UNIVERSIDADE FEDERAL DO PARANÁ

LUIZA SANTOS BARRETO

ESTUDO ECOTOXICOLÓGICO E DE IMPLICAÇÕES EM DINÂMICA POPULACIONAL DE ESPÉCIES DE PEIXES AMEAÇADAS DE EXTINÇÃO APÓS EXPOSIÇÃO À ÁGUA DO RIO ATUBA/CURITIBA-PR

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Orientador: Prof. Dr. Ciro Alberto de Oliveira Ribeiro

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A outorga do título de mestre está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 12 de Março de 2019.

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RESUMO

A poluição proveniente de efluentes lançados nos corpos hídricos é uma das principais causas da perda de biodiversidade aquática, onde os ensaios ecotoxicológicos se apresentam como uma ferramenta valiosa de avaliação dos riscos para a biota nesses ecossistemas. O objetivo desse trabalho foi avaliar o efeito tóxico da água de um rio altamente influenciado por despejos urbanos (Rio Atuba, Curitiba/PR) para os estágios iniciais de desenvolvimento do dourado (*Salminus brasiliensis*), curimbatá (*Prochilodus lineatus*), jundiá (*Rhamdia quelen*) e pintado (*Pseudoplatystoma corruscans*). Essas quatro espécies de peixe são nativas brasileiras e apresentam variáveis níveis de conservação e vulnerabilidade. Os embriões das quatro espécies foram expostos de 8 horas pós fertilização (hpf) até 96 hpf à água do rio Atuba, coletada à jusante do despejo da maior estação de tratamento de esgoto da Região Metropolitana de Curitiba (ETE Atuba Sul), em concentrações de 30% e 50%, mais o grupo controle. As taxas de sobrevivência e deformidades foram verificadas a cada 24 horas. Os estágios iniciais de desenvolvimento do dourado e do curimbatá apresentaram as maiores taxas de mortalidade em todas as diluições da água do rio Atuba, mostrando ser altamente sensíveis aos poluentes presentes nos despejos urbanos. Através de modelagem matemática baseada no indivíduo, foi possível avaliar o risco de exposição para as populações dessas duas espécies, mostrando que condições semelhantes de poluição poderiam extinguir suas populações em poucos anos de exposição, considerando diferentes cenários de sensibilidade dos juvenis e adultos. O embriões e larvas do jundiá e do pintado expostos à água do mesmo rio mostraram ser mais resistentes aos eventos de mortalidade, mas assim como as outras duas espécies, também apresentaram alta frequência e severidade de deformidades, reforçando a toxicidade das amostras de água testadas. Os resultados enfatizam a importância de testar a sensibilidade de diferentes espécies nativas e aplicar modelos teóricos para fins de conservação da biodiversidade e da qualidade dos corpos hídricos neotropicais.

Palavras-chave: Ecotoxicologia. Estágios iniciais de desenvolvimento. Espécies nativas. Modelagem ecológica.

ABSTRACT

Pollution from effluents discharged into water bodies is a major cause of aquatic biodiversity loss and ecotoxicological testing represents a valuable tool for assessing the risks to the biota in these ecosystems. The objective of this study was to evaluate the toxic effect of the water from a river highly influenced by urban effluents (Atuba River, Curitiba, Paraná state) for the early stages of development of dourado (*Salminus brasiliensis*), curimbatá (*Prochilodus lineatus*), jundiá (*Rhamdia quelen*), and pintado (*Pseudoplatystoma corruscans*). These four Brazilian native species present variable levels of conservation and vulnerability. The embryos of the four species were exposed from 8 hours post fertilization (hpf) to 96 hpf to the Atuba River water, collected downstream of the largest wastewater treatment plant in the Metropolitan Region of Curitiba (ETE Atuba Sul), Southern Brazil, at concentrations of 30% and 50%, plus the control group. Survival rates and deformities were checked every 24 hours. The early life stages of dourado and curimbatá presented the highest mortality rates in both dilutions of river water, showing to be highly sensitive to the pollutants present in the effluents. Through individual-based mathematical modeling, it was possible to evaluate the risk of exposure to the populations of these species, showing that similar pollution conditions could extinguish their populations in only a few years of exposure, considering different sensitivity scenarios of juveniles and adults. The embryos and larvae of jundiá and pintado exposed to the river water showed to be more resistant to mortality events, but like the other two species, they also presented high frequency and severity of deformities, reinforcing the toxic role of the water samples tested. The results emphasize the importance of testing the sensitivity of different native species and using theoretical models for the conservation of biodiversity and the quality of Neotropical water bodies.

Keywords: Ecotoxicology. Early life stages toxicity. Native species. Ecological modeling.

SUMÁRIO

1. INTRODUÇÃO GERAL

1.1. POLUIÇÃO DOS RECURSOS HÍDRICOS

A água é um recurso natural abundante e fundamental para a manutenção da vida e dos ecossistemas terrestres. No entanto, apenas 0,0067% de toda a água no planeta se encontra efetivamente disponível para uso em rios e lagos, representando a principal fonte de abastecimento de água potável para a população humana (SHIKLOMANOV, 1993; CASSARDO; JONES, 2011; DU PLESSIS, 2017). Contraditoriamente, estes ambientes aquáticos também são o principal destino para o lançamento e descarte de efluentes decorrentes das atividades humanas, como os despejos urbanos. Ao longo da história recente, tais despejos têm causado a poluição química dessas águas e o comprometimento da qualidade e disponibilidade desse recurso já bastante limitado no planeta. Considerando ainda o crescimento populacional e o aumento do consumo *per capita*, o problema tem ganhado proporções cada vez maiores devido à quantidade crescente de efluentes e sedimentos que são carreados para esses corpos hídricos (BRAGA et al., 2005).

A autodepuração se refere a um fenômeno natural de recuperação de rios e lagos que transforma compostos orgânicos em substâncias inertes e não prejudiciais do ponto de vista ecológico por meio de processos físicos (diluição e sedimentação), químicos (oxidação e estabilização) e biológicos (decomposição), restabelecendo um equilíbrio no meio aquático. No entanto, a decomposição da matéria orgânica poluidora envolve a ação de bactérias e o consumo de oxigênio pelas mesmas, fazendo com que esses sistemas aquáticos apresentem uma capacidade de assimilação de nutrientes limitada (BRAGA et al., 2005; ANDRADE, 2010). Além disso, compostos orgânicos biorresistentes e compostos inorgânicos não são afetados pelo mecanismo de autodepuração (BRAGA et al., 2005). Assim, a degradação de corpos hídricos através do lançamento de efluentes com altas cargas de matéria orgânica e poluentes pode afetar a própria eficiência da autodepuração dos mesmos, comprometendo a capacidade de suporte desses ecossistemas, afetando a diversidade biológica local e o provisionamento de serviços ecossistêmicos (VON SPERLING, 1996).

1.2. SANEAMENTO AMBIENTAL NO BRASIL

Além de ser um recurso natural limitado, a água é considerada um bem de domínio público dotado de valor econômico. Partindo dessa ideia fundamental, a Política Nacional de Recursos Hídricos, Lei nº 9.433 (BRASIL, 1997), objetiva assegurar à atual e às futuras gerações a disponibilidade de água em padrões de qualidade adequados aos diferentes usos. Essa lei estabelece alguns instrumentos para a gestão das águas, como a outorga de direito de uso, o enquadramento dos corpos d'água em classes de uso e a cobrança pelo uso. Esse último determina que o uso dos recursos hídricos para o lançamento de esgotos e demais resíduos líquidos precisa considerar o volume lançado, seu regime de variação e as características físico-químicas, biológicas e de toxicidade.

A Resolução Nº 357/2005, complementada e alterada parcialmente pela Resolução Nº 430/2011, do Conselho Nacional do Meio Ambiente (CONAMA) dispõe sobre a classificação dos corpos d'água, impondo limites de parâmetros específicos conforme enquadramento. As classes vão de 1 a 4, desde os corpos d'água destinados aos usos mais exigentes, como proteção das comunidades aquáticas e abastecimento para consumo humano, que requerem excelente qualidade de água, até os usos menos exigentes, como navegação e paisagismo. Além disso, a Resolução estabelece as condições e diretrizes para o controle e gestão do lançamento de efluentes em corpos d'água receptores: efluentes de qualquer fonte poluidora podem ser lançados em corpos hídricos receptores somente após o tratamento adequado e desde que obedeçam às condições e exigências dispostas. Assim, os padrões de qualidade de efluentes a serem acatados pelas fontes poluidoras devem ser aqueles da classe na qual o corpo receptor estiver enquadrado.

O tratamento de águas residuais tem sido empregado com o objetivo de atender esses parâmetros de emissão, minimizando o impacto desses lançamentos sobre os sistemas aquáticos. Porém, ainda que 50% da população brasileira tenha acesso à coleta de esgoto, menos da metade recebe um tratamento adequado antes de ser lançado nos corpos hídricos (BRASIL. Secretaria Nacional de Saneamento Ambiental (SNSA), 2018). Águas residuais podem ser diretamente responsáveis pela entrada de uma série de contaminantes nos corpos receptores quando não há tratamento ou quando os sistemas de tratamento utilizados não são eficientes em remover esses compostos (FATTA-KASSINOS et al., 2010). Para fins de fiscalização, a caracterização de efluentes tem sido realizada principalmente através de análises físico-químicas, como vazão, pH, temperatura, demanda química e bioquímica de oxigênio (DBQ e DBO), presença de sólidos, óleos e uma série de poluentes comuns, apesar de os critérios de ecotoxicidade também estarem presentes na Resolução (LIED; RODRIGUES, 2011). No entanto, mesmo quando essas características físico-químicas atendem aos padrões de emissão estabelecidos, os efluentes ainda podem apresentar toxicidade para a biota (BERTOLETTI, 2013). É preciso considerar que a produção global de produtos químicos aumentou de um milhão de toneladas em 1930 para 400 milhões de toneladas em 2001 (COMMISSION OF THE EUROPEAN COMMUNITIES, 2001). Devido à grande variedade e complexidade de substâncias encontradas atualmente nesses efluentes, apenas as análises físico-químicas não são suficientes para sua completa caracterização, pois elas falham em detectar micropoluentes tais como hormônios, naturais e sintéticos, e em prever o efeito conjunto e a toxicidade desses poluentes sobre a biota (CHAPMAN, 2006; LAITANO; MATIAS, 2006).

A Resolução do CONAMA supracitada estabelece que o efluente não deve causar efeitos tóxicos aos organismos aquáticos no corpo receptor, mas atribui a responsabilidade de definir os critérios de ecotoxicidade aos órgãos ambientais competentes de cada estado. No Paraná, a Resolução 81/2010 do Conselho Estadual do Meio Ambiente dispõe sobre esses critérios e padrões de ecotoxicidade para o controle de efluentes líquidos lançados nas águas superficiais do estado. Porém, a exigência de avaliações ecotoxicológicas, bem como a definição dos organismos-teste a serem usados, foi aplicada somente aos efluentes industriais. Ficou definido que as indústrias deveriam reduzir a toxicidade de seus efluentes até 2018, atingindo um fator de toxicidade, no máximo, igual a dois. O fator de toxicidade é representado pelo fator de diluição mínimo do efluente que não causa efeito deletério agudo aos organismos testados, onde um fator igual a dois equivale a ½ da solução original. Isto é, o fator de toxicidade representa, basicamente, quantas vezes um corpo receptor precisaria diluir certo volume de efluente para que ele deixe de ser tóxico para aqueles organismos, expressando a concentração máxima segura de um efluente (ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS (ABNT), 2016). No entanto, não foram ainda estabelecidos os valores máximos permitidos para efluentes domésticos. Em um inciso separado para os efluentes de estações de tratamento, foi determinado, apenas:

> II - Das estações de tratamento de esgoto sanitário
> Art. 9º Os efeitos ecotoxicológicos dos efluentes das Estações de Tratamento de Esgotos Sanitários serão avaliados durante um período mínimo de 2 anos, para posterior definição dos padrões e limites máximos de ecotoxicidade, por meio de norma complementar.
> § 1° O automonitoramento, que subsidiará a avaliação, será efetuado em Estações de Tratamento de Esgotos Sanitários (ETE's) com vazões nominais superiores a 400 L/s, de acordo com a Resolução CONAMA 377/0 (Resolução CEMA 81/2010).

O automonitoramento referido consiste no controle e acompanhamento periódico dos sistemas de tratamento de esgoto, por meio de coleta e análise do efluente final, por parte da própria companhia de saneamento. Passados oito anos da publicação dessa Resolução, ainda não existem normas específicas exigindo a avaliação e regulamentando dos limites de toxicidade dos efluentes de estações de tratamento de esgoto. Assim, apesar da avaliação de toxicidade em efluentes urbanos ter sido citada em 1997, na Política Nacional de Recursos Hídricos, essa ferramenta de análise ainda não é uma exigência para todas as companhias de saneamento do Brasil.

