UNIVERSIDADE ESTADUAL DO MARANHÃO
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MESTRADO EM RECURSOS AQUÁTICOS E PESCA

LUCENILDE CARVALHO DE FREITAS

BIOMARCADORES MORFOLÓGICOS EM *Hoplias malabaricus* (PISCES, CHARACIFORMES, ERYTHRINIDAE) EM AMBIENTE DULCÍCOLA DA ÁREA DE PROTEÇÃO DA BAIXADA MARANHENSE (BRASIL)

SÃO LUÍS – MA
2017

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Dissertação de mestrado apresentada em cumprimento às exigências do Programa de Pós-Graduação em Recursos Aquáticos e Pesca da Universidade Estadual do Maranhão, para obtenção do grau de Mestre.

Orientadora: Profa. Dra. Raimunda Nonata Fortes Carvalho Neta

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“Todo pensamento emocionalizado, unido à fé, tende a se realizar, a se materializar”. (Napoleon Hill)

“Se minha mente consegue imaginar, então eu consigo realizar”. (Napoleon Hill)

Dedico este trabalho, especialmente, à minha mãe (*in memoriam*) e ao meu pai, a toda minha família, bem como a todos que acreditaram e contribuíram para sua realização.

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RESUMO

Neste estudo objetivou-se comparar biomarcadores morfológicos em traíras (*Hoplias malabaricus*) capturadas em dois trechos do rio Mearim nos municípios de Arari e Vitória do Mearim da Área de Proteção Ambiental da Baixada Maranhense. Inicialmente, realizou-se um levantamento bibliográfico sobre o uso de peixes como bioindicadores para o monitoramento ambiental em rios. Após esta etapa, os peixes foram coletados trimestralmente em dois povoados da Baixada Maranhense, Engenho Grande (A1) no município de Vitória do Mearim, e Curral da Igreja (A2) no município de Arari. Os peixes foram transportados ao laboratório, para registro dos dados biométricos de comprimento total (LT), comprimento padrão (LP), peso total (WT) e das gônadas (WG). Dos 91 exemplares foram retirados o segundo arco branquial direito e o fígado, que foram fixados em formol a 10%. O arco branquial foi colocado em ácido nítrico a 10% para facilitar a descalcificação. Posteriormente, ambos os tecidos foram mantidos em álcool a 70% até a realização do processamento histológico usual. Cortes histológicos de cada órgão, com aproximadamente 5µm de espessura, foram corados com Hematoxilina Eosina (HE). As lâminas confeccionadas foram analisadas e fotomicrografadas em microscópio de luz. As lesões histológicas foram classificadas e seus índices calculados. A água do rio também foi coletada para análises físico-química e microbiológica, sendo os parâmetros físico-químicos observados foram cálcio, magnésio, alcalinidade total, cloretos, conductividade, sólidos dissolvidos totais, e os parâmetros microbiológicos observados foram coliformes totais e *Escherichia coli*. Os dados biométricos e o índice gonadossomático obtidos foram expressos como média ± desvio padrão. As diferenças significativas entre os grupos foram verificadas através do test *t Student* com $p < 0{,}05$ aceito como significativo. Foi realizada a análise de Cluster nas lesões encontradas nos peixes coletados nas duas áreas, visando obter agrupamentos similares. De acordo com a revisão bibliográfica, foi constatado que os biomarcadores enzimáticos foram os mais utilizados em peixes para o monitoramento ambiental em rios. Em relação à pesquisa em campo, foram observadas várias lesões branquiais nos peixes coletados nas duas áreas de estudo, tais como: fusão lamelar, deslocamento do epitélio, aneurisma, congestão, hiperplasia, desorganização lamelar e proliferação das células de muco. Também foram identificadas lesões hepáticas, como: vacuolização citoplasmática, núcleos na periferia, centros de melanomacrófagos, necrose, dilatação dos sinusóides, dilatação dos vasos, infiltração leucocitária e hiperemia. Os parâmetros abióticos da água como: turbidez, pH, oxigênio dissolvido, ferro e os dados microbiológicos apresentaram valores em desconformidade com a legislação brasileira. A biometria dos espécimes mostrou que no período seco os machos coletados na área de referência (A1) apresentaram maior comprimento e foram pesados, sendo os valores significativos ($p < 0,05$). As fêmeas coletadas na área contaminada (A2) nesse período apresentaram valores maiores de comprimento e peso. No período chuvoso, as fêmeas e machos coletados na área contaminada (A2) mostraram valores elevados de comprimento e peso. O índice gonadossomático (GSI) apresentou valores maiores para machos e fêmeas coletados na área de referência (A1) durante a estação seca, com significância para os valores em fêmeas ($p < 0,05$) na estação chuvosa. Em relação aos estádios de maturação, no período seco, o maior percentual foi para fêmeas de estádio EG3 (maduro), e para os machos EG2 (em maturação ou repouso). No período chuvoso, o maior percentual foi para fêmeas em estádio EG2 (em maturação ou repouso), e os machos para EG1 (imaturos). A espécie *H. malabaricus* mostrou ser satisfatória para analisar as condições ambientais do rio Mearim em Engenho Grande (A1) e Curral da Igreja (A2), uma vez que os peixes apresentaram alterações morfológicas em brânquias e fígado, indicando possível contaminação aquática deste recurso nestes dois locais, com maior intensidade na área contaminada (A2).

Palavras-chave: Peixe; Biomonitoramento aquático; bioindicadores; lesões branquiais e hepáticas; rio Mearim; contaminação aquática; espécies dulcícolas.

ABSTRACT

In this study, the objective was to compare morphological biomarkers in traíras (*Hoplias malabaricus*) captured in two stretches of the Mearim river in the municipalities of Arari and Vitória do Mearim of the Baixada Maranhense Protection Area. Initially, a bibliographic survey was conducted on the use of fish as bioindicators for environmental monitoring in rivers. After this stage, the fish were collected quarterly in two villages of (A1) in the municipality of Vitória do Mearim, and Curral da Igreja (A2) in the municipality of Arari; total length (TL), standard length (SL), total weight (WT) and gonads (WG) were recorded in the laboratory. Of the 91 specimens the second right branchial arch and the liver were removed, being fixed in 10% formaldehyde. After, both tissues were maintained in 70% alcohol until the usual histological processing. Histological sections of each organ, approximately 5 μm thick, were stained with Hematoxylin Eosin (HE). Histological lesions were classified and their indexes were calculated. The water of the river was also collected for physico-chemical and microbiological analyzes, and the physico-chemical parameters observed were calcium, magnesium, total alkalinity, chlorides, conductivity, total dissolved solids and microbiological parameters were total coliforms and *Escherichia coli*.The biometric data and gonadosomatic indexes obtained were expressed as mean ± standard deviation. Significant differences between the groups were verified through the *t Student* test with $p < 0.05$ accepted as significant. Cluster analysis was performed to the lesions found in fish collected in the two areas, in order to obtain similar groupings. According to the bibliographic review, it was found that enzymatic biomarkers were the most used in fish for environmental monitoring in rivers. In relation to field research, several gill lesions were observed in the fish collected in the two study areas. This lesions were lamellar fusion, epithelial displacement, aneurysm, congestion, hyperplasia, lamellar disorganization and mucus cell proliferation. Hepatic lesions or also been identified, such cytoplasmic vacuolization, peripheral nuclei, melanomacrophagous centers, necrosis, sinusoid dilatation, vessel dilation, leukocyte infiltration, and hyperemia. The abiotic parameters of the water such turbidity, pH, dissolved oxygen, iron and microbiological data presented values in disagreement with the Brazilian legislation. The biometry of the specimens showed that in the dry period the males collected in the reference area (A1) were longer and weighed, the values being significant ($p < 0.05$), and the females collected in the contaminated area presented higher values of length and weight. In the rainy season, females and males collected in the contaminated area (A2) showed high values for length and weight. The gonadosomatic index (GSI) presented higher values for males and females collected in the reference area (A1) during the dry season, with significance for females values ($p < 0.05$) in the rainy season. Regarding maturation stages, in the dry period, the highest percentage was for GS3 (mature) females, and GS2 to males (maturing or resting); in the rainy season, the highest percentage was for GS2 (maturing or resting) to females, and GS1 (immature) to males. The *H. malabaricus* species was satisfactory for analyzing the environmental conditions of the Mearim River in Engenho Grande (A1) and Curral da Igreja (A2), since the fish presented morphological alterations in gills and liver, indicating possible aquatic contamination of this resource in these locations, however, with greater intensity in the contaminated area (A2).

Keywords: Fish; aquatic biomonitoring; bioindicators; gill and hepatic lesions; river Mearim; water contamination; freshwater species.

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SUMÁRIO

1 INTRODUÇÃO

A Área de Proteção Ambiental (APA) da Baixada Maranhense é uma Unidade de Conservação (UC) de Uso Sustentável, criada pelo Decreto Estadual de nº 11.900 de 11 de junho de 1991 (MARANHÃO, 1991). O Sistema Nacional de Unidades de Conservação (SNUC) define APA como sendo "uma área em geral extensa, com um certo grau de ocupação humana, dotada de atributos abióticos, bióticos, estéticos ou culturais especialmente importantes para a qualidade de vida e o bem estar das populações humanas, e tem como objetivos básicos proteger a diversidade biológica, disciplinar o processo de ocupação e assegurar a sustentabilidade dos recursos naturais" (BRASIL, 2000).

Além disso, esta região é participante da lista de áreas úmidas internacionais da Convenção de Ramsar que ocorreu em 1971, na cidade de Ramsar, Irã. A realização desta reunião intergovernamental objetivou a conservação das áreas úmidas mundiais por meio de ações locais, nacionais e internacionais que priorizassem o seu uso mais sustentável. E essas áreas foram denominadas de Sítios Ramsar (RAMSAR CONVENTION MANUAL, 2013). Neste sentido, a APA da Baixada Maranhense é uma das regiões estratégicas para conservação e sustentabilidade da biodiversidade, em nível estadual, nacional e mundial (ALMEIDA et al., 2013; CARVALHO NETA et al., 2015), necessitando de monitoramento ambiental eficiente dos seus recursos naturais.

Entre os recursos naturais desta região, os ecossistemas hídricos da Baixada Maranhense vêm sofrendo impactos abundantes pelas atividades antrópicas como a pecuária intensiva, a agricultura com uso de fertilizantes, a piscicultura, a construção de barragens, os projetos de irrigação, dentre outros, os quais geram prejuízos à biota local (CANTANHEDE et al., 2014; CANTANHEDE et al., 2016). O uso de peixes como bioindicadores de contaminação aquática, bem como as alterações histológicas em brânquias e fígado como biomarcadores podem ser eficientes no monitoramento ambiental desses recursos aquáticos, facilitando a gestão dessas áreas pelos órgãos competentes (PINHEIRO-SOUSA; ALMEIDA; CARVALHO-NETA, 2013; CANTANHEDE et al., 2014; SANTOS et al., 2014; CANTANHEDE et al., 2016). Existem pesquisas científicas que apontam resultados satisfatórios na gestão ambiental de UC's, principalmente, em APA's a partir de peixes como bioindicadores e seus órgãos como biomarcadores histológicos (NASCIMENTO et al., 2012; CASTRO et al., 2014; SANTOS-FILHO et al., 2014; OLIVEIRA-RUBIO; VEGA-LÓPEZ, 2016).

Entre os peixes de importância econômica encontrados na APA da Baixada Maranhense, destaca-se a espécie *Hoplias malabaricus* (traíra). Esse táxon é considerado um bioindicador, principalmente por ser predador de topo da cadeia alimentar e apresentar ampla distribuição geográfica, bem como abundância e resistência física, resultando numa importante fonte de proteína e renda para as comunidades pesqueiras locais (BARBIERI, 1989; LINS et al., 2010; PETRY et al., 2010; PESSOA et al., 2013). Com isso, na literatura constam vários estudos com *H. malabaricus* sendo bioindicadora de contaminação, apresentando resultados eficientes para o monitoramento ambiental local (CASTRO et al., 2014; SILVA et al., 2011; PEREZ, 2008).

Neste sentido, no presente estudo, objetivou-se comparar biomarcadores morfológicos em traíras (*Hoplias malabaricus*) capturadas em dois trechos do rio Mearim nos municípios de Arari e Vitória do Mearim, Área de Proteção da Baixada Maranhense.

A presente dissertação está organizada da seguinte forma: Objetivo geral e específicos; fundamentação teórica abordando os temas biomarcadores morfológicos, *Hoplias malabaricus* e APA da Baixada Maranhense. Os resultados serão apresentados na forma de três artigos: 1) Capítulo I, artigo de revisão que aborda os peixes para monitoramento ambiental em rios; 2) Capítulo II, que discute as lesões branquiais em *Hoplias malabaricus* como biomarcadores para monitoramento de impactos ambientais numa zona úmida de interesse internacional; 3) Capítulo III, que discute as lesões hepáticas em *H. malabaricus* como biomarcadores de impactos antrópicos em dois trechos do rio Mearim (nas cidades de Arari e Vitória do Mearim).

2 OBJETIVOS

2.1 Objetivo geral

- Comparar biomarcadores morfológicos em traíras (*Hoplias malabaricus*) capturadas em dois trechos do rio Mearim nos municípios de Arari e Vitória do Mearim da Área de Proteção da Baixada Maranhense.

2.2 Objetivos específicos

- Identificar lesões branquiais e hepáticas em *Hoplias malabaricus* do rio Mearim na Baixada Maranhense;
- Quantificar as principais lesões branquiais e hepáticas indicativas de impacto ambiental em *H. malabaricus* em trechos do rio Mearim na Baixada Maranhense;
- Relacionar os graus de severidade das lesões branquiais e hepáticos encontradas em *H. malabaricus* coletadas em trechos do rio Mearim na Baixada Maranhense.

3 FUNDAMENTAÇÃO TEÓRICA

3.1 Biomonitoramento de recursos hídricos

Os recursos aquáticos são utilizados pelos seres humanos em várias atividades como transporte, lazer, alimentação, higiene pessoal, geração de energia, irrigação, abastecimento de água, aquicultura dentre outras (SPERLING, 1993; RAZZOLINI; GUNTHER, 2008; GIATTI; CUTOLO, 2012; MANNARINO et al., 2013). Porém, o uso demasiado destes recursos pelo homem resulta num grande desequilíbrio ambiental, podendo minimizar a sobrevivência de muitas espécies, da flora e fauna (DE OLIVEIRA, 1958; MORAES; JORDÃO, 2002; CANTANHEDE et al., 2014).

Além disso, o constante crescimento econômico dos centros urbanos, principalmente o industrial e o agrícola, proporciona uma perda acelerada da biodiversidade local (LINS et al., 2010; SILVA et al., 2014). Isso pode ser explicado pela grande quantidade de esgotos de origem doméstica e industrial sendo lançados em corpos d’ água, e também pelo despejo de pesticidas e herbicidas que são utilizados em cultivos próximos aos ecossistemas aquáticos (GARCIA, 1995; ARAÚJO; COSTA; CARREIRA, 2011; SILVA et al., 2014).

O monitoramento ambiental é definido como uma análise repetida e temporal de determinados parâmetros biológicos, químicos e físicos de uma área utilizando-se de parâmetros específicos, comparáveis e padronizados (WHO, 1989; STRIEDER et al., 2006; KIRBY; GIOIA; LAW, 2014; WANG; MACHADO; BAPTISTA, 2016; SINGHASEMANON; GOH, 2016). Segundo Van Der Oost; Beyer; Vermeulen (2003) o monitoramento ambiental é composto por cinco métodos de diagnóstico dos efeitos de poluentes no ambiente: 1) Monitoramento químico - é caracterizado pela análise de poluentes nos compartimentos ambientais; 2) Monitoramento da bioacumulação - é explicado pela análise da acumulação destes na biota regional; 3) Monitoramento de ecossistemas - é caracterizado pela análise das espécies presentes naquele ambiente; 4) Monitoramento da saúde - são os danos causados à saúde daquele animal; 5) Monitoramento dos efeitos biológicos - caracteriza-se pelos efeitos que podem ser reversíveis ou irreversíveis nos organismos.

O monitoramento dos efeitos biológicos ou biomonitoramento pode ser definido como o uso das reações biológicas nos organismos vivos na avaliação das alterações ambientais nos ecossistemas, estas muitas vezes ocasionadas pelos seres humanos (MATTEWS; BUKEMA; JUNIOR, 1982; FOSSI; MARSILI, 1997; OLIVARES – RUBIO; VEGA – LÓPEZ, 2016). O biomonitoramento aquático é bastante realizado pelos pesquisadores nacionais e internacionais, com resultados satisfatórios para o gerenciamento ambiental da região (CAPELA et al., 2015; ALVES et al., 2016), podendo ser utilizado vários organismos dentre eles, os moluscos, os crustáceos e os peixes (LINS et al., 2010). Estes organismos são caracterizados como bioindicadores ambientais, podendo ser bastante sensíveis a contaminação ambiental (SOUZA; KONRAD; JÚNIOR, 2016; SADAUSKAS-HENRIQUE et al., 2017). As respostas biológicas desses táxons a estas alterações do meio podem ser denominadas biomarcadores.

3.1.1 Biomarcadores

Segundo Huggett; Kimerie; Bergman (1992) os biomarcadores são descritos como mudanças bioquímicas, fisiológicas e morfológicas indicativas de exposição ou efeito dos organismos a um poluente naquele ecossistema. É utilizado em um sentido complexo, porque refere-se à medição de compostos perigosos de origem física, química ou biológica num sistema ecológico (WHO, 1993). Desta forma, os biomarcadores em bioindicadores ambientais são metodologias utilizadas para gerenciamento de uma área, seja ela aquática e terrestre (VAMPRÉ; FUCCILLO; ANDRÉA, 2010; PINHEIRO-SOUSA, 2015).

Existem três tipos de biomarcadores: exposição, suscetibilidade e efeito (NAS / NRC, 1987; WHO, 1993; AMORIM, 2003).

- Biomarcador de exposição é caracterizado por avaliar a presença de um organismo ou de um grupo de organismos a algum contaminante, relacionando a concentração externa observada no ambiente e a concentração interna que é verificada nas células, tecidos e órgãos dos animais, um exemplo é a observação de metais organoclorados no sangue, supondo a acumulação desses tipos de contaminantes no tecido adiposo (SUTER, 1993; KUNO; ROQUETTI; GOUVEIA, 2010).
- Biomarcadores de suscetibilidade é influenciado pelas características genéticas, idade, estas características interferem direta e indiretamente na exposição química dos animais aos contaminantes, bem como no desenvolvimento de efeitos nos seus organismos (LOURENÇO et al., 2015).
- Biomarcador de efeito é observado em nível biológico, sendo caracterizado pelas respostas internas dos organismos em relação ao contato com xenobióticos, é considerado o biomarcador ideal aquele que evidencia uma resposta precoce da doença no organismo, objetivando a prevenção da patologia, exemplo deste tipo são as respostas bioquímicas e, dentre as enzimas utilizadas estar a glucationa-S-transferase e a catalase, bem como respostas histológicas como as lesões encontradas, principalmente nos órgãos de alguns indivíduos, como nas brânquias e no fígado de peixes (RIBEIRO et al., 2015; ARANTES et al., 2016).

Os biomarcadores são altamente sensíveis para indicar a biodisponibilidade de poluentes nos meios naturais, sendo capazes de elucidar as relações causa/efeito/dose num ambiente, e assim avaliar e monitorar o risco à saúde daquele ecossistema, tem a capacidade de fornecer informações sobre a quantificação dos níveis de contaminação naquele ambiente e evidenciar se as lesões têm efeitos reversíveis ou irreversíveis nos organismos, obtendo respostas importantes sobre o estresse dos animais no ambiente (VAN DER OOST; BEYER; VERMEULEN, 2003). Vale destacar que existem algumas características ambientais e do próprio organismo que podem favorecer ou interferir nas alterações nos tecidos, dentre elas temperatura, sexo, idade e reprodução (MCCARTHY et al., 2000).

Segundo Stegeman et al. (1992) para a escolha do biomarcador em peixes é necessário observar alguns fatores importantes como: protocolo de ensaio barato e fácil de executar; impacto do biomarcador na morfofisiologia do animal; sensibilidade do biomarcador aos contaminantes, mostrando respostas precoces; e facilidade na identificação das alterações morfofisiológicas. Tendo em vista esta facilidade, uma das formas de se analisar as alterações morfológicas nos órgãos é a histologia.

