Cecília Bertacini de Assis

Co-exposição do cálcio e do neurofármaco carbamazepina sobre a qualidade seminal de machos de *Astyanax lacustris*

Co-exposure of calcium and the neurodrug carbamazepine on the seminal quality of *Astyanax lacustris* males

São Paulo

2024

Cecília Bertacini de Assis

Versão Corrigida

Co-exposição do cálcio e do neurofármaco carbamazepina sobre a qualidade seminal de machos de *Astyanax lacustris*

Co-exposure of calcium and the neurodrug carbamazepine on the seminal quality of *Astyanax lacustris* males

Dissertação apresentada ao Instituto de Biociências da Universidade de São Paulo, para a obtenção de Título de Mestre em Ciências, na Área de Fisiologia Geral.

Orientadora: Profa. Dra. Renata Guimarães Moreira Whitton
Coorientador: Dr. João Paulo Silva Pinheiro

São Paulo

2024

Ficha Catalográfica

Ficha catalográfica elaborada pelo Serviço de Biblioteca do Instituto de Biociências da USP, com os dados fornecidos pelo (a) autor (a) no formulário: "https://ficha-catalografica.ib.usp.br/ficha.php"

Assis, Cecília Bertacini de
Co-exposição do cálcio e do neurofármaco carbamazepina sobre a qualidade seminal de machos de *Astyanax lacustris* / Assis Cecília Bertacini de ; orientador Moreira Renata Guimarães ; coorientador Pinheiro João Paulo Silva -- São Paulo, 2024.
42 p.

Dissertação (Mestrado) -- Instituto de Biociências da Universidade de São Paulo. Ciências Biológicas (Fisiologia) .

1. Contaminante de preocupação emergente. 2. Toxicidade. 3. Água doce. 4. Teleósteo. 5. Espermatozoide. I. Moreira, Renata Guimarães, orient. II. Pinheiro, João Paulo Silva, coorient. Título.

Bibliotecária responsável pela catalogação:

Elisabete da Cruz Neves – CRB – 8/6228

Comissão Julgadora:

________________________ ________________________

Prof(a). Dr(a). Prof(a). Dr(a).

____

Prof(a). Dr.(a). Renata Guimarães Moreira

Orientador(a)

Dedicatória

Às minhas mães, Odete Cecília Bertacini e Benedita Custódio.

Epígrafe

In omnia paratus

"Você ganha força, coragem e confiança através de cada experiência em que você realmente para e encara o medo de frente".

Eleanor Roosevelt

Agradecimentos

Queria fazer um agradecimento padrão, começar pelas agências de fomento e depois seguir com as palavras bonitas dedicadas aos meus amigos e família, mas a grande verdade sobre essa sessão é que venho evitando-a por muitos motivos, que talvez sejam até grandes dramas internos que só são intensos dentro de mim. Procrastinei até em escrever a dissertação toda porque isso anuncia o fim de um ciclo que tenho grande dificuldade em fechar. Eu culpo meu ascendente em câncer por tudo isso.

Conscientemente eu sei que fiz/faço uma tempestade em uma xícara de café (não considero nem um copo d’agua, pois seria grande demais), e que não é o fim do mundo e muito menos o fim das relações que construí com tanto orgulho desde o segundo semestre da minha graduação. Tento consolar meu drama pensando na minha trajetória até aqui, porque foi no LAMEROA que eu cresci, foi lá que eu alimentei meu amor pelo que faço e onde dei meus primeiros passos como pesquisadora. Foi no LAMEROA que tive grandes mentores me guiando em todo meu processo de aprendizagem, onde tomei coragem para mudar de curso e principalmente foi onde deixei minhas melhores gargalhadas.

Entrar na Universidade de São Paulo como aluna de iniciação cientifica e sair como mestre é o meu maior orgulho! Nenhuma universidade ou trabalho no mundo vão ocupar o lugar que a USP ocupa dentro de mim, e qualquer oportunidade que venha depois dessa fase é consequência do que é lutar por um sonho que cultivei desde os meus 13 anos. Eu consegui! Mas não sozinha, nunca sozinha...Aline Dal’Olio, Amanda Guerreiro, Natalia Pires e Valquíria Pereira são grandes nomes que correram do meu lado durante essa estrada. João Paulo Pinheiro, Giovana Branco e Renata Guimarães foram os meus mentores em todo o percurso, e por isso eu não tenho como agradecer a troca que sempre tivemos. Se eu conseguir ser metade da profissional/ser humano que vocês são, já estarei realizada em minha vida. A família LAMEROA, os que já foram e os que ficaram, também são parte crucial em tudo isso, pois foi na nossa rotina que tudo se tornou melhor.

Apesar do tanto que sou grata por essas pessoas, existe uma em especial que merece um parágrafo individual, Renato Massaaki Honji. Ele atuou por trás das câmeras e aguentou com maestria essa barra que é gostar de mim, rs. Todas as vezes que achei que não iria aguentar mais, que eu disse que não servia para nada disso, ele pegou na minha mão e me mostrou que eu posso tudo que eu quiser, me mostrou que eu sou mais do que eu penso ser, me incentivou a voar alto e me amou incondicionalmente mesmo quando eu perdi o controle de mim mesma. Ele foi/é a minha maior inspiração! Me espelho na integridade, na ética, na lealdade e na bondade que ele emprega em tudo que ele faz, dentro e fora da vida profissional. Ele é O cara para mim (mesmo com todos os defeitos que todos nós conhecemos bem rs) e que sorte a minha me casar com o meu herói. Te amo para toda uma vida.

Unindo forças com o Renato, minha mãe Odete Cecilia Bertacini me carrega no colo desde 1997 e nunca me deixou cair. Eu não tenho palavras suficientes para expressar tudo que eu quero aqui, mas mãe esse trabalho também é seu! A gente conseguiu! Obrigada por fazer de mim quem eu sou e por me dar a honra de ser a sua filha. Obrigada por ser a luz que me guia todos os dias. Obrigada por ser minha melhor amiga. Obrigada por ser colo quando eu mais preciso. Obrigada por acreditar nos meus sonhos e obrigada por lutar para que eu tivesse a chance de realizá-los. Você é tudo que eu sempre quis ser. Te amo nessa vida e nas próximas.

Por fim, gostaria de agradecer as agências de fomento Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP - 2020/14252-5;

2022/03440-0), CNPq (Projeto universal, 404705/2021-9) e CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), ao Instituto de Biociências da Universidade de São Paulo, em especial Erika, e todos os funcionários envolvidos na minha formação. Sou feliz e orgulhosa por fazer parte dessa história.

Índice

Resumo

O neurofármaco Carbamazepina (CBZ), que está presente em corpos hídricos em altas concentrações, e é resistente à biodegradação, aliado à presença de cálcio (Ca), pode ter grande influência na fisiologia de organismos não-alvo. No entanto, não se sabe se a co-exposição de Ca e CBZ pode influenciar os parâmetros espermáticos dos peixes. Portanto, o objetivo do presente estudo foi avaliar o papel do CBZ na morfologia, cinética, concentração espermática e pH seminal de machos de *A. lacustris*, e a influência do Ca nos efeitos do CBZ nos mesmos parâmetros após exposição. Após o término da exposição dos machos à combinação de diferentes concentrações ambientais de CBZ com cálcio, amostras de sêmen foram coletadas para avaliar parâmetros de qualidade seminal. De modo geral, os resultados de morfologia mostraram queda significativa na porcentagem de espermatozoides normais em animais expostos ao CBZ nas concentrações de 250 e 500 ng L^{-1} e expostos a 21 mg L^{-1} Ca + 250 ng L^{-1} CBZ em relação ao DMSO. Da mesma forma, houve redução da velocidade curvilínea (VCL), velocidade em linha reta (VSL) e velocidade média de deslocamento (VAP), além do percentual de retilinearidade (STR) nos mesmos grupos experimentais. A motilidade espermática (MOT) foi reduzida quando os peixes foram expostos apenas a 250 e 500 ng L^{-1} de CBZ. O pH seminal e a concentração espermática não foram afetados pelo CBZ e/ou Ca. Considerando os resultados obtidos, ao contrário do que foi hipotetizado, observou-se que a CBZ sozinha tem maiores efeitos na qualidade seminal de *A. lacustris* em comparação ao CBZ+Ca. Portanto, estes resultados contribuem para ações urgentes na compreensão da toxicidade do CBZ, uma droga com ações perturbadoras da reprodução em peixes teleósteos.

Abstract

The neuropharmaceutical Carbamazepine (CBZ), which is present in water bodies in high concentrations, and is resistant to biodegradation, combined with the presence of calcium (Ca), can have a great influence on the physiology of non-target organisms. However, it is not understood whether co-exposure of Ca and CBZ can influence fish sperm parameters. Therefore, the objective of the present study was to evaluate the role of CBZ in the morphology, kinetics, sperm concentration, and seminal pH of *A. lacustris* males, and the influence of Ca on the effects of CBZ on the same parameters, after exposure. After the end of the males' exposure to the combination of different environmental concentrations of CBZ with calcium, semen samples were collected to evaluate seminal quality parameters. In general, the morphology results showed a significant drop in the percentage of normal spermatozoa in animals exposed to CBZ at concentrations of 250 and 500 ng L^{-1} and exposed to 21 mg L^{-1} Ca + 250 ng L^{-1} CBZ in relation to DMSO. Similarly, there was a reduction in curvilinear speed (VCL), straight line speed (VSL) and average travel speed (VAP), in addition to the percentage of straightness (STR) in the same experimental groups. The spermatozoa motility (MOT) was reduced when fish was exposed to 250 and 500 ng L^{-1} of CBZ only. Seminal pH and sperm concentration were not affected by CBZ and/or Ca. Considering the results obtained, contrary to what was hypothesized, it was observed that CBZ alone has greater effects on the seminal quality of *A. lacustris* compared to CBZ+Ca. Therefore, these results contribute to urgent action in understanding the toxicity of CBZ, a drug with reproductive disrupting actions in teleost fish.

Capítulo 1

1. Introdução Geral

Diversas substâncias como pesticidas, metais, resíduos industriais e fármacos estão presentes no ambiente aquático, e a origem dessas substâncias pode estar relacionada com urbanização, desenvolvimento das atividades de grandes indústrias e práticas agrícolas intensas (Pinheiro et al., 2021; Wilkinson et al., 2022). Levando em consideração apenas os produtos farmacêuticos e seus princípios ativos, esses poluentes são exemplos que vem cada vez mais ganhando destaque na literatura especializada (Pinheiro et al., 2021), principalmente no que diz respeito à qualidade de água, pois durante a fabricação, uso, liberação de metabólitos (sistema urinário por exemplo) de fármacos, e o descarte (descarte inadequado de produtos vencidos e/ou inutilizados), esses compostos têm como destino o ecossistema aquático (Wilkinson et al., 2022).

No que concerne o ambiente dulcícola, compostos farmacológicos vem sendo encontrados em ambiente de água doce por décadas (Alrashood, 2016) e muitos fármacos são considerados contaminantes ambientais de preocupação emergente, que segundo o Conselho de Meio Ambiente dos Estados Unidos (do inglês *The Environmental Council of the States* - ECOS) são substâncias de origem natural ou sintética frequentemente detectadas no ambiente e não há legislação ou base cientifica bem definidos para estabelecer padrões na relação produção *versus* descarte (ECOS, 2014). Além disso, podem apresentar um risco à saúde humana e do meio ambiente, pois grande parte delas não são biodegradáveis e tem como consequência a bioacumulação nos tecidos dos organismos e biomagnificação ao longo da cadeia trófica (Ruhí et al., 2016; Rajeshkumar e Li, 2018; Bergmann e Graça, 2020; Windsor et al., 2020).

Dentre os fármacos presentes no ambiente aquático, podemos destacar a Carbamazepina (CBZ) (Figura 1), que é um neurofármaco considerado um contaminante ambiental de preocupação emergente. A CBZ, conhecida como *benzo[b][1]benzazepine-11-carboxamide* segundo as normas da *International Union of Pure and Applied Chemistry* (IUPAC), é representada quimicamente pela fórmula $C_{15}H_{12}N_2O$, tem o ponto de fusão entre 189-193°C, apresenta polimorfismo, peso molecular igual a 236.27 g mol^{-1} (NCBI, 2024) e seu principal metabólito é o *carbamazepine-10,11-epoxide* (Abou-Khalil, 2016). Esse agente farmacêutico é usado principalmente em tratamentos de epilepsia (Beutler et al., 2005; Lee et al., 2007), mas também em transtorno bipolar (Beutler et al., 2005; Lee et al., 2007) e neuralgia trigeminal (Brunton et al., 2012).