1.3. BACIA HIDROGRÁFICA DO ALTO IGUAÇU

Formado pela confluência dos rios Atuba e Iraí, o rio Iguaçu é o maior e um dos mais importantes rios do estado do Paraná, tendo acompanhado a história do desenvolvimento do estado e de sua maior economia, a Região Metropolitana de Curitiba (RMC), por ser o principal manancial de abastecimento para a região (ANDREOLI et al., 1999; CASTRO NETO, 2002; COORDENAÇÃO DA REGIÃO METROPOLITANA DE CURITIBA, 2006). Considerando o Brasil e a Argentina, a bacia hidrográfica do Rio Iguaçu cobre uma superfície aproximada de 70.800 km², ocupando cerca de 28% da área total do estado do Paraná, enquanto apresenta em torno de 43% da população total do estado, demonstrando sua influência para a ocupação da região: são 4,5 milhões de habitantes, dos quais cerca de 2,5 milhões vivem na região metropolitana. No total, o rio Iguaçu possui um curso de 1.320 km dividido em três Unidades Hidrográficas de Gerenciamento de Recursos Hídricos: Alto, Médio e Baixo Iguaçu, quando finalmente desagua no Rio Paraná. Somente a bacia do Alto Iguaçu possui 6.382 km² e é responsável por 78% da demanda total para abastecimento público da bacia, sendo marcada, predominantemente, por diferentes usos urbanos e intensa atividade industrial da RMC (SUPERINTENDÊNCIA DE DESENVOLVIMENTO DE RECURSOS HÍDRICOS E SANEAMENTO AMBIENTAL (SUDERHSA), 2007; PARANÁ. Secretaria de Estado do Meio Ambiente e Recursos Hídricos (SEMA), 2010). Ao mesmo tempo, as áreas de várzeas e cabeceiras presentes da bacia do Alto Iguaçu possuem muitos endemismos e espécies ameaçadas, sendo identificadas como uma das áreas prioritárias de alta importância para conservação e uso sustentável da biodiversidade brasileira (BRASIL. Ministério do Meio Ambiente (MMA), 2007).

Apesar de sua importância, o Rio Iguaçu encontra-se degradado em vários pontos devido, principalmente, ao baixo percentual e eficácia do tratamento de esgoto lançado no rio durante sua passagem por cidades de médio e grande porte, grandes áreas urbanas e zonas industrializadas (INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA (IBGE), 2012). A maioria dos rios que compõe a bacia apresenta índices de qualidade de água muito inferior ao estipulado para a classe dois à qual eles foram originalmente enquadrados, fazendo com que novas propostas de enquadramento os identifiquem como classe quatro ou pior (SUPERINTENDÊNCIA DOS RECURSOS HÍDRICOS E MEIO AMBIENTE (SUREHMA), 1992; MARIN et al., 2007; SUDERHSA, 2007; CURITIBA. Secretaria Municipal do Meio Ambiente (SMMA), 2010). Uma das situações mais críticas foi observada no Alto Iguaçu, na RMC, que apresentou o segundo pior índice de qualidade de água do Brasil, ficando atrás apenas do rio Tietê, na Região Metropolitana de São Paulo (IBGE, 2012). Essa baixa qualidade da água do Alto Iguaçu é influenciada, sobretudo, pelos afluentes que drenam a RMC, como os Rios Atuba, Belém, Ivo, Bacacheri, Padilha, Barigui, Água Verde, Fanny e Parolin, que recebem poluição de fontes pontuais e difusas e são atualmente caracterizados como "Poluídos e Extremamente Poluídos" (SEMA, 2010).

Os serviços de coleta e tratamento de esgoto da cidade de Curitiba são operados pela Companhia de Saneamento do Paraná (SANEPAR) e as estações de tratamento de esgoto (ETEs) atuam predominantemente por meio de um sistema de biodegradação anaeróbia em Reatores Anaeróbios de Lodo Fluidizado (RALF). Esse sistema promove a percolação do esgoto através de um leito de lodo denso e rico em bactérias anaeróbias dentro dos reatores, onde o próprio fluxo ascensional do líquido e as bolhas de gases geradas no processo de decomposição impulsionam a mistura do sistema. Por meio da ação dessas bactérias, a matéria orgânica presente nas águas residuais é degradada, estabilizada e retida no manto de lodo (LETTINGA et al., 1980). O sistema RALF ganhou notoriedade na década de 70, quando o aumento acentuado dos preços de energia redirecionou os esforços de pesquisa para alternativas mais econômicas, como é o caso do tratamento anaeróbio, que além de não necessitar de energia para a aeração do processo, ainda gera subprodutos que podem ser utilizados para outros fins (SEGHEZZO et al., 1998). Além do baixo consumo de energia, as vantagens desse sistema de tratamento são: baixos investimentos e custos operacionais; tolerância a elevadas cargas orgânicas; excesso de produção de lodo é baixo e bem estabilizado, produz metano que poderá ser utilizado para fins energéticos. Porém, como todo método de tratamento, o sistema RALF também apresenta limitações: as bactérias anaeróbias podem ser inibidas por uma grande variedade de produtos químicos; o processo pode ser mais lento devido à baixa taxa de crescimento dessas bactérias; apresenta remoção limitada de DQO e DBO, entre 40-60% e 50-

70%, respectivamente; baixa remoção de patógenos, coliformes e nutrientes, como nitrogênio e fósforo; necessidade de um pós-tratamento adequado antes que o efluente possa ser despejado no corpo de água receptor (LETTINGA et al., 1980; SEGHEZZO et al., 1998).

A SANEPAR foi uma das companhias de saneamento pioneiras na implementação do sistema RALF em larga escala no Brasil, com mais de 200 unidades construídas (VON SPERLING, 2016; SANEPAR, 2018). A maior delas, a ETE Atuba Sul, opera com uma capacidade de tratamento média de 1.120 L/s, atendendo mais de 580 mil habitantes (CURITIBA. SMMA, 2017). Além dos efluentes de sua própria bacia, a ETE Atuba Sul recebe também os resíduos provenientes das bacias do Irai, do Palmital e do Itaqui (CURITIBA. SMMA, 2012). Os efluentes tratados na ETE Atuba Sul são lançados no rio Atuba e têm intensificado a degradação do principal tributário formador do rio Iguaçu (ANDREOLI et al., 1999; RACHWAL; CAMATI, 2001; MENDONÇA, 2004). Amostragens realizadas ao longo da bacia hidrográfica do Alto Iguaçu indicaram grande influência de esgotos domésticos, com altos valores de DBO e concentrações de nitrogênio amoniacal e fósforo total, principalmente nos pontos amostrais dos rios Atuba e Iguaçu localizados à jusante do despejo da ETE Atuba Sul, apresentando alta degradação (YAMAMOTO, 2012). Outro fato preocupante é a presença de áreas úmidas ou *wetlands* nas proximidades da ETE, uma vez que essas zonas úmidas são usadas para fornecer água às populações humanas durante períodos de seca (IWAMURA et al., 2011; YAMAMOTO, 2012).

De fato, estudos tem mostrado a nítida influência da descarga de efluentes urbanos sobre a qualidade da água do rio Atuba. Numerosas análises têm sugerido a ineficiência do sistema RALF da ETE Atuba Sul em remover uma ampla gama de compostos e contaminantes emergentes das águas residuais, como hormônios sexuais femininos, cafeína, esteróis, agentes anti-hipertensivos, antissépticos e anti-inflamatórios (KRAMER, 2015; OSAWA, 2013; IDE, 2012, 2013, 2017; KRAWCZYK, 2016; MIZUKAWA, 2016). Além disso, o sistema RALF não mostrou ser eficiente na remoção de nitrogênio amoniacal e fósforo, cujas concentrações foram encontradas acima dos valores máximos permitidos por lei (INSTITUTO AMBIENTAL DO PARANÁ (IAP), 2005; KRAMER, 2012; YAMAMOTO, 2012). Tais compostos provenientes de despejos urbanos podem ter efeitos tóxicos sobre os organismos aquáticos expostos (KLAASSEN, 2008; HODGSON, 2010), mas o controle desses contaminantes no Rio Atuba e sua toxicidade para a biota têm sido amplamente ignorados (IAP, 2009).

1.4. ECOTOXICOLOGIA

Os testes de toxicidade com organismos aquáticos têm sido utilizados em muitos países como uma ferramenta obrigatória e complementar às análises físico-químicas para a avaliação do tratamento de efluentes domésticos (ENDERLEIN; ENDERLEIN; WILLIAMS, 1997; EMBRY et al., 2010; CONNON; GEIST; WERNER, 2012). Esses testes possibilitam avaliar o potencial efeito tóxico desses efluentes sobre os organismos aquáticos testados, permitindo a extrapolação e estabelecimento de limites seguros para outros organismos envolvidos e o desenvolvimento de ações corretivas apropriadas. Por permitirem o controle da toxicidade em efluentes líquidos, esses bioensaios representam um instrumento importante para os órgãos de controle ambiental (WALL; HANMER, 1987; LEBLANC; BUCHWALTER, 2010). Apesar dessa importância, a aplicação de testes toxicológicos no Brasil em programas de monitoramento ambiental e de controle de qualidade da água tem recebido pouca atenção se comparada às tradicionais análises físico-químicas (LIED; RODRIGUES, 2011).

Dentre os organismos-teste, os peixes representam os vertebrados mais utilizados em testes de toxicidade aguda para avaliações de risco ambiental, pois são importantes elos nas cadeias tróficas, especialmente sensíveis à presença de agentes tóxicos no meio aquático e respondem de forma semelhante a outros vertebrados, incluindo mamíferos, permitindo uma avaliação do risco de exposição também para humanos (DE FLORA et al., 1991; LEMOS et al., 2007; ROCHA et al., 2011). Contudo, testes agudos com peixes, além de envolverem altos custos, têm mostrado resultados muito variáveis e questionáveis quanto à exatidão e relevância ecológica: dados de toxicidade podem diferir não apenas entre espécies diferentes, mas também para a mesma espécie em laboratórios diferentes (BRAUNBECK et al., 2005; LAMMER et al., 2009). Além de questões econômicas e científicas, existe uma séria preocupação ética relacionada ao sofrimento desses organismos submetidos a altas concentrações de substâncias tóxicas, incompatível com as leis de bem-estar animal (NAGEL, 2002; CHANDROO et al., 2004), dando origem a uma demanda para o desenvolvimento e promoção de métodos alternativos baseados no princípio dos três Rs (redução, refinamento e substituição) (RUSSELL; BURCH, 1959; EMBRY et al., 2010).

Uma das alternativas mais promissoras para a substituição do teste clássico de toxicidade aguda em peixes é o teste de toxicidade em embriões de peixes (*Fish Embryo Toxicity Test*, FET) (MCKIM, 1977; BRAUNBECK et al., 2015). Seus resultados apresentam uma alta correlação com os dados de toxicidade de indivíduos adultos para uma ampla gama de substâncias (LAMMER et al., 2009; KNÖBEL et al., 2012) e, por não serem considerados animais experimentais, os embriões não estão sujeitos a questões éticas de bem-estar animal (BRAUNBECK et al., 2005). Além disso, o uso de embriões de peixes é particularmente vantajoso por permitir a realização de testes em pequena escala, mais rápidos e acessíveis, cujos resultados podem ser extrapolados para os adultos, além de apresentarem sensibilidade, reprodutibilidade e adaptabilidade ideais aos testes laboratoriais (WOLTERING, 1984; ROCHA et al., 2011). Devido a sua imobilidade, imaturidade fisiológica e alta demanda energética exigida para sua formação, os embriões e larvas em desenvolvimento são geralmente considerados os estágios mais sensíveis do ciclo de vida de um peixe, onde a toxicidade de muitos poluentes pode ser verificada através de alterações fisiológicas, má formação e mortalidade (EATON; MCKIM; HOLCOMBE, 1978; HALLARE et al., 2005). Em 2005, a Alemanha substituiu legalmente o teste de toxicidade de efluentes domésticos em peixes adultos pelo teste em embriões de peixe-zebra (*Danio rerio*), tornando-o ferramenta obrigatória de avaliação (INSTITUTO ALEMÃO DE NORMALIZAÇÃO (DIN), 2001). Tal teste foi padronizado e representa um dos testes internacionalmente aceitos mais relevante, sendo usado pelo governo, indústria e laboratórios para identificar e caracterizar potenciais efeitos tóxicos de diversos produtos químicos e suas misturas (ORGANIZAÇÃO INTERNACIONAL DE NORMALIZAÇÃO (ISO), 2007; ORGANIZAÇÃO PARA A COOPERAÇÃO E DESENVOLVIMENTO ECONÔMICO (OCDE), 2013; ABNT, 2016).

No âmbito da ecotoxicologia, bioensaios devem fornecer resultados ecologicamente relevantes para a conservação da biodiversidade e dos processos ecossistêmicos, buscando entender as consequências de efeitos observados no indivíduo para as populações, em diversas espécies (CHAPMAN, 2002). Pequenos efeitos subletais observados nos indivíduos em ensaios laboratoriais, por exemplo, podem ser considerados inofensivos, porém podem causar grandes impactos sobre a dinâmica populacional em longo prazo (HALBACH et al., 1983; BANKS; STARK, 1998). Assim, uma das principais críticas aos ensaios toxicológicos comumente utilizados é que eles falham em prever situações como essa, pois os resultados obtidos para os indivíduos dificilmente podem ser interpretados em nível populacional (FORBES; SIBLY; CALOW, 2001; KRULL; BARROS, 2012). Nesse sentido, modelos matemáticos preditivos representam uma ferramenta valiosa para simular a resposta potencial das populações à toxicidade observada nos organismos (HUSTON; DEANGELIS; POST, 1988; MURRAY, 2002; GIACOMINI, 2007; JAGER; DEANGELIS, 2018).