A histologia de órgãos é uma maneira morfológica eficaz de diagnosticar lesões de efeitos diversos causados por determinado poluente presente no meio (FIGUEIREDO-FERNANDES et al., 2007). Dessa forma, a observação dessas lesões pode ser indício que o ambiente está contaminado. Vale destacar que, somente as análises histológicas não determinam o contaminante, nem qual é o local de despejo e nem se a fonte é pontual ou difusa: Logo é necessário a utilização de outros métodos científicos para averiguação de tal objetivo (LINS et al., 2010). As lesões em brânquias e fígado são as mais utilizados em estudos com biomarcadores morfológicos.

As brânquias apresentam características fundamentais que propiciam o seu uso em respostas biológicas, já que apresenta grande superfície de contato com o ambiente externo, por causa das lamelas, bem como estão em contato direto e permanente com o ambiente aquático; além disso, este órgão realiza a respiração, osmorregulação, excreção, dentre outras funções vitais para os organismos (GARCIA, 1995; NOGUEIRA; CASTRO; SÁ, 2011). A morfologia geral das brânquias dos animais é constituída por arcos branquiais e filamentos branquiais, sendo que estes últimos apresentam as lamelas secundárias, que funcionam como local de trocas gasosas (HIBIYA, 1982; FERGUSON, 1989; MACHADO, 2015).

O fígado tem a função primordial de biotransformar os metais presentes no organismo dos animais, bem como reduzi-los a pequenas moléculas, afim de os eliminar para o meio (BERVOETS et al., 2013; ARANTES et al., 2016). Esta glândula digestiva é composta pelos hepatócitos que são as células responsáveis pelo metabolismo dos xenobiontes, fibras para a sustentação, vasos sanguíneos, ductos biliares, tecido pancreático, entre outros componentes (HIBIYA, 1982).

A literatura apresenta muitos estudos utilizando como biomarcadores as lesões branquiais e hepáticas de peixes teleósteos, porque estes táxons apresentam características fundamentais para serem utilizados como bioindicadores, já que são resistentes, mostram-se abundantes nos ambientes aquáticos, adaptam-se facilmente às técnicas laboratoriais, a maioria é representante do topo da cadeia alimentar, podendo acumular os contaminantes transportados pelos organismos aquáticos, via alimentação, tem ampla distribuição, dentre outras características (OVERSTREET, 1988; ARIAS et al., 2007; JIA; CHEN, 2013). Além disso, são importantes recursos comerciais e econômicos para muitas comunidades ribeirinhas, às vezes, sendo as únicas fontes de subsistência e renda familiar (AJIACO-MARTÍNEZ, RAMÍREZ-GIL; ARIAS-CASTELLANOS, 2012; MATERA, 2016), a exemplo de *Hoplias malabaricus* (traíra) que apresenta relativo valor comercial na região da APA da Baixada Maranhense.

3.2 *Hoplias malabaricus*

Entre os peixes teleósteos utilizados como bioindicadores têm-se a espécie *Hoplias malabaricus* pertencente à ordem Characiformes, família Erythrynidae, chamada popularmente de traíra, apresenta como características morfológicas o corpo cilíndrico, cabeça achatada, boca larga com maxilar inferior proeminente, dentes fortes e pontiagudos, lábios finos, coloração intensa e variada ao longo do corpo, sendo principalmente, negro na parte dorsal, pardacento na lateral e esbranquiçado na região ventral, narinas duplas, escamas presentes e grandes do tipo ciclóide, e nadadeiras homocercas (PEREZ, 2008; BIALETZKI et al., 2008; PESSOA et al., 2013). Esta morfologia influencia nas suas características ecológicas.

A ecologia dessa espécie é descrita como sendo um organismo planctófago no período larval, insetívoro na fase juvenil e piscívoro quando adulto, indicando um peixe predador de topo de cadeia, podendo mostrar consequências constantes bioacumulativas transportados através da cadeia trófica (AZEVEDO; GOMES, 1942; MIRANDA, 2006). Possui ampla distribuição geográfica, apresentando indivíduos em todas as bacias da América do Sul, mostrando ampla diferença cariotípica, isto é, indicando uma complexidade de espécies e desenvolvimentos evolutivos variados (VICARI; ARTONI; BERTOLLO, 2005). Somente não é observada no oeste dos Andes e nos rios da Patagônia (BURGUESS, 2004). É espécie bem adaptada aos aquáticos lênticos como lagos, lagoas, porém é mais encontrada em ambientes lóticos como rios (BIALETZKI et al., 2008). Prefere habitar regiões com vegetação a qual favorece sua alimentação (PETRY et al., 2010).

Estes organismos mostram-se muito resistentes nos ambientes em que estão inseridos, podendo suportar períodos de seca e frio intensos e pouco oxigenados, essa resistência à hipóxia pode ser explicada pela grande influência desses animais nos seus sistemas cardíaco e respiratório, ou seja, a traíra apresenta auto controle sobre o metabolismo dos órgãos destes sistemas, adaptando-se facilmente às alterações ambientais (PETRY et al., 2007; LINS et al., 2010) bem como aos hábitats muito contaminados (COSTA et al., 2007).

Além disso, tem importância na pesca tradicional brasileira, tanto com a finalidade de subsistência, quanto comercial (NOVAES; CARVALHO, 2011). Muitas comunidades ribeirinhas do Nordeste utilizam esta espécie para obter renda, e para alimento de suas famílias; isto é explicado pela sua prevalência nos hábitats dulciaquícolas desta região (PESSOA et al., 2013).

Diante das informações apresentadas anteriormente, é perceptível que a espécie *Hoplias malabaricus* se mostra um organismo bioindicador ideal, podendo ser uma importante referência das condições do meio. Além disso, é necessário analisar a situação sanitária deste recurso que é tão utilizado pelos ribeirinhos da APA da Baixada Maranhense.

3.3 Área de Proteção Ambiental (APA) da Baixada Maranhense

A Área de Proteção Ambiental da Baixada Maranhense foi originada pelo Decreto Estadual nº 11.900, de 11 de junho de 1991, justificada por ser uma região importante que acomoda "uma complexa interface de ecossistemas ou incluindo manguezais, babaçuais, campos abertos e inundáveis, uma série de bacias lacustres em sistema de "rosário", um conjunto estuarino e lagunar e matas ciliares – todos abrigando rica e complexa fauna e flora aquática e terrestre, com destaque à ictiofauna, à avifauna migratória e permanente e às variedades de espécies da flora local e regional considerados alguns daqueles ecossistemas como Reservas Biológicas" (MARANHÃO, 1991).

Esta é uma Unidade de Conservação de Uso Sustentável, definida segundo o Sistema Nacional de Unidades de Conservação (SNUC) como sendo "uma área em geral extensa, com certo grau de ocupação humana, dotada de atributos abióticos, bióticos, estéticos ou culturais, especialmente importantes para a qualidade de vida e o bem-estar das populações humanas, e tem como objetivos básicos proteger a diversidade biológica, disciplinar o processo de ocupação e assegurar a sustentabilidade dos recursos naturais" (BRASIL, 2000).

Vale destacar a grande importância internacional desta região, já que foi incluída na lista de zonas úmidas mundiais e de relevância para a sustentabilidade ambiental e social, esta lista foi realizada pela Convenção Ramsar, no ano de 1971 em Ramsar, Irã, este documento foi originado através de um tratado intergovernamental, o qual discutia sobre estratégias diretivas que auxiliassem a conservação e preservação das áreas úmidas mundiais, a fim de alcançar a sustentabilidade dos recursos naturais encontrados nestes locais, bem como melhoria e qualidade de vida das populações viventes próximas a elas (RAMSAR CONVENTION SECRETARIAT, 2013).

Além dessa região, foram integradas outras regiões maranhenses protegidas: a APA das Reentrâncias Maranhenses e o Parque do Parcel de Manoel Luís (RAMSAR CONVENTION MANUAL, 2013). O Brasil aderiu, oficialmente, a este tratado em 1993, alegando que a inclusão dessas regiões poderia favorecer sua gestão e fiscalização, resultando numa maior eficiência na conservação da biodiversidade local priorizando o uso bem mais sustentável dos recursos, e preservando a sua existência para as gerações futuras (RAMSAR CONVENTION MANUAL, 2013).

A APA da Baixada Maranhense apresenta uma área de 1.775.035,6 hectares, localizando-se a oeste do estado, sendo dividida em três sub-áreas, a Baixo Pindaré; a Baixo Mearim-Grajaú e a Estuário Mearim-Pindaré Baía de São Marcos – incluindo a Ilha dos Caranguejos abrangendo vários municípios como Alcântara, Arari, Bequimão, Cajari, Cedral do Maranhão, Conceição do Lago Açu, Guimarães, Igarapé do Meio, Monção, Penalva, Pinheiro, Presidente Sarney, São Bento, São João Batista, São Vicente Ferrer, Olinda Nova do Maranhão, Viana, Vitória do Mearim (MARANHÃO, 1991). Entre outros municípios que a APA engloba parcialmente seus territórios, têm-se Anajatuba, Bacurituba, Bacabal, Bela Vista do Maranhão, Cajapió, Cedral, Lago Verde, Mirinzal, Pedro do Rosário, Pindaré Mirim, Santa Helena, São Mateus do Maranhão, Serrano do Maranhão, Turilândia (CARVALHO NETA et al., 2015).

Essa APA é caracterizada pelas planícies e campos inundáveis, bem acentuados principalmente no período chuvoso, podendo ser permanentes ou temporários, possuindo clima úmido e quente, envolvem um complexo e vasto ecossistema faunístico e florístico de transição, destacando a grande quantidade de peixes, por apresentar extensas regiões aquáticas (lagos, rios, estuários, regiões alagáveis) e possui o maior conjunto de bacias lacustres do nordeste brasileiro (LIMA et al., 2009; MITAMURA et al., 2012; CARVALHO NETA et al., 2015). Com isso, é observada a prática de várias atividades econômicas pela população residente, como exemplos têm-se a pesca, o comércio, agricultura, pecuária, dentre outras atividades (SILVA et al., 2015; SILVA et al., 2016).

A pesca é muito praticada nesta APA, principalmente de forma artesanal pela população ribeirinha, tendo como finalidade a comercialização e subsistência de suas famílias (ARAÚJO; PINHEIRO, 2008; FUNO; PINHEIRO; MONTELES, 2010). Nesta atividade vários petrechos de pesca são utilizados; o mais observado é a rede de emalhar. As espécies estuarinas e dulcícolas mais capturadas são: *Anableps anableps* (tralhoto), *Sciades herzbergii* (bagre), *Bagre bagre* (bagre), *Cathrorops spixii* (bagre), *Genyatremus luteus* (caicanha), *Hassar wilderi* (mandi bico de flor), *Plagioscion squamosissimus* (pescada branca), *Pygocentrus nattereri* (piranha vermelha), *Prochilodus lacustres* (curimatá) (CARVALHO NETA et al., 2015; SILVA; ALMEIDA; PIORSKI, 2015; CANTANHEDE et al., 2016).

Atualmente, é verificado pela comunidade científica o desequilíbrio ambiental dos recursos aquáticos da Baixada Maranhense. Vale destacar que esta APA ainda não tem plano de manejo, o que dificulta ainda mais a conservação das espécies estuarinas e dulcícolas (CARVALHO NETA et al., 2015; CANTANHEDE et al., 2016). Desta forma, a análise dos biomarcadores nos peixes destas regiões do rio Mearim proporciona aos pescadores ribeirinhos um diagnóstico das espécies com a saúde mais comprometida, e subsídios de gestão ambiental e manejo eficiente da pesca nesse recurso hídrico.

3.3.1 Rio Mearim e sua importância

O rio Mearim nasce na região norte da Serra da Menina com altitude de aproximadamente 400 a 500 m, esta região localiza entre os municípios de Formosa da Serra Negra, Fortaleza dos Nogueiras e São Pedro dos Crentes no sul do Estado, o rio deságua na Baía de São Marcos, entre os municípios de São Luís e Alcântara, após percurso de cerca de 930 Km de extensão, dividindo-se em baixo Mearim, médio Mearim e alto Mearim, tem como principais afluentes os rios Pindaré e Grajaú (LIMA, 2013; MARANHÃO, 2014). O Mearim é caracterizado por apresentar o fenômeno da pororoca e no período chuvoso as enchentes, cerca de 30% dele é navegável, principalmente nas suas regiões média e baixa, possui cerca de 2 metros de profundidade e uma largura extensa (SEMATUR, 1991; MARANHÃO, 2014).

Este recurso hídrico é o principal rio integrante da Bacia Hidrográfica do rio Mearim que compõe uma área de cerca de 99 mil Km^2 (representando 30% do território maranhense), é localizada inteiramente no Maranhão (MARANHÃO, 2014). Esta rede hidrográfica é composta, principalmente pelos rios Mearim, Pindaré, Das Flores e o Corda, englobando 83 municípios, 22 deles são pertencentes a APA da Baixada Maranhense, dentre as cidades estão Conceição do Lago Açu, Penalva, Viana, Cajapió, Arari e Vitória do Mearim (IBGE, 2000; LIMA, 2013; MARANHÃO, 2014).

Vale ressaltar que o rio Mearim é importante para a região desde a época colonial, já que ele era o único acesso para estas localidades, com o advento da estrada, o seu uso como transporte foi diminuído; hoje a população utiliza este recurso para realização da atividade pesqueira, tanto para a subsistência quanto para o comércio, sendo um uso frenquente e crescente; para a irrigação no cultivo de arroz, milho e melancia; lazer; esporte e turismo (CUNHA & SILVA, 2002; GASPAR et al., 2005; COSTA NETO et al., 2008; SILVA & ROSA, 2012).

Atualmente, o avanço no processo urbano das cidades adjacentes ao rio Mearim ocasiona a degradação ambiental deste recurso, levando ao despejo dos resíduos domésticos e industriais (CUNHA & SILVA, 2002). Também provoca o assoreamento, a redução da mata ciliar e a pesca predatória, bem como intensificando o desenvolvimento da agricultura, principalmente o cultivo de arroz, acentuando a contaminação por agrotóxicos (CUNHA & SILVA, 2002). Além de impulsionar o desenvolvimento da pecuária e da exploração de minerais como a gipsita e a bauxita em regiões próximas ao rio, resultando em uma grande perda da biodiversidade deste recurso hídrico, necessitando, ainda mais, de estudos de biomonitoramento que possam auxiliar na gestão ambiental do mesmo (CUNHA & SILVA, 2002; LIMA, 2013).

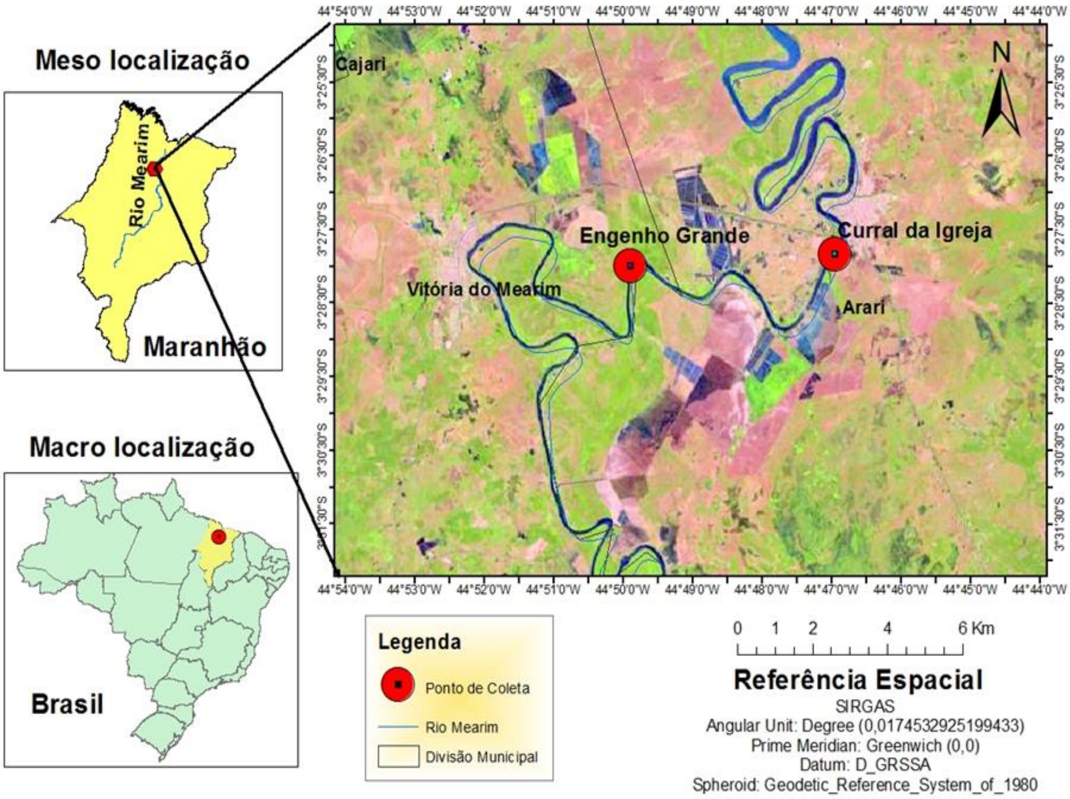
4 METODOLOGIA

4.1 Área de estudo

O município de Arari apresenta uma área de 1.100.275 Km^2 e população com, aproximadamente, 29.297 habitantes; o município de Vitória do Mearim possui área de 716.719 Km^2 e população com, aproximadamente, 32.161 habitantes (IBGE, 2016). Ambas as cidades são pertencentes à sub – área do Baixo Mearim – Grajaú (MARANHÃO, 1991).

Os povoados escolhidos para realização das coletas dos peixes foram Curral da Igreja com coordenadas 03° 27' 64.1" S e 044° 46' 79.7" W localizado cerca de 10 Km do centro de Arari, e o Engenho Grande com coordenadas 03° 27' 59.3" S e 044° 49' 55.9" W localizado cerca de 5 Km do centro de Vitória do Mearim (IBGE, 2010) (Figura 1).

Figura 1: Mapa de localização das coletas dos peixes nos povoados de Curral da Igreja (Arari) e Engenho Grande (Vitória do Mearim).



A hidrografia da região é originada das bacias dos rios Mearim, Pindaré, Aurá, Pericumã, Turiaçu, dentre outros; eles podem formar lagos sendo temporários ou permanentes, como o de Viana, Maracacumé, São José, Cajari, Capivari, Aquiri, Lontra e Formoso; apresentando baixa altitude, com relevo predominantemente, do tipo planície (CONCEIÇÃO; MOREIRA; FARIAS-FILHO, 2012).

Esta região tem elevadas temperaturas e chuvas abundantes, caracterizando clima tropical semiúmido com temperatura média anual de 26° C e índice pluviométrico entre 1000 a 2000 mm, o período chuvoso se estende de janeiro a julho, favorecendo um solo intemperizado, com granulometria argilosa, estas características proporcionam a APA uma superumidade na estação chuvosa, originando lagos com pequena profundidade e uma diminuição na quantidade de água no solo na estação de estiagem (CONCEIÇÃO; MOREIRA; FARIAS-FILHO, 2012).

A flora apresenta-se de forma transicional entre o clima semiárido do Nordeste e os climas úmido e subúmido do Norte, caracterizando um sistema vegetacional constituído por floresta ombrófila densa e aberta, manguezais, campos inundáveis e cerrado (FEITOSA; TROVÃO, 2006; LIMA et al., 2009; CARVALHO; SILVA; CORDEIRO, 2011).

A fauna é representada por animais nativos e exóticos, como exemplos tem-se os peixes *Anableps anableps* (tralhoto), *Sciades herzbergii* (bagre), *Hassar wilderi* (mandi bico de flor), *Pygocentrus nattereri* (piranha vermelha), *Prochilodus lacustres* (curimatá) (CARVALHO NETA et al., 2015; SILVA; ALMEIDA; PIORSKI, 2015), dentre outras; os mamíferos *Dasyprocta aguti* L. (cutia), *Cuniculus paca* L. (paca), *Cavea aperea*(préa), entre outras espécies; os répteis como camaleões, cobras, jacarés e lagartos; anfíbios como sapos e rãs; e aves como *Oryzoborus angolensis* (curió), *Crotophaga ani* (anum preto), *Phorphyrio martinica* (jaçanã), entre outras espécies (CONCEIÇÃO; MOREIRA; FARIAS-FILHO, 2012; RODRIGUES; SOUSA; SOUSA, 2013). Vale destacar, os cultivos de várias espécies com finalidade pecuária como búfalos, bovinos, equinos e suínos (SILVA et al., 2015). Além disso, predomínio de insetos tais como as abelhas (DUTRA et al., 2008).