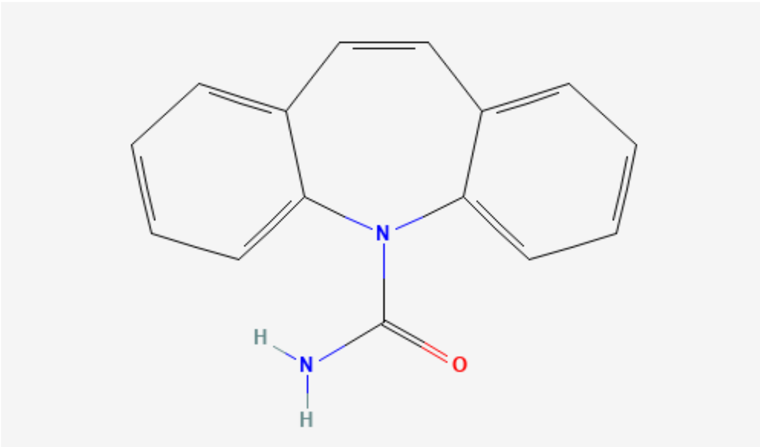

Figura 1: Estrutura química da carbamazepina (CBZ) ($C_{15}H_{12}N_2O$). Comercialmente a CBZ é conhecida como *Biston, Finlepsin, Stazepine, Tegretal, Telesmin* ou *Timonil*. *National Center for Biotechnology Information* (2024).

A atuação da CBZ no sistema nervoso central é realizada por diferentes mecanismos fisiológicos, como por exemplo: 1) diminuição da excitabilidade neuronal pela inativação dos canais de sódio (Na^+) e cálcio (Ca^{2+}) dependentes de voltagem (Potschka, 2013); 2) na transmissão dopaminérgica; 3) na elevação das concentrações de serotonina extracelular; 4) na inibição da liberação de glutamato (Okada et al., 1997; Okada et al., 1998; Schumacher et al., 1998; Mizuno et al., 2000; Kawata et al., 2001; Ambrósio et al., 2002; Sitges et al., 2016); e 5) é um forte indutor enzimático, podendo reduzir níveis de substâncias endógenas metabolizadas pelo sistema enzimático P450 (Abou-Khalil, 2016), dentre outros mecanismos. Em relação a fisiologia reprodutiva, a CBZ está entre as substâncias que interfere nos parâmetros reprodutivos de seres humanos, incluindo ambos os sexos, masculino e feminino (Hamed, 2016; Wu et al., 2018), principalmente nas pessoas que utilizam medicamentos antiepiléticos, pois esses medicamentos e as "convulsões ocasionadas no indivíduo" podem alterar a liberação de hormônios hipofisários e hipotalâmicos e consequentemente a produção de distintos esteroides sexuais (Khan et al., 2023). De acordo com Hamed (2016) e Wu e colaboradores (2018), entre 38 e 71% de homens com epilepsia apresentam alterações no processo reprodutivo devido a essa condição (epilepsia) e devido ao uso de medicamentos para o tratamento de epilepsias, como a CBZ.

No ambiente aquático, a CBZ é comumente detectada em águas residuais por ser altamente recalcitrante às técnicas padrão de tratamento de água com meia vida relativamente longa de 82 ± 11 dias (Brandão et al., 2013) e menos de 30% do composto é removido durante esse processo (Ji et al., 2016). No Brasil, a CBZ foi encontrada nas águas do Rio Belém (Curitiba – PR) com concentrações variando de 670 a 856 ng L^{-1} (Böger et al., 2018), no Rio Igarapé (AM) com 652 ng L^{-1} (Thomas et al., 2014) e no estado de São Paulo, no reservatório Guarapiranga, observa-se a faixa de 12 a 358 ng L^{-1} deste fármaco na água (Shihomatsu et al., 2017). Devido à esta presença da CBZ nos ecossistemas aquáticos, efeitos tóxicos podem ser esperados em diversos organismos. Neste contexto, foi visto que a CBZ age negativamente na fisiologia reprodutiva de teleósteos, como observado em *Danio rerio*, pois animais expostos a CBZ, apresentaram alterações na concentração plasmática de esteroides gonadais, como a 11-cetotestosterona e 17-β-estradiol (Fraz et al., 2018), prejudicando a formação de gametas. Qiang e colaboradores (2016) expuseram *D. rerio* a CBZ (1 μg/L) e concluíram que este fármaco pode afetar o desenvolvimento embrionário e crescimento de embriões e larvas. Já Silva Santos e colaboradores (2018) sugerem que a CBZ diminuiu a porcentagem de ovos viáveis à fertilização de *D. rerio* expostos a CBZ nas concentrações de 0, 10 e 10,000 μgL^{-1}. Em *Gobiocypris rarus* exposto a CBZ nas concentrações de 1, 10 e 100μg/L, esse fármaco foi capaz de alterar a expressão gênica de distintos genes do eixo reprodutivo (cérebro-hipófise-gônadas) e de esteroides sexuais, sugerindo que a CBZ tem atividade estrogênica homóloga e induzem toxicidade reprodutiva nessa espécie (Yan et al., 2018). Somado a esses estudos descritos acima, a CBZ tem efeitos deletérios na qualidade seminal, alterando a morfologia dos espermatozoides (Fraz et al., 2017), reduzindo a cinética espermática e causando estresse oxidativo nos gametas masculinos (*in vitro*) de *Cyprinus carpio* (Li et al., 2010).

Além dos poluentes, como a CBZ, as características físicas e químicas da água (variação de pH e temperatura, a salinidade e a concentração de minerais) também podem interferir na fisiologia dos organismos (Valdebenito et al., 2015; Kowalski e Cejko, 2019). Todos os organismos aquáticos necessitam de minerais para a regulação de processos fisiológicos e bioquímicos para manter a homeostase. Os peixes, por exemplo, absorvem pelas brânquias (sistema respiratório e osmorregulatório) esses minerais através da água circundante e pela dieta (sistema digestório) como os demais vertebrados (Lall e Kaushik, 2021) e a absorção dessas substâncias dependem da biodisponibilidade no ambiente (Lall e Kaushik, 2021). Dentre os macros minerais (por exemplo, fósforo (P), magnésio (Mg), sódio (Na), potássio (K), cloreto (Cl^-) e cálcio (Ca)), esse último (Ca) pode ser destacado como essencial, pois desempenha importante função como mensageiro celular secundário, necessário para diversos processos fisiológicos e celulares, incluindo a regulação da expressão gênica, contração muscular e transporte molecular, bem como a amplificação da ação de ligantes na superfície celular (Butts et al., 2013). O cálcio também participa ativamente no desencadeamento da motilidade dos espermatozoides, pois em geral, o início da batida axonemal da cauda está associado a um aumento intracelular de íons Ca^{2+} nos espermatozoides de teleósteos (Parodi et al., 2017; Alavi et al., 2019). Além disso, este íon tem suma importância para a espermatogênese, regulando funções importantes durante diferentes estágios da formação dos gametas, como o crescimento, proliferação e diferenciação (Ravindranath et al., 1994; Treviño et al., 1998).

Além da influência nos processos fisiológicos descritos acima, estudos mostram que a qualidade da água, como a dureza (quantidade total de íons cátions divalentes dissolvidos – Ca^{2+}, magnésio (Mg^{2+}) e/ou ferro (Fe^{2+})), pode agir na toxicidade de substâncias químicas presentes no ambiente aquático (Soucek et al., 2011; Marchand et al., 2013; Baldisserotto et al., 2014; Hundt et al., 2016). No entanto, o que não está claro é a ação desses cátions separadamente (Pinheiro et al., 2021) e como essa interação pode interferir em aspectos importantes do processo reprodutivo como a qualidade seminal. Em condições adequadas, este processo fisiológico ocorre naturalmente nos testículos resultando na produção de espermatozoides viáveis à fertilização, porém, a presença de poluentes no ambiente aquático pode alterar a fisiologia reprodutiva de machos de teleósteos (Kowalski e Cejko, 2019).

Em resumo, sabendo da problemática da CBZ como um contaminante aquático de preocupação emergente (do inglês, *contaminants of emerging concern* - CECs); da presença e importância do Ca^{2+} na qualidade de água; e a possibilidade de eles interagirem, é possível sugerir que a presença desse íon em concentração ambiental possa aumentar a toxicidade da CBZ. No entanto, não existem estudos com objetivo de investigar as consequências dos efeitos da CBZ e do Ca^{2+}, de forma isolada ou associada, na biologia reprodutiva de espécies nativas (neotropical) brasileiras, tanto em bioensaios quanto em ambiente natural.

1.1. *Astyanax lacustris*

Astyanax lacustris (Figura 2) (Lucena e Soares, 2016), antes classificado como *Astyanax altiparanae* (Garutti e Britiski, 2000), é um pequeno peixe teleósteo onívoro e popularmente conhecido como lambari-do-rabo-amarelo. *A. lacustris* faz parte da família *Characidae* (Characiformes) e tem ampla distribuição geográfica pelos rios do Brasil, além disso, apresenta um alto valor econômico e, por conta da facilidade de manejo em condições laboratoriais, *A. lacustris* vem atraindo cada vez mais atenção da comunidade cientifica como modelo experimental de peixe neotropical (Porto-Foresti et al., 2010; Gonçalves et al., 2014; Chehade et al., 2014; Siqueira Silva et al., 2020; Pinheiro et al., 2019, 2020; Peñuela et al., 2021, 2022, 2024; Branco et al., 2021; Assis et al., 2021; Godoi et al., 2024). Esta espécie apresenta várias características que facilitam a utilização como modelo experimental, dentre estas, facilidade no manejo em cativeiro, período reprodutivo conhecido (geralmente na primavera e verão na região sudeste de São Paulo), dimorfismo sexual aparente durante o período reprodutivo (os machos apresentam pequenos "ganchos" na nadadeira anal, tornando-os ásperos ao toque manual), o que torna a identificação e seleção de reprodutores facilitada (Porto-Foresti et al., 2010). Os gametas masculinos também são usados como modelos em bioensaios ecotoxicológicos em decorrência da alta sensibilidade aos efeitos antagônicos dos compostos presentes no meio (Fabbrocini et al., 2013), bem como ao fato da fertilização dos gametas ocorrer externamente (água) na maioria das espécies.

Figura 2: Macho de *Astyanax lacustris* (lambari-do-rabo-amarelo) (Characiformes, Characidae) utilizado no presente estudo.

1.2. Hipótese

Sabendo-se da importância da qualidade dos gametas na fertilização, eclosão, no desenvolvimento embrionário e consequentemente no sucesso reprodutivo da espécie, e da ampla presença da CBZ nos corpos d'água, a hipótese do presente estudo é que a CBZ (em concentrações ambientais) causa danos à qualidade seminal de *A. lacustris* e que o Ca potencializa estes danos.

1.3. Objetivo geral

O objetivo geral do presente estudo foi avaliar os efeitos de diferentes concentrações ambientais de CBZ na água, na qualidade seminal de *A. lacustris*, bem como os efeitos deste fármaco quando co-exposto com o Ca.

1.4. Objetivos específicos

Para alcançar este objetivo geral e testar a nossa hipótese, os seguintes objetivos específicos foram propostos:

1) Avaliar a morfologia, cinética, concentração espermática, e pH seminal de *A. lacustris* expostos a diferentes concentrações de CBZ;

2) Avaliar a influência do Ca, associado à diferentes concentrações de CBZ, na morfologia, cinética, concentração espermática, e pH seminal de *A. lacustris*.

2. Referências Bibliográficas

Abou-Khalil, B. W. 2016. Antiepileptic drugs. Continuum (Minneap Minn). 22: 132-56.

Alavi, S. M. H.; Cosson, J.; Bondarenko, O. & Linhart, O. 2019. Sperm motility in fishes:(III). Diversity of regulatory signals from membrane to the axoneme. Theriogenology. 136: 143-165.

Alrashood, S. T. 2016. Carbamazepine. Profiles of Drug Substances, Excipients and Related Methodology. 41: 133-321.

Ambrósio, A. F.; Soares da Silva, P.; Carvalho, C. M. & Carvalho, A. P. 2002. Mechanisms of action of carbamazepine and its derivatives, oxcarbazepine, BIA 2-093, and BIA 2-024. Neurochemical Research. 27: 121-130.