Outra crítica envolve a escolha do organismo-teste. Além do peixe-zebra, nativo do leste asiático, o medaka (*Oryzias latipes*), do sudeste asiático, a truta-arco-íris (*Oncorhynchus mykiss*) e o "fathead minnow" (*Pimephales promelas*), da América do Norte, também são considerados organismos-modelo para ensaios toxicológicos, recomendados por organizações de normalização internacionais (OCDE, 1992; ANKLEY; JOHNSON, 2004; BRAUNBECK et al., 2005; EMBRY et al., 2010). Isto é, as espécies mais utilizadas globalmente são aquelas que possuem seus testes em protocolos padronizados, o que é compreensível em estudos que exigem comparações de resultados e, portanto, maior exatidão nos métodos. No entanto, para fins de conservação, é importante considerar situações realísticas e ecologicamente relevantes. As espécies mais usadas em testes ecotoxicológicos não necessariamente representam a sensibilidade das espécies de determinada comunidade, principalmente quando consideramos as espécies raras e ameaçadas de extinção (DWYER et al., 2005; RAIMONDO et al., 2008). Na América do Norte, estima-se que a exposição a contaminantes ambientais tenha contribuído com até 38% das extinções de peixes no último século (MILLER et al., 1989). O Brasil possui a maior diversidade de peixes do planeta e um número significativo de espécies está ameaçado de extinção (BRASIL. MMA, 1999; BUCKUP et al., 2007), porém, poucos ensaios ecotoxicológicos foram realizados com esse objetivo e um número restrito de espécies nativas foram testadas. Considerando essa megadiversidade e a grande variedade de ecossistemas aquáticos no Brasil, existe uma necessidade urgente de desenvolvimento de estudos ecotoxicológicos com diferentes espécies nativas, escolhidas principalmente com base em questões ecológicas, por serem importantes na cadeia alimentar ou espécies-chave no ecossistema, por exemplo (CHAPMAN, 2002; MARTINS; BIANCHINI, 2011).

1.5. PESQUISAS DESENVOLVIDAS NO LABORATÓRIO DE TOXICOLOGIA CELULAR

Atendendo a essa demanda global, o grupo de pesquisa do Laboratório de Toxicologia Celular da UFPR, contando com a parceria da Piscicultura Panamá, em Paulo Lopes, Santa Catarina, tem realizado diversos estudos ao longo dos últimos anos com os estágios iniciais de desenvolvimento do jundiá, *Rhamdia quelen* (QUOY & GAIMARD, 1824). Essa espécie de peixe siluriforme é sul americana e apresenta ampla distribuição geográfica, principalmente no sudeste e sul do Brasil. Nagamatsu et al. (2018) expuseram os embriões e larvas a diferentes misturas de chumbo, manganês, mercúrio e nanopartículas de prata, em concentrações ambientalmente relevantes, resultando em eclosão prematura, mortalidade e alterações morfológicas, especialmente em estruturas sensoriais, onde foi destacado o potencial neurotóxico desses metais para essa fase do ciclo de vida da espécie. López-Barrera et al. (2018), considerando apenas nanopartículas de prata em ensaios com a mesma espécie, mostram que a concentração e o tempo de exposição são fatores que afetam a absorção das nanopartículas, a frequência de deformidades e a taxa de sobrevivência das larvas.

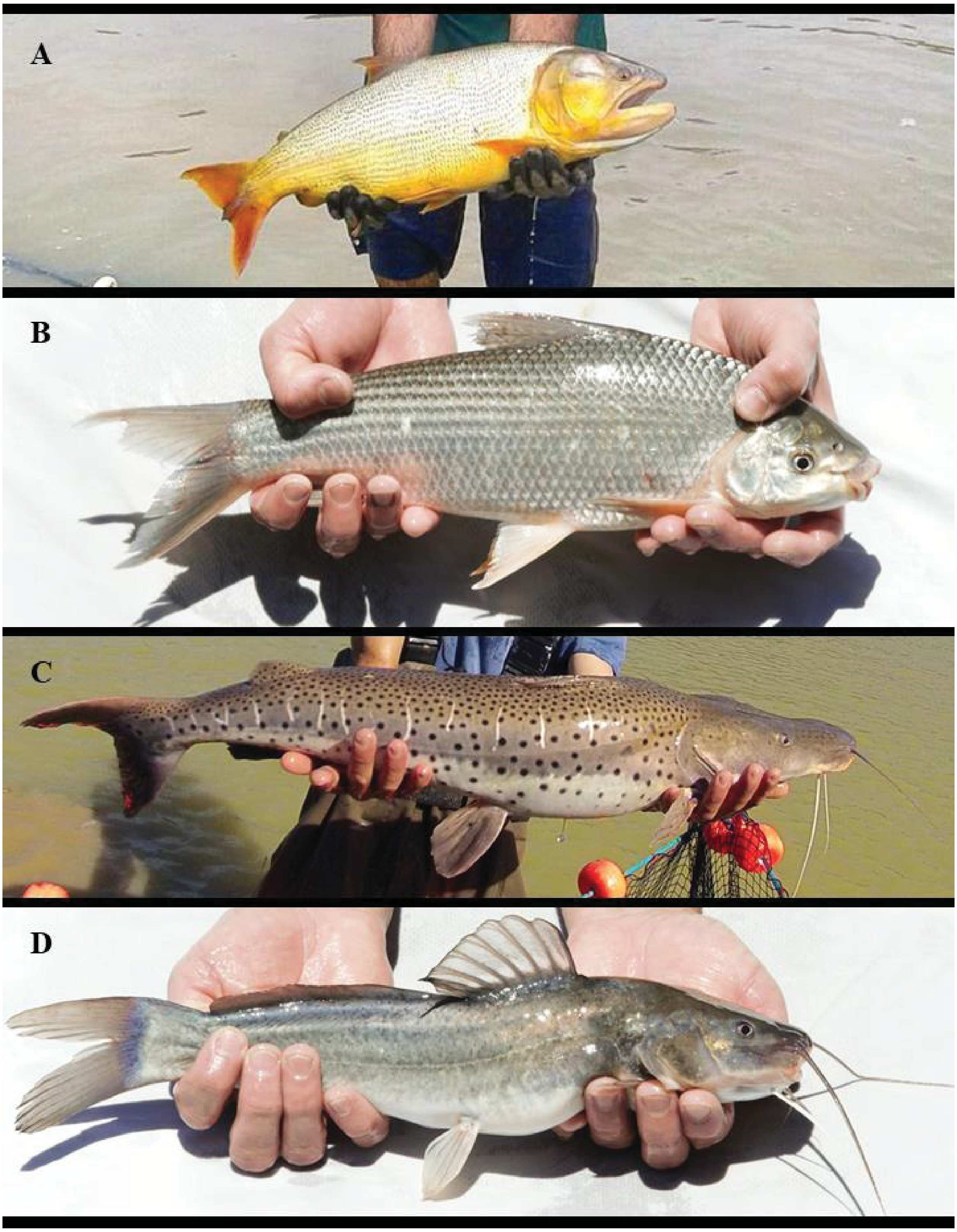
Considerando ainda, a necessidade de investigar as consequências de efeitos toxicológicos a nível populacional, Brito et al. (2017) desenvolveram um modelo matemático teórico preditivo, estocástico e baseado no indivíduo, para avaliar o efeito de poluentes na dinâmica populacional do jundiá. O estudo foi baseado nos resultados de sobrevivência dos embriões e larvas de jundiá expostos por 96 horas aos diferentes efluentes da ETE Atuba Sul: esgoto não tratado, esgoto tratado com tecnologia RALF e esgoto tratado com tecnologia de reúso de água (com e sem adição de cloro). Os diferentes impactos desses efluentes na sobrevivência das larvas foram impostos aos juvenis e adultos, através do modelo, considerando diversos cenários. Assim, foi observado que a tecnologia de reúso da água sem adição de cloro foi o melhor tratamento recebido, enquanto que o mesmo tratamento com adição de cloro e o esgoto sem tratamento levariam uma população hipotética do jundiá a extinção em apenas alguns anos de exposição. Estudos como esse oferecem subsídio para o desenvolvimento de políticas de conservação de espécies e ações regulatórias adequadas para o tratamento de águas residuais e o seu lançamento em corpos receptores.

O grupo de pesquisa tem aplicado o mesmo modelo com sucesso em diversos estudos de toxicidade com as larvas de jundiá. Por exemplo, para a avaliação da qualidade da água da bacia hidrográfica do Alto Iguaçu utilizando a sobrevivência das larvas expostas à água coletada em vários pontos da bacia (BRITO et al., 2018). Folle et al. (2018) mostraram o efeito tóxico do tribromofenol, um poluente ambiental persistente, para as larvas de jundiá, prevendo o impacto a nível populacional através do modelo. Azevedo-Linhares et al. (2018) investigaram os efeitos da cianotoxina microcistina, do inseticida piriproxifeno e da associação entre os dois para os estágios iniciais de desenvolvimento e populações do jundiá, apontando o risco representado pela interação toxicológica entre esses dois compostos que podem coexistir em corpos d'água eutrofizados.

Nesse contexto, considerando que a escolha dos organismos-teste é uma das questões-chave da ecotoxicologia aquática no Brasil, a relevância ecológica de tais estudos pode ser maximizada através do uso de diferentes espécies e níveis tróficos e da escolha de espécies ecológica e economicamente importantes (CHAPMAN, 2002; KRULL; BARROS, 2012).

Considerando ainda a viabilidade de obtenção dos ovos, três espécies de peixe, além do jundiá, se destacam como bons modelos para a avaliação dos efeitos tóxicos de efluentes urbanos, sendo elas selecionadas para o presente estudo (Figura 1): dourado, *Salminus brasiliensis* (CUVIER, 1816), pintado, *Pseudoplatystoma corruscans* (SPIX & AGASSIZ, 1829), e curimbatá, *Prochilodus lineatus* (VALENCIENNES, 1837), sendo as três migratórias, as duas primeiras espécies piscívoras, enquanto o curimbatá é uma espécie detritívora (CAROLSFELD et al., 2003). As três espécies são ameaçadas pela degradação de seus habitats naturais e pela presença de obstáculos à migração reprodutiva anual, como as barragens. Além disso, o dourado e pintado são alvos de sobrepesca por apresentarem alto valor comercial, o que tem causado uma redução gradual de suas populações nativas e extinções locais, fazendo com que essas espécies frequentemente figurem nas listas de espécies ameaçadas da fauna brasileira em diversos estados (MACHADO; DRUMMOND; PAGLIA, 2008; MELLO et al., 2009; DELLA-FLORA et al, 2010). Por fim, um incremento no número de espécies nativas avaliadas em relação à sensibilidade a uma das fontes de poluentes mais preocupantes, os despejos urbanos, certamente ajudaria agências regulatórias a controlar a emissão desses efluentes nos corpos d'água (MARTINS; BIANCHINI, 2011).

FIGURA 1 - INDIVÍDUOS ADULTOS DE (A) DOURADO, S*alminus brasiliensis*, (B) CURIMBATÁ, *Prochilodus lineatus*, (C) PINTADO, *Pseudoplatystoma corruscans*, E (D) JUNDIÁ, *Rhamdia quelen*.



FONTE: Piscicultura Panamá (www.pisciculturapanama.com.br).

2. OBJETIVOS

2.1. OBJETIVO GERAL

O objetivo desse trabalho foi avaliar o efeito tóxico da exposição dos estágios iniciais de desenvolvimento de quatro espécies de peixe nativas brasileiras à água do rio Atuba e investigar as consequências a nível populacional e longo prazo para as espécies analisadas.

2.2. OBJETIVOS ESPECÍFICOS

- Realizar testes de toxicidade com os embriões e larvas de:
    - Dourado (*Salminus brasiliensis*)
    - Curimbatá (*Prochilodus lineatus*)
    - Pintado (*Pseudoplatystoma corruscans*)
    - Jundiá (*Rhamdia quelen*)

- Determinar taxas de eclosão, sobrevivência e deformidades dos organismos expostos.

- Adaptar um modelo matemático teórico baseado no indivíduo às espécies estudadas.

- Investigar o potencial impacto a nível populacional de uma possível exposição dessas espécies a condições semelhantes de poluição.

- Realizar análises para caracterização química da água do rio, correlacionando os resultados com os efeitos tóxicos observados nos embriões e larvas das espécies.

ARTIGO

Urban effluents affect the early development stages of endangered Brazilian fish species with implications for their population dynamics

Urban effluents affect the early development stages of endangered Brazilian fish species with implications for their population dynamics

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Abstract

The pollution from urban effluents discharged into natural waters is a major cause of aquatic biodiversity loss. Ecotoxicological testing contributes significantly to understand the risk of exposure to the biota and to establish conservation policies. The objective of the current study was to assess the water toxicity of a river highly influenced by urban effluents (Atuba River, Curitiba city, Southern Brazil) to the early stages of development of four native species of variable levels of conservation and vulnerability. *Salminus brasiliensis*, *Prochilodus lineatus*, *Rhamdia quelen*, and *Pseudoplatystoma corruscans* were considered in the study. The embryos of the four species were exposed from 8 hours post fertilization (hpf) to 96 hpf to the Atuba River water, collected downstream of the largest wastewater treatment plant in the Metropolitan Region of Curitiba, at concentrations of 30% and 50%, and compared to a control group. Survival rates and deformities were registered every 24 hours. The species *S. brasiliensis* and *P. lineatus* presented the highest mortality rates in both tested dilutions, showing high sensitivity to the pollutants present in the water. According to the individual-based mathematical model, these species showed high vulnerability and risk of extinction under the tested experimental conditions. The model also considered different sensitivity scenarios of juveniles and adults. The other two species, *R. quelen* and *P. corruscans*, showed a more resistant condition to mortality, but also presented high frequency and severity of deformities. These results emphasize the importance of testing the sensitivity of different Brazilian native species for the conservation of biodiversity and the application of models to predict the effects of pollutants on the population level.

Keywords: ecotoxicology, early life stages toxicity, native species, ecological modeling.