4.2 Determinação dos locais de coleta de peixes e amostras de água

As coletas ocorreram em dois locais no rio Mearim, no povoado Engenho Grande (03° 27' 59.3" S, 044° 49' 55.9" W) localizado no município de Vitória do Mearim, onde ainda são preservados a flora e fauna nativas, apresentando pouca urbanização e atividades antrópicas em comparação com o outro povoado, sendo a área de referência (A1); e no povoado Curral da Igreja (03° 27' 64.1" S, 44° 46' 79.7" W) localizado no município de Arari, é um povoado com intensa urbanização que apresenta diversas ações antrópicas como agricultura, piscicultura, construção civil próximas ao rio, causando impactos aos ecossistemas, sendo a área contaminada (A2). Estes povoados foram escolhidas por serem relevantes na atividade pesqueira ribeirinha da região a partir de conversas informais com os pescadores locais. Esses locais de coleta foram georreferenciados pelo GPS (Global Position System).

Foram coletados, 91 espécimes de *Hoplias malabaricus* (Figura 2) em quatro coletas, duas no período seco (Agosto e Novembro/2016) e duas no período chuvoso (Maio/2016 e Junho/2017). No povoado Curral da Igreja foram coletados 69 indivíduos, e em Engenho Grande 22 indivíduos, sendo 56 machos e 35 fêmeas. No período chuvoso foi coletado 37 espécimes e no período de estiagem 54 espécimes. Os organismos foram pescados através da rede de emalhar.

As amostras de água foram coletadas trimestralmente, nos períodos chuvoso e de estiagem, utilizando recipientes adequados doados pelo Laboratório de Físico - Química de Alimentos e Água do curso de Medicina Veterinária da Universidade Estadual do Maranhão (UEMA) para análises físico-química e microbiológica.

Figura 2. Exemplar de *Hoplias malabaricus* coletado na APA da Baixada Maranhense, Maranhão, Brasil.

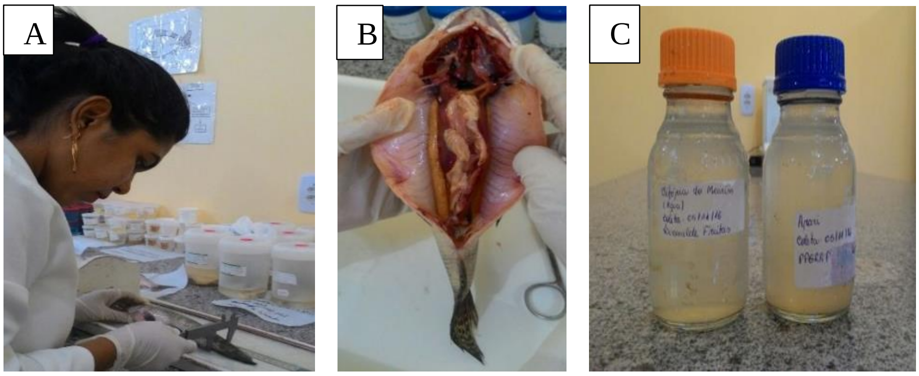
Fonte: Arquivo pessoal, 2017.

4.3 Biometria, análises físico-químicas e microbiológicas

Os peixes foram armazenados em caixas isotérmicas com gelo, e posteriormente, transportados ao Laboratório de Biomarcadores em Organismos Aquáticos (LABOAq) da Universidade Estadual do Maranhão (UEMA) para obter os dados biométricos de cada exemplar (Figura 3). Foram mensurados comprimento total (LT) e comprimento padrão (LP) em centímetros (cm), peso total (WT) e peso das gônadas (WG) em gramas (g). O estádio de maturação gonadal foi classificado segundo Vazzoler (1996) em: EG1 (imaturo), EG2 (em maturação ou repouso), EG3 (maduro) e EG4 (esgotado).

A água coletada foi utilizada para análises das características físico-químicas como cálcio, magnésio, dureza total, alcalinidade em OH-, alcalinidade em CO3-, alcalinidade total, cloretos (Cl-), condutividade, sólidos totais dissolvidos, NaCl, Ph, turbidez, oxigênio dissolvido, nitrito, nitrato, ferro. As características microbiológicas foram realizadas para determinação do número mais provável de coliformes totais e *Escherichia coli*, ambos pelo método Colilert que é considerado simples, rápido e eficiente. Estas análises foram realizadas no Laboratório de Físico – Química de Alimentos e Água do curso de Medicina Veterinária da Universidade Estadual do Maranhão (UEMA).

Figura 3: A) Biometria dos espécimes coletados; B) Observação dos órgãos internos de *Hoplias malabaricus*; C) Amostras de água coletadas nas áreas de estudo.



Fonte: Arquivo pessoal, 2017.

4.4 Análises histológicas

Foram retirados o segundo arco branquial direito e fragmentos de fígado de cada exemplar coletado. Estes materiais foram colocados em formol a 10% por 24 a 48 horas, para que os tecidos fossem clivados, e colocados em cassetes. Somente os tecidos branquiais foram colocados em em ácido nítrico a 10% por aproximadamente 4 horas. Depois disso, os materiais foram desidratadas em série crescente de álcoois, diafanizados em xilol, impregnados e incluídos em parafina. Foram realizados 3 cortes transversais para cada órgão do animal, com aproximadamente 5 μm de espessura e corados com Hematoxilina e Eosina (HE) (LUNA, 1968). As lâminas foram analisadas em microscópio de luz e as lesões histológicas visualizadas foram fotomicrografadas em fotomicroscópio (Figura 4). As alterações histológicas observadas nestes órgãos foram analisadas em relação ao seu grau de severidade conforme a escala sugerida por Poleksic & Mitrovic – Tutundzic (1994) e Bernet et al. (1999).

A coleta de peixes foi autorizada por uma licença emitida pela Secretaria Estadual de Meio Ambiente (SEMA) com número (18208/2014), por ser uma região legalmente protegida. O protocolo de pesquisa foi aprovado pelo Comitê de Ética da Universidade Estadual do Maranhão (CRMV-MA) com número 13/2017. Os peixes foram eutanasiados através do choque térmico, isto é, eles eram mantidos em baixas temperaturas (caixas isotérmicas com gelo), logo após a coleta ainda em campo, e durante o transporte até ao laboratório.

Figura 4: A) Tecidos histológicos em cassetes; B) Álcoois e xilóis; C) Lâminas histológicas confeccionadas; D) Visualização das lâminas histológicas no microscópio de luz.

Fonte: Arquivo pessoal, 2017.

O índice de alteração histológica (IAH) proposto por Poleksic & Mitrovic-Tutundzic (1994) é baseado nos níveis de severidade de cada lesão, sendo I referente às lesões de estágio I que não comprometem as funções do órgão; II, às alterações de estágio II, que são lesões severas e que danificam as funções do órgão, III; as alterações de estágio III, mais severas e irreversíveis.

As alterações observadas nas brânquias foram incluídas nos padrões de reação: Hipertrofia e hiperplasia do epitélio branquial, alterações nas células de cloreto e de muco, alterações nos vasos sanguíneos e lesões em estágio terminal (Tabela 1) (POLEKSIC; MITROVIC – TUTUNDZIC, 1994). As lesões histológicas observadas no fígado foram incluídas nos padrões de reação: alterações nos hepatócitos, alterações nos vasos sanguíneos e necrose (Tabela 2) (POLEKSIC; MITROVIC – TUTUNDZIC, 1994).

Os valores médios do IAH dos órgãos foram divididos em cinco categorias: 0-10 indica a normalidade do funcionamento do órgão; 11-20 indica alteração leve no órgão; 21-50 indica alteração moderada no órgão; 51-100 indica alteração severa no órgão; > 100 indica uma alteração irrecuperável no órgão (POLEKSIC; MITROVIC – TUTUNDZIC, 1994).

A fórmula utilizada para o cálculo do índice de alteração histológica (IAH), conforme proposto por Poleksic & Mitrovic-Tutundzic (1994), foi:

IAH: $1\times\sum \mathbf{I} + 10\times\sum \mathbf{II} + 100\times\sum \mathbf{III}$

Sendo:

I, II, III: Estágios das alterações encontradas nos órgãos

Tabela 1: Classificação das alterações histológicas branquiais baseada na metodologia proposta por Poleksic e Mitrovic-Tutundzic (1994).

Alterações branquiais	Estágio
Grupo 1: Hipertrofia e Hiperplasia do Epitélio branquial	
Hipertrofia do epitélio lamelar	I
Elevação do epitélio respiratório	I
Hiperplasia do epitélio lamelar	I
Desorganização das lamelas secundáriasI	
Fusão das lamelas secundárias	I
Encurtamento das lamelas secundárias	I
Grupo 2: Alterações nas células de cloreto e de muco	
Proliferação de células de cloreto	II
Proliferação de células de muco	II

Grupo 3: Alterações nos vasos sanguíneos	
Dilatação do seio venoso	I
Congestão vascular	I
Hemorragia com ruptura do epitélio	II
Aneurisma	II
Grupo 4: Estágio terminal	
Necrose	III
Fibrose	III

Fonte: Elaborado com modificações a partir da dissertação intitulada Alterações histológicas de peixes como biomarcadores da contaminação aquática (SILVA, 2004).

Tabela 2: Classificação das alterações histológicas hepáticas baseada na metodologia proposta por Poleksic e Mitrovic-Tutundzic (1994).

Alterações hepáticas	Estágio
Grupo 1: Alterações nos hepatócitos	
Núcleo na periferia da célula (esteatose)	I
Deformação no contorno celular	I
Deformação no contorno nuclear	I
Hipertrofia celular	I
Hipertrofia nuclear	I
Atrofia celular	I
Atrofia nuclear	I
Centros de melanomacrófagos	I
Vacuolização citoplasmática	I
Vacuolização nuclear	II
Degeneração citoplasmática	II
Degeneração nuclear	II
Rompimento celular	II
Estagnação biliar	II
Grupo 2: Alterações nos vasos sanguíneos	
Hiperemia	II

Grupo 3: Necrose	
Necrose	III

Fonte: Elaborado com modificações a partir da dissertação intitulada Alterações histológicas de peixes como biomarcadores da contaminação aquática (SILVA, 2004).

Em relação ao índice das alterações proposto por Bernet et al. (1999) (Tabela 3), cada alteração encontrada em cada órgão (brânquias e fígado) foi incluída em um padrão de reação (rp): distúrbios circulatórios, mudanças regressivas, mudanças progressivas, inflamação e tumor. Cada alteração foi pontuada com um fator de importância (w): 1 (lesões reversíveis), 2 (lesões moderadas) e 3 (lesões irreversíveis). Também pontuou o grau de extensão da alteração (a): 0 (sem mudanças), 2 (leve ocorrência), 4 (ocorrência moderada), e 6 (ocorrência grave). Os valores do fator de importância e do grau de extensão da alteração para cada lesão encontrada no órgão da lâmina analisada são multiplicados. Caso seja observada mais de uma lesão no órgão naquela lâmina, os resultados são somados, para obtenção do índice do órgão na lâmina analisada.

A fórmula utilizada para o cálculo do índice das alterações, conforme proposto por Bernet et al. (1999), foi:

$$I_{org} \sum rp = \sum alt\ (a.w)$$

Sendo:

I org: Índice do órgão avaliado

Rp: Padrão de reação

Alt: Alteração encontrada no órgão

a: Grau de extensão da alteração

w: Fator de importância

Tabela 3: Classificação das lesões histológicas brânquiais e hepáticas com o fator de importância (w), baseada na metodologia proposta por Bernet et al. (1999).

Padrão de Reação	Tecido Branquial	w	Tecido Hepático	w
Distúrbios Circulatórios	Hemorragia	1	Hemorragia	1
	Hiperemia	1	Hiperemia	1
	Aneurisma	1	Aneurisma	1
	Edema Intercelular	1	Edema Intercelular	1
Mudanças Regressivas	Alterações Estruturais	1	Alterações estruturais	1
	Atrofia	2	Atrofia	2
	Depósitos	1	Depósitos	1
	Necrose	3	Necrose	3
	Alterações no plasma	1	Alterações no plasma	1
	Alterações nucleares	2	Alterações nucleares	2
Mudanças Progressivas	Hiperplasia	2	Hiperplasia	2
	Hipertrofia	1	Hipertrofia	1
Inflamação	Exsudase	1	Exsudase	1
	Ativação do sistema reticuloendotelial	1	Ativação do sistema reticuloendotelial	1
	Infiltração	2	Infiltração	2
Tumor	Benigno	2	Benigno	2
	Maligno	3	Maligno	3

Fonte: Elaborado com modificações a partir do artigo intitulado Histopathology in fish: proposal for a protocol to assess aquatic pollution, por Bernet et al. (1999) publicado no **Journal of Fish Diseases**.

4.5 Tratamento estatístico dos dados

As informações registradas na revisão bibliográfica (capítulo 1) foram indicadas em forma de porcentagem. Os dados biométricos e os valores do índice gonadossomático foram expressos como média ± desvio padrão. Os estádios gonadais e as lesões histológicas encontradas foram apresentadas em porcentagem e frequência. O cálculo do índice gonadossomático foi realizado por meio da fórmula GSI = 100 x Wg / Wt, onde Wg é o valor do peso das gônadas e o Wt é o valor do peso corporal (VAZZOLER, 1996).

As diferenças significativas entre os grupos foram verificadas utilizando o test t de Student ($p < 0,05$). Foi realizada a análise de Cluster para os dados das lesões branquiais e hepáticas observadas nos peixes coletados nas áreas de referência (A1) e contaminada (A2) para analisar similaridade entre estas alterações.

5 RESULTADOS

Os resultados desta dissertação estão apresentados em três capítulos. No primeiro capítulo apresenta-se uma revisão bibliográfica acerca do uso de peixes como bioindicadores de contaminação ambiental em rios, utilizando três bases de dados e artigos científicos nacionais e internacionais, proporcionando melhor compreensão sobre o tema abordado. No segundo capítulo, discute-se os dados sobre as lesões branquiais encontradas na espécie *Hoplias malabaricus* (traíra) a fim de subsidiar programas de monitoramento ambiental regional numa zona úmida de interesse internacional. No terceiro capítulo, analisa-se os resultados das alterações hepáticas em *H. malabaricus* coletadas em dois trechos do rio Mearim, Baixada Maranhense.

5.1 CAPÍTULO I: RIVER MONITORING: FISH AS BIOINDICATORS OF ENVIRONMENTAL CONTAMINATION[1]

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ABSTRACT

The increase of river contamination raises concerns about the possible consequences for aquatic biodiversity, requiring effective methods for the environmental monitoring of these regions. In this study, a systematic review was carried out on the methodologies that have fish as bioindicators of environmental contamination in the monitoring of rivers, in three databases, Scielo, Wiley and Science Direct in articles published between 2007

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and 2017. Our results showed 42 publications, which the species *Oreochromis niloticus* was the most observed as a bioindicator of environmental contamination. The rivers Paraíba do Sul and Sinos in Brazil were the most studied in the scientific works used. Copper and zinc were the most cited chemical agents as aquatic contaminants. Enzymatic biomarkers were the most used in these articles. Through the present literature review, it indicates the need for other analyzes capable of further expanding knowledge about the use of fish in environmental biomonitoring in rivers.

Key words: Biomarkers; Biomonitoring; Enzyme; Chemical Agents; Review; Freshwater Species.

1 INTRODUCTION

It noted the increase in water pollution of the world's natural resources, caused by the growth of human intervention in ecosystems (Saltveit et al., 2012). The constant economic growth of urban centers, intensified by civil, industrial and agricultural processes, provides an accelerated loss of aquatic biodiversity (Lins et al., 2010). This is explained by the large amount of domestic sewage, industrial and agricultural chemicals released into water bodies (Benito et al., 2017).

It is evident the exacerbated growth of the amount of contaminants in the rivers, intensify the loss of flora and fauna of these natural resources (Castro et al., 2015; Guan et al., 2016). Xenobionts are mainly derived from diffuse sources, such as heavy metals, organic compounds, agrochemicals, fertilizers, among others (Rashed et al., 2001 and Arias et al., 2007). These compounds can be carried to river ecosystems through rainwater, irrigation or infiltration into groundwater (Evans and Wilcox, 2014).

Thus, the water environmental monitoring aims to minimize and predict future biological impacts (Batista et al., 2016). That defined as a repeated and temporal analysis of certain biological, chemical and physical an area through specific, comparable and standard parameters (WHO, 1993; Wang et al., 2016).

According to Van der Oost et al. (2003) the environmental monitoring of an area consists of five methods of diagnosis of the effects of pollutants on the environment: 1) chemical monitoring - characterized by the analysis of pollutants in the environmental compartments; 2) monitoring of bioaccumulation - explained by accumulation of these pollutants of the regional biota; 3) monitoring of biological effects - determined by reversible or irreversible effects on organisms; 4) health monitoring - are the damage to the health of that animal; 5) monitoring of ecosystems - described by the analysis of the species present in that environment.

The use of biological organisms in environmental monitoring is essential, called biomonitoring or biological monitoring (De Zwart, 1995). Worldwide, this type of environmental analysis for the detection of contamination in aquatic ecosystems has been gaining attention for presenting satisfactory results for ecosystem management (Muposhi et al., 2015; Gupta et al., 2014).

The biological groups used are characterized as environmental bioindicators, that is, they indicate changes in their habitat through morphological, physiological or biochemical responses in their organism, these are known as biomarkers (Sadauskas-Henrique et al., 2017). According to the literature, several taxa may be bioindicators of aquatic contamination such as mollusks, crustaceans and fish (Lins et al., 2010; Chandurvelan et al., 2015).

This methodology of environmental monitoring is applied to rivers and guarantees efficiency in the management of these resources (Arias et al., 2007). The rivers along its route have high physical, chemistry, geomorphological and biological, diferences, which hinder further environmental management (Nestler et al., 2011). In addition, they portray various types of contamination from different sources (Saltveit et al., 2012; Rawtenberg et al., 2014). According to Arias et al. (2007) to determine the organism used as bioindicator in certain river. Ecosystems it is necessary to analyze the characteristics of the river, in some cases the use of fish is more recommended.

As the rivers have several characteristics that determine the specific type of bioindicators, it is important to conduct an analysis of papers showing fundamental information about usefull species, advantages and disadvantages of fish species as bioindicators in rivers. Based on this, this work aimed to carry out a systematic review on the methodologies that have fish as bioindicators of environmental contamination in the monitoring of rivers, in three databases, Scielo, Wiley and Science Direct in articles published between 2007 and 2017.

2 MATERIALS AND METHODS

This study is a bibliographic review, whose information was collected of the scientific articles produced between 2007 and 2017, using three data bases, Scielo (Scientific Electronic Library Online), Science Direct and Wiley (Wiley Online Library), containing the following descriptors in Portuguese and English languages: "environmental monitoring"; "Biomonitoring"; "Bioindicator"; "Biomarker", "fish", "rivers" and "environmental contamination". To specify the articles of interest by the authors were used Boolean Operators: and, or and and not.

Firstly, for the organization of the data, the readings of the abstracts of the scientific articles were done, in order to identify the information of interest for the present work (Rechenmacher et al., 2010). Subsequently, the relevant scientific papers were archived for complete reading. In these articles, were observed useful topics as: bioindicators (species) used in the studies, country and continent where the research took place, which the river was conducted environmental monitoring, types of biomarkers and the methodological reference adopted in the study, contaminants found in the rivers, as well as the year of publication.The scientific papers used in this article are listed by database (Scielo, Wiley, Science Direct) and in chronological order in table 1.

Table 1. Articles published between 2007 to 2017 in the three databases (Scielo, Wiley Science Direct), mentioned above and used in a bibliographic review in the present study.

