a fatores externos (BRUSLÉ; ANADON, 1996, DYK et al., 2007), o comprometimento da saúde do peixe pode afetar o equilíbrio da biota, principalmente se esta contaminação afetar a cadeia trófica, como de fato ocorre. O resultado disso pode comprometer inclusive a saúde humana, uma vez que estas espécies são continuamente consumidas pelos habitantes da região.

Os índices de integridade são metodologias que integram informações sobre a comunidade, através da utilização de atributos que representam a estrutura biológica e o funcionamento da comunidade indicados por métricas reprodutivas, tróficas, saúde das espécies, diversidade e abundância (FERREIRA; CASATTI, 2006; ROSET et al., 2007). Neste trabalho, várias famílias de índices foram utilizadas com o objetivo de fornecer informações agregadas sobre a integridade ambiental de Vila do Conde.

O uso de integridade ecológica para classificar a qualidade da água tem sido bastante utilizado por outros países, através de iniciativas e criação de leis para a proteção dos ecossistemas aquáticos, como forma de monitorar as regiões estuarinas (PÉREZ-DOMÍGUEZ et al., 2009). No caso do estuário amazônico, especificamente o estuário do rio Pará, algumas métricas utilizadas ainda merecem mais estudos antes que possam ser completamente eficientes como indicadores.

Adotar critérios biológicos pode ser mais eficiente do que adotar critérios exclusivamente físico-químicos para avaliar a qualidade da água, uma vez que estes últimos apesar de serem bons indicadores da qualidade da água, fornecendo importantes informações e de forma imediata sobre o estado de integridade do ambiente, nem sempre eles, sozinhos, responde de forma satisfatória a avaliação da integridade ecológica (GOULART; CALLISTO, 2003; SILVEIRA, 2004). Isso de deve à capacidade de autodepuração e do fluxo de ecossistemas lóticos, os efluentes carreados podem ser diluídos antes dos dados serem coletados resultando por fim em poucas modificações em suas variáveis. Este foi o caso do presente estudo. Locais como o estuário Amazônico, rio Pará, baía do Guajará entre outras regiões, o sedimento parece refletir melhor os efeitos da poluição, pois os metais pesados tendem a se acumular nesta porção do ambiente. Neste estudo, não foram feitas análises de metais pesados, mas trabalhos anteriores já realizados na região (BERREDO et al., 2001; CARNEIRO et al., 2007; LIMA et al., 2007) mostram valores alterados de metais pesados nos sedimentos na área mais próxima ao porto e as industrias (Cádmio, Ferro, Zinco, Alumínio e Sódio) e que estes estão acima dos limites permitidos pela legislação brasileira (CONAMA 357/2005). A presença de metais pesados acima do limite de tolerância pode causar doenças e mortandade de peixes, como já observado em acidentes ocorrido anteriormente em Vila do Conde.

Os três tipos de abordagem utilizados neste estudo foram complementares e eficientes em responder às hipóteses formuladas na pesquisa, indicando: que a comunidade de peixe que vive no entorno do terminal portuário e industrial de Vila do Conde está sofrendo alterações na estrutura de sua comunidade e que o canal de maré sofre mais com os efeitos antrópicos (**hipótese 1**) , pois são corpos de água mais fechados e que passam mais tempo para depurar e diluir qualquer contaminante. Como resultado da exposição crônica aos contaminantes químicos as espécies passaram a adquirir histopatologias demonstrada através do estudo do fígado (**hipótese 2**), principalmente para a espécie carnívora cujas alterações foram mais severas (**hipotese 3**).

As metodologias utilizadas estão aptas para ser replicadas em outros ambientes estuarinos. Pesquisas posteriores podem ser agora comparadas com os dados obtidos no presente estudo. Este foi o primeiro trabalho em Vila do Conde que utilizou diferentes mecanismos para avaliar a qualidade da água através da ictiofauna. Em nível de estuário no Brasil, também foi o primeiro estudo que integrou informações de várias naturezas (ecológico, químico, histopatológico, biológico) para avaliar a qualidade ambiental.

Estudos mais detalhados e por um maior período de tempo ainda são necessários em Vila do Conde, principalmente relacionadas à bioacumulação de metais pesados nas principais espécies consumidas. Este trabalho pode ser considerado como um protocolo de monitoramento biológico, mas que deve ser ainda mais aprimorado através da inclusão de outros táxons, tais como o bentos e o plâncton. Adicionalmente, novas metodologias devem ser inseridas e adaptadas para o ambiente estuarino amazônico correlacionando estes diferentes grupos de organismos.

O monitoramento ambiental de uma área é uma atividade que deve ser constantemente realizada por parte do governo e dos órgãos privados para que ocorra a remediação dos efeitos da poluição que rebatem negativamente na comunidade que usufrui dos recursos naturais. Adicionalmente, as conseqüências da contaminação sobre a atividade pesqueira, considerada como um setor rentável economicamente além de ser uma atividade de subsistência, geraria desemprego e prejuízos para a região.

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