Introduction

Water pollution constitutes one of the most representative causes of aquatic biodiversity loss (Malmqvist and Rundle, 2002; Dudgeon et al., 2006; Vörösmarty et al., 2010). All over the world, species have to face the consequences of large loads of urban and agricultural runoff, domestic and industrial wastewaters, and an ever-increasing number of emerging chemicals being released into water bodies every day (Chapman, 2006; Templeton et al., 2009; Raghav et al., 2013). Although many methods of wastewater treatment have been developed, they vary in the removal efficiency of different pollutants and environmental agencies face the challenge of monitoring the toxicity of the resulting effluents (Bolong et al., 2009; Ahmed et al., 2016). Besides, many developing countries lack proper sanitation, especially in areas where rapid and unplanned urban growth led to informal residential settlements (Hutton and Haller, 2004; Elimelech, 2006; Montgomery and Elimelech, 2007). Consequently, watersheds surrounding big metropolis generally present the worst water quality indexes, with rivers polluted by a complex mixture of effluents from point and diffuse sources (Sliva and Williams, 2001; Carle et al., 2005; Ouyang et al., 2006; Kannel et al., 2007). Such high levels of pollution can affect the self-depuration efficiency of water bodies for long stretches of the river basin, compromising the carrying capacity of these ecosystems, affecting local biodiversity and the provision of ecosystem services (Von Sperling, 1996; Braga et al., 2005). The scenario is even more critical for developing countries, where the water pollution levels are expected to increase in the next years (Liu et al., 2012).

Since those mixtures are extremely complex, physicochemical analyses alone are not enough to predict and prevent toxic effects on aquatic organisms exposed (Laitano and Matias, 2006). First, the identification of all toxicants and thus the establishment of their limit values may not be possible. In addition to being costly, measuring individual toxicants does not account for their bioavailability and the interactions between them. Bioassays represent a relatively simple laboratory procedure, having the advantage of reflecting the aggregate toxicity of all chemicals present in a complex effluent mixture and providing evidence of environmental impact that is easily understood by the general public (Hall and Golding, 1998). Although many developed countries have taken a step forward making toxicity assays a mandatory component in effluent testing, unfortunately, those tests are not as required as the classic physicochemical analyses in most countries (Enderlein, et al., 1997; Embry et al., 2010; Connon, et al., 2012).

The acute fish toxicity test has been one of the most used to assess water toxicity. However, economical, scientific, and ethical concerns about the use of adult organisms have given rise to the fish embryo-larval test as an alternative assay (Braunbeck et al., 2005; Lammer et al., 2009; Embry et al., 2010). The use of embryos is particularly advantageous because it allows for small-scale, faster and less costly tests that are generally easy to adapt and reproduce in laboratory conditions (Woltering, 1984; Rocha et al., 2011). Because of their immobility, physiological immaturity, and high energy demand, the developing embryos and larvae are very sensitive to disturbances in water quality, which toxicity can be verified through their physiological changes, malformation, and mortality (Eaton et al., 1978; Hallare et al., 2005a). Besides, the toxic effects observed in embryos and larvae can be extrapolated for other stages of the life cycle, since they show a high correlation with the toxicity data of adult individuals for a wide range of substances (Lammer et al., 2009; Knöbel et al., 2012; Belanger et al., 2013). Thus, bioassays with the early life stages of fish represent a relevant and internationally accepted test, which is used by the government, industries, and laboratories to identify and characterize the potential toxic effects of numerous chemicals and their mixtures to the aquatic life (Braunbeck and Lammer, 2006; Braunbeck et al., 2015).

In the field of ecotoxicology, bioassays must provide ecologically relevant results for the conservation of biodiversity and ecosystem processes, seeking to understand the consequences of toxic effects observed at the individual level for populations and for several species (Chapman, 2002). One of the main criticisms of commonly used toxicology tests is the lack of interpretation of their results at the population level (Forbes et al., 2001; Krull and Barros, 2012). In this sense, predictive mathematical models represent a valuable tool to simulate the potential response of populations to the toxicity observed in organisms (Huston et al., 1988; Murray, 2002; Giacomini, 2007). Another point involves the species used, which normally are those that have their tests described by standardized protocols. The fish embryo-larval test has been normalized by international organizations and described for many species, mainly zebrafish, *Danio rerio*, fathead minnow, *Pimephales promelas*, and Japanese medaka, *Oryzias latipes* (ISO, 2007; OECD, 2013). While these species perfectly fit the goal of studies that require rigorous method for comparative results, they are often not expressive of native and endangered fish species sensitivity for conservation purposes (Buhl, 1997; Dwyer et al., 2005; Raimondo et al., 2008). Hence, there is a need for testing the sensitivity of these species and establishing their risk of exposure to pollution (Chapman, 2002; Martins and Bianchini, 2011).

Just as in the global scenario, Brazilian rivers undergo serious degradation due to uncontrolled urban wastewater disposals, which has affected several species and caused the depletion of their natural populations (Marques et al., 2004; Clevelario Junior et al., 2005). Only half of the Brazilian population has access to a wastewater collection network and even less than that receives proper treatment before being discharged into receiving water bodies, making irregular disposals very common (Von Sperling, 2016). For regular disposals, Brazilian environmental legislation establishes that effluents should not cause toxic effects to aquatic organisms in the receiving water body, in accordance with ecotoxicological tests using organisms of two different trophic levels, at least (Brazilian National Environmental Council, 2005). Nevertheless, physicochemical analyses are still the focus of monitoring programs and bioassays have not yet become a common practice for assessing the toxicity of effluents, especially because they are not mandatory in the case of domestic sewage (CONAMA, 2005; Laitano and Matias, 2006).

Despite having the largest fish diversity in the world and a significant number of fish species listed as threatened or endangered because of water pollution (Machado et al., 2008; Buckup et al., 2007), few ecotoxicological assays have been conducted in Brazil and a limited number of native species have been tested (Martins and Bianchini, 2011). So, considering such megadiversity and the great variety of aquatic ecosystems, there is a need to develop ecotoxicological studies with native species, chosen mainly based on ecological issues, for being important in a food chain or key species in an ecosystem, for example (Chapman, 2002; Martins and Bianchini, 2011).

Four fish species stand out as good models for the evaluation of the toxic effects of urban effluents, being selected for the present study: dourado, *Salminus brasiliensis* (Cuvier, 1816) and curimbatá, *Prochilodus lineatus* (Valenciennes, 1837), Characiformes; pintado, *Pseudoplatystoma corruscans* (Spix & Agassiz, 1829) and jundiá, *Rhamdia quelen* (Quoy & Gaimard, 1824), Siluriformes. The first three species are long-distance potamodromous migrators and have a wide range occurrence in southern South America, especially at the Platine basin, which includes Paraná, Paraguay, and Uruguay Rivers and their tributaries. *S. brasiliensis* and *P. corruscans* are top predators, while *P. lineatus* is a detritivorous species and a natural prey for the first two. They show high fertility and total spawning, which occurs annually as they migrate in running waters to upper stretches of large tributaries. The eggs drift in the river current to floodplains while completing development (Carolsfeld et al., 2003; Castro and Vari, 2004; Zaniboni-Filho et al., 2017). *R. quelen* has a wider geographical distribution, from Southern Mexico to Central Argentina, is mostly sedentary, benthic, omnivorous, and show low fecundity, with partial spawning throughout the year in calm and clean waters (Gomiero et al., 2007; Olaya-Nieto et al., 2010). Regarding ecotoxicology, *R. quelen* has been more studied than the others, including water quality assessments. All of them are commonly raised in fish farms and their eggs can be easily obtained.

The four species are subject to the degradation of their natural habitats, poor water quality, and/or the presence of obstacles to their reproductive migrations, such as dams. In addition, because of the high commercial value of migratory fishes, overfishing has caused an abrupt reduction of their native populations and local extinctions. Most large fishes, such as *S. brasiliensis*, *P. lineatus*, and *P. corruscans*, are extinct in almost the entire Paraná River basin, but they are not included in the national list because they also occur in the Paraguay river basin, where, although also subject to a high fishing pressure, they cannot be considered as endangered due to the relatively preserved state of the Pantanal ecosystem (Machado et al., 2008). Finally, an increase in the number of native species evaluated in relation to their sensitivity to one of the most serious sources of pollution, urban effluents, would certainly help regulatory agencies to control these emissions in receiving water bodies.

Hence, the objective of the present study was to evaluate the water quality and the toxic effects of a highly impacted urban river to the initial stages of development of four South American native species, being three of them threatened, investigating the consequences of long-term exposure to their populations through theoretical mathematical modeling.

Methods

Characterization of the studied area

The Iguaçu River is the largest and most important river of the Paraná state, Southern Brazil, with a course of 1.320 km until the Iguaçu Falls, when it joins the Paraná River. The upper portion of the Iguaçu River, located in the metropolitan region of Curitiba city (Fig. 1), was classified as the second most polluted river in Brazil due to the urban and industrial wastewaters discharged into the tributaries draining the region (IBGE, 2012). The Metropolitan Region of Curitiba, state capital and most populous city, is responsible for 78% of the total demand for public water supply of the whole basin (SEMA, 2010). The Atuba River is one of the two tributaries forming the Iguaçu River and was selected for the present study because it receives the effluents from the largest Wastewater Treatment Plant of Curitiba (ETE Atuba Sul). The station employs the Upflow Anaerobic Sludge Blanket (UASB) technology, with limited removal of nutrients, oxygen demand, pathogens, coliforms, and micropollutants, causing intense degradation of the Atuba River (Rachwal and Camati, 2001; Mendonça, 2004; Ide et al., 2012, 2013, 2017; Yamamoto, 2012; Machado et al., 2014; Kramer et al., 2015; Brito et al., 2017, 2018). An aggravating factor is that the wetlands in the vicinity of the WWTP are used for water supply during periods of drought, besides being identified as one of the high priority areas for Brazilian biodiversity conservation and sustainable use (MMA, 2007; Iwamura et al., 2011; Yamamoto, 2012).

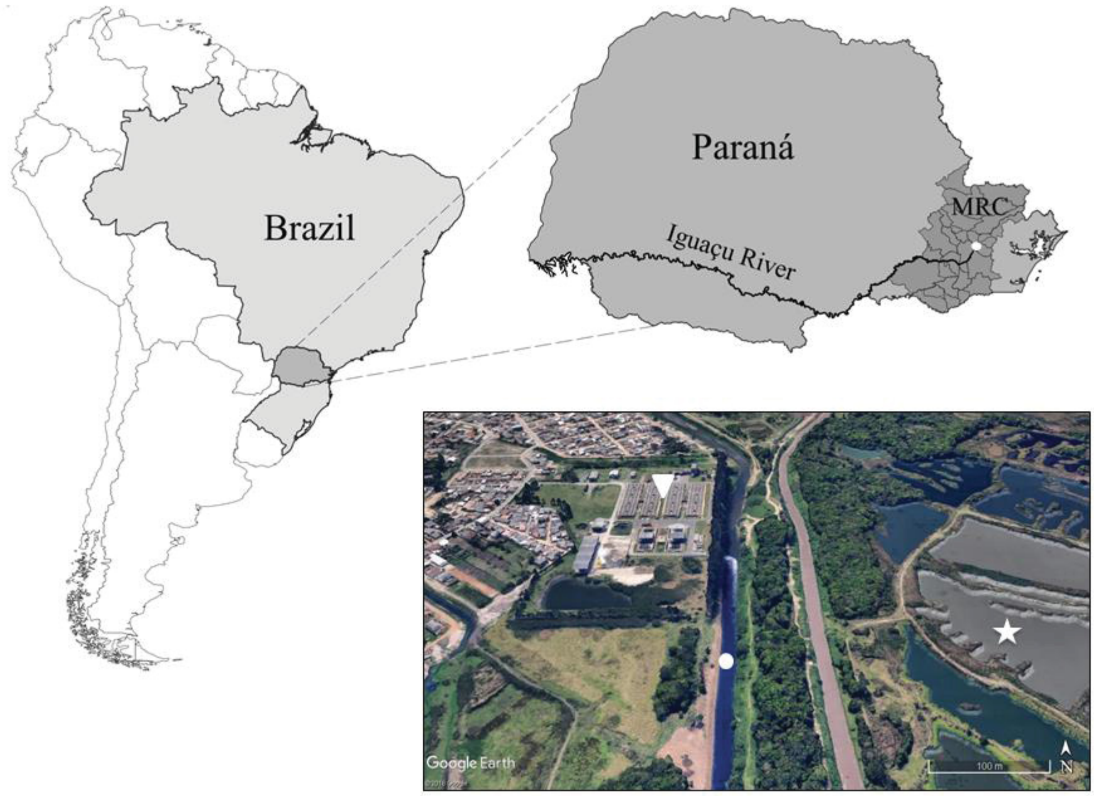

Figure 1. Study area. The maps illustrate the Paraná state in Brazil and the Iguaçu River flowing from the Metropolitan Region of Curitiba (MRC). The circle indicates the sampling point at the Atuba River (25°28'31.95"S, 49°11'7.58"W). The triangle points the Atuba Sul Wastewater Treatment Plant, while the star shaped symbol marks the wetland area in the vicinity.

Water Sampling and Test Solutions

The water samples were collected about 200 meters downstream from the Atuba Sul WWTP discharge into the Atuba River, representing a realistic mixture with the river water. Due to differences in the reproduction season of the four species, two water samplings were carried out in the study (December 12, 2017 and January 30, 2018), being the first sample used for *S. brasiliensis* and *P. lineatus* tests and the second used for *R. quelen* and *P. corruscans* tests. The samples were collected about 30 cm deep and transported to the Cellular Toxicology Laboratory at the Federal University of Paraná. For each sampling, two liters of river water were filtered (0.45 µm), reserved in sterile amber glass bottles, kept refrigerated at 4ºC and protected from light for further chemical analysis. Apart from that, at the same day that the tests initiated, another fraction of the river water samples was filtered (12 µm pore size paper filter) and diluted in reconstituted water (6.5 mg L^{-1} $CaCl_2$, 133.5 mg L^{-1} $MgSO_4$, 0.4 mg L^{-1} KCl, 10.5 mg L^{-1} $NaHCO_3$; 0.45 μm-filtered) for the preparation of the two test solutions with final concentrations of 30% and 50% of river water.