Authors	**Bioindicators**	**Types of biomarkers**	**River (s)**
		SCIELO	
Arias et al. (2007)	*Geophagus brasiliensis; Oreochromis niloticus*	Genotóxico; Enzimático	Paraíba do Sul; Guandu
Mota et al. (2009)	*Cichidae sp*	Genotóxico	Paraguaçu
Scalon et al. (2010)	*Hyphessobrycon luetkenii*	Genotóxico	Sinos
Paulo et al. (2012)	*Poecilia vivípara*	Histológico	Cachoeira
Furnus et al. (2014)	*Steindachnerine brevipinna; Astyanax asuncionensis Astyanax schubarti; Leporinus obtusidens Schizodon nasutus; Apareiodon affinis; Crenicichla niederleinii; Pimelodella cf grifini; Loricaria simillima*	Genotóxico	Paraná
Batista et al. (2014)	*Astyanax bimaculatus*	Enzimático	Una
Muposhi et al. (2015)	*Oreochomis niloticus*	Enzimático, Protéico	Pote
Ribeiro et al. (2015)	*Astyanax bimaculatus*	Enzimático	Una
Bianchi et al. (2015)	*Astyanax jacuhiensis*	Genotóxico, Citotóxico	Sinos

Loro et al. (2015)	*Astyanax jacuhiensis*	Genotóxico, Enzimático, Protéico	Uruguai
Steffens et al. (2015)	*Astyanax jacuhiensis*	Genotóxico	Sinos
		WILEY	
Ergene et al. (2007)	*Barbus barbus, Acipenser ruthens*	Genotóxico	Berdan
Thilakaratne et al. (2007)	*Notropis hudsonius*	Histológico	St Lawrence
Jeffries et al. (2008)	*Rhinichths cataractae*	Citotóxico	Oldman, Bow
Hanson et al. (2009)	*Oncorhynchus mykiss*	Genotóxico, Enzimático	Gata Alv
Britvic et al. (2010)	*Rutilis pigus virgo, Rutilus rutilis, Leuciscus cephalus, Chondrostoma nasus, Alburnas alburnas*	Enzimático	Sava, Mereznica
Mower et al. (2011)	*Geophagus brasiliensis*	Enzimático	Androscoggin
Nascimento et al. (2012)	*Oligosarcus hepsetus, Hypostomus auroguttatus,*	Histológico	Paraíba do Sul
Scarcia et al. (2012)	*Catostomus commersoni*	Enzimático	Luján
Otter et al. (2012)	*Cyprinus carpio, Pimelodella laticeps*	Genotóxico, Enzimático	Reedy
Dane et al. (2014)	*Capoeta capoeta*	Histológico	Karasu
Raphael et al. (2014)	*Micropterus salmoides*	Histológico; Genotóxico	PTherain, Reveillon, Rhonelle
Raskovic et al. (2014)	*Gasterosteus aculeatus* L	Histológico	Danube
Benerjee et al. (2015)	*Barbus barbus; Acipenser ruthenus*	Histológico	Subarnarekha
Liu et al. (2015)	*P. pontius; L bata*	Enzimático	Yellow
Young et al. (2016)	*Ictalurus puctatus*	Comportamental	Kaskaski

	SCIENCE DIRECT		
Hinck et al.(2007)	*Cyprinus carpio; Micropterus spp.; Ictalurus puctatu*	Enzimático, Histológico	Colorado, Gila Yampa Green, San Juan
Linde-Arias et al. (2008)	*Oreochromis niloticus*	Enzimático, Genotóxico	Paraíba do Sul
Hinck et al. (2008)	*Micropterus salmoides, Cyprinus carpio*	Enzimático, Histológico	Tombigbee, Coosa, Alabama, Mobile, Chattaboo, Chee, Flint Apalachicola
Hoshina et al. (2008)	*Oreochromis niloticus*	Genotóxico	Atibaia
Otte et al. (2008)	*Gasterosteus aculeatus L.*	Enzimático	Danube
Parente et al. (2008)	*Oreochromis niloticus, Geophagus brasiliensis*	Enzimático	Guandu
Ruas et al. (2008)	*Oreochromis niloticus, Tilapia rendalli, Geophagus brasiliensis*	Enzimático	Monjolinho
Pereira et al. (2013)	*Luciobarbus bocagei, Squalius carolitertii, Pseudochondrostoma* sp	Enzimático, Histológico	Cávado, Este, Ave, Vizela
Mierzejewski et al. (2014)	*Lepomis sp., Micropterus salmoides*	Enzimático	Saluda
Procópio et al. (2014)	*Prochilodus argenteus*	Enzimático, Histológico	São Francisco
Zheng et al. (2014)	*Carassius auratus*	Enzimático	Hun
Bueno-Krawczyk et al. (2015)	*Astyanax bifasciatus*	Enzimático, Genotóxico Protéico	Iguaçu
Hackenberger et al. (2015)	*Cyprinus carpio*	Enzimático, Protéico	Drava
Rautenberg et al. (2015)	*Gambusia affinis*	Histológico	Suquía
Ghisi et al. (2016)	*Hypostomus ancistroides*	Enzimático, Genotóxico Histológico	Pirapó
Kumar et al. (2017)	*Oreochromis mossambicus*	Enzimático, Histológico	Bhima

3 RESULTS

In the cited databases, a total of 2,892 articles were collected, of which only 42 publications were used in the present study, both in national and international journals, divided into 11 (26%) papers in the Scielo database, 15 (36%) publications in Wiley and 16 (38%) in Science Direct.

Brazil and the United States published the highest numbers of scientific articles during the required period, 55% and 18%, respectively.

The most cited rivers in the scientific articles studied were the Paraíba do Sul and Sinos in Brazil with 30% of total (table 2).

Table 2. The main rivers with the countries most found in the analyzed scientific articles.

River (s)	Country (es)	Number of papers	%
Paraíba do Sul	Brazil	3	30
Sinos	Brazil	3	30
Danube	Germany, Serbia	2	20
Guandu	Brazil	2	20

The most widely used fish species as bioindicators of environmental pollution were *Oreochromis niloticus* with 22%, followed by *Cyprinus carpio* and *Geophagus brasiliensis* with 14% (table 3).

Table 3. The fish species most used as bioindicators of environmental contamination in rivers observed in the analyzed scientific articles.

Species of bioindicators	Number of papers	%
Oreochromis niloticus	6	22
Cyprinus carpio	4	14
Geophagus brasiliensis	4	14
Astyanax jacuhiensis	3	11
Micropterus salmoides	3	11
Astyanax bimaculatus	2	7
Ictalurus punctatus	2	7
Gasterosteus aculeatus	2	7
Rutilis rutilis	2	7

The contaminants most cited as the possible cause of damage to the fish were copper and zinc with 15%, followed by chromium with 12% and lead with 11%. The years of 2014 and 2015 had the highest number of articles published, being 9 articles (figure 1).

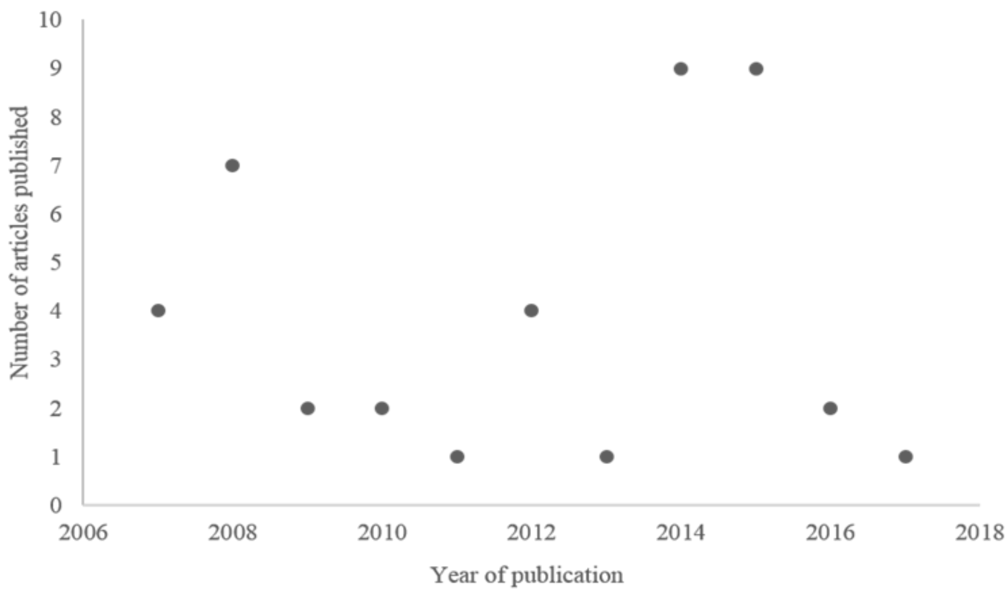

Figure 1. Number of articles published between 2007 and 2017 on the use of fish as bioindicators of environmental contamination in rivers.

The types of biomarkers most observed in the analyzed studies were enzymatic (47%), followed by histological (27%) and genotoxic (26%) (table 4)

Table 4. The types of biomarkers most observed in the published scientific articles together with the methodology and the reference.

Types of biomarkers	Numbers of papers	Methodology	Methodological reference
Enzymatic	25	Glucationa S-Transferase (GST)	Habig et al. (1974)
		Lipidperoxidation analysis (LPO)	Jiang et al. (1992)
		Catalase (CAT)	Aebi (1984)
		Acetylcholinesterase (AChE)	Ellman et al. (1961)
		EROD	Hinck et al. (2006)
		Metallothionein (MT)	Viarengo et al. (2000)
Genotoxic	14	Nuclear alterations	Carrasco et al. (1990)
		Comet Assay	Anderson et al. (1994) Palus et al. (1999) Pitarque et al. (1999)
Histological	14	Hepatic Lesions	PoleKsic and Mitrovic-Tutundzic (1994) Bernet et al. (1999)
		Branchial Lesions	PoleKsic and Mitrovic-Tutundzic (1994) Bernet et al. (1999)

The most used methodologies in these scientific articles followed by the most cited methodological references were: Glucationa S-transferase (GST) referred by Habig et al. (1974), lipidperoxidation analysis by Jiang et al. (1992), catalase (CAT) by Aebi (1984), acetylcholinesterase (AChE) by Ellman et al. (1961), ethoxyresorufin-O-deethylase (EROD) by Hinck et al. (2006), metallothionein (MT) by Viarengo et al. (2000); hepatic and branchial lesions referenced by Poleksic and Mitrovic-Tutundzic (1994) and Bernet et al. (1999); the nuclear alterations by Carrasco et al. (1990) and the comet assay by Anderson et al. (1994), Pitarque et al. (1999) and Palus et al. (1999).

4 DISCUSSION

The species *Oreochromis niloticus*, *Cyprinus carpio* and *Geophagus brasiliensis* present favorable characteristics for the study as environmental bioindicators, since they show a wide geographical distribution and abundance, both adapting easily to the environmental changes, as well as showing great sensitivity to contaminants (Arias et al., 2007; Hinck et al., 2007; Parente et al., 2008). According Grisolia et al. (2009), these species are able to change their eating habits in response to environmental stresses in order to survive them, being a good bioindicator of environmental contamination.

Fish are very sensitive organisms to pollution of the aquatic environment and are characterized as efficient in environmental monitoring, since they present tissue alterations in their biological systems, even in low concentrations of contaminants; participate in the highest level of the food chain, accumulating contaminants in the body (Van der Oost et al., 2003; Souza et al., 2013; Dalzochio et al., 2016).

The biomarkers in aquatic organisms represent the biological responses caused by the presence of the contaminating agents in the environment. These effects can be at the cellular, molecular, physiological and structural level (Depledge et al., 1995; Hook et al., 2014). Enzymatics biomarkers result of chemical reactions between the body of the fish with contaminants, causing rapid and specific effects on oxidative metabolism of the organism (Arias et al., 2007; Lushchk et al., 2011). Histologicals biomarkers can be used to verify toxic effects of xenobiotics, as well as changes in some fish organs, featuring as important and efficient environmental monitoring method (Yasser and Naser, 2011). Genotoxics evidence the damage caused to fish DNA, mainly the failures in cellular genetic division, using fast and less costly techniques (Carrasco et al., 1990).

The most importante enzymatic biomarkers cited in papers analyzed were glutationa s-transferase (GST), catalase (CAT), acetylcholinesterase, ethoxyresorufin-o-deethylase, metallothionein, are examples of antioxidant enzymes which degrade reactive oxygen species (ROS), besides being fundamental in the control, production and elimination of ROS, however, when in large quantity, these enzymes can impair the vital functions of the cell, affecting organelles such as mitochondria (Batista et al., 2014). Environmental changes cause oxidative stresses in the cell, accelerating, increasing and unbalancing the activities of these enzymes (Lushckak, 2011).

The most importante histological biomarkers cited in papers showed that liver and gills are easily affected by the contamination in the environment, presenting several changes in their tissues, some of them, are reversible or irreversible (Bernet et al., 1999). Nuclear alterations (genotoxic biomarkers) were cited in papers with the advantage of evidencing the low exposure of xenobionts in the environment in several species (Scalon et al., 2010). The comet assay (genotoxic biomarkers) also known as single-cell electrophoresis gel is usefulness as a simple and sensitive method able to verify the DNA damage caused by contaminants in fish (Carrasco et al., 2009).

The toxic effects of the chemical contaminants can be evidenced in the different levels of organization of the animals, both individually, as well as in the communities (Dane et al., 2014). These xenobionts bioaccumulate along the trophic levels of the food chain, concentrating on top predators and damaging the health of these organisms (Mackay et al. 2016). Elevated exposure of zinc to fish interferes mainly with cellular biochemical processes, impairing the equilibrium of Ca, Na and K ions activating the animal's defense mechanisms such as mucus secretion (Skidmore, 1970; Loro et al. 2014). According Grassi et al. (2000) the excess of organic matter in the water interferes with the distribution of some metals, among them copper.

The most papers cited Sinos and Paraíba do Sul rivers, located in the south and southeast regions, respectively. There characterized by being two of the most polluted rivers in Brazil (Arias et al., 2007; Linde-Arias et al., 2008; Scalon et al., 2010; Schulz and Costa, 2010; Nascimento et al., 2012; Bianchi et al., 2015; Steffens et al., 2015). In the Sinos river the domestic and industrial effluents of about 32 cities are dumped, seldom do not receive prior treatment, making the pollution situation of this resource even more worrying (Rubio et al., 2015). Disregard by public environmental agencies is observed with the contamination situation in this river, as well as with the various populations of dead fish (Nascimento and Naime, 2009). Thus, various studies of environmental contamination biomarkers in this river reinforces the serious situation of Brazilian water resources.

The Paraíba do Sul river also suffers from large amounts of agricultural, domestic and industrial releases, as it covers a region with a very demographic and highly industrialized density, covering 184 municipalities in the states of São Paulo, Rio de Janeiro and Minas Gerais, according literature, other environmental factors that entail losses to this resource are the dumping of solid waste near its banks, deforestation of the ciliary forest, erosion causing river siltation and predatory fishing, these have been further intensified over the years (Barcellos et al., 2011).

Brazil presents an intense infrastructure for studies focused on the use of bioindicators of aquatic contamination, mainly in the universities of the South and Southeastern regions of the country, worth highlighting, that these regions have the most polluted rivers in Brazil, requiring a greater environmental monitoring of them (Dalzochio et al., 2016). Some rivers in the United States are affected contamination domestic and industrial effluents from large cities, as well as accidents with oil spills, causing the loss of several species of fish and macroinvertebrates (Otter et al., 2012; Kubach et al., 2010).

In this way, it is interesting to know the enzymatic stress reactions caused by certain contaminants, in order to indicate which is the chemical agent causing damages in that environment. In addition, some methodologies used in bioindicators often reinforce their data associated to water and chemical analyzes, generating greater reliability in results (Raphael et al., 2014). In this sense, the fish present a certain support capacity (K), characterized by conditions conducive to the development of the organismo. In a contaminated environment this K is affected, requiring further studies, in order to seek other biomonitoring strategies for the conservation of river species.

5 CONCLUSIONS

The analysis of scientific articles published between the years 2007 to 2017 in three databases shows that fish as bioindicators of environmental contamination in rivers is widely used in academic studies, since they have efficient results in monitoring the region as well as a lower cost.It is worth mentioning that some species present much more satisfactory results for certain rivers, because they adapt more to contaminated sites, being more resistant. Moreover, the most contaminated rivers show more scientific studies. Enzymatic biomarkers are very useful and effective in fish surveys as bioindicators.It is necessary to further study this line to increase knowledge about the use of fish in rivers in environmental biomonitoring.

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CONFLICT OF INTEREST

The above authors declare that they do not have any potential conflict of interest in this study.

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5.2 CAPÍTULO II: MORPHOLOGICAL BIOMARKERS IN *Hoplias malabaricus* (PISCES, CHARACIFORMES, ERYTHRINIDAE): A CASE STUDY IN A WETLAND OF INTERNATIONAL INTEREST (MARANHÃO, BRAZIL)[2]

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ABSTRACT

Environmental management through the use of morphological biomarkers in fish is widely used and with effective results. The aim of the study was to identify branchial lesions in *Hoplias malabaricus* as an environmental impact biomarker in a wetland of international interest, Baixada Maranhense Protected Area, (Maranhão, Brazil). Fish were collected in two areas of the river Mearim, reference area (A1 = Engenho Grande/Vitória do Mearim) and contaminated area (A2 = Curral da Igreja/Arari). Abiotic analysis of water, biometry of the specimens were performed, as well as the usual histological routine and light microscope observation. The gill lesions observed in the contaminated area (A2) were lamellar fusion, epithelial displacement, congestion, lamellar disorganization, mucous cell proliferation, hyperplasia, aneurysm. These changes were classified as low and moderate severity by the methodologies used. Analysis of contaminants such as magnesium, calcium, iron and chlorides corroborated the results of the survey, as well as most of the biometric data. The species *Hoplias malabaricus* proved to be a suitable bioindicator, since it presented branchial lesions capable of evading the environmental conditions of the region.

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Key words: Aquatic biomonitoring; branchial lesions; Mearim river; contamination of rivers; international protected areas; freshwater species.

1 INTRODUCTION

Aquatic ecosystems are strongly impacted by human activities (Green et al., 2016). This can be explained mainly by the large number of domestic and industrial sewage releases in water bodies, pesticides originating from agricultural crops adjacent to water resources (Viganò et al., 2016). In addition, eutrophication, deforestation of riparian forest and silting of rivers greatly aggravate the loss of biodiversity in these resources (Pantalena and Maia 2014). In order to alleviate this situation, and to contribute to the conservation of ecosystems, protected areas were created.

Protected areas have the function of balancing the anthropic uses of natural resources and the conservation of biodiversity (Laschefski and Costa 2008; Abdullah et al., 2015). Among the various wetlands of international interest, there is the Baixada Maranhense Environmental Protection Area (APA), that presents a vast and complex fauna and flora, aquatic and terrestrial. This area, in addition to beingconsidered an area of great relevance for international environmental and social sustainability, is part of the list of global wetlands, as indicated by the Ramsar Convention Manual (2013). This convention is an intergovernmental treaty that advocates policy strategies that promote improvements in the conservation and preservation of global wetlands in order to achieve the sustainability of significant natural resources at the international level and the quality of life of adjacent populations (Ramsar Convention Manual, 2013). In this sense, the biomonitoring of these wetlands with the use of morphological biomarkers in fish can be a great ally in the conservation and preservation of these resources.

The use of morphological biomarkers in fish has been widely to contribute to the management of aquatic areas (Saleh and Marie 2016). Biomarkers are described as biochemical, physiological and morphological changes indicative of a pollutant on an organism in that ecosystem (Dalzochio et al., 2016). They are able to indicate the availability of natural resources pollutants in elucidating the cause/effect/dosage of a xenobionte an environment, and thus, assess and monitor the health risk that ecosystem (Van der Oost et al., 2003). Gill lesions are used as morphological biomarkers, since this organ presents direct and permanent contact with the aquatic environment and are fundamental in respiration, osmoregulation, excretion, among other vital functions for fish (Bernet et al., 1999; Kumar et al., 2017). These biomarkers are especially suitable to assess the effects of human impact on predatory species, such as the species *Hoplias malabaricus*.

H. malabaricus is a fish predator chain-end type, facilitating bioaccumulative process of throughout the food chain (Rossi et al., 2014), besides having importance in fishing held in rivers several wetlands (Novaes and Carvalho 2011). From these characteristics, *H. malabaricus* can be used in studies with morphological biomarkers, with satisfactory results for environmental monitoring in freshwater areas. However, there are no studies of this kind carried out in the Baixada Maranhense Environmental Protection Area. Thus, in this study, it was intended to identify branchial lesions in *H. malabaricus* as an environmental impact biomarker in a wetland of international interest, Baixada Maranhense, Brazil.