Assis, C. B.; Branco, G. S.; Moreira, R. G. & Pinheiro, J. P. S. 2021. Aluminum at environmental concentrations affects the sperm quality of the freshwater teleost *Astyanax altiparanae*: an in vitro study. Comparative Biochemistry and Physiology C - Toxicology & Pharmacology. 243: 108983.

Baldisserotto, B.; Martos-Sitcha, J. A.; Menezes, C. C.; Toni, C.; Prati, R. L.; Garcia, L. D. O.; Salbego, J.; Mancera, J. M. & Martínez-Rodríguez, G. 2014. The effects of ammonia and water hardness on the hormonal, osmoregulatory and metabolic responses of the freshwater silver catfish *Rhamdia quelen*. Aquatic Toxicology. 152: 341-352.

Bergmann, M. & Graça, M. A. 2020. Bioaccumulation and dispersion of uranium by freshwater organisms. Archives of Environmental Contamination and Toxicology. 78: 254 -266.

Beutler, A. S.; Li, S.; Nicol, R. & Walsh, M. J. 2005. Carbamazepine is an inhibitor of histone deacetylases. Life Sciences. 76: 3107–3115.

Böger, B.; Amaral, B. D.; Estevão, P. L. D. S.; Wagner, R.; Peralta-Zamora, P. G. & Gomes, E. C. 2018. Determination of carbamazepine and diazepam by SPE-HPLC-DAD in Belém River water, Curitiba-PR/Brazil. Revista Ambiente & Água. 13: e2196.

Branco, G. S.; Moreira, R. G.; Borella, M. I.; Paiva, C. M.; Muñoz-Peñuela, M.; Gomes, A. D. O. & Tolussi, C. E. 2021. Nonsteroidal anti-inflammatory drugs act as endocrine disruptors in *Astyanax lacustris* (Teleostei: Characidae) reproduction: an ex vivo approach. Aquatic Toxicology. 232:105767.

Brandão, F. P.; Rodrigues, S.; Castro, B. B.; Gonçalves, F.; Antunes, S. C. & Nunes, B. 2013. Short-term effects of neuroactive pharmaceutical drugs on a fish species: biochemical and behavioural effects. Aquatic Toxicology. 144−145: 218−229.

Brunton, L. L.; Chabner, B. A. & Knollmann, B. C. 2012. Las bases farmacológicas de la terapéutica 12° edición. Ed. 21. McGraw Hill, México, D.F.

Butts, I. A.; Alavi, S. M. H.; Mokdad, A. & Pitcher, T. E. 2013. Physiological functions of osmolality and calcium ions on the initiation of sperm motility and swimming performance in red side dace, *Clinostomus elongatus*. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology. 166: 147-157.

Chehade, C.; Cassel, M.; Borella, M. I. & Costa, F. G. 2014. Morphologic study of the liver of lambari (*Astyanax altiparanae*) with emphasis on the distribution of cytokeratin. Fish Physiology and Biochemistry. 40: 571–576.

ECOS. 2014. The Environmental Council of the States. Retrieved March 11, 2024 from: https://www.ecos.org.

Fabbrocini, A.; D'adamo, R.; Del Prete, F.; Langellotti, A. L.; Barone, C. M. A.; Rinna, F.; Sessa, R.; Silvestri, F.; Villani, G.; Vitiello, V.; Vittielo, V. & Sansone, G. 2013. Motility of cryopreserved spermatozoa for the ecotoxicological evaluation of aquatic environments. Journal of Chemical Ecology 29: 660-667.

Fraz, S. 2017. Carbamazepine & Gemfibrozil affect zebrafish reproduction. Doctoral dissertation – McMaster University, Canadá.

Fraz, S.; Lee, A. H. & Wilson, J. Y. 2018. Gemfibrozil and carbamazepine decrease steroid production in zebrafish testes (*Danio rerio*). Aquatic Toxicology. 198: 1-9.

Garutti, V. & Britiski, H. A. 2000. Descrição de uma espécie nova de *Astyanax* (Teleostei: Characidae) da bacia do alto rio Paraná e considerações sobre as demais espécies do gênero na bacia. Comunicações do Museu de Ciências e Tecnologia Serie Zoológica Porto Alegre. 13:65–88.

Godoi, F. G. A.; Dias, M. A.; Guerreiro, A. S.; Branco, G. S.; Montagner, C. C.; Moreira, R. G. & Lo Nostro, F. L. 2024. Physiological responses on the reproductive, metabolism and stress endpoints of *Astyanax lacustris* females (Teleostei: Characiformes) after diclofenac and ibuprofen exposure. Comparative Biochemistry and Physiology C-Toxicology & Pharmacology. 278: 109846.

Gonçalves, L. U.; Parisi, G.; Bonelli, A.; Sussel, F. R. & Viegas, E. M. M. 2014. The fatty acid compositions of total, neutral and polar lipids in wild and farmed lambari (*Astyanax altiparanae*) (Garutti & Britski, 2000) broodstock. Aquaculture Research. 45: 195–203.

Hamed, S. A. 2016. The effect of epilepsy and antiepileptic drugs on sexual, reproductive and gonadal health of adults with epilepsy. Expert Review of Clinical Pharmacology. 9: 807-819.

Hundt, M.; Schreiber, B.; Eckmann, R.; Lunestad, B. T.; Wünneman, H. & Schulz, R. 2016. The effect of water hardness on mortality of zebrafish (*Danio rerio*) during exposure to oxytetracycline. Bulletin of Environmental Contamination and Toxicology. 96: 144-9.

Ji, C.; Hou, J.; Wang, K.; Zhang, Y. & Chen, V. 2016. Biocatalytic degradation of carbamazepine with immobilized laccase-mediator membrane hybrid reactor. Journal of Membrane Science. 502: 11-20.

Kawata, Y.; Okada, M.; Murakami, T.; Kamata, A.; Zhu, G. & Kaneko, S. 2001. Pharmacological discrimination between effects of carbamazepine on hippocampal basal, Ca^{2+} and K^{+} evoked serotonin release. British Journal of Pharmacology. 133: 557–567.

Khan, M.; Bankar, N. J.; Bandre, G. R.; Dhobale, A. V. & Bawaskar, P. A. 2023. Epilepsy and issues related to reproductive health. Cureus. 15: e48201.

Kowalski, R. K. & Cejko, B. I. 2019. Sperm quality in fish: determinants and affecting factors. Theriogenology. 135: 94-108.

Lall, S. P. & Kaushik, S. J. 2021. Nutrition and metabolism of minerals in fish. Animals. 11: 2711.

Lee, H. J.; Rao, J. S.; Rapoport, S. I. & Bazinet, R. P. 2007. Antimanic therapies target brain arachidonic acid signaling: lessons learned about the regulation of brain fatty acid metabolism. Prostaglandins, Leukotrienes & Essential Fatty Acids. 77: 239–246.

Li, Z. H.; Li, P.; Rodina, M. & Randak, T. 2010. Effect of human pharmaceutical Carbamazepine on the quality parameters and oxidative stress in common carp (*Cyprinus carpio L.*) spermatozoa. Chemosphere. 80: 530-534.

Lucena, C. A. S. & Soares, H. G. 2016. Review of species of the *Astyanax bimaculatus* “caudal peduncle spot” subgroup sensu Garutti & Langeani (Characiformes, Characidae) from the rio La Plata and rio São Francisco drainages and coastal systems of southern Brazil and Uruguay. Zootaxa. 4072: 101-125.

Marchand, P. A.; Straus, D. L.; Wienke, A.; Pedersen, L. F. & Meinelt, T. 2013. Effect of water hardness on peracetic acid toxicity to zebrafish, *Danio rerio*, embryos. Aquaculture International. 21: 679-686.

Mizuno, K.; Okada, M.; Murakami, T.; Kamata, A.; Zhu, G.; Kawata, Y.; Wada, K. & Kaneko, S. 2000. Effects of carbamazepine on acetylcholine release and metabolism. Epilepsy Research. 40: 187–195.

NCBI. 2024. National Center for Biotechnology Information. PubChem Compound Summary for CID 2554, Carbamazepine. Retrieved March 11, 2024 from https://pubchem.ncbi.nlm.nih.gov/compound/Carbamazepine.

Okada, M.; Hirano, T.; Mizuno, K.; Chiba, T.; Kawata, Y.; Kiryu, K.; Wada, K.; Tasaki, H. & Kaneko, S. 1997. Biphasic effects of carbamazepine on the dopaminergic system in rat striatum and hippocampus. Epilepsy Research. 28: 143–153.

Okada, M.; Kawata, Y.; Mizuno, K.; Wada, K.; Kondo, T. & Kaneko, S. 1998. Interaction between Ca^{2+}, K^{+}, carbamazepine and zonizamide on hippocampal extracellular glutamate monitored with a microdialysis electrode. British Journal of Pharmacology. 124: 1277–1285.

Parodi, J.; Guerra, G.; Cuevas, M.; Ramírez-Reveco, A. & Romero, F. 2017. Effects of storage time on the motility, mortality and calcium levels of Atlantic salmon *Salmo salar* spermatozoa. Journal of Fish Biology. 90: 1506-1516.

Peñuela, M. M.; Lo Nostro, F. L.; Gomes, A. D.; Tolussi, C. E.; Branco, G. S.; Pinheiro, J. P. S.; Godoi, F. G. A. & Moreira, R. G. 2021. Diclofenac and caffeine inhibit hepatic antioxidant enzymes in the freshwater fish *Astyanax altiparanae* (Teleostei: Characiformes). Comparative Biochemistry and Physiology C - Toxicology & Pharmacology. 240: 108910.

Peñuela, M. M.; Moreira, R. G.; Gomes, A. D.; Tolussi, C. E.; Branco, G. S.; Pinheiro, J. P. S.; Zampieri, R. A. & Lo Nostro, F. L. 2022. Neurotoxic, biotransformation, oxidative stress and genotoxic effects in *Astyanax altiparanae* (Teleostei, Characiformes) males exposed to environmentally relevant concentrations of diclofenac and/or caffeine. Environmental Toxicology and Pharmacology. 91: 103821.

Peñuela, M.; Lo Nostro, F. L.; Gomes, A. D. O.; Tolussi, C. E.; Lozano, I.; Moreira, R. G. 2024. A biomarker approach to study the effects of polluted Brazilian urban reservoirs in a native fish. Science of the Total Environment. 923: 171500.

Pinheiro, J. P. S.; Assis, C. B.; Peñuela, M. M.; Júnior, F. B.; Correia, T. G. & Moreira, R. G. 2019. Water temperature and acid pH influence the cytotoxic and genotoxic effects of aluminum in the freshwater teleost *Astyanax altiparanae* (Teleostei: Characidae). Chemosphere. 220: 266-274.

Pinheiro, J. P. S.; Assis, C. B.; Sanches, E. A. & Moreira, R. G. 2020. Aluminum, at an environmental concentration, associated with acidic pH and high water temperature, causes impairment of sperm quality in the freshwater teleost *Astyanax altiparanae* (Teleostei: Characidae). Environmental Pollution. 262: 114252.

Pinheiro, J. P. S.; Windsor, F. M.; Wilson, R. W. & Tyler, C. R. 2021. Global variation in freshwater physico-chemistry and its influence on chemical toxicity in aquatic wildlife. Biological Reviews. 96: 1528-1546.

Porto-Foresti, F.; Castilho-Almeida, R. B.; Senhorini, J. A. & Foresti, F. 2010. Biologia e criação do lambari-do-rabo-amarelo (*Astyanax altiparanae*). In: Baldisserotto, B. G. (ed) Espécies nativas para piscicultura no Brasil. Editora UFSM, Santa Maria.

Potschka, H. 2013. Pharmacological treatment strategies: mechanisms of antiepileptic drugs. Epileptology. 1: 31-37.

National Center for Biotechnology Information (2024). PubChem Compound Summary for CID 2554, Carbamazepine. Retrieved March 11, 2024 from https://pubchem.ncbi.nlm.nih.gov/compound/Carbamazepine.

Qiang, L.; Cheng, J.; Yi, J.; Rotchell, J. M.; Zhu, X. & Zhou, J. 2016. Environmental concentration of carbamazepine accelerates fish embryonic development and disturbs larvae behavior. Ecotoxicology. 25: 1426-1437.