Chemical analysis

The analyzes were carried out in the Laboratory of Organic Geochemistry and Marine Pollution (LaGPoM) of the Center for Marine Studies (CEM) at the Federal University of Paraná. The extraction of organic contaminants (polycyclic aromatic hydrocarbons - PAHs, organochlorine pesticides - OCPs, polybrominated diphenyl ethers – PBDEs, and polychlorinated biphenyls - PCBs) followed the protocol EPA 3510C (Separatory Funnel Liquid-Liquid Extraction). The quantification and identification of the organic compounds were performed using an Agilent GC 7890A gas chromatograph equipped with an Agilent 19091J-433 capillary fused-silica column coated with 5% diphenyl/dimethylsiloxane (30 m length, 0.25 mm ID, 0.25 mm film thickness) and coupled with an Agilent 5975C inert MSD with a Triple-Axis Detector Mass Spectrometer. The PAHs quantification was performed by use of analytical curves based on the concentration range of 0.1 to 1.50 ng μL^{-1}. The OCPs, PCBs, and PBDEs quantification was based on calibration curves of external standards in a range of 1 to 200 pg μL^{-1}. The identification was made by matching their retention times with external standard solutions of known composition for all classes of organic compounds after full scan (m/z 50-550) and selected ion monitoring (SIM).

Fish Embryo-Larval Toxicity Tests

The fertilized eggs were obtained from a local fish farm (www.pisciculturapanama.com.br) that performs the induced reproduction of several native fish species. The gametes were obtained from a single pair of progenitors for each species in order to reduce the variability of the egg batches. After fertilization, the developing embryos were transported in properly aerated plastic bags to the Cellular Toxicology Laboratory.

Meanwhile, 24 and 96-well culture plates were prepared with 2 ml or 200 μl of the test solution per well, respectively. The experiment was conducted in triplicate: there were three culture plates for each exposure time (24, 48, 72, and 96h) and treatment (30%, 50%, and control) considered, totalizing 36 plates for each species. Once in the lab, viable embryos at the gastrula stage were selected and individually transferred to the culture plates wells (one embryo per well) about 8 to 10 hours post fertilization (hpf), following Santos and Godinho (2002), Ninhaus-Silveira et al. (2006), Rodrigues-Galdino et al. (2009), and Marques et al. (2008). Due to the embryo size, *S. brasiliensis*, *R. quelen* and *P. corruscans* embryos were placed in 24-well plates, while those of *P. lineatus* were placed in 96-well plates. Embryos were exposed to the two concentrations of river water (30% and 50%) and reconstituted water (control) for 96 hpf. The plates were maintained in the incubator at 24 °C throughout the experiment, with half of the test solution volume from each well being replaced every 24 hours, promoting better water oxygenation, excretion removal and maintenance of solution concentration. Also, at each of those water renewals, the dead individuals were removed from the wells.

The plates were checked daily up to 96 hpf, with hatching rates determined 24 hpf and survival rates and frequency of deformities assessed every 24 hours. These observations were used for the determination of lethality: coagulation of embryos, lack of somite formation, and lack of heartbeat. At each count, larvae with deformities were collected, anesthetized with eugenol and immersed in Karnovsky fixative solution (2.5% glutaraldehyde, 4% paraformaldehyde in 0.1 mol L^{-1} sodium phosphate buffer, pH 7.3) for 24 hours. Then, the embryos were washed with a 0.1 mol L^{-1} sodium phosphate buffer and stored at 4 °C until analysis in an inverted microscope Leica Microsystems (DMIL LED). The images were captured using Leica Application Suite 3.0.0 operational software.

The classification of the deformities observed was adapted from Powers et al. (2011), consisting of three categories, depending on the species: 1) spine (e.g. stunted skeletal growth and kink in tail), 2) cranial (e.g. absence/atrophy of eyes and/or barbels), and 3) thorax (distension and pericardial/yolk sac edema). For each type of malformation presented by an individual, a score of 1 to 3 was attributed to that deformity, according to its severity level: 1 = low, 2 = moderate, and 3 = high. Thus, each individual presented a deformity score that was equal to the sum of their scores in each category of malformation presented. Finally, the sum of scores established for each culture plate was divided by the number of surviving organisms, resulting in a deformity index.

Data analysis

Survival and deformity data were checked for the normality and homoscedasticity assumptions of parametric tests. When these assumptions were met, ANOVA followed by Tukey tests were performed; if not, Kruskal-Wallis followed by Dunn's test with Bonferroni correction method was used. These *post hoc* pairwise multiple comparisons were performed to analyze the differences between treatments and control and between exposure times of the same treatment. The difference in the deformity index between the treatments was analyzed considering the different exposure time culture plates as replicates. The analyses were performed in RStudio statistical program (R Core Team, 2018) with a significance level of 5%.

Mathematical model

The stochastic individual based model developed for *R. quelen* by Brito et al. (2017) was parametrized for *S. brasiliensis* and *P. lineatus* with the purpose of evaluating the impact of the toxic effects observed on the larvae to the population dynamics of these species. These two species were chosen based on the larvae survival results obtained here. As the model was already described in detail in the original work, only a short explanation is provided here.

The individuals were explicitly modeled from population parameters of the species found in the literature, being characterized by their age in three phases. Phase A is the embryonic-larval stage, characterized by the presence of the yolk sac and, theoretically, absence of intraspecific competition, corresponding, approximately, to the first 96 hours for the two species. Phase B is the juvenile stage, from the complete absorption of the yolk sac (96 hours) and beginning of exogenous feeding (and competition) until sexual maturity (2 years), and phase C is the adult reproductive stage, which includes individuals of various ages between the first sexual maturation and the highest reproductive age (variable among species) that reproduce annually.

Based on the survival rate of larvae exposed to the different river water dilutions observed experimentally, impact values were calculated by dividing the survival in river water by the survival in the control. Then, each river water dilution tested resulted in an impact value which was then imposed on the life phases to investigate its effect on population dynamics. The effect of 30% and 50% river water was modeled by multiplying the maximum survival probability of each phase to its respective impact value.

- Phase A

Each embryo presents a S^A probability of surviving until the juvenile stage. S^A values found in the literature for the two species were very conservative as they represent larval survival in fish farms; these values represented the maximum survival rate in the absence of pollutants, where, under ideal conditions, individuals die only from natural causes. At this stage, mortality may occur due to natural causes and due to the impact of pollutants, considering the impact values of the scenarios investigated.

- Phase B

Each juvenile has a S^B probability of surviving into adulthood that is proportional to the density of juvenile individuals competing for the same resources, once they are limited by a carrying capacity. That is, due to limited resources, S^B decreases as the number of juvenile increases. The impact of the pollutants in this phase is modeled by the reduction of the survival rate (b), regardless of the number of competitors. Then, at this stage, mortality may occur due to natural causes, competition and also due to the impact of pollutants.

- Phase C

Adults present an S^C probability of surviving each year of life (reproductive cycle). Surviving individuals reproduce and add new individuals to the phase A. As in the previous phase, the effect of pollutants is investigated by multiplying the S^C value by the impact factor observed experimentally at phase A.

This phase is very important for the population dynamics created by the model, since it determines the addition of individuals to the population at each reproductive event of the surviving adults. The model was parameterized with information regarding the growth and reproduction of these species, such as weight, age, fertility (number of eggs produced by females of different ages), fertilization rate, sex ratio and maximum reproductive age. The data and references used can be found in table 1.

Beginning with an arbitrary adult population of 10% of the maximum population size (asymptotic behavior was not sensitive to different initial population sizes) and a juvenile carrying capacity of 75, the simulations were performed for 200 years, being the first 100 years based on maximum survival probabilities, without imposing any impact, so the population dynamic could achieve asymptotic behavior. Then, at year 101, an impact value proportional to the survival experimentally observed in each treatment was applied. For that, it was assumed that younger individuals are equally or more sensitive to pollutants than older individuals (larvae $\geq$ juveniles $\geq$ adults) (Hutchinson et al., 1998). Although some authors have tried to establish a comparative for the toxicity of chemicals to different life stages of fish, there is no such formula on how an effect observed on one life stage would translate to another, especially when the species have never been tested. Therefore, since the effect of polluted waters is unknown for the juvenile and adult life stages, an exploratory approach was taken, according to two different scenarios investigated: (I) the impact of the river water on juveniles and adults would be the same of that observed experimentally on the larvae and (II) the impact of the river water on juveniles and adults would be, respectively, 75% and 50% of that observed experimentally on the larvae. The model does not consider environmental variability or biological interactions, so, being a simplification of reality, it probably overestimates the survival that would be observed if these species were exposed to similar pollution conditions in nature.

Table 1

Parameters used for modeling the dynamic of S*alminus brasiliensis* and *Prochilodus lineatus* populations.

Parameters	Estimative Parameter	Reference
Salminus brasiliensis		
Larval survival probability (S^A)	0.50	Dumont-Neto et al. (1997)
Juvenile survival probability (b)	0.77	Fracalossi et al. (2004)
Adult survival probability (S^C)	0.60	Angelini and Agostinho (2005)
Sexual maturity	2 years	Barbieri et al. (2001)
Maximum reproductive age	15 years	Zuliani et al. (2016)
Female weight per age	$W_i = 10226.8\ (1 - e^{-0{,}17\,(i + 0.41)})^{3.05}$	
Fertility per weight	$F_i = 9501.43\ W_i^{0.48}$	*
Fertilization rate	0.57	Sanches et al. (2009)
Prochilodus lineatus		
Larval survival probability (S^A)	0.71	Hernández-Cuadrado (2013)
Juvenile survival probability (b)	0.5	Sverlij et al. (1993)
Adult survival probability (S^C)	0.64	Baigún et al. (2013)
Sexual maturity	2 years	Sverlij et al. (1993)
Maximum reproductive age	15 years	Avigliano et al. (2017)
Female weight per age	$W_i = 4.3 \cdot 10^{-5}\ (Ls)^{2.93}$ $Ls = 457.8\ (1 - e^{-0.45(i+0.93)})$	Resende et al. (1995)
Fertility per weight	$F_i = 11.918 + 79.65\ W_i$	
Fertilization rate	0.50	Silva et al. (2009)

*Once this equation could not be found on the literature, it was obtained through regression analysis of female weight and fertility data from Weingartner (2010) and Dumont-Neto et al. (1997).

The simulations resulted in the number of adults over the years for each scenario, which was relativized to make the values interpretable. The relative population densities over the years were calculated as the adult population size of each year divided by the mean population size in a non-perturbative scenario (without any impact on the maximum survival probabilities of the three phases). Thus, the mean relative population density of the control treatment is equal to 1, while it varies for the different impact values of the scenarios considered.

Results

Chemical analysis

The polycyclic aromatic hydrocarbons (PAH) present in the samples were associated with petrogenic source, indicated by the presence of alkylated PAHs and lower molecular weight compounds (2 to 3 aromatic rings). Organochlorine pesticides (OCP), polychlorinated biphenyls (PCB), and polybrominated diphenyl ethers (PBDE) were present in very low or undetectable concentrations, basically because these contaminants are no longer used in large scale (Table 2). Both samples presented significant amounts of plastic materials (phthalates and bisphenol-A derivatives), which could not be quantified due to analytical limitations.

Table 2

Organic pollutants detected in two water samples from the Atuba River. The last column shows, when present, the maximum value allowed by the Brazilian legislation (The National Environmental Council Resolution 357/2005). LoD = limit of detection.

Pollutant classification	Water Sample		Limit
	Dec 2017	Jan 2018	($\mu g\ L^{-1}$)
Polycyclic aromatic hydrocarbons (PAH)	$\mu g\ L^{-1}$		-
Naphthalene (Naph)	0.078	0.121	-
2-Methylnaphthalene	0.014	0.016	-
C_2-Naphthalenes	0.055	0.047	-
C_3-Naphthalenes	0.043	0.040	-
Phenanthrene (Phen)	0.011	0.010	-
Total PAHs	0.202	0.234	-
Organochlorine pesticides (OCP)	$\mu g\ L^{-1}$		
Hexachlorobenzene (HCB)	0.002	0.004	0.0065
γ-Hexachlorocyclohexane (γ-HCH)	0.001	0.008	0.020
Oxychlordane	0.002	0.002	
γ-Chlordane	0.001	< LoD	0.040
α-Chlordane	0.000	< LoD	
Total OCPs	0.006	0.013	
Total Polychlorinated biphenyls (PCB)	< LoD	< LoD	0.001
Total Polybrominated diphenyl ethers (PBDE)	< LoD	< LoD	-

Survival and deformities

For the four species, all living individuals observed at 24 hpf had hatched, so the hatching rate equals to the survival rate 24 hpf for all the experiments.

The embryos and larvae of *S. brasiliensis* exposed to the Atuba River water at both tested dilutions presented the highest mortality rate among the three species, differing significantly from the control at all exposure periods (Fig. 2A). Most of these embryos were found dead at 24 hpf still presenting the chorion on, so most of them did not even hatch. Conversely, individuals exposed to the control treatment showed high survival until the end of the experiment, with a mean survival rate of 77.1% at 96 hpf, which is even higher than that found in fish farms (Table 1). The deformity index was higher among the larvae exposed to the river water, differing significantly from the control (Fig. 2B). The most common deformities in *S. brasiliensis* larvae were spinal torsions and pericardial edema (Fig. 3). No cranial deformities were observed.