2 METHODS AND MATERIALS

2.1 Sampling sites

Two stretches of the Mearim river were selected for collection, both located in the Baixada Maranhense Environmental Protection Area (Figure 1). The first stretch was located in the village of the town of Engenho Grande (03° 27' 53.3 "S e 44° 49' 55.9" W) (Figure 1), located in the municipality of Vitória do Mearim, still has native flora and fauna, with little urbanization and rare anthropic activities, considered as the reference area (A1).

The second stretch of the river was located in the village Curral da Igreja (03° 27' 64.1" S e 44° 46' 79.7" W) (Figure 1), municipality of Arari, a town with intense urbanization that presents various anthropic actions such as agriculture, being considered as a potentially contaminated area (A2). The two villages are bathed by the river Mearim, an important aquatic resource of the region.

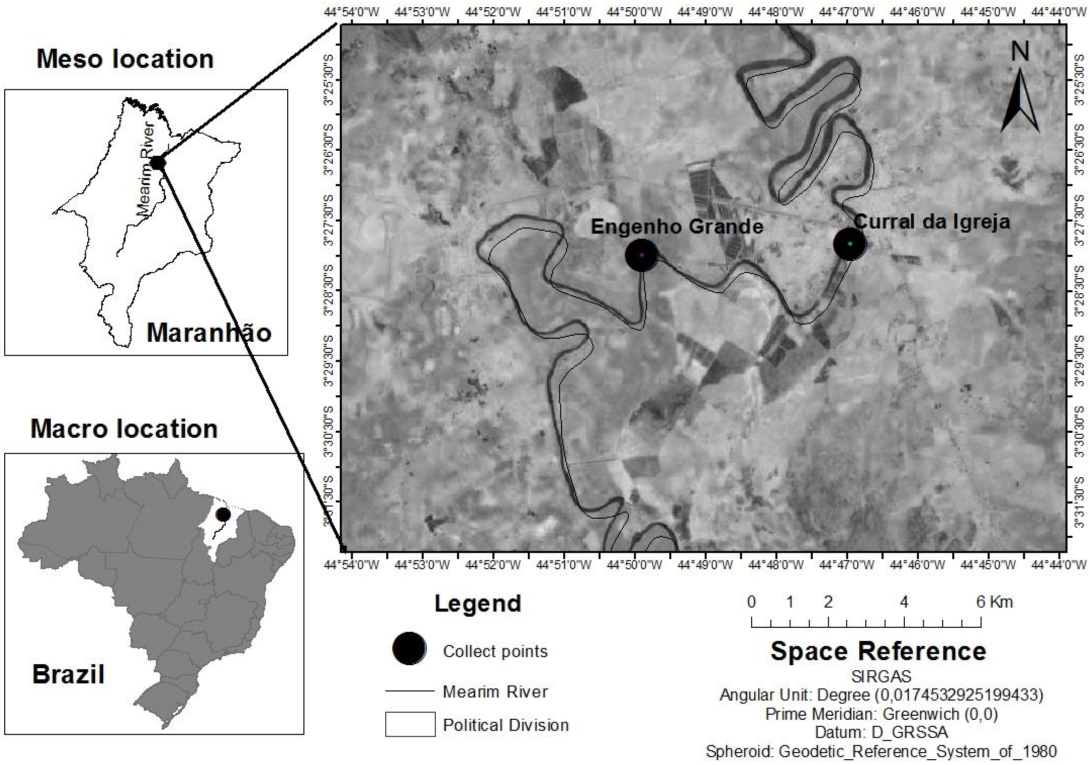


Figure 1. Map of the Baixada Maranhense Environmental Protected Area (Arari and Vitória do Mearim, Maranhão, Brazil) showing the sampling sites.

2.2 License and ethics declaration

The taking of fish samples was authorized by a permit issued by the State Department of Natural Resources and Environment (SEMA, 18208/2014). The protocol was ap-proved by the Ethics Committee of Maranhão State University (13/2017 CRMV-MA) and met the guidelines of the Brazilian College for Animal Experimentation (COBEA; http://www.cobea.org.br).

2.3 Sampling of *H. malabaricus*

The *H. malabaricus* specimens were collected from two sites along the river Mearim within the Environmental Protection Area of Baixada Maranhense, totaling 91 fish being 56 males and 35 females, 54 specimens collected in the dry period and 37 in the rainy season. Sampling was performed in 2016 (August and November) during the dry season, and May/2016 and June/2017 during the rainy season.

2.4 Enviromental and microbiological parameters

Physicochemical parameters-calcium, magnesium, hardness, alkalinity in OH-, alkalinity in CO3-, alkalinity in HCO3-, total alkalinity, chlorides (Cl-), conductivity, total solids dissolved, NaCl, pH, turbidity, dissolved oxygen, nitrite, nitrate, iron - were measured at each site during the dry and rainy season when fish were sampled.This measurement was performed through laboratory analysis.

Microbiological characteristics - the most probable number of total coliforms and *Escherichia coli* were analyzed by the Colilert method through the presence or absence and quantification of these characteristics in the sample.

2.5 Biometric data

Biometric data of individual *H. malabaricus* specimens-total length (TL), standard length (SL), total weight (BW) and gonads weight (GW)-were recorded. All fish were juveniles, and gonads were categorized macroscopically into four classes in accordance with Vazzoler (1996): immature (GS1) or maturing (GS2), mature (GS3) and exhausted (GS4).

2.6 Histological and gonadossomatic analysis

The second right gill arch of all fish were removed, and placed in 10% formaldehyde for approximately 1 and 2 days, placed in 10% nitric acid for approximately 4 hours. In the sequence was followed the usual histological routine. Slides with sections of approximately 5 μm thickness were stained with Hematoxylin and Eosin (HE) (Luna, 1968) for analysis under an light microscope.

The histological changes were observed displaying the methodology as suggested by Poleksic & Mitrovic-Tutundzic (1994) and Bernet et al. (1999). The Poleksic & Mitrovic-Tutundzic (1994) methodology calculates the index of histological changes (IAH), by the formula IAH: $1 \times \Sigma I + 10 \times \Sigma II + 100 \times \Sigma III$, where I, II and III indicate the degree of severity lesion in each gill analyzed. The methodology of Bernet et al. (1999) is $Iorg\Sigma rp = \Sigma alt (a.w)$ being rp the reaction pattern, alt the alterations found and w the importance factor of the lesion.The gonadosomatic index (GSI) was calculated according to Vazzoler (1996): GSI = 100 x GW / TW, where GW is the weight of the gonads and TW is fish body weight.

2.7 Statistical analysis

Biometric data, gonadosomatic indexes obtained and values of histological changes index aren expressed as mean ± standard deviation. Stage of gonadal maturation gonadais a histological lesions found are presented in percentage. Significant differences between groups were checked by the t-test and $p < 0.05$ accepted as significant. The analysis of similarity of the branchial lesions between the studied areas was observed through Cluster analysis, based on the values in paired groups (similarity) measured by Bray-Curtis, being ANE: aneurysm, CON: congestion, HIP: hyperplasia, DESL: displacement of the epithelium, DESO: lamellar disorganization, DIL: venous sinus dilation, PRO: proliferation of mucus cells, FUC: complete lamellar fusion, FUI: incomplete lamellar fusion.

3 RESULTS

3.1 Environmental conditions

The abiotic and microbiological data of the collected water samples are shown in table 1. The turbidity showed higher values in the contaminated area (A2) and in the dry season. Conductivity and alkalinity also had its highest values in the contaminated area (A2) and pH presented differences in both areas, especially in the dry period; already dissolved oxygen showed differences in values in the two seasons, in both areas, mainly in the dry period.

Regarding the chemical compounds examined, calcium and magnesium had higher values in the reference area (A1) and during the rainy season. Dissolved solids presented higher values in the contaminated area (A2) in all periods.The iron presented values not allowed by the legislation in both areas and periods, with the highest values in the contaminated area (A2). The nitrate remained constant in both areas and during both periods, as well as NaCl that did not show variations during the periods.

Microbiological analyzes determined the most probable number of total coliform showed higher values in the contaminated area (A2) in the rainy season. *Escherichia coli* showed higher values in the contaminated area (A2) and during the dry season.

Table 1. Abiotic and microbiological analysis of the water collected in the APA Baixada Maranhense (Brazil).

Parameters	**Reference (A1)**				**Contaminated (A2)**				**Resolução CONAMA 357-2005/430-2011**
	Dry		Rainy		Dry		Rainy		
	Collect 1	Collect 2	Collect 1	Collect 2	Collect 1	Collect 2	Collect 1	Collect 2	
Calcium (mg/L CaCO3)	0	0	1.058	0	0	0	561.6	0	-
Magnesium (mg/L CaCO3)	0	0	1.058	0	0	0	561.6	0	-
Alkalinity total (mg/L CaCO3)	62	32	0	40	90	34	0	38	-
Chlorides (Cl-) (mg/L Cl-)	32.53	31.9	40.78	6.9	35.32	51.9	44.8	8.9	250 mg/L Cl-
Conductivity (µJ/cm)	470	74.6	115.7	-	606	104.9	116	-	-
Total dissolved solids (ppm)	231	37.4	58	-	303	52.6	60	-	-
NaCl (%)	1	0.2	0.2	-	1.3	0.2	0,2	-	-
pH	6.2	5.21*	6.8	6.2	6	5.67*	6,8	6.18	6.0 a 9.0
Dissolved oxygen (mg/ LO_2)	0.7*	0*	0.3*	10.8	0.6*	0*	0,3*	14.3	<5,0 mg/ L O_2
Turbidity (U.N.T)	26.86	369*	1.75	0.51	113*	376*	1.87	0.73	100 U.N.T
Nitrite (mg/L N)	0	0	0	0	0	0	0	0	1.0 mg/L N
Nitrate (mg/L N)	4.43	0	2.21	0	4.43	0	2.21	0	10,0 mg/L N
Iron (mg/L Fe)	2.61*	1.66*	2.13*	0.68*	5.01*	2.53*	3.77*	0.12	0,3 mg/L Fe
T. coliforms (NMP)	3.410*	2.481*	24.196*	4.352*	5.940*	12.033*	24.196*	9.208*	1.000/NMP/100 mL
Escherichia coli	900	175	20	20	1730*	816	41	108	1.000/NMP/100 mL

*Values in deformity of the allowed by resolution CONAMA 357/2005, fresh water, class II.

3.2 Biometric parameters, gonadosomatic index (GSI) and gonadal maturation development

The values of the biometric analysis and the gonadosomatic index are presented in tables 2 e 3 and the gonadal stages of maturation in table 4. Regarding the dry period, the males collected in the reference area (A1) presented higher values of weight and body length with significance by the test t (Student). The females collected in the contaminated area (A2) were shown to be longer and heavier.

In relation to the rainy season, females and males collected in the contaminated area (A2) were longer and heavier. It is worth mentioning that a female was collected during this period in the reference area, interfering with the comparison of the data.

GSI presented in the dry period, higher values in the reference area (A1) for both males and females in the dry period with the for values of the significant females by the test t (Student). In the rainy season, the females of the contaminated area (A2) presented the highest value, while the males in the reference area (A1).

The data from the gonadal stages are shown in table 4. During the dry period, the females of the GS3 stage and the males of the GS2 stage were more observed. In relation to the rainy season, females of stage GS2 and males of stage GS1 were more verified.

Table 2. Biometric data of the *H. malabaricus* collected in dry season in the APA Baixada Maranhense, Brazil.

Parameters	A1 (reference area)		A2 (contaminated area)		t Test (females / males)
	Females	Males	Females	Males	
LT (cm)	23,32 ± 3,04	26,31 ± 3,91	25,18 ± 3,87	19,96 ± 3,34	0,26 / 0,00012*
LP (cm)	18,36 ± 2,31	20,81 ± 2,90	21,02 ± 2,73	16,10 ± 2,88	0,039* / 0,00051*
WT (g)	131,6 ± 52,83	198,42 ± 120,87	172,12 ± 91,20	101,46 ± 73,12	0,25 / 0,0099*
WG (g)	5,23 ± 2,86	0,88 ± 0,99	3,06 ± 1,37	0,36 ± 0,21	0,06 / 0,013*
GSI	4,01 ± 1,69	0,61 ± 0,59	2,04 ± 1,32	0,41 ± 0,27	0,015* / 0,19

*Indicates statistical difference in relation to the reference area ($p < 0.05$). LT: total length; LP: standard length; BW: body weight; WG: weight of gonads; GSI: gonadosomatic index; cm: centimeters; g: grams.

Table 3. Biometric data of the *H. malabaricus* collected rainy season in the APA Baixada Maranhense, Brazil.

Parameters	A1 (reference area)		A2 (contaminated area)		t Test (females / males)
	Females	Males	Females	Males	
LT (cm)	16,4 ± 0	16,07 ± 0,87	23,6 ± 1,86	21,46 ± 2,23	0 / 0,00020*
LP (cm)	12,3 ± 0	12,87 ± 0,99	18,42 ± 1,53	17,56 ± 1,66	0 / 0,00004*
WT (g)	48 ± 0	50,75 ± 7,97	140,46 ± 24,40	121,43 ± 38,71	0 / 0,0022*
WG (g)	0,36 ± 0	0,87 ± 0,69	2,31 ± 0,93	1,30 ± 1,44	0 / 0,57
GSI	0,75 ± 0	1,84 ± 1,55	1,66 ± 0,71	0,95 ± 0,81	0 / 0,12

*Indicates statistical difference in relation to the reference area ($p < 0.05$). LT: total length; LP: standard length; BW: body weight; WG: weight of gonads; GSI: gonadosomatic index; cm: centimeters; g: grams.

Table 4. Stages of gonadal maturation of males and females *H. malabaricus* collected during the rainy season and dry season in the APA Baixada Maranhense, Brazil.

GS	Dry		Rainy	
	Females	Males	Females	Males
GS1	10%	40%	37%	90%
GS2	32%	57%	63%	10%
GS3	58%	0%	0%	0%
GS4	0%	3%	0%	0%

GS: Gonadal stages: GS1 (immature), GS2 (maturing or resting), GS3 (mature) and GS4 (depleted) (Vazzoler, 1996).

3.3 Gill histological lesions

The percentages of gill lesions (Figura 2) seen in each area are shown in table 5. Histological changes in the gills, according to the methodology proposed by Bernet et al. (1992) shows that the two areas (A1 and A2) changes in architecture and structure of the gill epithelium were the most significant being included in the group of regressive changes.

Regarding the methodology suggested by Poleksic & Mitrovic-Tutundzic (1994), the displacement changes of the lamellar epithelium inserted in the pattern of hypertrophic reaction and hyperplasia of the gill epithelium, and congestion included in the pattern of reaction changes in blood vessels were lesions with higher percentages in the reference area (A1). The aneurysm injury included in the pattern of reaction changes in blood vessels, and lesion displacement of the lamellar epithelium were the most observed in the contaminated area (A2).

The data of the histological indices proposed by the methodologies used in this study are in grafhic 1. In the reference area (A1), according to Bernet et al. (1992), the highest index was 16.4 in the rainy season; according to Poleksic & Mitrovic - Tutundzic (1994) (IAH) the highest value was 15.6 also in the rainy season. In the contaminated area (A2), according to Bernet et al. (1992) the highest value was 22.6 in the rainy season; and for IAH the highest value was 19.4 in the dry season.

The analysis of similarity (Cluster Analysis) performed for the morphological lesions of the fish presented differentiated groups for the two APA Baixada Maranhense (Figure 3). The most similar groups of gill lesions (90%) were venous sinus dilation (DIL) and complete lamellar fusion (FUC); fusão lamellar incomplete (FUI) and hyperplasia (HIP), as well as congestion (CON) and aneurysm (ANE).

Table 5. Observed lesions and their importance factor (w) relating to the methodologies used, as well as predominance of occurrence (%) in each area.

Metodology	Reaction pattern (groups)	Alterations	w	% (A1)	% (A2)
1	Circulatory disorders	Aneurysm	1	17	16
	Regressive changes	Structural alterations	1	73	73
	Progressive chances	Hyperplasia	2	10	11
2	Hypertrophy and hyperplasia of gill epithelia	Hyperplasia	1	10	11
		Incomplete fusion	1	8	10
		Complete fusion	2	8	7
		Displac. epithelium	1	26	28
		Lamelar disorg.	1	2	7
	Changes in mucous / chloride cells	Muco. cells prolif.	2	1	1
	Blood vessel changes	Dilation venous sinus	1	7	8
		Congestion	1	21	12
		Aneurysm	2	17	16

w: Factor of importance; 1: Bernet et al. (1999); 2: Poleksic & Mitrovic-Tutundzic (1994). Displac.: Displacement; Disorg.: Disorganization; Muco.: Mucous cele; Prolif.: Proliferation.

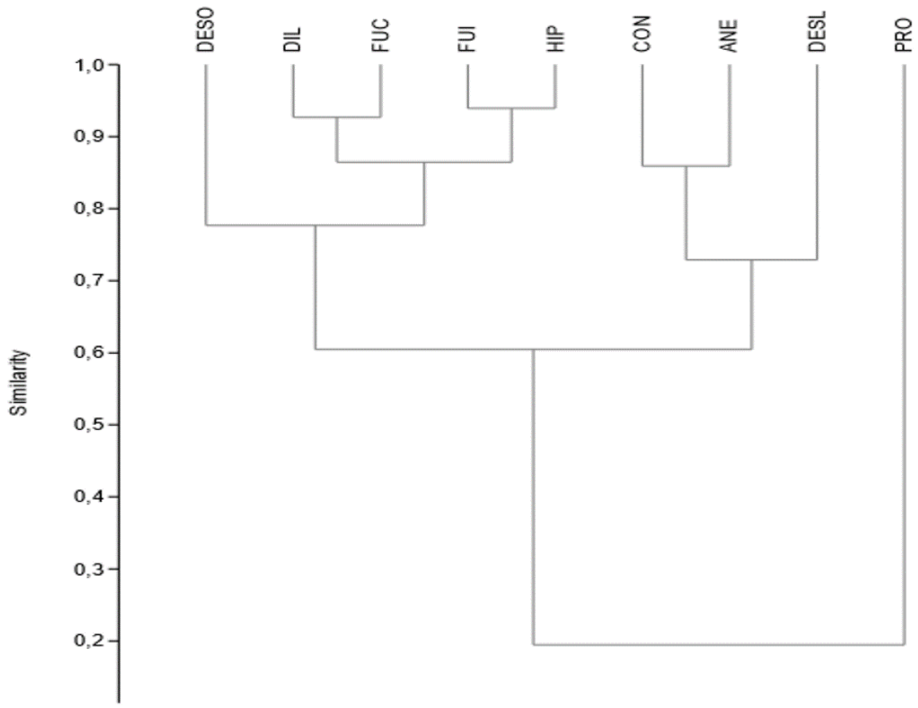
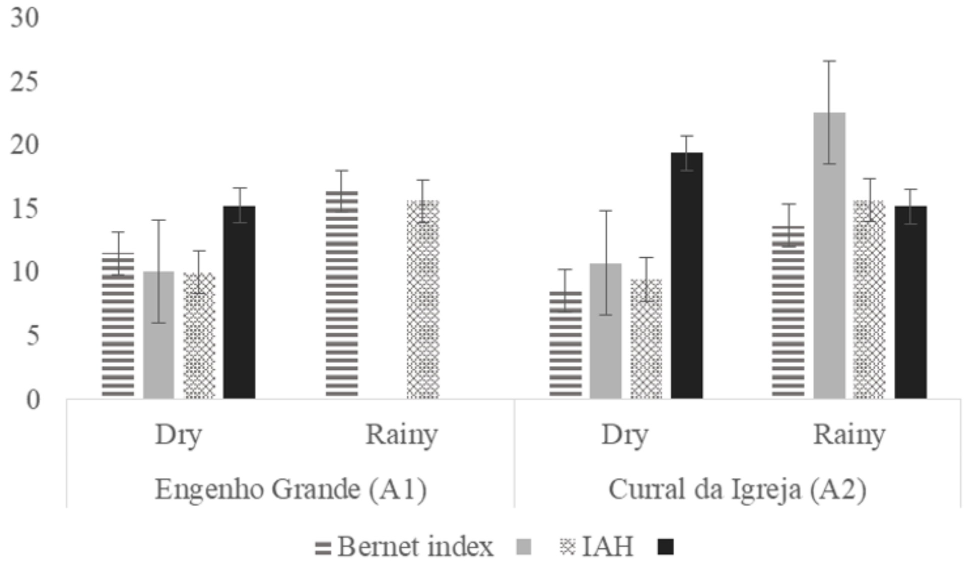


Figure 3. Tree of similarity of the branchial lesions observed in the specimens collected in the two study areas, Maranhão, Brazil. ANE: aneurysm, CON: congestion, HIP: hyperplasia, DESL: displacement of the epithelium, DESO: lamellar disorganization, DIL: venous sinus dilation, PRO: proliferation of mucus cells, FUC: complete lamellar fusion, FUI: incomplete lamellar fusion.