Rajeshkumar, S. & Li, X. 2018. Bioaccumulation of heavy metals in fish species from the Meiliang Bay, Taihu Lake, China. Toxicology Reports. 5: 288-295.

Ravindranath, N.; Papadopoulos, V.; Vornberger, W.; Zitzmann, D. & Dym, M. 1994. Ultrastructural distribution of calcium in the rat testis. Biology of Reproduction. 51: 50–62.

Ruhí, A.; Acuña, V.; Barceló, D.; Huerta, B.; Mor, J. R.; Rodríguez-Mozaz, S. & Sabater, S. 2016. Bioaccumulation and trophic magnification of pharmaceuticals and endocrine disruptors in a Mediterranean river food web. Science of the Total Environment. 540: 250-259.

Schumacher, T. B.; Beck, H.; Steinhäuser, C.; Schramm, J. & Elger, C. E. 1998. Effects of phenytoin, carbamazepine, and gabapentin on calcium channels in hippocampal granule cells from patients with temporal lobe epilepsy. Epilepsia. 39: 355–363.

Shihomatsu, H.; Martins, E.; Cotrim, M.; Lebre, D.; Ortiz, N. & Pires, M. 2017. Guarapiranga Reservoir - pharmaceuticals and historical urban occupation in a water source. Journal of Geoscience and Environment Protection. 5: 1-17.

Silva Santos, N.; Oliveira, R.; Lisboa, C. A.; Pinto, J. M.; Sousa-Moura, D.; Camargo, N. S.; Perillo, V.; Oliveira, M.; Grisolia, C. K. & Domingues, I. 2018. Chronic effects of carbamazepine on zebrafish: behavioral, reproductive and biochemical endpoints. Ecotoxicology and Environmental Safety. 164: 297-304.

Siqueira-Silva, D. H.; Bertolini, R. M.; Pereira, N. L.; Nascimento, N. F.; Senhorini J. A.; Piva, L. H.; Ferraz, J. B. F. & Yasui, G. S. 2020. Factors affecting secondary sex characteristics in the yellowtail tetra *Astyanax altiparanae*. Fish Physiology and Biochemistry. 47: 737–746.

Sitges, M.; Chiu, L. M. & Reed, R. C. 2016. Effects of levetiracetam, carbamazepine, phenytoin, valproate, lamotrigine, oxcarbazepine, topiramate, vinpocetine and sertraline on presynaptic hippocampal Na^+ and Ca^{2+} channels permeability. Neurochemical Research. 41: 758-769.

Soucek, D. J.; Linton, T. K.; Tarr, C. D.; Dickinson, A.; Wickramanayake, N.; Delos, C. G. & Cruz, L. A. 2011. Influence of water hardness and sulfate on the acute toxicity of chloride to sensitive freshwater invertebrates. Environmental Toxicology and Chemistry. 30: 930-938.

Thomas, K. V.; Silva, F. M. A.; Langford, K. H.; Souza, A. D. L.; Nizzeto, L. & Waichman, A. V. 2014. Screening for selected human pharmaceuticals and cocaine in the urban streams of Manaus, Amazonas, Brazil. Journal of the American Water Resources Association. 50: 302-308.

Treviño, C. L.; Santi, C. M.; Beltrán, C.; Hernández-Cruz, A.; Darszon, A. & Lomeli, H. 1998. Localization of inositol trisphosphate and ryano-dine receptors during mouse spermatogenesis: Possible functional implications. Zygote. 6: 159–172.

Valdebenito, I.; Gallegos, P. C. & Effer, B. R. 2015. Gamete quality in fish: evaluation parameters and determining factors. Zygote. 23: 177e97.

Wilkinsona, J. L.; Boxall, A. B. A.; Kolpin, D. W.; Leung, K. M. Y.; Lai, R. W. S.; Galbán-Malagón, C.; Adell, A. D.; Mondon, J.; Metian, M.; et. al., 2022. Pharmaceutical pollution of the world's rivers. Proceedings of the National Academy of Sciences (PNAS). 119: 8 e2113947119.

Windsor, F. M.; Pereira, M. G.; Morrissey, C. A.; Tyler, C. R. & Ormerod, S. J. 2020. Environment and food web structure interact to alter the trophic magnification of persistent chemicals across river ecosystems. Science of the Total Environment. 717: 137271.

Wu, D.; Chen, L.; Ji, F.; Si, Y. & Sun, H. 2018. The effects of oxcarbazepine, levetiracetam, and lamotrigine on semen quality, sexual function, and sex hormones in male adults with epilepsy. Epilepsia. Official Journal of the International League Against Epilepsy. 59: 1344-1350.

Yan, S.; Wang, M.; Zha, J.; Zhu, L.; Li, W.; Luo, Q.; Sun, J. & Wang, Z. 2018. Environmentally relevant concentrations of carbamazepine caused endocrine-disrupting effects on nontarget organisms, Chinese rare minnows (*Gobiocypris rarus*). Environmental Science & Technology. 52: 886-894.

Capítulo 2

The influence of the neuropharmaceutical carbamazepine on the seminal quality of the neotropical fish *Astyanax lacustris*.

Cecilia Bertacini de Assis[1], Giovana S. Branco[1], Renato M. Honji[1], Amanda S. Guerreiro[1], Lorena Pacheco[2], Alexandre Ninhaus[2], Susanne Rath[3], Larissa D. Caminhas[3], Renata G. Moreira[1], João Paulo S. Pinheiro[1]

[1]Instituto de Biociências, Departamento de Fisiologia Geral, Universidade de São Paulo - USP, São Paulo, SP, Brazil.
[2]Programa de Pós-Graduação em Ciência e Tecnologia Animal, Universidade Estadual Paulista "Júlio de Mesquita Filho" - FEIS/UNESP, Ilha Solteira, SP, Brazil.
[3]Instituto de Química, Departamento de Química Analítica, Universidade de Campinas - UNICAMP, Campinas, SP, Brazil.

Abstract

The neuropharmaceutical Carbamazepine (CBZ), which is present in water bodies in high concentrations, and is resistant to biodegradation, combined with the presence of calcium (Ca), can have a great influence on the physiology of non-target organisms. However, it is not understood whether co-exposure of Ca and CBZ can influence fish sperm parameters. Therefore, the objective of the present study was to evaluate the effect of CBZ (250 and 500 ng L^{-1}) in the water, as well as the co-exposure with Ca (21 mg L^{-1} Ca + 250 ng L^{-1} CBZ and 21 mg L^{-1} Ca + 500 ng L^{-1} CBZ), in the seminal quality of *Astyanax lacustris*, using DMSO (dimethyl sulfoxide) as vehicle. After the end of exposure of the males to the combination of different environmental concentrations of CBZ with calcium, semen samples were collected to evaluate seminal quality parameters such as pH, morphology, kinetics, and sperm concentration. In general, the morphology results showed a significant drop in the percentage of normal spermatozoa in animals exposed to CBZ at concentrations of 250 and 500 ng L^{-1} and exposed to 21 mg L^{-1} Ca + 250 ng L^{-1} CBZ in relation to DMSO. Similarly, there was a reduction in curvilinear speed (VCL), straight line speed (VSL) and average travel speed (VAP), in addition to the percentage of straightness (STR) in the same experimental groups. The spermatozoa motility (MOT) was reduced when fish was exposed to 250 and 500 ng L^{-1} of CBZ only. Seminal pH and sperm concentration was not affected by CBZ and/or Ca. Considering the results obtained, contrary to what was hypothesized, it was observed that CBZ alone has greater effects on the seminal quality of *A. lacustris* compared to CBZ+Ca. Therefore, these results contribute to urgent action in understanding the toxicity of CBZ, a drug with reproductive disrupting actions in teleost fish.

Keywords: contaminants of emerging concern, toxicity, freshwater, teleost, spermatozoa.

1. Introduction

Pharmacological compounds are emerging pollutants that are gaining increasing attention from the scientific community, since they have been detected in bodies of water in different concentrations for decades (Alrashood, 2016), and yet there is no theoretical/scientific basis and well-established legislation for the control of production and disposal in the environment (ECOS, 2014). Many of these compounds can pose a risk to health, both human and ecosystems, as they can bioaccumulate in the tissues of organisms and biomagnifies along the trophic chain (Ruhí et al., 2016; Rajeshkumar and Li, 2018; Bergmann and Graça, 2020; Windsor et al., 2020).

Carbamazepine (CBZ; $C_{15}H_{12}N_2O$) is a drug aimed at treating epilepsy, bipolar disorder (Beutler et al., 2005; Lee et al., 2007) and trigeminal neuralgia (Brunton et al., 2012) among other neurological disorders. They are among the most found medicines in freshwater environments (Li et al., 2010; Hampel et al., 2014; Yan et al., 2018; Gasca-Perez et al., 2019) as they are highly recalcitrant to water treatment residuals (Ji et al., 2016). In Brazil for example, the concentration can vary between 12 and 358 ng L^{-1} in the Guarapiranga reservoir located in the State of São Paulo (Shihomatsu et al., 2017), in addition to the states of Paraná and Amazonas, in Belém (670 to 856 ng L^{-1} (Böger et al., 2018)) and Igarapé (652 ng L^{-1} (Thomas et al., 2014)) rivers, respectively.

In vertebrates, the reproductive process is controlled by the brain-pituitary-gonad (BHG) axis and modulated by environmental variables, such as temperature, photoperiod, and water quality (Alix et al., 2020; Servili et al., 2020). Under appropriate conditions, this axis synthesizes and releases internal factors (neurohormones and neurotransmitters) and hypothalamic, pituitary, and gonadal hormones naturally, which in males, induce the testicles to produce sperm viable for fertilization. However, the presence of pollutants and contaminants in the aquatic environment can alter the reproductive physiology of teleost males (Kowalski and Cejko, 2019), as seen in the concentrations of 11-ketotestosterone (11-KT) in *Danio rerio* (Fraz et al., 2018, 2019) and in *Gobiocypris rarus* (Yan et al., 2018), a gonadal steroid that decreased in the plasma of animals exposed to CBZ. In addition, sperm from *Cyprinus carpio* exposed to CBZ *in vitro* showed a reduction in sperm kinetics, as well as oxidative stress (Li et al., 2010), and Silva Santos and collaborators (2018) also suggest that CBZ rendered *D. rerio* eggs unviable.

Beyond the contaminant substances, such as CBZ, the physical and chemical characteristics of water (pH, temperature, salinity, and concentration of minerals, as available ions) can also interfere with the physiology of organisms (Valdebenito et al., 2015; Kowalski and Cejko, 2019). All aquatic organisms are dependent of minerals for the regulation of physiological and biochemical processes to maintain homeostasis. Fishes for example, absorb these minerals through their gills and skin through the surrounding water, in addition to their diet like other vertebrates (Lall and Kaushik, 2021) and the absorption of this substance depends on bioavailability in the environment (Lall and Kaushik, 2021). Among the macro minerals, calcium (Ca) can be highlighted as essential as it plays an important role as a secondary cellular messenger necessary for complex functions (*i.e.* regulation of gene expression) in different types of cells (Butts et al., 2013). In the reproductive process, Ca actively participates in the ability of sperm to move, as in general, this process is associated with an intracellular increase in Ca ions in teleost sperm (Parodi et al., 2017; Alavi et al., 2019). In addition to being extremely important for spermatogenesis, Ca interferes with the regulation of different stages of gamete formation (growth, regulation, and differentiation) (Ravindranath et al., 1994; Treviño et al., 1998).

In a natural environment, substances coexist and can interact with each other that can harm the health of aquatic organisms. Therefore, it is important to consider the relationship between physical and chemical characteristics of water versus harmful substances present in the environment (Soucek et al., 2011; Marchand et al., 2013; Baldisserotto et al., 2014; Hundt et al., 2016) in the reproductive process of the species. These substances can harm reproductive processes, such as the formation/quality of sperm, since they are the main key to the success of gametes fertilization and its sensitivity to the antagonistic effects of compounds present in the environment (Fabbrocini et al., 2013). In the environment, Ca concentrations can vary temporally and along the course of rivers, as observed in the Tietê River Basin (São Paulo, Brazil) according to data obtained from reports from the São Paulo State Water Company (CETESB). CETESB (2018, 2019, 2020, 2021) reports of inland waters in the state of São Paulo show a variation in Ca concentration in some locations considered polluted (*e.g.* 21 mg L^{-1}). Environments contaminated with pharmaceuticals, and the presence of high concentrations of ions can alter fish homeostasis and, consequently, the entire food chain of the aquatic environment, therefore it is a topic of environmental concern.