The *P. lineatus* embryos exposed to the river water showed low hatching rates (survival rate 24 hpf) and high mortality, consistently differing from the control up to 48 hpf at both tested dilutions. Thereafter, only the survival in 50% river water differed significantly from the control, due to a decline in survival also in the reconstituted water treatment (Fig. 4). Since only three larvae, one in each treatment, presented spinal malformations, the deformity index was not calculated.

The survival of *R. quelen* larvae did not differ significantly between the treatments and across time (Fig. 5A). There were no significant differences in the deformity indexes, but a concentration-dependent response could be observed, with pericardial edema and spinal torsions being the most common malformations, and abnormal barbel development and eye pigmentation being the most common cranial deformities (Fig. 5B, 6).

The *P. corruscans* larvae exposed to the different treatments showed mixed results (Fig. 7A). One of the three control 24 hpf culture plates showed very low survival, which dragged the mean survival rate down, so it differed from the 30% treatment, which showed high survival. However, survival rate in the 30% river water treatment declined at 72 and 96 hpf, showing significant difference from 24 and 48 hpf. The larvae survival of 50% river water at 48 hpf was significantly lower than the control and 30% groups. The survival at 72 and 96 hpf did not differ significantly between the treatments. The larvae with malformations were very frequent in the river water treatments at both concentrations, but only 50% differed significantly from control (Fig. 7B). The larvae showed severe edema and spinal torsion deformities, with abnormal barbel development and eye pigmentation being the most common cranial deformities (Fig. 8).

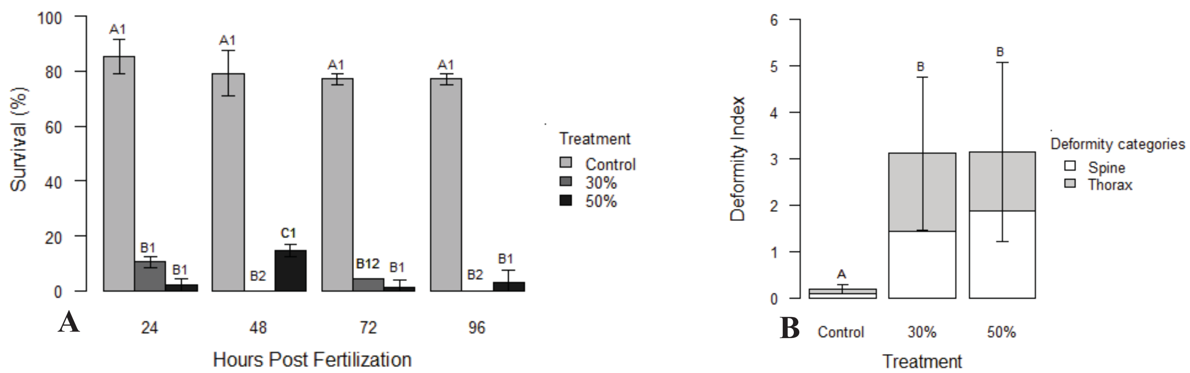

Figure 2. Fish embryo test with *Salminus brasiliensis* exposed to reconstituted water (control) and to the Atuba River water at concentrations of 30% and 50%. (A) Survival rate of larvae observed every 24 hours until 96 hours post fertilization. (B) General deformity index, showing the scores of spine and thorax malformations of larvae exposed to the different treatments. Different letters indicate significant differences between the treatments, while different numbers indicate differences in the same treatment across different exposure times ($p < 0.05$).

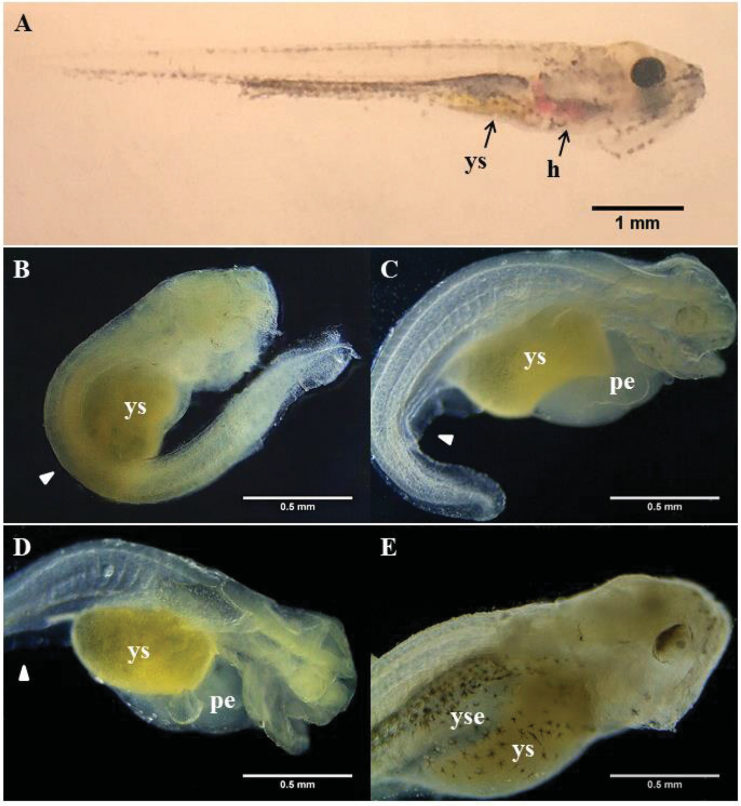

Figure 3. *Salminus brasiliensis* larvae. (A) Normal larva at 96 hpf showing remaining yolk. (B, C, D) Larvae exposed to 50% river water at 48 hpf showing severe spinal torsions (spinal scoliosis and kyphosis) and pericardial edema. (E) Larva exposed to 30% river water at 72 hpf showing yolk sac edema. Arrowheads point to spinal torsions and atrophy. Ys = yolk sac, h = heart, pe = pericardial edema, yse = yolk sac edema.

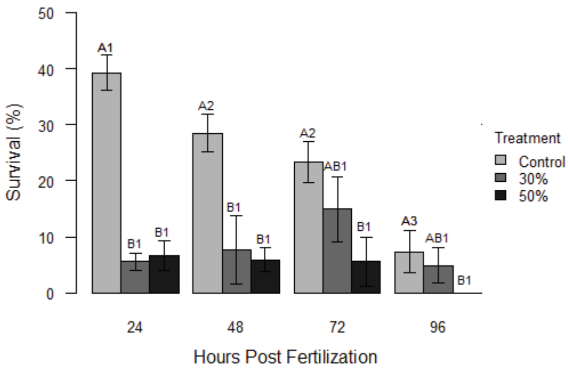

Figure 4. Survival rate of *Prochilodus lineatus* larvae exposed to reconstituted water (control) and to the Atuba River water at concentrations of 30% and 50%, observed every 24 hours until 96 hours post fertilization. Different letters indicate significant differences between the treatments at the same exposure time, while different numbers indicate differences in the same treatment across different exposure times ($p < 0.05$).

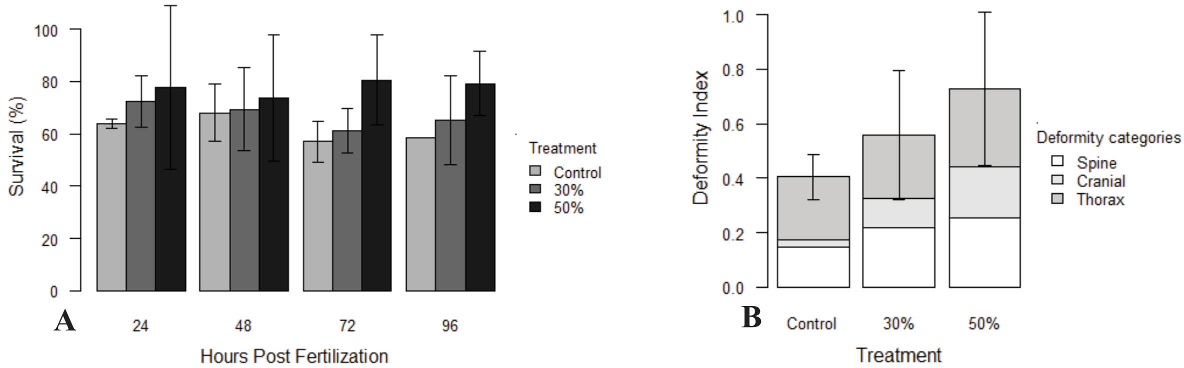

Figure 5. Fish embryo test with *Rhamdia quelen* exposed to reconstituted water (control) and to the Atuba River water at concentrations of 30% and 50%. (A) Survival rate of larvae observed every 24 hours until 96 hours post fertilization. (B) General deformity index, showing the scores of spine, cranial and thorax malformations of larvae exposed to the different treatments. There was no significant difference between treatments ($p < 0.05$).

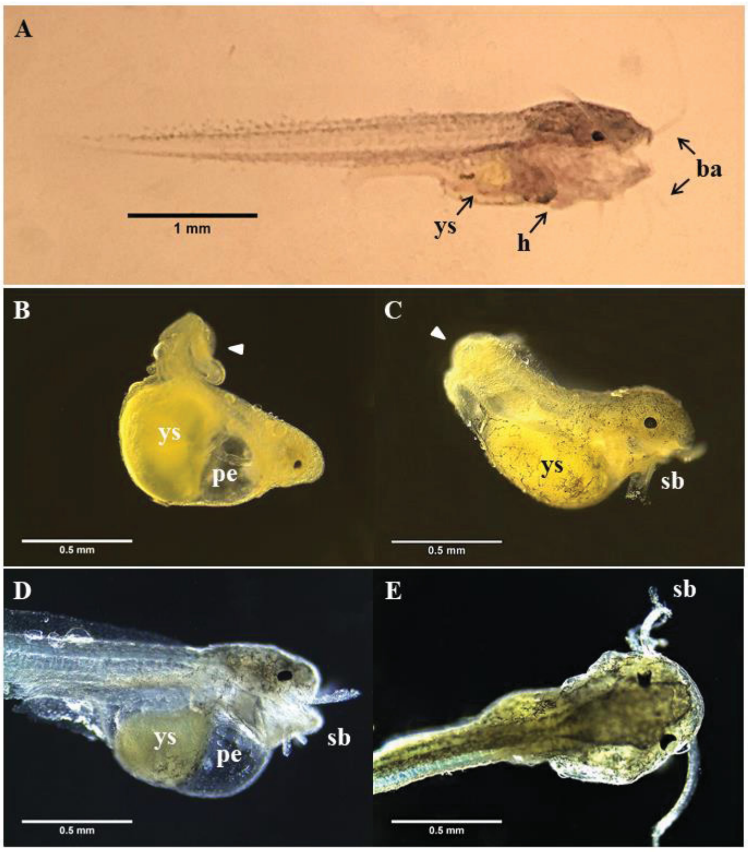

Figure 6. *Rhamdia quelen* larvae. (A) Normal larva at 96 hpf. (B) Larva exposed to 50% river water at 48 hpf showing severe spinal torsion, general body atrophy, and pericardial edema with unlooped heart tube. (C) Larva exposed to 50% river water at 72 hpf showing stunted barbels and severe body atrophy. (D, E) Larvae exposed to 50% river water at 96 hpf showing pericardial edema and stunted barbels. Arrowheads point to spinal torsions and atrophy. Ys = yolk sac, h = heart, ba = barbels, pe = pericardial edema, sb = stunted barbels.

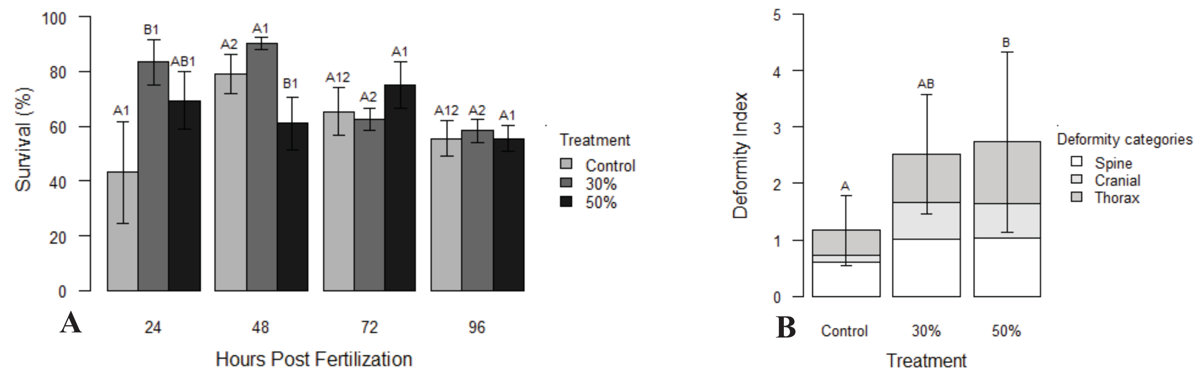

Figure 7. Fish embryo test with *Pseudoplatystoma corruscans* exposed to reconstituted water (control) and to the Atuba River water at concentrations of 30% and 50%. (A) Survival rate of larvae observed each 24 hours until 96 hours post fertilization. (B) General deformity index, showing the scores of spine, cranial and thorax malformations of larvae exposed to the different treatments. Different letters indicate significant differences between the treatments, while different numbers indicate differences in the same treatment across different exposure times ($p < 0.05$).