Graphic 1. Index values of Poleksic & Mitrovic-Tutundzic (1994) (IAH) and Bernet et al. (1999) for the branchial lesions in dry and rainy periods.



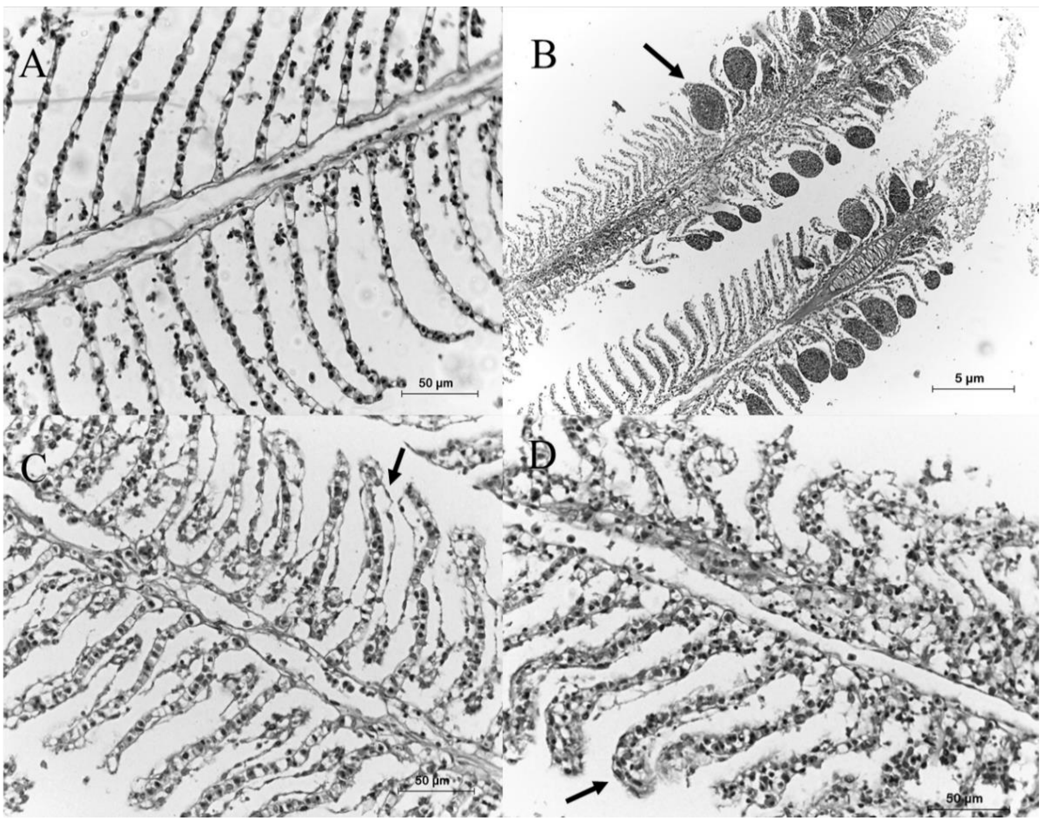


Figure 2. Gill-related lesions. A) Normal gill tissue; B) Aneurysm (arrow); C) Epithelium displacement (arrow); D) Congestion (arrow). B, C, D: 50 µm; B: 5 µm.

4 DISCUSSION

The presence of gill lesions in fish collected in Engenho Grande (A1) and Curral da Igreja (A2), indicate that fish from both Mearim river areas are presenting biological responses caused by contamination. Despite this, the village of Engenho Grande (A1) still has a relatively better environmental quality.The impacts caused in Mearim river by rural, household and industrial, waste can affect the amount of oxygen dissolved in water, reducted of nutrients and temperature variations. These environmental conditions cause imbalances in fish morphology and physiology. In a study by Castro et al. (2014) with the same species in river, also recorded some branchial lesions described in the present study as: lamellar fusion, epithelial displacement, dilation of the epithelium, proliferation of mucous cells and aneurysm.

The lamellar fusion and the hyperplasia are formed in the gill tissue in order to reduce the stress promoted by the contaminated environment, since the lesion displacement of the epithelium when formed in the cells greatly affects the respiratory and osmoregulatory functions of the fish (Nogueira et al., 2011). In the congestion, a grouping of erythrocytes occurs, which will initiate an aneurysm, affecting the pillar cells and the structural arrangement of the cell, which may result in a blood leakage, this lesion may be much more severe, making it irreversible for the organ if the animal remains in a contaminated environment (Heath, 1987; Meletti et al., 2003; Flores-Lopes and Thomaz, 2011). Dilation of the gill epithelium can be caused by irritation of the walls of blood vessels caused by chemical elements present in the medium (Fiuza et al., 2011). The aneurysm lesion was the most representative. This can seriously compromise the respiratory system of fish because it affects the vascular integrity, and epithelial rupture may occur, making it difficult for oxygen uptake by cells (Alazemi et al., 1996; Cantanhêde et al. 2016).

Lesions called mucous cell proliferation and lamellar disorganization were also found in the fish collected in both areas.The intense mucus production is influenced by the stress of the fish in the environment, being responsible for the protection of the lamellae against microorganisms, contaminants and other particles found in the water (Malatt, 1985; Menezes et al., 2015). The cell disorganization is characterized by imbalance in the structure of the gill cells, affecting the normal operation gill mainly in O_2 absorption of water by the fish (Machado, 1999). Changes in the gill tissues can be used to analyze the effects of specific and non-specific pollutants in the watercourse, in addition to predicting future effects in the ichthyological communities of chronically contaminated rivers (Nogueira et al., 2008).

The results of the indexes of the branchial lesions in the collected fish show a low and moderate degree of severity. Martins et al. (2016) found results similar to the data showed in the present study, and argued that these changes, even at low levels, have significant effects on the respiratory balance of the animal. The normal functioning of the respiratory and osmoregulatory systems of fish can be affected by minimal structural changes in the gills, which may become irreversible for animal health and be fatal (Galat et al., 1985).

In relation to the indexes of histological changes, the rainy season presented prominence, both in the reference area (A1) and in the contaminated area (A2), in this, especially the index proposed by Bernet et al. (1999), indicating a higher prevalence of lesions in *Hoplias malabaricus* during this time and in this region, since the environmental changes in this period are intensified, among them, the abiotic and microbiological changes, ions such as nitrate and phosphate can be intensified through the leaching of fertilizers and pesticides into rivers during the rainy season (Iida et al., 2011; Robert et al., 2016; Ballesteros et al., 2017),

Influencing the use of some mechanisms of defense by the organisms, therefore, being able to prevail the histological alterations (Ballesteros et al., 2017).The methodologies of Bernet et al. (1999) and Poleksic & Mitrovic-Tutundzic (1994) used to ascertain the histological lesions were satisfactory for their analysis, being both the most used in studies with histological biomarkers, but the first methodology is more comprehensive, since, besides the level of severity of the lesion, this index presents a degree of extension of the lesion, that is, it highlights the severity and intensity of that alteration In the organ.Cluster analysis showed that there are slight and moderate lesions forming similar groups in different histological patterns, noting that gills are very vulnerable to the effects of contaminants in the environment (Costa et al., 2009; Carvalho-Neta et al., 2014; Oliveira et al., 2016).

The contaminants are one of the fundamental agents that interfere in the normal structure of the gills, interfering in the physiological dynamics of the lamellae (Maggioni et al., 2012), because these xenobionts persist in the medium, providing their bioaccumulation along the food chain (Otter et al., 2012; Cantanhêde et al., 2016). In the present study, calcium and magnesium values were higher in the rainy season. The presence of calcium and magnesium in the environment directly affects the ion exchange in gills of teleosts (Wurts & Stickney, 1989).

The iron showed high values in all collections and in the two areas studied. According, the CONAMA Resolution 357/2005 para água doce e classe II, (Brazilian legislation that regulates the quality of water bodies), the level of iron can not exceed 0,3 mg/L. Iron is a natural component of the soil of this region, since it participates in the geological formation of the Mearim River Basin (Lima, 2013), and the acidic pH of the surface waters of the Baixada Maranhensemay favor the solubilization of this element in the soil (Marques et al., 2010). Excessive amounts of iron in the fish's body can cause imbalance in the body's normal functioning, DNA damage, oxidative stress, and inflammatory tissue damage (Singh et al., 2010). Iron in water also causes changes in its coloration, interfering with turbidity, and survival of living beings (Richter & Azevedo-Neto, 1999).

Furthermore, the values of solid and dissolved chlorides were higher in all samples in the contaminated area (A2).The dissolved oxygen showed lower values in the two areas and in the dry period. Chlorides in the water may suggest a possible contamination by domestic sewage, as this contaminant is present in the urine, also causing change in the taste of water (Fernandes et al., 2012). The rivers that drain cities, intensely antropized regions, have a greater influence of nutrients and dissolved solids in the water (Biggs et al., 2004; Scorsafava et al., 2010). The low amount of dissolved oxygen impairs the metabolic process of aquatic organisms, especially respiration and photosynthesis (Null et al., 2017). The excess dissolved oxygen in the water is related to the abundance of organic sediments in the water column, influencing the intensity of photosynthetic organisms, impairing the survival of other aquatic species, and interfering with water turbidity (Breitburg et al., 1997).

The turbidity is characterized by the high amount of organic material in suspension in the water column, these can be originated by the particles from domestic and industrial sewage discharges released into the aquatic environment (Robert et al., 2016; Salvinelli et al., 2016). A high turbidity greatly affects photosynthesis vegetation of water bodies, affecting the supply of herbivorous fish, causing imbalance in the biological community that ecosystem (Alves et al., 2008). High values in turbidity are directly linked to the high incidence of fecal coliform bacteria in the water and (Robert et al., 2016).

The data of total coliforms and *Escherichia coli* showed higher values, mainly in the contaminated area (A2), especially in the rainy season. According CONAMA (2005) maximum allowable values for fecal coliforms are 1,000 in 100 ml of 80% of the samples. Fecal coliforms and *Escherichia coli* are most commonly found in environments with intense discharge of domestic effluents, human and animal faeces (Alves et al., 2008). Cassaro & Carreira (2001) in a study in Pirapó river, found high fecal coliform and *E. coli* values, and argued that the tributaries of this river received waste from various pollution sources, mainly domestic and industrial waste, and automotive lubricants in addition to suffer siltation and erosion processes. As a result, these characteristics point to environmental contamination, especially by domestic and industrial sewage from diffuse sources along the Mearim river. Modifications in aquatic environmental conditions, especially microbiological and chemical, influence the health of the fish, causing histological lesions, mainly in the gills, affecting the respiratory and osmoregulatory functioning of these organisms (Reis et al., 2009). In addition to affecting the growth and reproduction of individuals (Vazzoler, 1996; Santos et al., 2016).

The length and weight of the male fish collected in the reference area (A1) showed high values in the dry period; the females and males were longer and heavier during the rainy season in the contaminated area (A2). High amount of organic matter in water leads to higher volumes of feed for animals, favoring weight gain (Esenowo & Ugwumba, 2010). Animals present healthy conditions in environments that can function better than individuals living in environments with contaminants (Carvalho Neta et al., 2017; Viganò et al., 2016). In a study by Santos et al. (2016) concluded that contaminants afetaram o comprimento the length of individuals of the species *Hoplias malabaricus.* Organisms that coexist with xenobiont tend to spend more energy on detoxification processes, rather than investing energy in their reproduction (Saborido-Rey et al., 2007; Esenowo & Ugwumba, 2010).It is noteworthy that the amount of fish collected was higher in the dry season than in the rainy season in these stretches of the Mearim river.

GSI of the male and female fish collected in the reference area (A1) and in the dry season presented the highest values. In the rainy season the females collected in the contaminated area showed high values and the males collected in the reference area showed the highest values. Higher GSI values indicate a greater development of the gonads of male and female individuals of that environment (Vazzoler, 1996). The presence of contaminants in the water may interfere with the gonadal maturation of the fish, especially in sexual maturation, egg pigmentation, gonadosomatic index and gonadal stages (Hansson et al., 2014).

In relation to the gonadal stages, in the dry period females and males presented stages GS3 (mature) and GS2 (in maturation), respectively; in the rainy season females and males presented stages GS2 (in maturation) and GS1 (immature), respectively. According to Azevedo & Gomes (1943), the peak of the gonadal maturation of the *H. malabaricus* species occurs between october and november in some regions of the brazilian northeast, that may explain the higher stages of maturation of this species during the dry season in the Mearim river, different from the data found by Marques et al. (2001) in a work carried out with the same species in a Gramame river, Paraíba, Brazil, in which the individuals collected had the highest stages of maturation in the rainy season.

According to Farias et al. (2010) in the rainy season the amount of effluent discharges in river is higher, and consequently higher contamination in these environments.This may interfere in the reproductive process of the *Hoplias malabaricus* species in the Mearim river, because this species presents a split spawning, occurring the release of its oocytes in periods with less environmental turbulence, like little discharge of waste, soon avoiding the drag of their eggs and larvae, preventing the high mortality of their offspring, being a strategy of maintaining the species (Barbieri, 1989; Prado et al., 2006; Barros et al., 2016). Thus, this research may contribute to studies of morphological biomarkers using this species as a bioindicator of environmental impact in the Mearim river, as well as actions for the conservation of the aquatic ecosystems of the APA Baixada Maranhense.

5 CONCLUSIONS

The two areas are being relatively impacted, since the environmental and histological analyzes presented concerning results, however the contaminated area (A2) still stands out in relation to the reference area (A1). *H. malabaricus* species presented branchial lesions which could differentiate the health quality of the fish in these two stretches of the river Mearim.

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CONFLICT OF INTEREST

The above authors declare that they do not have any potential conflict of interest in this study.

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5.3 CAPÍTULO III: HISTOLOGICAL BIOMARKERS IN *Hoplias malabaricus* (PISCES, CHARACIFORMES, ERYTHRINIDAE) CAPTURED IN TWO STRETCHES OF THE MEARIM RIVER, BAIXADA MARANHENSE, BRAZIL[3]

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ABSTRACT

This study aimed to identify hepatic lesions in *Hoplias malabaricus* as biomarkers of anthropic impacts in the Mearim river, Baixada Maranhense, Brazil.Two stretches of the river were used as sampling sites, Engenho Grande (reference area = A1) and Curral da Igreja (contaminated area = A2). Specimens were collected at each site, as well as water for abiotic and microbiological evaluation. Two methodologies were used for histological analysis. The species collected in the two areas presented hepatic lesions, some of them cytoplasmic vacuolation, leukocyte infiltration, sinusoid dilation, peripheral nuclei, hyperemia, melanomacrophage centers. The parameters iron, pH, dissolved oxygen, turbidity, total coliforms and *Escherichia coli* presented values that were in disagreement with the legislation. The male fish collected in the reference area (A1) were longer and heavier, with statistical difference. GSI values of the fish collected in the reference area (A1), especially the males, obtained higher and significant values.The maturing peaks for both females and males were in the dry season. In this way, results indicate that the health of the species of these two stretches of the river is possibly affected by contaminants present in the region.

Key words: Aquatic biomonitoring; Hepatics lesions; Mearim river; Contamination of rivers; Baixada Maranhense; Freshwater species.

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1 INTRODUCION

The rivers are formed by a complex variety of chemical components which can come mainly from the anabolic and catabolic processes of living beings and the disposal of domestic and industrial effluents in these ecosystems (Jordão et al., 1999; Coraway et al. 2012). The rivers of the Baixada Maranhense are affected by aquatic environmental contamination, especially by the launching of toxic residues from agricultural development in the region (Gaspar et al., 2005).

The Baixada Maranhense is a Brazilian region that presents an immense terrestrial and aquatic, faunistic and floristic biodiversity, since it is one of the Brazilian legally protected areas and a member of the list of World Wetlands by the Ramsar Convention (Lima et al., 2009; Mitamura et al., 2012; Ramsar Convention Manual, 2013). Among the many important rivers for the people of the Baixada Maranhense, there is the Mearim river, used by residents for commercial and subsistence fishing in increasing development; irrigation in rice, corn and watermelon cultivacion (Cunha & Silva, 2002; Costa Neto et al., 2008; Silva & Rosa, 2012).

Nowadays, the advance in the urban process of the cities adjacent to the Mearim River has aggravated the environmental degradation of this resource, intensified by the dumping of domestic and industrial waste in the river, by the silting, by the reduction of riparian forest (Cunha & Silva, 2002). The exploration of minerals such as gypsum and bauxite in areas adjacent to the river results in a great loss of biodiversity in this resource, requiring even more studies of biomonitoring that can assist in the environmental management of the same (Lima, 2013).

Aquatic biomonitoring presents satisfactory results for the environmental management of the region, since it uses bioindicators and biomarkers in the evaluation of environmental changes in ecosystems (Mattews et al., 1982; Van Der Oost et al., 2003; Alves et al., 2016; Sadauskas-Henrique et al., 2017). The liver is a fabric widely used in studies with histological biomarkers, since it has direct contact with the contaminants and is responsible for the process of biotransformation of substances in the body, so they can be eliminated (Bervoets et al., 2013; Arantes et al., 2016). Among fish species that present studies with hepatic biomarkers, we can highlight *Hoplias malabaricus*.

The species *Hoplias malabaricus*, commonly known as traíra, is a top-chain predator, has a facility for the bioaccumulation of contaminants along the food chain

(Santos et al., 2016), besides being important in the fishing of the rivers of the Baixada Maranhense, especially the Mearim river (Novaes & Carvalho, 2011). From these characteristics, *H. malabaricus* can be used in studies with histological biomarkers, with satisfactory results for environmental monitoring in freshwater. Thus, this study aims to identify liver damage in *H. malabaricus* as biomarkers of anthropic impacts in two stretches of the river Mearim, Baixada Maranhense, Brazil.

2 MATERIALS AND METHODS

2.1 Sampling sites

Two stretches of the Mearim River (Figure 1) were selected for collection, both stretches located in the Baixada Maranhense. The first stretch was located in the village of Engenho Grande (03 ° 27 '64.1 "S, 044 ° 46' 79.7" W) (Figure 1), municipality of Vitória do Mearim, still has native flora and fauna, with little urbanization and rare anthropic activities, considered as the reference area (A1).

The second stretch of the river was located in the village of Curral da Igreja (03 ° 27 '64.1 "S, 044 ° 46' 79.7" W) (Figure 1), municipality of Arari, a town with intense urbanization and presents various anthropic actions such as agriculture, being considered as a potentially contaminated area (A2). The two villages are bathed by the river Mearim, an important aquatic resource of the region.