Considering the importance and concentration of Ca in the environment and the toxicity of CBZ on animal physiology, we hypothesized that the *Astyanax lacustris,* popularly known as *lambari-do-rabo-amarelo,* exposed to concentrations of CBZ and an environmental concentration of Ca would harm the sperm quality. Therefore, the aim of the present study was to evaluate the effect of CBZ, in different concentrations, on the seminal quality of *A. lacustris*, including morphology, and kinetics spermatic parameters, and the influence of Ca on the effects of CBZ.

2. Material and methods

2.1. Chemicals

Due to the low solubility in water, CBZ (Sigma-Aldrich, Saint Louis, MO, USA - purity ≥ 98%) was dissolved in DMSO (<0.001% - Sigma-Aldrich, Saint Louis, MO, USA). The concentration of Ca in the water was obtained based on a previous analysis of all salts (anions and cations) present in the water and the addition of calcium nitrate tetrahydrate – $Ca(NO_3)_2(4H_2O)$ (Sigma-Aldrich, Saint Louis, MO - Jia et al., 2020). CBZ and Ca values corresponded to the combination of environmental concentration of calcium 21 mg L^{-1} Ca (CETESB, 2018) and the drug Carbamazepine 0, 250 (Shihomatsu et al., 2017) and 500 ng L^{-1} (Hai et al., 2018).

2.2. Experimental design

For this study, mature males of the species *A. lacustris* were donated by *Universidade Estadual Paulista* "Júlio de Mesquita Filho" (UNESP) - Ilha Solteira campus. The animals were kept at the university facilities in 36 L aquariums, which was covered with brown Ethylene-vinyl acetate (E.V.A.), in a natural photoperiod, for a period of 7 days for acclimatization. Additionally, the animals were fed (*ad libitum* with 32% crude protein) every 48 h before water renewal (70% - semi-static experiment), to maintain water quality and the aquariums were constantly aerated at the temperature of 27 ± 2 ºC. After the acclimatization period, the animals were exposed to the following experimental groups (in quadruplicate) during seven days (natural photoperiod, fed every 48 h before water renewal (70% - semi-static experiment)): 1) Dimethyl sulfoxide (DMSO) – control; 2) 250 ng L^{-1} CBZ (250CBZ); 3) 500 ng L^{-1} CBZ (500CBZ); 4) 21 mg L^{-1} Ca + 0 ng L^{-1} CBZ (21Ca); 5) 21mg L^{-1} Ca + 250 ng L^{-1} CBZ (21Ca250CBZ); 6) 21 mg L^{-1} Ca + 500 ng L^{-1} CBZ (21Ca500CBZ). Dissolved oxygen and water temperature (YSI, model 55; respectively, mg L^{-1} and ºC), as well as pH (LAQUAtwin pH-11 Horiba, Axios Brazil Operations) were monitored during the 7-day exposure period (Table 2). In the present study, we also quantified the concentration of Ca and CBZ according to the protocols described below.

With the aim of increasing semen volume, *A. lacustris* males were artificially induced to spermiation with 5 mg Kg^{-1} of carp pituitary extract, eleven hours before semen collection and the end of exposure, according to previous studies carried out by our research group (Assis et al., 2021). To collect the samples, the fish were sedated with a solution based on Eugenol (clove oil) in a proportion of 1 mL of Eugenol: 10 mL of absolute ethanol: 10,000 mL of aquarium water. After sedation, the animals had their abdominal region dried with a paper towel, to avoid contamination and seminal activation with blood, faeces, urine, and water, and then an abdominal massage in the cephalic-caudal direction was performed for semen collection with an automatic pipette. The samples were deposited in graduated polyethylene tubes and kept refrigerated in a polystyrene thermal box (4 ºC) until processing. Additionally, fresh aliquots of semen were used for seminal pH analyses and sperm kinetics. For sperm morphology and concentration analyses, semen aliquots were fixed in 4% formaldehyde citrate solution, according to the protocols described below.

All procedures were performed and approved by the *Comissão de Ética no Uso de Animais, Instituto de Biociências, Universidade de São Paulo,* Brazil (protocol number: 374/2021).

2.3. Quantification of CBZ in water

CBZ in water was measured using the ACQUITY ultra-efficiency liquid chromatography method (Waters, USA), equipped with a binary and quaternary pumping system, automatic injection system, coupled to a Xevo TQD triple quadrupole mass spectrometry detector (Waters, USA), with electro foil ionization (ESI) interface. Data acquisition was performed using the MassLynx computer program (Waters, USA). According to the developed protocol, the accuracy of the results obtained was ±10%.

2.4. Quantification of Ca in water

The quantification of Ca in the water was performed with Atomic Absorption Spectrophotometry using a Calcium Hollow Cathode lamp, readings expressed in part per million (PPM).

2.5. Seminal pH

For this analysis, seminal pH was evaluated using portable equipment LAQUAtwin pH-11 (Horiba) with fresh semen.

2.6. Sperm morphology

An aliquot of 1 µL of semen from each animal was fixed in 100 µL of 4% formaldehyde citrate solution, and then 10 µL of the solution was mixed with 3 µL of Rose Bengal stain (Sigma-Aldrich, St. Louis, MO, USA). From this mixture (2 slides per fish), 4 µL were removed and then dripped onto glass slides. After drying, 100 sperm per slide were analysed to evaluate the flagellum morphology (normal, curled, corrugated or folded flagellum) using an optical microscope (400 x) and a computerized image capture system (LEICA DM1000 light microscope, LEICA DFC295 photo camera and LEICA Application Suite professional LAS V 3.6 image capture). The protocol from Galo et al., (2011) was adjusted for *A. lacustris* semen, and based on these criteria the percentage of normal spermatozoa was calculated.

2.7. Sperm kinetics

In sperm kinetics, 1 µL of the semen sample was administered and activated with 100 µL of distilled water to evaluate the following sperm parameters: curvilinear speed (VCL), straight line speed (VSL), average travel speed (VAP), percentage motility (MOT) and straightness (STR). The results were obtained using a computerized system (Computer Assisted Sperm Analysis - CASA) with the Integrated Semen Analysis System software, ISAS®, Proiser, Valencia, Spain, coupled to a UB200i phase contrast microscope (UOP/Proiser) with an objective 10x negative phase contrast.

2.8. Sperm concentration

Semen samples from each animal were fixed in a 4% formaldehyde citrate solution (1 µL of semen: 4 mL of fixative (1:4,000)). From each diluted sample, 20 µL was deposited in a Neubauer camera and counted using an optical microscope (400x). The calculation of sperm concentration was based on the method of Wirtz and Steinmann (2006).

2.9. Statistical analysis

The results were expressed as mean ± standard error of the mean. The data were initially checked for normality and homoscedasticity and then a one-way analysis of variance (One-way ANOVA) followed by Tukey's post-hoc test was performed. If data were non-parametric, a Kruskal-Wallis test followed by Dunns test was used. Significant differences were considered to exist when $p \leq 0.05$. Statistical analyses were performed using statistical software Graphpad Prism, version 8.0.1 (San Diego, California, USA) for windows.

3. Results

3.1. Biometric and weight data of experimental animals

The morphometric and weight data of *A. lacustris* during the period of exposure to environmental concentrations of Ca and CBZ were presented in Table 1. All animals were adult males and maintained homogeneity in both total length (cm) and total body mass (g).

Table 1. Morphometric and weight data during exposure of *Astyanax lacustris* to Calcium (Ca) and Carbamazepine (CBZ). Dimethyl sulfoxide (DMSO) – control. Data were expressed as Mean ± Standard Error of the Mean (SEM).

Treatments	**Total length (mm)**	**Body mass (g)**
DMSO	96.57 ± 1.85	11.93 ± 0.88
21Ca	93.90 ± 2.79	11.97 ± 1.18
250CBZ	92.82 ± 1.52	12.12 ± 0.73
500CBZ	96.38 ± 3.25	13.30 ± 1.40
21Ca250CBZ	94.77 ± 2.70	12.21 ± 1.22
21Ca500CBZ	94.71 ± 1.15	13.02 ± 0.74

There is no statistical difference between the experimental groups DMSO, 21Ca, 250CBZ, 500CBZ, 21Ca250CBZ e 21Ca500CBZ in Total length (mm) and Body mass (g). $p \leq 0.05$.

3.2. Water Quality, CBZ and Ca quantification

Regarding water quality, the parameters of dissolved oxygen concentration, pH and temperature in the different experimental groups were presented in Table 2. There were no significant differences between the experimental groups for these parameters. Thus, the minimum and maximum values for the entire data set were: 8.42 and 8.52 for pH; 6.12 and 6.53 mg L^{-1} for dissolved oxygen; 27.56 and 28.54 ºC for temperature.

Table 2. Water quality parameters (pH, dissolved oxygen (mg L^{-1}) and average temperature (ºC)) during exposure of *Astyanax lacustris* to Calcium (Ca) and Carbamazepine (CBZ). Dimethyl sulfoxide (DMSO) – control. Data were expressed as Mean ± Standard Error of the Mean (SEM).

Treatments	**pH**	**Dissolved oxygen (mg L^{-1})**	**Temperature (°C)**
DMSO	8.52 ± 0.05	6.53 ± 0.12	27.95 ± 0.48
21Ca	8.44 ± 0.05	6.14 ± 0.15	28.54 ± 0.42
250CBZ	8.49 ± 0.04	6.39 ± 0.11	27.71 ± 0.50
500CBZ	8.46 ± 0.05	6.23 ± 0.15	27.70 ± 0.53
21Ca250CBZ	8.42 ± 0.05	6.12 ± 0.10	27.56 ± 0.51
21Ca500CBZ	8.44 ± 0.05	6.19 ± 0.12	28.25 ± 0.45

There is no statistical difference between the experimental groups DMSO, 21Ca, 250CBZ, 500CBZ, 21Ca250CBZ e 21Ca500CBZ in pH, Dissolved oxygen (mg L^{-1}) and Temperature (°C). $p \leq 0.05$.

The results of CBZ and Ca quantification from the different treatments throughout the exposure were described in Tables 3 and 4, respectively. The concentration of CBZ in the water was below the detection limit of the method for the DMSO (control group) and 21Ca groups.

Table 3. Concentration of carbamazepine (CBZ) in water in different treatments during exposure of *Astyanax lacustris* to Calcium (Ca) and CBZ. Dimethyl sulfoxide (DMSO) – control. Data were expressed as Mean ± Standard Error of the Mean (SEM).

Treatments	CBZ concentration (ng L^{-1})
DMSO	<LOQ
21Ca	<LOQ
250CBZ	237.22 ± 4.83
500CBZ	471.11 ± 5.95
21Ca250CBZ	221.05 ± 3.01
21Ca500CBZ	443.94 ± 5.64

LOQ = *Limit of quantification*

Table 4. Concentration of calcium (Ca) in water in different treatments during exposure of *Astyanax lacustris* to Ca and Carbamazepine (CBZ). Dimethyl sulfoxide (DMSO) – control.

Treatments	Ca concentration (mg L^{-1})
Supply water	4.19
DMSO	4.09
21Ca	21.56
250CBZ	4.40
500CBZ	4.39
21Ca250CBZ	21.32
21Ca500CBZ	21.54

3.3. Seminal pH

In relation to seminal pH, no significant difference was identified between treatments during the exposure of the animals. The data were presented in Table 5.

Table 5. pH seminal data during exposure of *Astyanax lacustris* to Calcium (Ca) and Carbamazepine (CBZ). Dimethyl sulfoxide (DMSO) – control. Data were expressed as Mean ± Standard Error of the Mean (SEM).

Treatments	Seminal pH
DMSO	7.671 ± 0.094
21Ca	8.150 ± 0.144
250CBZ	7.700 ± 0.208
500CBZ	7.900 ± 0.058
21Ca250CBZ	7.975 ± 0.229
21Ca500CBZ	8.125 ± 0.075

There is no statistical difference between the experimental groups DMSO, 21Ca, 250CBZ, 500CBZ, 21Ca250CBZ e 21Ca500CBZ in Seminal pH. $p \leq 0.05$.