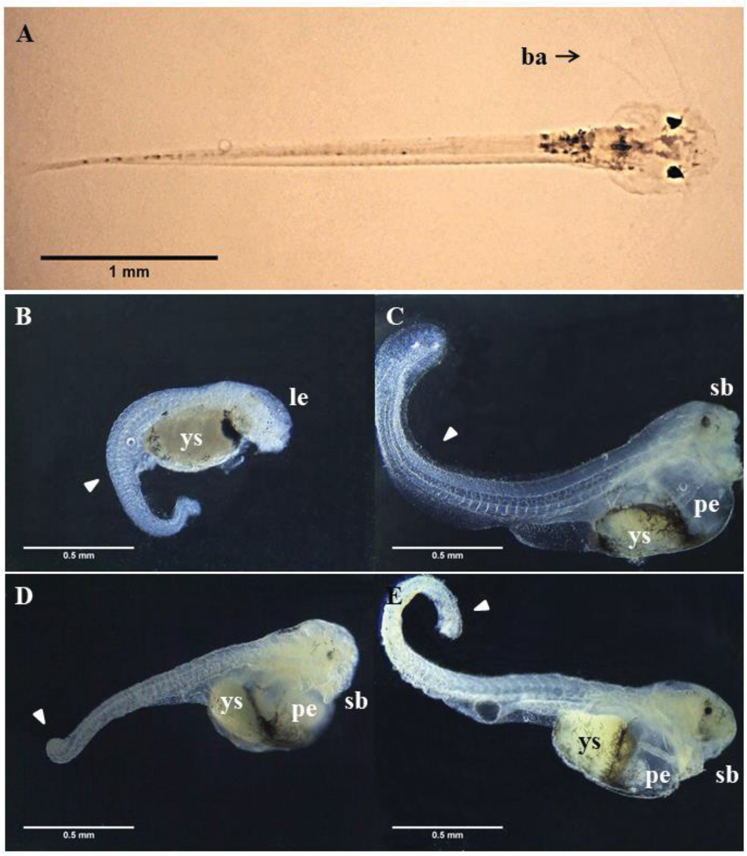

Figure 8. *Pseudoplatystoma corruscans* larvae. (A) Normal larva at 96 hpf. (B) Underdeveloped larva exposed to 50% river water at 48 hpf showing spinal torsion and general body atrophy, especially in the head (lack of eyes). (C) Larva exposed to 30% river water at 72 hpf showing stunted barbel development, pericardial edema with unlooped heart tube, and spinal torsion (lordosis). (D, E) Larvae exposed to 50% river water at 96 hpf showing pericardial edema with unlooped heart tube, stunted barbel development, body atrophy, and spinal torsions. Arrowheads point to spinal torsions and atrophy. Ba = barbels, ys = yolk sac, le = lacking eyes, sb = stunted barbels, pe = pericardial edema.

Mathematical model

After analyzing the different survival rates presented by the four species across experimental time, it was decided to model the population dynamics of those which experienced the greatest impact due to the exposure to polluted water: *S. brasiliensis* and *P. lineatus*. The survival rates used for the calculation of the impact values can be found in table 3. The survival rates of the two other species were not different from that observed in the control treatment, so there was not an impact to be imposed and explored by the mathematical model.

Table 3
Survival rates and impact values calculated for the modeled species (S*alminus brasiliensis* and *Prochilodus lineatus*) exposed to reconstituted water (control), 30% and 50% Atuba River water.

Treatment	Survival rate (%)	Impact value		
		Scenario I	Scenario II	
Salminus brasiliensis	(96 hpf)		Juveniles	Adults
Control	77.08	1	1	1
30%	0.00	0	0	0
50%	2.78	0.036	0.351	0.667
Prochilodus lineatus	(24 hpf)		Juveniles	Adults
Control	39.24	1	1	1
30%	5.56	0.142	0.743	1
50%	6.60	0.168	0.763	1

Experimental data revealed a consistent intense impact on the embryo-larval stage (phase A) of *S. brasiliensis* exposed to water from the Atuba River in both concentrations. Of course, as there was 100% mortality in 30% river water at 96 hpf, all the simulations imposing such impact were drastic. In the first modeled scenario (I), it was revealed that if juvenile and adult individuals were to experience an impact with the same intensity, the population would go extinct in the first year of impact for 30% river water and in the third year for 50% river water (Fig. 9A). Comparatively, in the second scenario (II), if the juveniles and adults were to be 25% and 50%, respectively, less sensitive than the embryo-larval stage, the population would obviously still go extinct in 30% river water or, considering the 50% river water impact, the population density would reduce to 16%, reaching values near extinction (Fig. 9B).

Since *P. lineatus* in the control water did not show a satisfactory survival at 96 hpf, probably due to experimental and test-organism limitations, the survival at 24 hpf was used to calculate the impact of the river water on the embryo-larval stage (phase A). In the first modeled scenario (I), the population would go extinct in just a few years of exposition to both dilutions of river water (Fig. 10A). Comparatively, in the second scenario (II), the average population density would reduce to 71% and 77% if they were exposed to 30% and 50%, respectively, of river water (Fig. 10B).

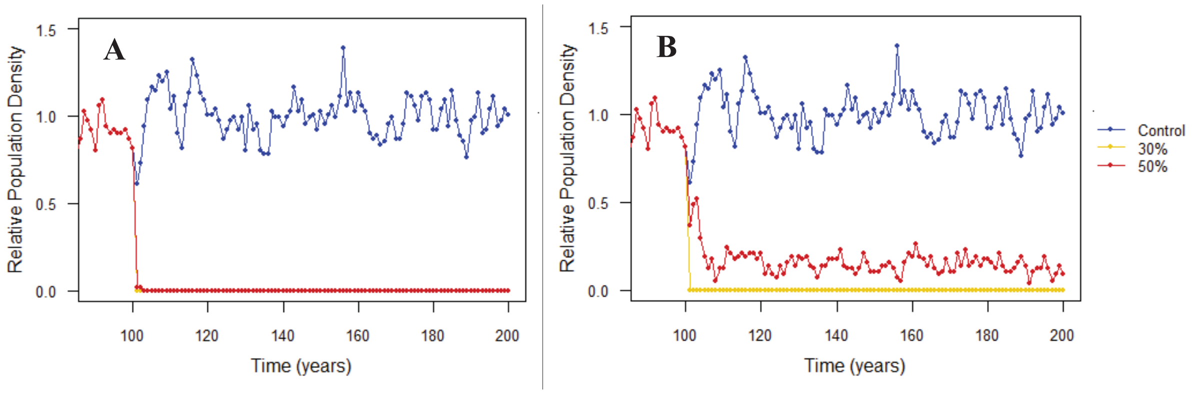

Figure 9. Temporal evolution of relative population size of *Salminus brasiliensis* in two different scenarios. In both cases, the blue line represents a situation without water pollution, the yellow line represents the impact of 30% river water and the red line represents the impact of 50% river water. In (A), the impact observed experimentally on the embryo-larval stage was the same imposed on juveniles and adults (control: $S^A = 0.5, b = 0.77, S^C = 0.6$; 30%: $S^A = 0, b = 0, S^C = 0$; 50%: $S^A = 0.02, b = 0.03, S^C = 0.02$). While in (B), only 75% and 50% of the impact observed experimentally on the embryo-larval stage were imposed on juveniles and adults, respectively (50%: $S^A = 0.02, b = 0.27, S^C = 0.4$).

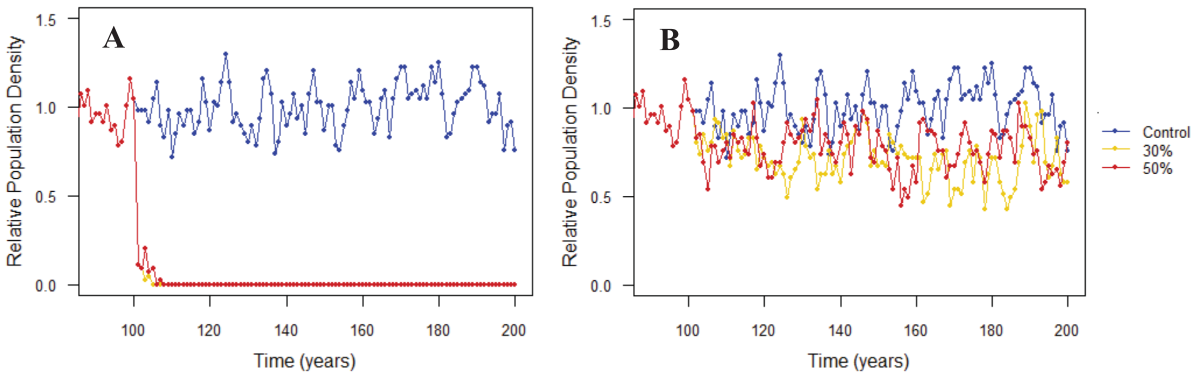

Figure 10. Temporal evolution of relative population size of *Prochilodus lineatus* in two different scenarios. In both cases, the blue line represents a situation without water pollution, the yellow line represents the impact of 30% river water and the red line represents the impact of 50% river water. In (A), the impact observed experimentally on the embryo-larval stage was the same imposed on juveniles and adults (control: $S^A = 0.71, b = 0.5, S^C = 0.54$; 30%: $S^A = 0.1, b = 0.07, S^C = 0.08$; 50%: $S^A = 0.12, b = 0.08, S^C = 0.09$). While in (B), only 75% and 50% of the impact observed experimentally on the embryo-larval stage were imposed on juveniles and adults, respectively (30%: $S^A = 0.1, b = 0.37, S^C = 0.54$; 50%: $S^A = 0.12, b = 0.38, S^C = 0.54$).

Discussion

Since the water of the Atuba River is highly influenced by the high loads of urban discharges received daily, its composition is quite variable, so were the results of the embryo-larval tests with the four species. Considering the mortality endpoint, the characiforms *S. brasiliensis* and *P. lineatus* were the most sensitive to the river water, while the siluriforms *R.*

quelen and *P. corruscans* were less sensitive, without consistent differences between dilutions. However, for the deformity analyses carried out, all species showed higher frequency and severity of deformities in the higher concentration of the river water. Although the chemical nature of the Atuba River water, as any highly impacted urban river, is really complex due to the multiple discharge sources, there are some characteristic compounds that are common to these kinds of water bodies worldwide, being linked to impacts on the biota.

The PAHs detected in the Atuba River are characteristic of urban rivers due to the disposal of oil, fuels, lubricants, and other petroleum derivatives in the wastewater collection network (Stogiannidis and Laane, 2015). There are many detailed studies with high consonance across species showing detrimental effects on developing fish embryos associated with petrogenic related PAHs like the ones found in the Atuba River water samples (naphthalenes and phenanthrene) (Carls et al., 1999, 2008; Heintz et al., 1999; Middaugh et al., 1996, 2002). Teleost early life stages may be particularly susceptible to PAHs exposure, resulting in mortality and developmental defects such as pericardial and yolk sac edema and skeletal deformities (Vines et al., 2000; Couillard et al., 2002; Le Bihanic et al., 2014a, 2014b). Incardona et al. (2004) showed that morphological abnormalities observed in zebrafish embryos exposed to different PAH mixtures, especially phenanthrene, are preceded by defects in cardiac function caused by these compounds. Pollino and Holdway (2002) found that waterborne PAHs, including naphthalene, were able to cross the chorion of crimson-spotted rainbowfish (*Melanotaenia fluviatilis*) embryos, reducing the hatching and survival rates and increasing the frequency of developmental abnormalities.

In addition to the chemicals measured in the samples, it has also been shown that plastic waste such as phthalate and bisphenol-A derivatives may cause lethal and sub-lethal effects on fish embryos. The associated lesions are pericardial edema, tail malformations, delayed hatching and development in *Japanese medaka* and zebrafish, besides bisphenol-A being identified as one of the most impactful estrogenic chemicals (Chikae et al., 2004; Duan et al., 2008; Perkins et al., 2017). Furthermore, many drugs commonly found in domestic wastewater and thus present in urban rivers may also be responsible for critical anomalies in the development of fish (Lange and Dietrich, 2002; Ptluger and Dietrich 2008; Corcoran et al., 2010; Sándor et al., 2012). Brannen et al. (2010) tested the teratogenicity of 31 compounds, including many commercial drugs, finding a high teratogenic potential, assessed via zebrafish embryo viability, morphological abnormalities, and mortality. Furthermore, the authors have found 89% concordance comparing these results with mammal teratogenicity data for the same compounds.

Among the common components in urban wastewater, toxic metals also have been associated with many fish deformities in natural populations and in the laboratory, affecting the survival, growth rates and fitness of exposed individuals (Authman et al., 2015; Sfakianakis et al., 2015). Brito et al. (2018) found that copper, manganese, iron, zinc, and lead were present in concentrations higher than the limits established by the Brazilian legislation at many sites across the Upper Iguaçu River basin, especially around the WWTP Atuba Sul. In general, it was also found that the water samples from the sites with the highest metal concentrations caused the lowest 96hpf survival rates in *R. quelen* larvae.