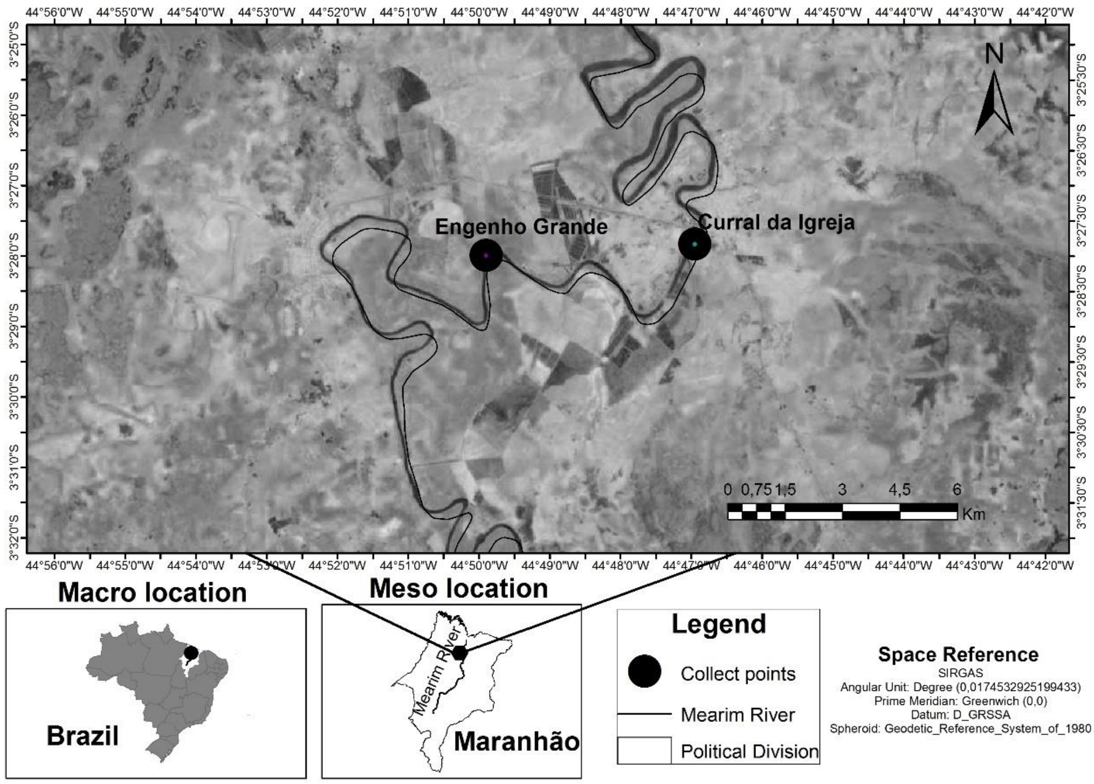


Figure 1. Map showing the sampling sites on the Mearim river (Curral da Igreja and Engenho Grande, Maranhão, Brazil).

2.2 License and ethics declaration

The taking of fish samples was authorized by a permit issued by the State Department of Natural Resources and Environment (SEMA, 18208//2014). The protocol was ap- proved by the Ethics Committee of Maranhão State University (13/2017 CRMV-MA) and met the guidelines of the Brazilian College for Animal Experimentation (COBEA; http://www.cobea.org.br).

2.3 Sampling of *H. malabaricus*

We sampled 91 fish specimens, being 56 males and 35 females, 54 specimens collected in the dry period and 37 in the rainy season. Sampling was performed in 2016 and 2017, twice in 2016 (August and November) during the dry season and in may/2016 and june/2017 during the rainy season.

2.4 Enviromental and microbiological parameters

Physicochemical parameters-calcium, magnesium, hardness, alkalinity in OH-, alkalinity in CO3-, alkalinity in HCO3-, total alkalinity, chlorides (Cl-), conductivity, total solids dissolved, NaCl, pH, turbidity, dissolved oxygen, nitrite, nitrate, iron-were measured at each site during the dry and rainy season when fish were sampled.This measurement was performed through laboratory analysis.

Microbiological characteristics - the most probable number of total coliforms and *Escherichia coli* were analyzed by the Colilert method through the presence or absence and quantification of these characteristics in the sample.

2.5 Biometric data

Biometric data of individual *H. malabaricus* specimens-total length (TL), standard length (SL), body weight (BW) and gonads weight (GW) - were recorded. All fish were juveniles, and gonads were categorized macroscopically into four classesonly in accordance with Vazzoler (1996): immature (GS1) or maturing (GS2), mature (GS3) and exhausted (GS4).

2.6 Histopathological and gonadossomatic analysis

Fragments of liver all fish were removed, and placed in 10% formaldehyde for approximately 1 and 2 days. Slides with cuts of approximately 5µm thickness were stained with Hematoxylin and Eosin (HE) (Luna, 1968) for analysis under an optical microscope.

The histological changes were observed displaying the methodology as suggested by Poleksic & Mitrovic - Tutundzic (1994) and Bernet et al. (1999). The Poleksic & Mitrovic - Tutundzic (1994) methodology calculates the index of histological changes (AHI) of each liver analyzed, calculated by the formula IAH: $1 \times \Sigma\, I + 10 \times \Sigma\, II + 100 \times \Sigma\, III$, where I, II and III indicate the degree of severity of the lesions observed. The methodology of Bernet et al. (1999) also calculates the rate of change for each animal observed, calculated by the formula: $Iorg\Sigma rp = \Sigma\, alt\, (a.w)$ being rp the reaction pattern, alt alterations found and w the importance factor of the lesion.The gonadosomatic index

(GSI) was calculated according to Vazzoler (1996): GSI = 100 x GW / TW, where GW is the weight of the gonads and TW is fish total weight.

2.7 Statistical analysis

Biometric data, gonadosomatic indexes obtained and values of histological changes index are expressed as mean ± standard deviation. Stages gonadais and histological lesions found are presented in percentage. Significant differences between groups were checked by the t-test and $p < 0.05$ accepted as significant. The analysis of similarity of the liver lesions between the studied areas was observed through Cluster analysis, based on the values in paired groups (similarity) measured by Bray-Curtis being VAC: cytoplasmic vacuolization, INF: inflammatory infiltrate, DILV: vessel dilatation, HIP: hyperemia, CEN: melanoma center, NUC: nuclei in the periphery, DILS: dilatation of sinusoids, NEC: necrosis.

3 RESULTS

3.1 Environmental conditions

The abiotic and microbiological data of the collected water samples are shown in table 1. The turbidity showed higher values in the contaminated area (A2) and in the dry season. Conductivity and alkalinity also had its highest values in the contaminated area (A2); pH and dissolved oxygen presented lower values those permitted by legislation in two areas.

Regarding the chemical compounds examined, calcium and magnesium had higher values in the reference area (A1) and during the rainy season. Dissolved solids presented higher values in the contaminated area (A2) in all periods. The iron presented values not allowed by the legislation in both areas and periods, with the highest values in the contaminated area (A2). The nitrate remained constant in both areas and during both periods, as well as NaCl that did not show variations during the periods.

Microbiological analyzes determined the most probable number of total coliform showed higher values in the contaminated area (A2) in the rainy season. *Escherichia coli* showed higher values in the contaminated area (A2) in during the dry season.

Table 1. Environmental analysis of the water collected in two stretches of the river Mearim, Maranhão, Brazil.

Parameters	**Reference (A1)**				**Contaminated (A2)**				**Brazilian Law CONAMA (2005/2011)**
	Dry		Rainy		Dry		Rainy		
	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	
Calcium (mg/L CaCO3)	0	0	1.058	0	0	0	561.6	0	-
Magnesium (mg/L CaCO3)	0	0	1.058	0	0	0	561.6	0	-
Alkalinity total (mg/L CaCO3)	62	32	0	40	90	34	0	38	-
Chlorides (Cl-) (mg/L Cl-)	32.53	31.9	40.78	6.9	35.32	51.9	44.8	8.9	250 mg/L Cl-
Conductivity (µJ/cm)	470	74.6	115.7	-	606	104.9	116	-	-
Total dissolved solids (ppm)	231	37.4	58	-	303	52.6	60	-	-
NaCl (%)	1	0.2	0.2	-	1.3	0.2	0,2	-	-
pH	6.2	5.21*	6.8	6.2	6	5.67*	6,8	6.18	6.0 a 9.0
Dissolved oxygen (mg/ L O_2)	0.7*	0*	0.3*	10.8	0.6*	0*	0,3*	14.3	< 5,0 mg/ L O_2
Turbidity (U.N.T)	26.86	369*	1.75	0.51	113*	376*	1.87	0.73	100.0 U.N.T
Nitrite (mg/L N)	0	0	0	0	0	0	0	0	1.0 mg/L N
Nitrate (mg/L N)	4.43	0	2.21	0	4.43	0	2.21	0	10,0 mg/L N
Iron (mg/L Fe)	2.61*	1.66*	2.13*	0.68*	5.01*	2.53*	3.77*	0.12	0,3 mg/L Fe
T. coliforms (NMP)	3.410*	2.481*	24.196*	4.352*	5.940*	12.033*	24.196*	9.208*	1.000/NMP/100 mL
Escherichia coli	900	175	20	20	1730*	816	41	108	1.000/NMP/100 mL

*Values in deformity of the allowed by resolution CONAMA 357/2005, fresh water, class II.

3.2 Biometric parameters, gonadosomatic index (GSI) and gonadal stadiums

The values of the biometric analysis and the gonadosomatic index are presented in table 2 and 3, gonadal stadiums in table 4. Regarding the dry period, the males collected in the reference area (A1) presented higher values of weight and length with significance by the test t (Student). The females collected in the contaminated area (A2) were shown to be longer and heavier.

Regarding the rainy season, females and males collected in the contaminated area (A2) were larger and heavier. GSI presented in the dry period, higher values in the reference area (A1) for men and women, the values for women being significant by t test (Student). In the rainy season, the females of the contaminated area (A2) presented the highest value, while the males in the reference area (A1).

The data from the gonadal stages are shown in table 4. During the dry period, the females of the GS3 stage and the males of the GS2 stage were more observed. In relation to the rainy season, females of stage GS2 and males of stage GS1 were more verified.

Table 2. Biometric data of the *H. malabaricus* collected in the dry season in the two stretches of the Mearim River, Maranhão, Brazil.

Parameters	A1 (reference area)		A2 (contaminated area)		t Test (females / males)
	Females	Males	Females	Males	
LT (cm)	23,32 ± 3,04	26,31 ± 3,91	25,18 ± 3,87	19,96 ± 3,34	0,26 / 0,00012*
LP (cm)	18,36 ± 2,31	20,81 ± 2,90	21,02 ± 2,73	16,10 ± 2,88	0,039* / 0,00051*
WT (g)	131,6 ± 52,83	198,42 ± 120,87	172,12 ± 91,20	101,46 ± 73,12	0,25 / 0,0099*
WG (g)	5,23 ± 2,86	0,88 ± 0,99	3,06 ± 1,37	0,36 ± 0,21	0,06 / 0,013*
GSI	4,01 ± 1,69	0,61 ± 0,59	2,04 ± 1,32	0,41 ± 0,27	0,015* / 0,19

*It indicates statistical difference in relation to the reference area ($p < 0.05$). LT: total length; LP: standard length; BW: body weight; WG: weight of gonads; GSI: gonadosomatic index; cm: centimeters; g: grams.

Table 3. Biometric data of the *H. malabaricus* collected in the rainy season in the two stretches of the Mearim river, Maranhão, Brazil.

Parameters	A1 (reference area)		A2 (contaminated area)		t Test (females / males)
	Females	Males	Females	Males	
LT (cm)	16,4 ± 0	16,07 ± 0,87	23,6 ± 1,86	21,46 ± 2,23	0 / 0,00020*
LP (cm)	12,3 ± 0	12,87 ± 0,99	18,42 ± 1,53	17,56 ± 1,66	0 / 0,00004*
WT (g)	48 ± 0	50,75 ± 7,97	140,46 ± 24,40	121,43 ± 38,71	0 / 0,0022*
WG (g)	0,36 ± 0	0,87 ± 0,69	2,31 ± 0,93	1,30 ± 1,44	0 / 0,57
GSI	0,75 ± 0	1,84 ± 1,55	1,66 ± 0,71	0,95 ± 0,81	0 / 0,12

*It indicates statistical difference in relation to the reference area ($p < 0.05$). LT: total length; LP: standard length; BW: body weight; WG: weight of gonads; GSI: gonadosomatic index; cm: centimeters; g: grams.

Table 4. Stage gonadal maturation of males and females *H. malabaricus* collected during the rainy season and dry in Mearim river, Maranhão, Brazil.

GS	Dry		Rainy	
	Females	Males	Females	Males
GS1	10%	40%	37%	90%
GS2	32%	57%	63%	10%
GS3	58%	0%	0%	0%
GS4	0%	3%	0%	0%

GS: Gonadal stages. GS1 (immature), GS2 (maturing or resting), GS3 (mature) and GS4 (exhausted) (Vazzoler, 1996).

3.3 Hepatic histological changes

The frequencies and severities of hepatic lesions according to the methodologies used are presented in figure 2 and 3. Some images of liver lesions are presented in figure 4.

According to the methodology proposed by Bernet et al. (1999) it was possible to identify more frequently the change of deposits in the two study areas, being classified as regressive changes and level of severity 1. Followed by leukocyte infiltration lesions and structural changes which showed high frequencies in the reference area (A1) and in the contaminated area (A2), respectively.The leukocyte infiltration grouped into the inflammation group and severity level 2. The architectural and structural changes included in this group dilation of the vessel and dilatation of the sinusoids, characterized as regressive changes and level of severity 1.In addition to these changes were observed in the liver the presence of vacuolated, nuclei in the periphery and melanomacrophages centers.

Regarding the methodology proposed by Poleksic & Mitrovic-Tutundzic (1994) cytoplasmic vacuolation and nuclei in the periphery had the highest frequency in both study areas, both in the group changes in hepatocytes and severity level 1. Note that some liver lesions were classified in this methodology, since they were not found in it, among them, the injuries dilation of sinusoids dilated vessel and leukocyte infiltration.

The values of histological changes index are shown in graphic 1. According to the methodology proposed by Bernet et al. (1999), the highest value (9) was presented in the dry period and for the reference area (A1), methodology for Poleksic & Mitrovic-Tutundzic (1994) the largest value (30.1) was made in the rainy season and to a contaminated area (A2); indicating the existence of changes with low and moderate levels of severity in the organ.

Cluster analysis (analysis of similarity) formed differentiated histological groups with lesions of mild to moderate levels of severity during the rainy and dry periods in Engenho Grande (A1) and Curral da Igreja (A2). The groups formed with great similarity were inflammatory infiltrate (INF) and vessel dilation (DILV); nucleus at the periphery (NUC) and dilation of sinusoids (DILS).

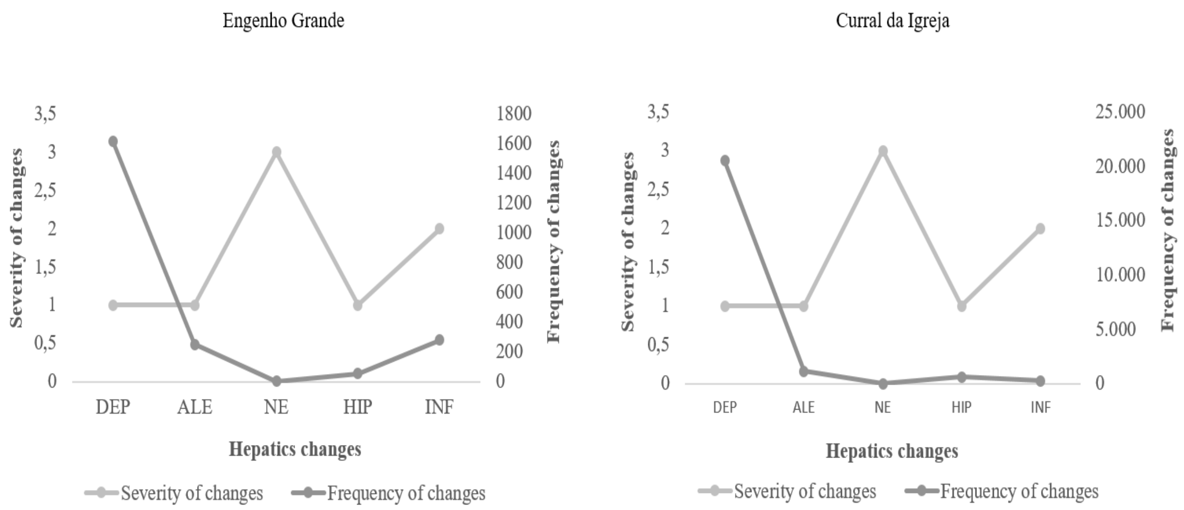


Figure 2. Frequency and severity of *Hoplias malabaricus* hepatic alterations in two sections of the Mearim river, Baixada Maranhense, Brazil, according to Bernet et al. (1999). DEP (deposits); ALE (structural changes); NE (necrosis); HYP (hyperemia); INF (leukocyte infiltration).

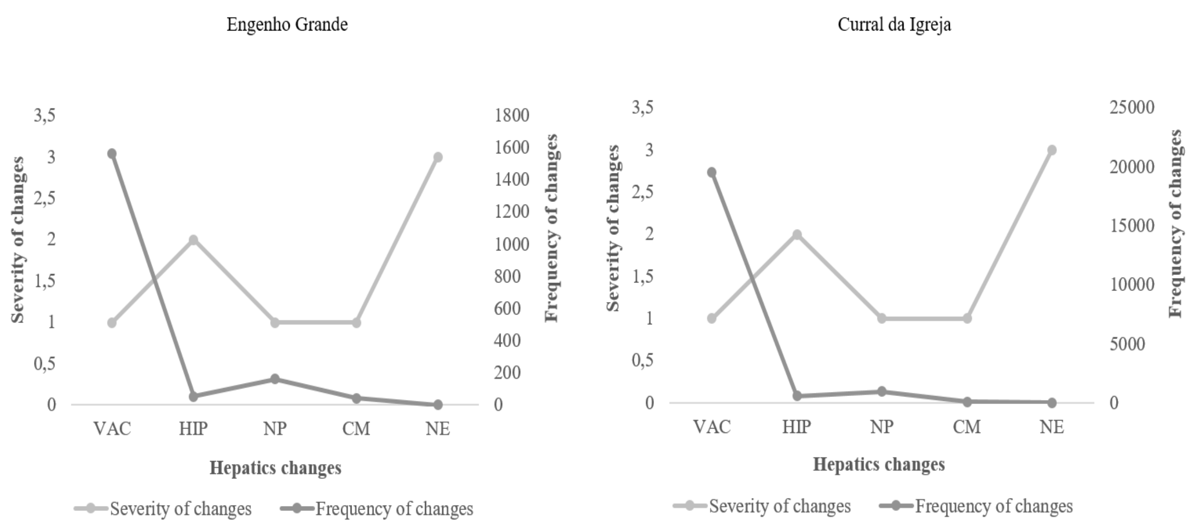


Figure 3. Frequency and severity of *Hoplias malabaricus* hepatic alterations in two sections of the Mearim river, Baixada Maranhense, Brazil, according to Poleksic & Mitrovic-Tutundzic (1994) methodology. VAC (cytoplasmic vacuolization); HYP (hyperemia); NP (nuclei in the periphery); MC (melanomacrophage center); NE (necrosis).