3.4. Sperm morphology

Morphological changes were observed in the tails of the sperm between treatments during the exposure of the animal (*i.e.,* normal flagellum, curled flagellum, corrugated flagellum and folded flagellum) (Fig. 1). On the other hand, the results obtained in the analysis of sperm morphology show that there is no difference in the percentage of normal sperm between the experimental groups: 21Ca and 21Ca500CBZ (p=0.9815) when compared with each other and with the DMSO group (21Ca, p>0.99; and 21Ca500CBZ, p=0.95). However, when observing the treatments 250CBZ (p=0.0007), 500CBZ (p=0.0445) and 21Ca250CBZ (p=0.0001), a decrease in the percentage of normal spermatozoa was noted when compared to the DMSO group. Finally, there were no statistical differences between the 500CBZ, 250CBZ and 21Ca250CBZ groups (p=0.9349 and p=0.7394, respectively) (Fig. 2).

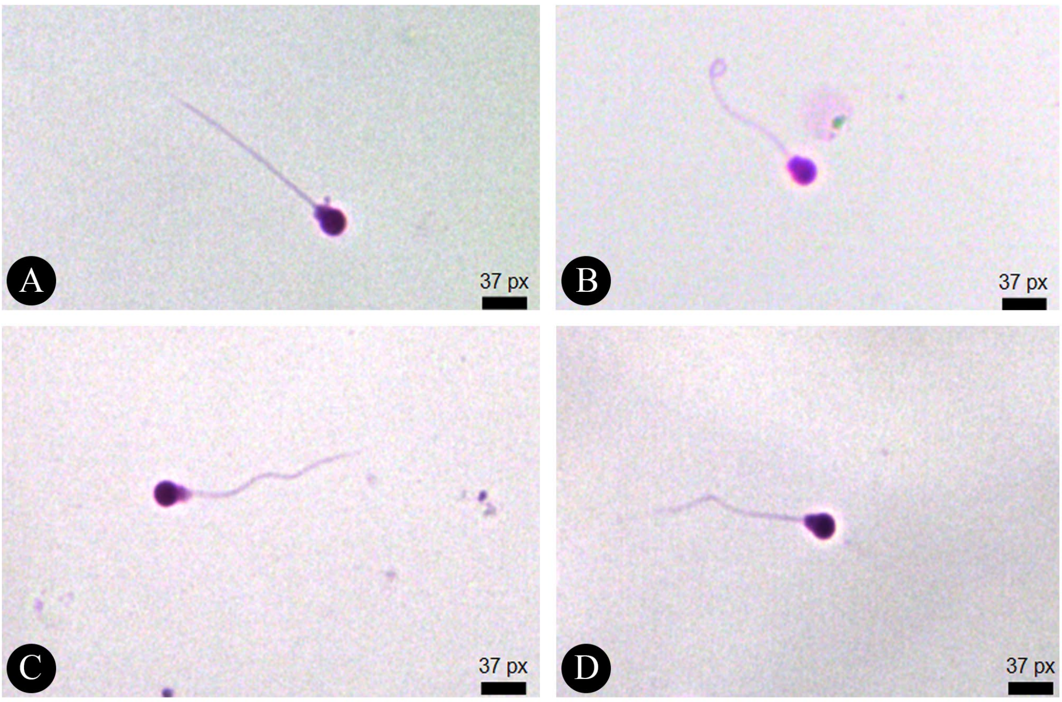

Figure 1. *Astyanax lacustris* sperm morphological changes in the flagellum of the sperm during the Calcium (Ca) and Carbamazepine (CBZ) exposure. A) normal flagellum; B) curled flagellum; C) corrugated flagellum; and D) folded flagellum. Stain: Rose Bengal.

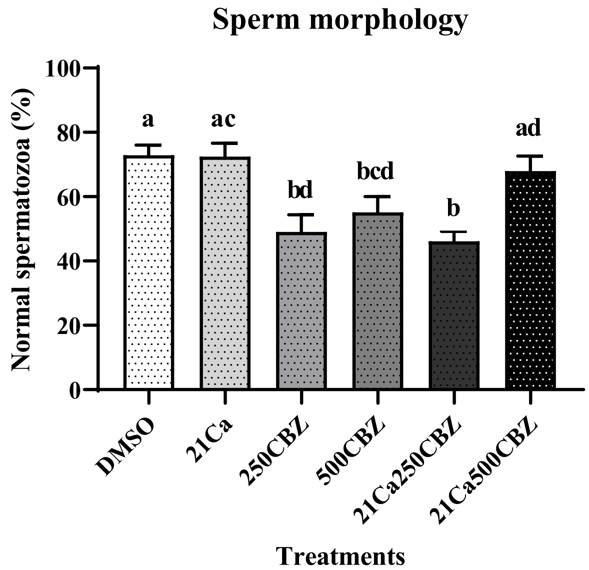

Figure 2. Percentage of normal *Astyanax lacustris* sperm exposed to Calcium (Ca) and Carbamazepine (CBZ). Dimethyl sulfoxide (DMSO) – control. Data were expressed as Mean ± Standard Error of the Mean (SEM). Experimental groups DMSO, 21Ca, 250CBZ, 500CBZ, 21Ca250CBZ and 21Ca500CBZ. [a, b, c, d] Different letters mean statistically different values between experimental groups.

3.5. Sperm kinetics

Considering MOT, the treatments with the lowest percentage of motile sperm were the 250CBZ and 500CBZ experimental groups when compared to the DMSO control group. These groups (250CBZ, 500CBZ) did not show statistical differences between them (p=0.9076) nor when compared to the 21Ca group (p=0.6813 and p=0.1759). The CBZ and Ca combination also did not alter sperm motility at any of the concentrations tested (21Ca250CBZ and 21Ca500CBZ) when compared to the DMSO control group (p=0.4698 and p=0.8361), to the 21Ca group (p=0. 9325 and p=0.8282) and the 250CBZ group (p=0.1702 and p=0.1354). However, in both cases, sperm MOT was higher than that of the 500CBZ group (p=0.0222 and p=0.0200) (Fig. 3A).

As for STR, the 250CBZ, 500CBZ and 21Ca250CBZ treatments showed a significant drop in the percentage of this parameter in relation to the DMSO treatment (p=0.0222; p=0.0016; p=0.038, respectively). Between these same treatments (250CBZ, 500CBZ and 21Ca250CBZ) no statistically significant differences were observed. Similarly, the DMSO, 21Ca and 21Ca500CBZ groups were not different from each other (Fig. 3B).

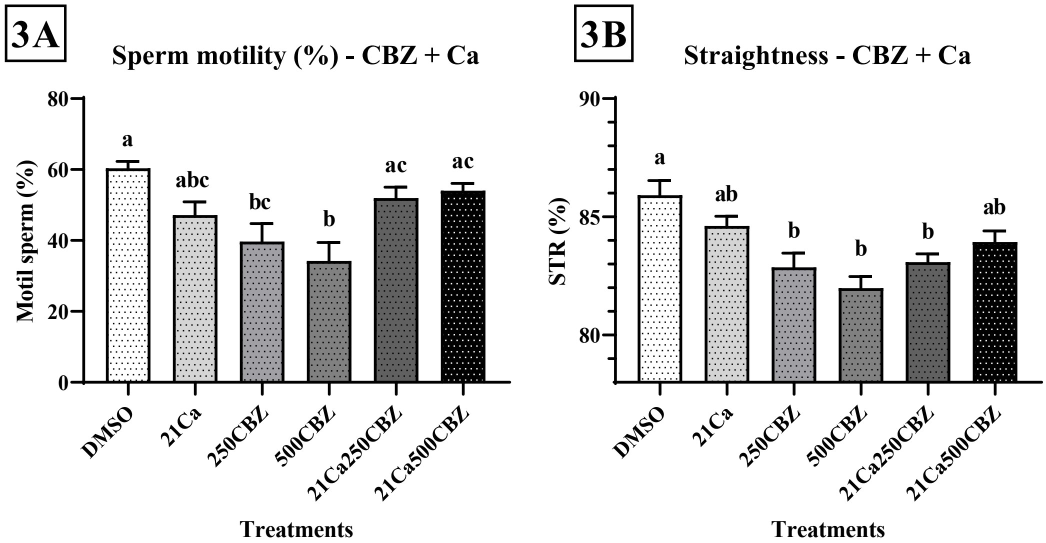

Figure 3. Data on sperm motility and straightness of *Astyanax lacustris* males exposed to Calcium (Ca) and Carbamazepine (CBZ). (A) Sperm motility (MOT, %) and (B) Straightness (STR) after exposure to the experimental groups DMSO, 21Ca, 250CBZ, 500CBZ, 21Ca250CBZ and 21Ca500CBZ. Dimethyl sulfoxide (DMSO) – control. Data were expressed as Mean ± Standard Error of the Mean (SEM). Experimental groups DMSO, 21Ca, 250CBZ, 500CBZ, 21Ca250CBZ and 21Ca500CBZ. [a, b, c] Different letters mean statistically different values between experimental groups**.**

Figures 4A, B and C show the VCL, VSL and VAP speeds, respectively. As described for STR percentage, a similarity was observed between the results obtained at the 3 speeds evaluated, that is, a consistency remained between the treatment relationships. Observing the treatments 250CBZ, 500CBZ and 21Ca250CBZ, it is noted that there was a significant drop in these measured velocities in relation to the DMSO group, while in the treatments 21Ca and 21Ca500CBZ there were no significant differences with the control treatment as well when compared with the experimental groups 250CBZ, 500CBZ and 21Ca250CBZ.

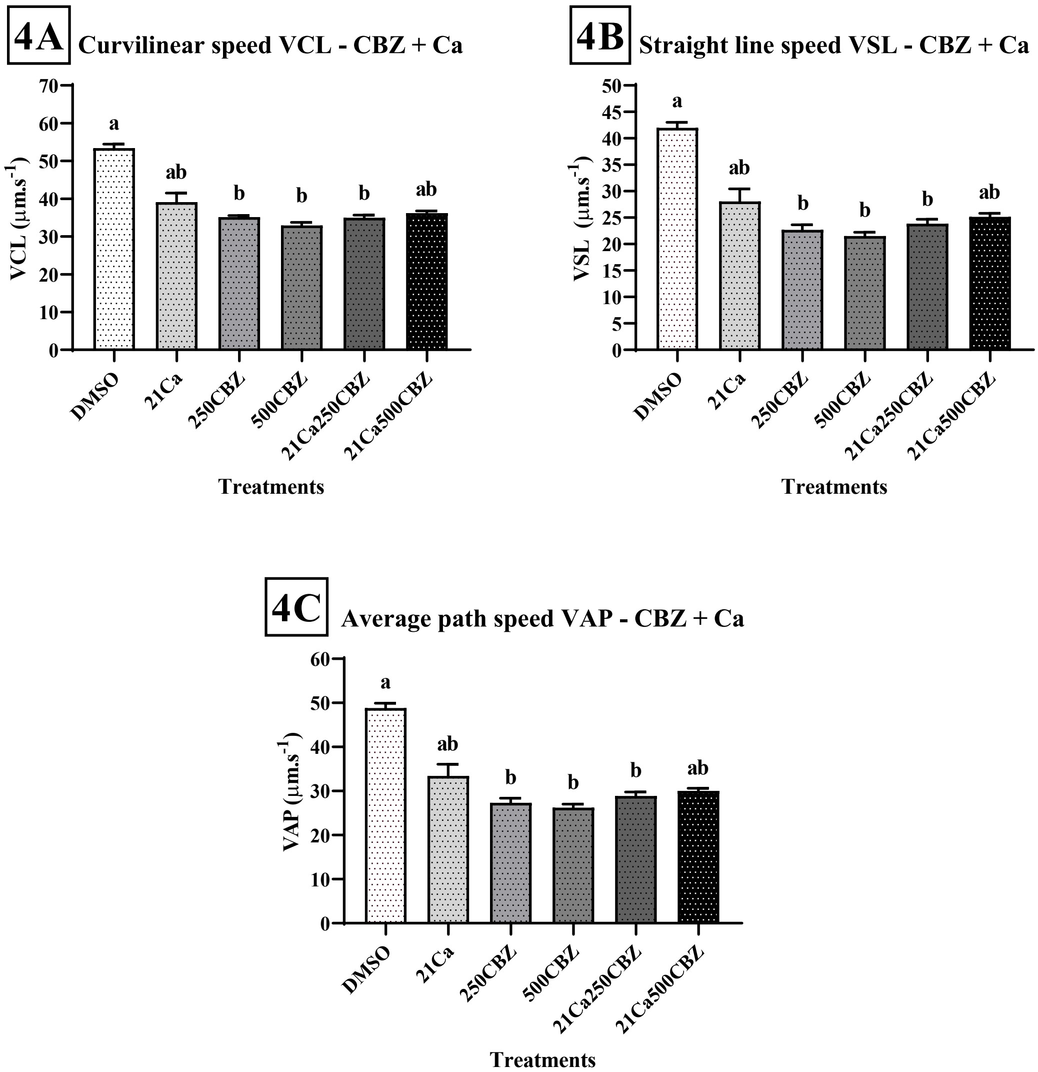

Figure 4. Data of (A) Curvilinear velocity (VCL), (B) Straight line velocity (VSL), and (C) Mean path velocity (VAP) of *Astyanax lacustris* males after exposed to Calcium (Ca) and Carbamazepine (CBZ). Dimethyl sulfoxide (DMSO) – control. Experimental groups DMSO, 21Ca, 250CBZ, 500CBZ, 21Ca250CBZ and 21Ca500CBZ. Data were expressed as Mean ± Standard Error of the Mean (SEM). [a, b] Different letters mean statistically different values between experimental groups.