Embryonic sensitivity varies throughout embryogenesis due to the formation of different tissues, organs, and systems, so death at different stages of embryonic development can be attributed to exposure to different chemicals in the water (Zha e Wang, 2005). Many of the *S. brasiliensis* and *P. lineatus* embryos exposed to the river water did not go through hatching, as they were found dead at the first day still with the chorionic membrane on, and some studies report that this may occur due to exposure to some types of toxic substances (Strmac and Brauunbeck, 1999; Villalobos et al., 2000). The release of the chorionase enzyme around the embryo degrades the inner layer of the chorion and allows the larva to perform movements related to hatching. Through different toxic mechanisms, some chemical substances may inhibit the release of this enzyme, deactivate its proteolytic activity, or impair the movements of the larvae and the rupture of the external nondigestible egg shell, delaying or even preventing the hatching (Hagenmaier, 1974; Hallare et al., 2005b; Cavalcante et al., 2017). Conducting toxicity tests on six marine fish species, Stene and Lonning (1984) observed that the resistance of fish embryos to 2-methylnaphthalene was related to chorion thickness, volume of the perivitelline space, and lipid percentage in egg yolk. Sharp et al. (1979) reported a decrease in the permeability of the chorionic membrane to naphthalene in mummichog (*Fundulus heteroclitus*) embryos during the time course of development, suggesting that the embryos in the first hours after fertilization show a greater uptake of chemicals present in the water. Thus, different permeability of the embryonic membranes of the four species tested may explain distinct survival rates at 24 hpf, since those of *R. quelen* and *P. corruscans* have indeed been reported as being very resistant. The eggs of both species show an extra thick layer of mucous coat, which acts primarily as a barrier to polyspermy, that is common for siluriform eggs, but rarely found on characiform eggs (Rizzo et al., 1998, 2002; Pereira et al., 2006).

Considering the strong impact observed on the embryos and larvae of *S. brasiliensis*, the hypothetical modeled scenarios were also extreme. Even if juvenile and adults were more tolerant than the embryo-larval phase, the population would still reduce to critical relative population density values near extinction. These results are in agreement with the fact that many natural populations of this species have practically disappeared, and it is aggravating considering its distribution range across La Plata basin, which water quality has been compromised (Natale, 2005; Barros et al., 2006; Wong et al., 2007; Baigún et al., 2012; FAO, 2016). Pollution is one of the main causes of local extinction of several large-sized migratory South American species such as *S. brasiliensis*. Besides being economically relevant, this species play an important role maintaining prey species diversity because of their top-down regulation effect (Agostinho et al., 2007). In the Mogi Guaçu River, Upper Paraná River basin in São Paulo state, the *S. brasiliensis* populations have reduce drastically in the last years. Added to the fact that the main fishing area of the region is organized around its breeding ground, there are many pollution sources along the basin in which the fish performs its migration (Esteves and Pinto Lôbo, 2001). In the Jacaré-Guaçu River, a Tietê River affluent, also in São Paulo state, studies have also linked the absence of this species with increasing level of pollution (Esguícero and Arcifa, 2011). Considering the existence of many spawning sites at different sections of the Paraná River basin, the wastewaters coming from affluent sub-basins with strong urban occupation and farming activities, as the Iguaçu River, actually represents a threat, not only for *S. brasiliensis* populations, but also for the other species studied that occur in this river as well (Agostinho et al., 1997; Rossi et al., 2007; Assumpção et al., 2016).

The modeled population dynamics of the second most sensitive species, *P. lineatus*, also led to rapid extinction if all life stages were proportionally impacted. In the second scenario, its population would undergo only a slight decrease in relation to the control. It is important to note that this species showed a reduced survival rate also in the control treatment, which consequently decreased the relative impact value of the river water on the fish and probably overestimated the relative density of the impacted population. For its ecological and economical importance, *P. lineatus* has been used for environmental monitoring and in many toxicity studies, showing high mortality due to copper exposure (Mazon and Fernandes, 1999), cell damages from nitrite (Martinez and Souza, 2002), genotoxic effects induced by glyphosate (Modesto and Martinez, 2010), biochemical, physiological, and histological alterations related to lead exposure (Martinez et al., 2004), polluted water from an urban stream (Camargo and Martinez, 2006), diesel oil (Simonato et al., 2008), river water impacted by diverse anthropogenic activities (Cazenave et al., 2009; 2014), and due to endosulfan exposure (Bacchetta et al., 2010).

Although numerous ecotoxicological studies have been carried out with *P. lineatus*, most of them have used juvenile or adult individuals in their experiments, so there is scarce information on embryo-larval performance of this species under test conditions for comparison. The only study found investigated the acute toxicity of an organophosphorus pesticide to *P. lineatus* embryos and larvae and, despite showing a satisfactory survival in the control treatment, it also exhibited a time-dependent increase in mortality rate (Campagna et al., 2006). Initial larval development represents an extremely sensitive phase and the mortality increase observed in the control can be explained by characteristics related to the specific individuals tested, such as spawning quality, which represents a great challenge for the success of the induced reproduction in fish farms (Woynarovich and Horváth, 1983). Variation in egg quality is one of the limiting factors for successful fingerling mass production in fish farms and may also cause some variability in wild stock recruitment, since poor egg quality may decrease larval survival probability (Kjørsvik et al., 1990). A study on *P. lineatus* cryopreserved semen reported mean fertilization rates between 47% and 83%, depending on the female from which the eggs were collected, which represents a considerable intraspecific variation (Viveiros et al., 2009).

Even with the unexpected mortality in the control treatment, the exposure to the urban river water was detrimental for *P. lineatus* in the first hours after fertilization and until the end of the experiment for the higher concentration tested. In fact, variation in water quality is one of the causes of the severe reductions observed in its natural populations (Agostinho and Gomes, 2002). Being a detritivorous, *P. lineatus* plays a very important role preventing sediment accumulation, allowing different species of algae and invertebrate to grow, and may be viewed as a keystone species, promoting the transfer of energy in the food chain for representing a common prey for many larger, top predator species (Castro & Vari, 2004; Agostinho et al., 2007). On the other hand, its trophic level also maximizes their exposition to toxicants, once many pollutants, such as heavy metals and persistent organic pollutants, concentrate in the sediments on which the species feed and then bioaccumulate (Mugetti et al., 2004; Weber et al., 2013). Many cases of massive fish mortality including *P. lineatus* in the Argentinian portion of the Platine basin have been attributed to the presence of toxicants, high loads of organic matter, and toxins from algal bloom (Gómez, 2014). Furthermore, because of years of domestic and industrial effluents being dumped into the Sino River basin, Rio Grande do Sul state, massive fish mortality events (as high as 90 thousand tons) due to chemical synergistic effects have become common, causing great impact on nursery areas of species like *S. brasiliensis* and *P. lineatus* (Barletta et al., 2010; Harres, 2018). Given this reality, the assessment of fish population status in South American rivers is urgent. Across the Platine basin, actions need to be taken in order to restrain the reduction of natural populations, considering all the possible threats, including low water quality, and ensure the conservation of this important species (Capatto et al., 2010; Baigún et al., 2012).

As discussed by Brito et al. (2017), although the embryo-larval stage may represent the most toxicant-sensitive individuals, the adult stage is the most decisive to the modeled population dynamic. Therefore, imposing a great impact on adults would obviously reduce adult population size. Although the impact values were calculated based on larvae survival, they do not necessarily mean mortality for adults. Even if adult fish is much more tolerant to mortality events than the embryo-larval stage, it is important to consider how pollutants may affect reproduction or how the deformities observed may reduce the fitness of adult fish. Severe malformations can cause large mortality when important functions are impaired (Woock et al., 1987) and even non-lethal abnormalities, which may persist in the juvenile and adult life stages, may decrease the survival probability by compromising the individual ability to feed and avoid predators (Lemly, 1993; 1997). For the population dynamics, these factors would mean the same as lethality, as they impair reproduction and population maintenance. For example, Yamamoto et al. (2017) described the impact on the reproductive physiology of native fish species present along the Iguaçu River reservoirs, probably due to endocrine disrupting chemicals released into the water through urban wastewater discharges. Additionally, Carvalho et al. (2008) observed reduced predation efficiency on *P. lineatus* by *S. brasiliensis* exposed to naphthalene and phenanthrene, while Wu et al. (2011) suggested that bisphenol-induced oxidative stress on zebrafish embryos would affect skeletal development and increase cell death on adult fish, decreasing their general fitness. Thus, the effects of pollutants on adults can be more complex, involving physiological and behavioral responses that may impair basic activities or vital functions and hence population dynamics.

The two siluriform species, *R. quelen* and *P. corruscans*, showed relatively high survival rates, similar in all treatments and exposure times, with a small decrease in *P. corruscans* survival at 72 and 96 hpf. It should be noted that the tests with these two species were conducted with a river water sample collected in a different day, so direct comparisons with the two other species tested should be taken with caution. Brito et al. (2018) exposed embryos and larvae of *R. quelen* to the water of different sites across the Upper Iguaçu River basin, being one of them just 1 km downstream from where the present study was conducted. The same pattern was observed, in which the hatching rate was high (almost 100%) for all treatments. This further suggests that this species egg and embryo are more resistant to disturbances in the water composition than the other species tested. In addition, the final survival of the larvae exposed to 50% river water at 96 hpf also did not differ from control, indicating this species may be actually more resistant to pollution at this stage of the cycle life. The fact that they presented high frequency and severity of deformities when exposed to the river water shows that the level of pollution tested was not enough to cause high mortality on these species, but deleterious enough to induce serious sub-lethal effects, which could affect juveniles or adult individuals. Considering these two similar species shared similar results, some of the biological and ecological characteristics they have in common may explain their higher survival and tolerance to the water from the Atuba River. Although both of them inhabit the bottom of rivers, *R. quelen* is sedentary and omnivorous, while *P. corruscans* is migratory and carnivorous, with more specific needs that can make the species more exigent and sensitive.

The consequences of these findings for the biota of the Iguaçu River must also be considered. Due to the geographic isolation imposed by the Iguaçu falls, the Iguaçu River presents a less diverse but highly endemic ichthyofauna, with more than 70% of endemic fishes among its native species, being recognized as a World Ecoregion of freshwater fish distribution (Abell et al., 2008; Baumgartner et al., 2012). Despite this importance, different impacts have threatened the fish fauna, causing local extinctions that, in this basin, may actually represent a global extinction (Delariva et al., 2018). Besides, considering the smaller number of species occurring in this river and a possible lower functional redundancy, the absence of a single species may be enough to trigger unexpected consequences for the aquatic communities. Therefore, given the serious ecotoxicological effects observed on the species tested on this study, there is a primary need to test and establish the sensitivity of the native and endemic species of the Iguaçu River, in addition to control the pollution sources in the basin, considering it is one of the major causes related to the extinction of the native fish species.

Finally, long distance migratory species, such as *S. brasiliensis*, *P. lineatus* and *P. corruscans*, have a great appeal with the general public, use different habitats during their life cycle, play key ecological roles, and showed to be very sensitive to water quality, especially *S brasiliensis*. Therefore, as suggested by Agostinho et al. (2007), they should be considered "umbrella species", useful not only for conservation programs, but also for long-term perspective environmental policies, as their conservation would involve the protection of a variety of habitats, many other species, and also promote better quality of Neotropical water bodies.

Conclusions

The variability of results obtained among the studied species demonstrates how extrapolations of sensitivity data of a single species may have implications for biodiversity conservation and emphasizes the importance of testing different native species. It was shown how embryo-larval tests offer valuable information on bioavailability and synergistic effects of the complex mixture of chemicals present in a highly impacted urban river. The theoretical model suggested serious risk of exposure of the most sensitive species to polluted waters through the alarming outcome for their populations in the long-term. Thus, the use of modeling applied to ecotoxicological tests should be more frequent, as they contribute to the investigation of the ecological significance of the toxic effects observed at different levels of biological organization. Lastly, Brazilian environmental protection agencies should recognize the importance of ecotoxicological tests for biodiversity conservation measures, directing the elaboration of more restrictive regulatory actions for the treatment and discharge of effluents and prioritizing actions related to the conservation of the megadiverse ichthyofuna and national aquatic ecosystems.

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3. CONSIDERAÇÕES FINAIS

A exposição dos estágios iniciais de desenvolvimento de dourado (*Salminus brasiliensis*), curimbatá (*Prochilodus lineatus*), jundiá (*Rhamdia quelen*) e pintado (*Pseudoplatystoma corruscans*) à água do rio Atuba produziram efeitos letais e subletais variáveis entre as espécies. Os resultados mais graves observados nos embriões e larvas expostos corroboram os estudos já realizados na região, indicando um alto índice de degradação da água e revelando os possíveis efeitos deletérios para a biota. Além disso, a grande variabilidade de resultados obtidos entre as espécies estudadas demonstra como as extrapolações de dados de sensibilidade de uma única espécie podem possuir implicações para conservação da biodiversidade e enfatizam a importância de testar diferentes espécies nativas.

A utilização da modelagem matemática revelou consequências drásticas a nível populacional para as espécies analisadas, reforçando seu papel como uma ferramenta complementar importante aos estudos ecotoxicológicos. Mesmo assumindo considerável tolerância dos estágios de vida mais tardios aos efeitos observados nas larvas, as populações poderiam, ainda assim, exibir uma diminuição crítica de densidade ou ser extintas em poucos anos de exposição à água do rio. Assim, o uso da modelagem aplicada aos ensaios ecotoxicológicos deve ser mais frequente, pois contribuem para a investigação do significado ecológico dos efeitos tóxicos observados em diferentes níveis de organização biológica.

Por fim, os programas de monitoramento e avaliação da qualidade da água no Brasil precisam adotar uma abordagem multidisciplinar, para além dos parâmetros físico-químicos. A ecotoxicologia integra diferentes linhas de evidência, proporcionando uma visão abrangente da relação entre a poluição e seus efeitos à biota. Portanto, os testes ecotoxicológicos devem compor a rotina de monitoramento de qualidade dos recursos hídricos, direcionando a elaboração de ações regulatórias mais restritivas para o tratamento e lançamento de efluentes e proporcionando a manutenção da qualidade dos recursos hídricos nacionais. Baseando-se em tais estudos, tais programas de monitoramento e avaliação poderiam adotar uma visão mais holística, voltada aos ecossistemas, priorizando as ações relacionadas à conservação da ictiofauna megadiversa e dos ecossistemas aquáticos do país.

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