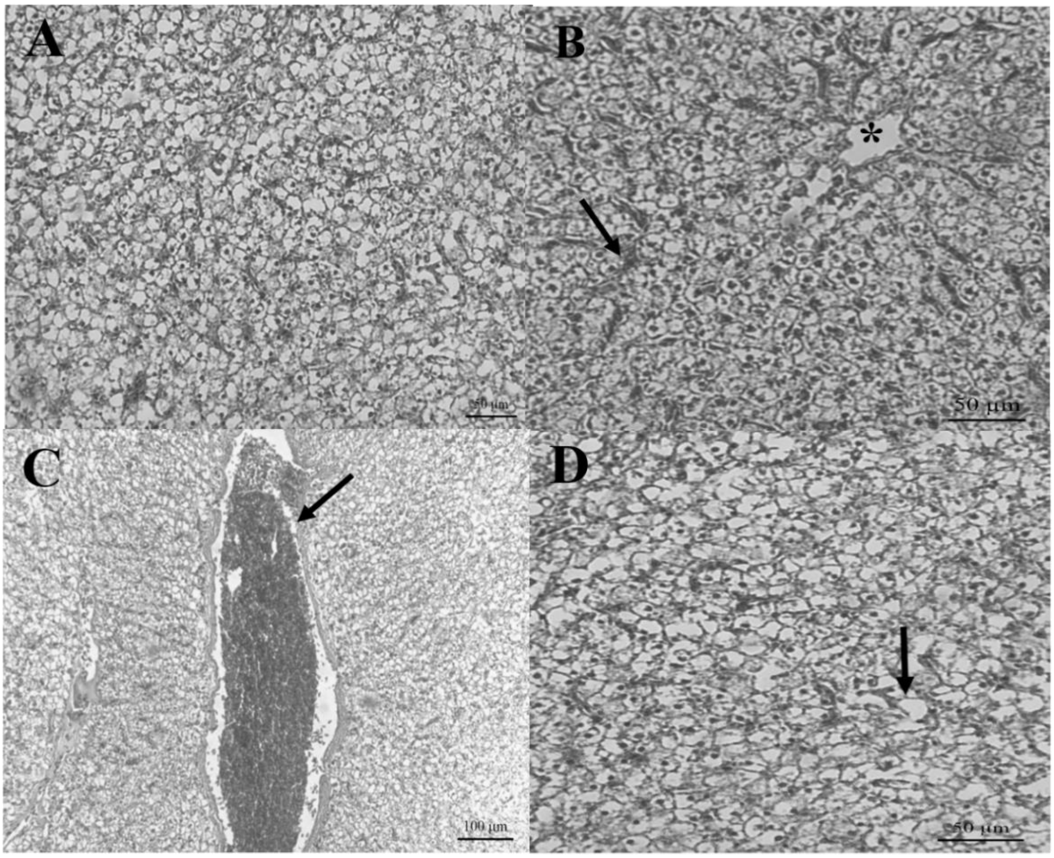
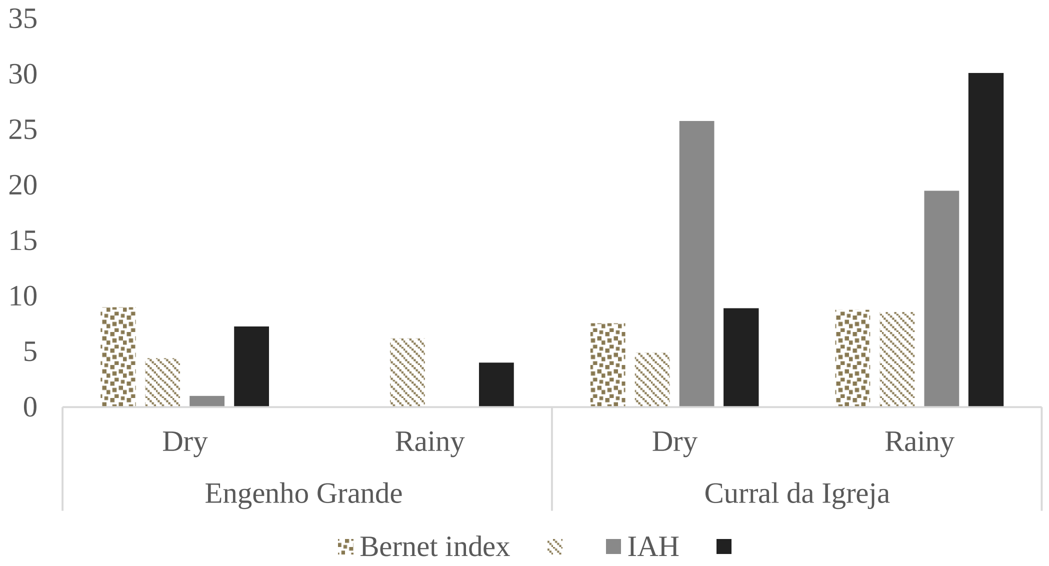


Figure 4. Liver histological alterations of *H. malabaricus*. A- normal tissue; B- vessel dilation (asterisk), sinusoids dilation (arrow); C- hyperemia (arrow); D- cytoplasmic vacuolation (arrow). A, B, D: 50 µm; C: 100 µm.

Graphic 1. Index values of Poleksic & Mitrovic - Tutundzic (1994) (IAH) and Bernet et al. (1999) for the hepatic lesions in dry and rainy periods.



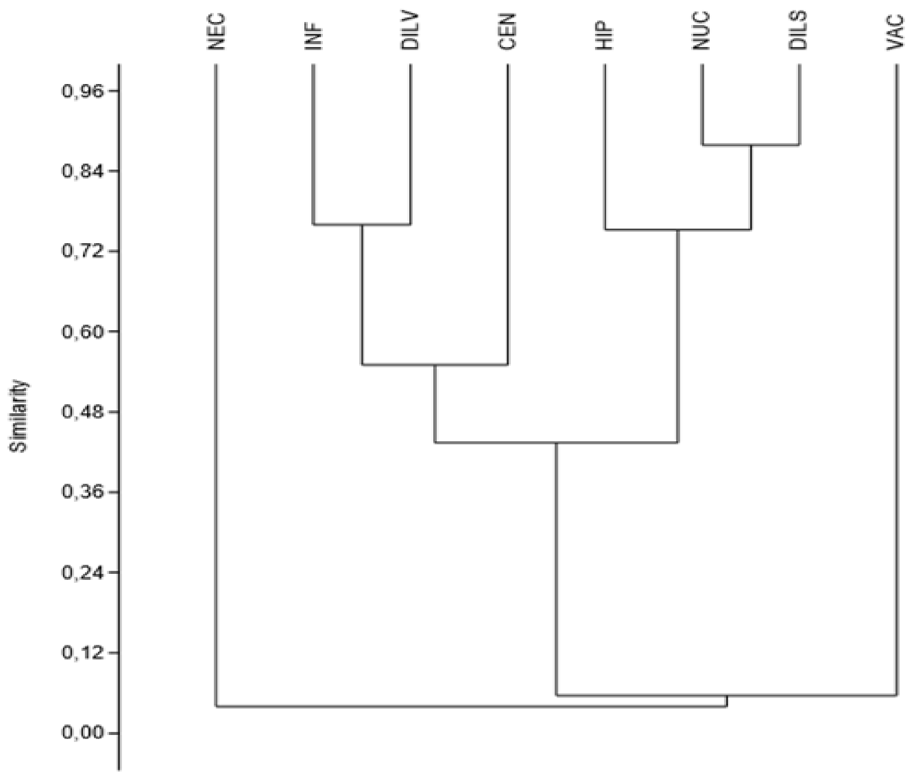


Figure 5. Cluster analysis of hepatic lesions observed in fish collected during the rainy and dry periods in Engenho Grande (A1) and Curral da Igreja (A2), Maranhão, Brazil. VAC: cytoplasmic vacuolization, INF: leukocyte infiltration, DILV: vessel dilatation, HIP: hyperemia, CEN: melanomacrophage center, NUC: nuclei in the periphery, DILS: dilatation of sinusoids, NEC: necrosis.

4 DISCUSSION

Hepatic alterations were observed in *Hoplias malabaricus* in the present study. They were cytoplasmic vacuolation, leukocyte infiltration, vessel dilation, hyperemia, melanomacrophage center, peripheral nucleus, sinusoidal dilatation and necrosis, presenting different levels of severity according to the methodologies of Poleksic & Mitrovic-Tutundzic (1994) and Bernet et al. (1999), indicating the presence of pollutants in these two stretches of the Mearim river, affecting the liver of these animals.

The liver is critical for fish since it is responsible for the basic functions of metabolism, such as biotransformation, the accumulation and excretion capacity of xenobiotics (Gingerich & Dalich, 1982; Bambonato et al., 2007; Dyk et al., 2012; Dane & Sisman, 2014), besides considered the main target of toxic substances in the organism, being important for studies using biomarkers of environmental contamination (Zelikoff, 1998; Hermenean et al., 2015).

Liver functions are performed primarily through hepatocytes, fibers and blood vessels, with hepatocytes being uninucleated cells essential in metabolic control, fibers being structures that ensure tissue support and blood vessels including sinusoids are responsible for vascularization (Hibiya, 1982). This gland has other components such as the bile ducts; the pancreatic tissue and melanomacrophage centers (Bombonato et al., 2007). In this sense, the xenobionts affect, firstly, the components of the hepatic tissue, and can cause histological alterations, being the effect of the lesions dependent on the duration and level of concentration of the contaminant in the liver, resulting in a metabolic imbalance in fish (Zelikoff, 1998; Jesus & Carvalho, 2008).

These methodologies present severity levels of severity for each lesion found in the organ, with differences: the Bernet et al. (1999), uses the extent of the change in the organ, that is, how much the lesion is found in that organ; the methodology of Poleksic & Mitrovic - Tutundzic (1994) presents a larger number of lesions that can be found in the organ. Thus, both methods are effective in assessing changes, but proposed by Bernet et al. (1999) is more complete, since it uses the degree of extension changes, beyond the level of severity to calculate the index of histological changes.

Vacuoluzation and nuclei in the periphery (steatosis) were frequent lesions in this study and has a low level of severity according to methods used for analysis, the Bernet et al. (1999) methodology included in the lesion deposits that characterize intracellular accumulations of substances. They show a likely accumulation of lipids in hepatocytes related to environmental contamination by heavy metals, warning the existence of degenerative processes and metabolic problems (Takashima & Hibiya, 1995; Pacheco & Santos, 2002; Figueiredo-Fernandes et al., 2007; Velma & Tchounwou, 2010).

High concentrations of fat in the liver decrease the amount of glycogen and protein synthesis, causes disruption of microtubules, changes in substrate utilization and displacement of the cell nucleus to the periphery of the cell (steatosis), impairing the metabolic functions performed by hepatocytes (Santos et al., 2004; Ahmed et al., 2013). Similar works using hepatic biomarkers also verified these lesions (Pedroso et al., 2007; Rocha et al., 2010; Paulino et al., 2014; Hermenean et al., 2015; Tabassum et al., 2016).

Leukocyte infiltration characterized by leukocyte proliferation in blood vessels is commonly present in the liver tissue of organisms exposed to environmental stress situations (Santos et al., 2004; Rodrigues et al., 2017). This lesion is considered first immunological response with a moderate level of severity by the Bernet et al. (1999), which can progress to tissue necrosis (Ding et al., 2010; Javed et al., 2016), not found in the methodology of Poleksic & Mitrovic-Tutundzic, 1994.

Necrosis is characterized by tissue death and represents a severe level of severity, being irreversible for the organism (Poleksic & Mitrovic-Tutundzic, 1994; Bernet et al., 1992). This lesion is frequent in the liver of fish exposed to chemical agents, like metals, for example copper (Figueiredo-Fernandes et al., 2007). Herbicides at elevated temperatures can cause synergistic effects in fish liver provoking extensive areas of necrosis (Salazar-Luzo et al., 2011). Heavy metals and pesticides help in the formation of free radicals in the cell, intensifying tissue necrosis (Ibuki &Goto, 2002; Azzalis et al., 1995; Abdel-Monein et al., 2012). Castro et al. (2014) in a study carried out with *Hoplias malabaricus* in the Ambude river and Lagoa Serena in a Protected Area in Maranhão, also detected these hepatic lesions as an immunological response of the fish to the xenobiotic compounds present in the environment.

Dilation of vessels and sinusoids are characterized as low level of severity by the two methodologies. In the methodology of Bernet et al. (1999) was included in the architectural and structural changes. These hepatic structures may exhibit structural modifications due to the contact of fish to high concentrations of metals and pesticides in water (Cengiz & Unlu, 2003; Albinati et al., 2017). If the perturbation is continuous and intense, the liver changes may progress to bleeding, intensified by high blood pressure in the blood vessels (Javed et al., 2016).

In this context, in relation to circulatory problems, the lesion found in the present study was hyperemia, representing low and moderate levels of severity by the methodology of Bernet et al. (1999) and Poleksic & Mitrovic-Tutundzic (1994), respectively.This change is a direct consequence of the enlargement of the tissue blood vessels causing accumulation of blood inside these vessels (Hibiya, 1982; Cengiz & Unlu, 2003). Marcon et al. (2015) and Kosti'c et al. (2017) performed studies with liver biomarkers for biomonitoring in fish, and found the lesions hyperemia and circulatory disorders.

The melanomacrophage center presents a low level of severity in both methodologies. In the method suggested by Bernet et al. (1999) this damage was included in the change deposits which designates the intracellular accumulations of substances.These centers are components of the normal hepatic tissue of the fish, which help in the accumulation of various compounds in the liver, among them lipofucsin, melanin, cersoid and hemosiderin (Bombonato et al., 2007).They are fundamental in the process of defense of the fish, showing immune effects against xenobiotic agents to the organism (Hartley et al., 1996). Changes in the number and size of melanomacrophages center in the liver may result in nutritional animal processes, age, health and environmental stresses (Ferguson, 1989; Hartley et al., 1996). Several studies indicate the cause of the increase of these structures in the liver of the fish the environmental contamination (Flores-Lopes & Malabarba, 2007; Poleksiv et al., 2010; Ahmed et al., 2013; Hermenean et al., 2015). Through the data of the liver lesions the indexes of histological alterations were calculated.

The highest indexes of histological changes were in the dry season for the reference area (A1) and in the rainy season for the contaminated area (A2), resulting in mild and moderate severity lesions. High total amounts of the histological index in the rainy season was found by Ballesteros et al. (2017) when analyzing the morphological biomarkers in the species *Jenynsia multidentata* fish in an Argentine river. Kosti´c et al. (2017) when they performed a study using fish liver biomarkers performed in the Sava river in the city of Belgrade, Serbia and observed high and significant values of the hepatic index during the dry season, arguing that the increase of contaminants in the river Sava and the increase of the metabolic rate of some species of fish that period may have influenced the presence of histological damage. Cluster analysis was performed to compile the index data in this article.

The cluster analysis indicated the formation of histological groups, also with lesions of mild to moderate severity levels for the wet and dry periods in the two areas.Although hepatic lesions are moderate, the toxic effects of organ contaminants are continuous (chronic), affecting the body functioning of the individual and compromising the sanity of the fish (Costa et al., 2009; Flores-Lopes & Thomaz, 2011; Ghisi et al., 2017). Values of abiotic parameters are essential in the analysis of possible interferences and causes of these lesions.

Iron values were higher than those permitted by Brazilian legislation, which is up to 0.3 mg/L, mainly in the contaminated area (A2) (Conama, 2005). This may have influenced the presence of hepatic changes in fish these regions (Hermenean et al., 2015), since it is one of the key chemicals in biological processes, however, when high concentrations may be toxic to animals (Heath, 1995). This can result in respiratory toxicity, reproductive and genetic damage, inflammation in the tissues, inhibition of ion channels, oxidative stress, and neurological disorders (Singh et al., 2010; Kadar et al., 2011; Chen et al., 2012; Khatri et al., 2017). Note that this metal is usually found in the soil of the region, since it is a constituent element of the geological formation of the Mearim (Lima, 2013).

The production of proteins essential for the proper functioning of the organism (Bombardelli et al., 2003), the pH of the water has a close relation with the dissolved oxygen (He et al., 2011). Dissolved oxygen was other abiotic data; in some water sampling values presented in disagreement with the Brazilian law which is greater than 5.0 mg/L (Conama, 2005). Lower amount of dissolved oxygen in water is of concern since this interferes with vital processes in aquatic animals, impairing photosynthesis and respiration (He et al., 2011). Other abiotic data which presented values above that allowed by the legislation were turbidity, total coliforms/*Escherichia coli* that are 100 U.N.T and 1000 NMP/100 mL, respectively. Excess nutrients, dissolved solids and bacteria in the water influence the high values of turbidity, affecting the trophic conditions of the ecosystem and the sanity of the fish, affecting growth and reproduction (Esenowo & Ugwumba, 2010; Shi et al., 2017).

The length and the body weight of male fish collected on the reference area (A1) showed high values during the dry period; females and males were longer and heavier during the rainy season in the contaminated area (A2). The growth of individuals of the species *H. malabaricus* is affected in a contaminated environment (Santos et al., 2016). Organisms living with xenobiotics spend more energy in detoxification processes, than in growth and reproduction processes (Saradha & Mathur, 2006; Saborido-Rey et al., 2007; Esenowo & Ugwumba, 2010). Chemicals in the water can affect the processes of fish gonads and sexual maturation, pigmentation of eggs and gonads gonosomatic stages (Hansson et al., 2014). The GSI of male and female fish listed in the reference area (A1) in the dry season had higher values; females collected in the contaminated area (A2) and males listed in the reference area (A1) had high values in the rainy season. High GSI values indicate greater and better gonadal maturation of males and females in this environment (Vazzoler, 1996).

Regarding gonadal stages in the dry period females and males showed GS3 stages (mature) and GS2 (maturing), respectively; in the rainy season, males and females showed GS2 stages (maturing) and GS1 (immature), respectively. According to Azevedo & Gomes (1943), the period of gonadal maturation of the *H. malabaricus* species occurs between October and November in some regions of the Brazilian northeast, clarifying the higher maturation stages of this species during the dry season in the Mearim river. However, in studies conducted by Marques et al. (2001) with the same species in the Gramame river, Paraíba, Brazil, the individuals collected had the highest maturation stages in the rainy season.

The effluent load in the river is higher in the rainy season. One reason is the ease of dissolution of the solid particles in the water (Farias et al., 2010; Horta-Puga et al., 2013). This may interfere with the reproductive process of *Hoplias malabaricus* in the Mearim river, since it prefers to release its oocytes in periods with less environmental turbulence, such as little effluent discharge, avoiding the trawl of its eggs and larvae, preventing the high mortality of its offspring, being a strategy of maintenance of the species (Barbieri, 1989; Barros et al., 2016).Therefore, this research may support future studies with histological biomarkers using fish liver to analyze anthropic impacts on the

Mearim river, in addition to contributing to the biomonitoring of the aquatic resources of the Baixada Maranhense.

5 CONCLUSIONS

From the results, the two areas are relatively contaminated, but the reference area (A1) is still less impacted.Thus, *Hoplias malabaricus* species collected in the two study sites showed histological damage to the liver, demonstrating be a suitable biomarker for evidence disturbing environmental conditions these stretches Mearim the river.

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CONFLICT OF INTEREST

The above authors declare that they do not have any potential conflict of interest in this study.

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6 CONCLUSÃO

As conclusões obtidas com este trabalho são:

- A análise dos artigos científicos publicados entre os anos de 2007 a 2017 em três bases de dados mostra que as metodologias que utilizam espécies de peixes como bioindicadores de contaminação ambiental em rios são amplamente aplicadas em estudos acadêmicos, pois elas têm resultados eficientes no monitoramento da região, além de um menor custo; as espécies *Oreochromis niloticus* e *Cyprinus carpio* apresentam resultados muito mais satisfatórios para certos rios, porque se adaptam mais a locais contaminados, sendo mais resistentes; os ambientes dulcícolas mais contaminados são mais investigados, e consequentemente mostram mais estudos científicos; e os biomarcadores enzimáticos são muito úteis e eficazes em pesquisas de peixes como bioindicadores;

- Várias lesões branquiais foram observadas em *Hoplias malabaricus*coletadas em Engenho Grande e Curral da Igreja, tais como: fusão lamelar, deslocamento do epitélio, aneurisma, congestão, hiperplasia, desorganização lamelar e proliferação das células de muco, constatando em uma possível contaminação do rio Mearim;

- Algumas lesões hepáticas foram encontradas em *Hoplias malabaricus* coletadas em Engenho Grande e Curral da Igreja, dentre elas: vacuolização citoplasmática, infiltração leucocitária, dilatação dos vasos, dilatação dos sinusóides, hiperemia, centro de melanomacrófagos, núcleo na periferia, necrose, sugerindo contaminação aquática do rio Mearim nestes dois trechos;

- As análises abióticas e microbiológicas mostraram impactos dos ambientes estudados o que reforça o estudo conduzido com o uso de peixes para o monitoramento desses ambientes aquáticos;

- Os dados biométricos mostraram que os machos da espécie *Hoplias malabaricus* coletados na área de referência (A1) durante o período chuvoso apresentaram mais compridos e pesados, com diferença significativa através do Test t Student. E as fêmeas e machos coletados na área contaminada (A2) apresentaram valores maiores de comprimento e peso;

- O GSI indicou que os peixes coletados na área de referência (A1) apresentaram valores elevados no período seco, com significância para as fêmeas através do Test t Student; enquanto que no período chuvoso, maiores valores para as fêmeas e machos coletados na área contaminada (A2) e na área de referência (A1), respectivamente;

- Os índices histológicos utilizados e a análise de Cluster branquial e hepática indicaram a prevalência de alterações com níveis leves e moderados de severidade, mesmo assim, estas lesões podem afetar o fisiologia dos peixes;

- *H. malabaricus* foi considerado bioindicador nos locais de estudos propostos por apresentar alterações histológicas dos órgãos estudados, o que indica preocupação com as condições dos recursos hídricos em questão.

- Engenho Grande (A1) e Curral da Igreja (A2) estão sendo impactadas, uma vez que as análises ambientais e histológicas apresentaram resultados preocupantes, no entanto, a área contaminada (A2) ainda se destaca em relação à área de referência (A1);

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