3.6. Sperm concentration

No significant difference was identified between treatments during the exposure of the animals (Table 6).

Table 6. Sperm concentration data during exposure of *Astyanax lacustris* to Calcium (Ca) and Carbamazepine (CBZ). Dimethyl sulfoxide (DMSO) – control. Data were expressed as Mean ± Standard Error of the Mean (SEM).

Treatments	Sperm concentration (10^6 - mm^3)
DMSO	4.94 ± 0.51
21Ca	6.74 ± 1.11
250CBZ	5.86 ± 0.73
500CBZ	4.43 ± 0.67
21Ca250CBZ	4.96 ± 0.91
21Ca500CBZ	5.77 ± 1.01

There is no statistical difference between the experimental groups DMSO, 21Ca, 250CBZ, 500CBZ, 21Ca250CBZ e 21Ca500CBZ in Sperm concentration (106 - mm^3). $p \leq 0.05$.

4. Discussion

Physico-chemical characteristics of natural freshwaters can directly and/or indirectly affect the solubility, speciation, bioavailability, and uptake of chemicals (Pinheiro et al., 2021). The existence of pharmaceuticals remainders (drugs and metabolites) in the aquatic ecosystem has been raising concerns regarding their potential effects to aquatic organisms considered non-target, considering that low concentrations of pharmaceuticals compounds may exert toxicity through unpredicted mechanisms of action in non-target aquatic biota (Malarvizhi et al., 2012). Exposure to CBZ in environmentally relevant concentrations causes changes in animal homeostasis and, therefore, can harm the organism's performance and survival. The present study is the first that combines the effects of the presence of Ca ions in water and the emerging drug of concern CBZ, in relation to the seminal quality of *A. lacustris*. Briefly, co-exposure of Ca and CBZ were not relevant to seminal quality. On the other hand, the effect of the isolated drug is notable, especially when we evaluate the sperm kinetics and morphology parameters of *A. lacustris*.

In this case, the present study was conducted with adult males of *A. lacustris* during the reproductive period of the species, where there is greater sexual maturation of the animals, as the age and stage of gonadal maturation used in ecotoxicological studies could also influence the results (Segner, 2011; Taslima et al., 2022) since the objective was to evaluate the quality of sperm from an animal capable of reproduction exposed to the mentioned substances. During the exposure of the animals, the physical-chemical parameters of the water analysed, such as dissolved O_2 and temperature, remained in accordance with what was expected and described in the literature for the species in question (Baldisserotto and Gomes, 2005), suggesting that the observed changes occurred due to the exposure to CBZ. Maintaining these parameters is important, since O_2 is an indispensable and limiting factor for the survival of animals, as it is necessary for vital functions, and temperature is closely related to physiological activities (reproduction, breathing, feeding, etc.) of ectothermic animals (Moyes and Schulte, 2014). Additionally, the results obtained are in accordance with CONAMA resolution 357/05(2), which establishes the minimum value of O_2 dissolved in fresh water of 5 mg L^{-1}, as well as the optimal conditions described in the specialized literature for *A. lacustris* (Baldisserotto and Gomes, 2005).

Water quality influences the physiological processes of aquatic organisms, since they are in constant absorption through breathing and feeding from the environment that surrounds them (Moyes and Schulte, 2014). The water contamination by CBZ in *A. lacustris*, in general, has shown a greater interference in sperm morphology and kinetic parameters at concentrations of 250 ng L^{-1} CBZ and 500 ng L^{-1} CBZ in relation to the control group where there was only DMSO, while pH and sperm concentration were not significantly affected. These results were different from those initially expected because it was possible to assess that CBZ alone was more toxic to sperm than when combined with Ca. One possibility of interpreting this result is that some cations compete with pollutants uptake process in freshwater animals or reduce gill permeability. Similar discussion was performed by Grosell (2011), who suggests that some cations compete with copper uptake processes or reduce gill permeability (Ca^{2+}), thereby reducing the toxic impacts of copper.

The absorption by animals (*A. lacustris*) of the neuro drug CBZ (250 and 500 ng L^{-1}) added to the water led to a significant drop in the percentage of spermatozoa classified as normal in relation to flagellum morphology. Similar changes were also reported in different generations of *D. rerio* (F_0, F_1, F_2 and F_3) exposed to a concentration of 10000 ng L^{-1} of CBZ, because these cells (spermatozoa) presented a longer flagellum, a smaller head diameter, and difference in the length of the midpiece, suggesting that the effects of CBZ can affect subsequent generations (Fraz et al., 2019). In addition to influencing the reproduction of teleosts, the effects of CBZ can also be extended to other vertebrates, such as *Mus musculus*, also known as house rat or mouse (mammals). After their exposure to the drug, sperm formation was compromised in relation to damage to the flagellum and size of the sperm head (Sabr and Karim, 2023). Additionally, CBZ is also harmful to human sperm, as after exposure to the drug (310 ng L^{-1}) there is an increase in the sperm DNA damage (by comet assay analysis), DNA fragmentation (expressed by TUNEL test - Terminal deoxynucleotidyl transferase UTPdriven Nick End Labeling), and sperm apoptotic cells (diffusion assay analysis) (Rocco et al., 2012).

Linked to the morphology of the flagellum, the present results of sperm kinetics strengthen the evidence of the influence of CBZ, groups 250 and 500 ng L^{-1}, on the quality of *A. lacustris* semen, since the percentage of motile sperm (MOT) was reduced significantly as well as the percentage of straightness (STR), in addition to the average, curvilinear and straight-line speeds. To understand the importance of this relationship, it is necessary to keep in mind that the sperm's ability to move is based on a highly organized structural/morphological arrangement of the flagellum to successfully fertilize the oocytes, as the movement and speed of these cells are dependent on the capacity flagellar ability to generate waves (Inaba, 2003; Wachten et al., 2017; Dzyuba et al., 2017; Boryshpolets, et al., 2018; Cosson, 2019). An example of this relationship was also suggested by Costa and collaborators (2022), who despite leading the study for a different focus, they discuss the consequences of poor sperm formation on the sperm kinetics of Piraputanga (*Brycon hilarii*), mainly to how does morphology affect sperm movement.

To date, knowledge about the influence of CBZ on the reproductive success of teleost has not been much explored, however there is a previous study that elucidates the interaction of this neuro drug, under *in vitro* conditions, with the parameters that translate into sperm kinetics of *Cyprinus carpio* (Li et al., 2010). In this study the cells were exposed to varying concentrations of CBZ (200000, 2000000 and 2000000 ng L^{-1}) and consequently significant drops in MOT were shown at the highest concentrations of CBZ when compared to the DMSO group (Li et al., 2010). Although the present study was carried out with *in vivo* exposure, the results of the percentage of MOT demonstrated lower values of motile sperm in relation to the DMSO group and the experimental groups with 250CBZ and 500CBZ, showing a reduction of 20.63% and 26.17%, respectively. Additionally, in *D. rerio* exposed to CBZ (10µg L^{-1}) for 6 weeks (Fraz et al., 2019), VCL and VAP swimming velocity of sperm remained unchanged, differently from the present study, as there was a decrease in these speeds in the animals exposed only to CBZ compared to the control group.

On the other hand, studies focused on the implications of CBZ on human male sexual health have been developed. In 2013, the concentrations of sexual hormones and seminal quality were evaluated in epileptic patients treated with CBZ and it was seen that luteinizing hormone and free androgens were lower compared to the control group, without CBZ treatment, and sperm motility together with sperm morphology were also reduced, although seminal concentration was not changed (Reis et al., 2013), as well as *A. lacustris* males treated with CBZ in the present study (in relation to seminal quality). Future studies are being carried out by our research group relating the influence of CBZ on the reproductive neuroendocrine axis of *A. lacustris*, mainly to sexual steroid hormones and expression genes of HHG axis.

Regarding the presence of Ca together with CBZ, it is known that physicochemical factors, that is, water quality, can vary during ecotoxicological studies and influence the toxicity of drugs and other contaminants on aquatic biota (Sumpter, 1997; Valdebenito et at., 2013; Kowalski and Cejko, 2019; Pinheiro et al., 2021). Added to this, it is known that CBZ is capable of directly modulating voltage-gated calcium channels (D'Onofrio et al. 2017; Iannaccone et al., 2021; Jia et al., 2022) and, according to the review carried out by Pinheiro and collaborators (2021), the role of calcium in water is discussed, which is closely linked to the regulation of processes physiologically important that can influence, for example, the absorption of ionized chemicals. The physiological/biochemical explanation for this lies in the participation of the ion in controlling the gills and skin of fish, decreasing the membrane permeability of molecules (Lauren & McDonald, 1985; Wood, 2020). Based on this, it was expected that the addition of the Ca ion would be a factor in increasing the toxic effects of CBZ, however, the results obtained go against the initial hypothesis. For future studies, it would be interesting to evaluate tight junctions, calcium channels, membrane permeability, among other physiological points to track the interaction between this important water parameter and the presence of CBZ in the aquatic environment. New studies are being carried out by our research group to understand the exposure of females of *A. lacustris* to CBZ. Therefore, all these studies must be interpreted together to solve the puzzle of the effects of this CEC in *A. lacustris* reproductive physiology.

5. Conclusion

Given the data presented, the hypothesis of this study was partially refuted, as the co-exposure of Ca and CBZ, even at the highest concentrations of CBZ, were not relevant to alter the seminal quality. When analysing the effects of CBZ without added Ca, however, the effect of the drug is notable, especially when we evaluated the sperm kinetics and morphology parameters of *A. lacustris*. Furthermore, future studies should be conducted to evaluate fertilization rate, embryonic development, occlusion junctions, Ca channels, membrane permeability, among other physiological points, thus being a starting point for future studies.

6. Acknowledgments

The authors would like to thank the *Fundação de Amparo à Pesquisa do Estado de São Paulo* (FAPESP), which funded the present study (Grants #2020/14252-5, #2022/03440-0 and 2022/11174-9), CAPES (*Coordenação de Aperfeiçoamento de Pessoal de Nível Superior*; CAPES: finance code 001) and CNPq (Universal Project, 404705/2021-9). The authors would like to thank Prof. Rosicleire Veríssimo for donating the fish and the infrastructure to run the experiment (UNESP - Ilha Solteira, SP - Brazil); to the LAMEROA (*Laboratório de Metabolismo e Reprodução de Organismos Aquáticos*) and LINEO (*Laboratório de Ictiologia Neotropical*) team for their help and *Instituto de Biociências* (*Universidade de São Paulo*) for providing the infrastructure and the facilities for the development of this study. Renata Guimarães Moreira is the recipient of the CNPq (*Conselho Nacional de Desenvolvimento Científico e Tecnológico*) productivity scholarship.

7. References

Alavi, S. M. H.; Cosson, J.; Bondarenko, O. & Linhart, O. 2019. Sperm motility in fishes:(III). Diversity of regulatory signals from membrane to the axoneme. Theriogenology. 136: 143-165.

Alix, M.; Kjesbu, O. S. & Anderson, K. C. 2020. From gametogenesis to spawning: how climate-driven warming affects teleost reproductive biology. Journal of Fish Biology. 97: 607-632.

Alrashood, S. T. 2016. Carbamazepine. Profiles of Drug Substances, Excipients and Related Methodology. 41: 133-321.

Assis, C. B.; Branco, G. S.; Moreira, R. G. & Pinheiro, J. P. S. 2021. Aluminum at environmental concentrations affects the sperm quality of the freshwater teleost *Astyanax altiparanae*: an in vitro study. Comparative Biochemistry and Physiology C - Toxicology & Pharmacology. 243: 108983.

Baldisserotto, B.; Martos-Sitcha, J. A.; Menezes, C. C.; Toni, C.; Prati, R. L.; Garcia, L. D. O.; Salbego, J.; Mancera, J. M. & Martínez-Rodríguez, G. 2014. The effects of ammonia and water hardness on the hormonal, osmoregulatory and metabolic responses of the freshwater silver catfish *Rhamdia quelen*. Aquatic Toxicology. 152: 341-352.

Baldisserotto, B. & Gomes, L. C. 2005. Espécies nativas para piscicultura no Brasil. Editora UFSM, Santa Maria.

Bergmann, M. & Graça, M. A. 2020. Bioaccumulation and dispersion of uranium by freshwater organisms. Archives of Environmental Contamination and Toxicology. 78: 254 -266.

Beutler, A. S.; Li, S.; Nicol, R. & Walsh, M. J. 2005. Carbamazepine is an inhibitor of histone deacetylases. Life Sciences. 76: 3107–3115.

Böger, B.; Amaral, B. D.; Estevão, P. L. D. S.; Wagner, R.; Peralta-Zamora, P. G. & Gomes, E. C. 2018. Determination of carbamazepine and diazepam by SPE-HPLC-DAD in Belém River water, Curitiba-PR/Brazil. Revista Ambiente & Água. 13: e2196.

Boryshpolets, S.; Kholodnyy, V.; Cosson, J. & Dzyuba, B. 2018. Fish sperm motility analysis: the central role of the flagellum. Reproduction, Fertility and Development. 30: 833-841.

Brunton, L. L.; Chabner, B. A. & Knollmann, B. C. 2012. Las bases farmacológicas de la terapéutica 12° edición. Ed. 21. McGraw Hill, México, D.F.

Butts, I. A.; Alavi, S. M. H.; Mokdad, A. & Pitcher, T. E. 2013. Physiological functions of osmolality and calcium ions on the initiation of sperm motility and swimming performance in red side dace, *Clinostomus elongatus*. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology. 166: 147-157.

CETESB (Companhia Ambiental do Estado de São Paulo), 2018. Relatório de qualidade das águas interiores do Estado de São Paulo, Governo do Estado de São Paulo, Secretaria do Meio Ambiente. São Paulo.

http://cetesb.sp.gov.br/aguasinteriores/publicacoes-e -relatorios/. (Acessado 27 Fevereiro 2024).

CETESB (Companhia Ambiental do Estado de São Paulo), 2019. Relatório de qualidade das águas interiores do Estado de São Paulo, Governo do Estado de São Paulo, Secretaria do Meio Ambiente. São Paulo. http://cetesb.sp.gov.br/aguasinteriores/publicacoes-e -relatorios/. (Acessado 27 Fevereiro 2024).

CETESB (Companhia Ambiental do Estado de São Paulo), 2020. Relatório de qualidade das águas interiores do Estado de São Paulo, Governo do Estado de São Paulo, Secretaria do Meio Ambiente. São Paulo. http://cetesb.sp.gov.br/aguasinteriores/publicacoes-e -relatorios/. (Acessado 27 Fevereiro 2024).

CETESB (Companhia Ambiental do Estado de São Paulo), 2021. Relatório de qualidade das águas interiores do Estado de São Paulo, Governo do Estado de São Paulo, Secretaria do Meio Ambiente. São Paulo. http://cetesb.sp.gov.br/aguasinteriores/publicacoes-e -relatorios/. (Acessado 27 Fevereiro 2024).

Cosson, J. 2019. Fish sperm physiology: structure, factors regulating motility, and motility evaluation. Biological Research in Aquatic Science. 1: 1-26.

Costa, B. B.; Povh, J. A.; Sanches, E. A.; Spica, L. N.; Rodrigues, R. B.; Teixeira, N. N. S.; França, T. S.; Benato, J. L.; Machado, T. L. F.; Brasileiro, L. O.; Kasai, R. Y. D. & Streit, D. P. 2022. Characterization of sperm quality in *Brycon hilarii:* how does morphology affect sperm movement? Theriogenology Wild. 1: 100007.

D'Onofrio, S.; Mahaffey, S. & Garcia-Rill, E. 2017. Role of calcium channels in bipolar disorder. Current Psychopharmacology. 6: 122-135.

Dzyuba, B.; Bondarenko, O.; Fedorov, P.; Gazo, I.; Prokopchuk, G. & Cosson, J. 2017. Energetics of fish spermatozoa: the proven and the possible. Aquaculture. 472: 60-72.

ECOS. 2014. The Environmental Council of the States. Retrieved March 11, 2024 from: https://www.ecos.org.

Fabbrocini, A.; D'adamo, R.; Del Prete, F.; Langellotti, A. L.; Barone, C. M. A.; Rinna, F.; Sessa, R.; Silvestri, F.; Villani, G.; Vitiello, V.; Vittielo, V. & Sansone, G.

2013. Motility of cryopreserved spermatozoa for the ecotoxicological evaluation of aquatic environments. Journal of Chemical Ecology 29: 660-667.

Fraz, S.; Lee, A. H.; Pollard, S.; Srinivasan, K.; Vermani, A.; David, E. & Wilson, J. Y. 2019. Paternal exposure to carbamazepine impacts zebrafish offspring reproduction over multiple generations. Environmental Science & Technology. 53: 12734-12743.

Fraz, S.; Lee, A. H. & Wilson, J. Y. 2018. Gemfibrozil and carbamazepine decrease steroid production in zebrafish testes (*Danio rerio*). Aquatic Toxicology. 198: 1-9.

Galo, J. M.; Streit Junior, D. P.; Sirol, R. N.; Ribeiro, R. P.; Digmayer, M.; Andrade, V. X. L. & Ebert, A. R. 2011. Spermatic abnormalities of piracanjuba *Brycon orbignyanus* (Valenciennes, 1849) after cryopreservation. Brazilian Journal of Biology. 71: 693 -699.

Gasca-Pérez, E.; Galar-Martínez, M.; García-Medina, S.; Pérez-Coyotl, I. A.; Ruiz-Lara, K.; Cano-Viveros, S.; Borja, R. P. P. & Gómez-Oliván, L. M. 2019. Short-term exposure to carbamazepine causes oxidative stress on common carp (*Cyprinus carpio*). Environmental Toxicology and Pharmacology. 66: 96-103.

Grosell, M. 2011. Copper. In: Wood, C. M.; Farrell, A. P. & Brauner, C. J. Fish Physiology. Academic Press, Cambridge.

Hai, F. I.; Yang, S.; Asif, M. B.; Sencadas, V.; Shawkat, S.; Sanderson-Smith, M.; Gorman, J.; Xu, Z. Q. & Yamamoto, K. 2018. Carbamazepine as a possible anthropogenic marker in water: occurrences, toxicological effects, regulations and removal by wastewater treatment technologies. Water. 10: 107.

Hampel, M.; Bron, J. E.; Taggart, J. B. & Leaver, M. J. 2014. The antidepressant drug carbamazepine induces differential transcriptome expression in the brain of Atlantic salmon, *Salmo salar*. Aquatic Toxicology. 151: 114-123.

Hundt, M.; Schreiber, B.; Eckmann, R.; Lunestad, B. T.; Wünneman, H. & Schulz, R. 2016. The effect of water hardness on mortality of zebrafish (*Danio rerio*) during exposure to oxytetracycline. Bulletin of Environmental Contamination and Toxicology. 96: 144-9.

Iannaccone, T.; Sellitto, C.; Manzo, V.; Colucci, F.; Giudice, V.; Stefanelli, B.; Iuliano, A.; Corrivetti, G. & Filippelli, A. 2021. Pharmacogenetics of carbamazepine and valproate: focus on polymorphisms of drug metabolizing enzymes and transporters. Pharmaceuticals. 14: 204.

Inaba, K. 2003. Molecular architecture of the sperm flagella: molecules for motility and signaling. Zoological Science. 20: 1043-1056.

Ji, C.; Hou, J.; Wang, K.; Zhang, Y. & Chen, V. 2016. Biocatalytic degradation of carbamazepine with immobilized laccase-mediator membrane hybrid reactor. Journal of Membrane Science. 502: 11-20.

Jia, D.; Li, X.; Du, S.; Xu, N.; Zhang, W.; Yang, R.; Zhang, Y.; He, Y. & Zhang, Y. 2020. Single and combined effects of carbamazepine and copper on nervous and antioxidant systems of zebrafish (*Danio rerio*). Environmental Toxicology. 35: 1091-1099.

Jia, L.; Eroglu, T. E.; Wilders, R.; Verkerk, A. O. & Tan, H. L. 2022. Carbamazepine increases the risk of sudden cardiac arrest by a reduction of the cardiac sodium current. Frontiers in Cell and Developmental Biology. 10: 891996.

Kowalski, R. K. & Cejko, B. I. 2019. Sperm quality in fish: determinants and affecting factors. Theriogenology. 135: 94-108.

Lall, S. P. & Kaushik, S. J. 2021. Nutrition and metabolism of minerals in fish. Animals. 11: 2711.

Lauren, D. J. & Mcdonald, D. G. 1985. Effects of copper on branchial ionoregulation in the rainbow-trout, *Salmo gairdneri* Richardson - modulation by water hardness and pH. Journal of Comparative Physiology B: Biochemical Systemic and Environmental Physiology. 155: 635–644

Lee, H. J.; Rao, J. S.; Rapoport, S. I. & Bazinet, R. P. 2007. Antimanic therapies target brain arachidonic acid signaling: lessons learned about the regulation of brain fatty acid metabolism. Prostaglandins, Leukotrienes & Essential Fatty Acids. 77: 239–246.

Li, Z. H.; Li, P.; Rodina, M. & Randak, T. 2010. Effect of human pharmaceutical Carbamazepine on the quality parameters and oxidative stress in common carp (*Cyprinus carpio L.*) spermatozoa. Chemosphere. 80: 530-534.

Malarvizhi, A.; Kavitha, C.; Saravanan, M. & Ramesh, M. 2012. Carbamazepine (CBZ) induced enzymatic stress in gill, liver and muscle of a common carp, *Cyprinus carpio*. Journal of King Saud University – Science. 24: 179-186.

Marchand, P. A.; Straus, D. L.; Wienke, A.; Pedersen, L. F. & Meinelt, T. 2013. Effect of water hardness on peracetic acid toxicity to zebrafish, *Danio rerio*, embryos. Aquaculture International. 21: 679-686.

Moyes, C. D. & Schulte, P. M. 2014. Principles of Animal Physiology. 3rd Edition.

Parodi, J.; Guerra, G.; Cuevas, M.; Ramírez-Reveco, A. & Romero, F. 2017. Effects of storage time on the motility, mortality and calcium levels of Atlantic salmon *Salmo salar* spermatozoa. Journal of Fish Biology. 90: 1506-1516.

Pinheiro, J. P. S.; Windsor, F. M.; Wilson, R. W. & Tyler, C. R. 2021. Global variation in freshwater physico-chemistry and its influence on chemical toxicity in aquatic wildlife. Biological Reviews. 96: 1528-1546.

Rajeshkumar, S. & Li, X. 2018. Bioaccumulation of heavy metals in fish species from the Meiliang Bay, Taihu Lake, China. Toxicology Reports. 5: 288-295.

Ravindranath, N.; Papadopoulos, V.; Vornberger, W.; Zitzmann, D. & Dym, M. 1994. Ultrastructural distribution of calcium in the rat testis. Biology of Reproduction. 51: 50–62.

Reis, R. M.; Angelo, A. G.; Sakamoto, A. C.; Ferriani, R. A. & Lara, L. A. S. 2013. Altered sexual and reproductive functions in epileptic men taking carbamazepine. The journal of Sexual Medicine. 10: 493-499.

Rocco, L.; Peluso, C.; Cesaroni, F.; Morra, N.; Cesaroni, D. & Stingo, V. 2012. Genomic damage in human sperm cells exposed in vitro to environmental pollutants. Journal of Environmental and Analytical Toxicology. 2: 117.

Ruhí, A.; Acuña, V.; Barceló, D.; Huerta, B.; Mor, J. R.; Rodríguez-Mozaz, S. & Sabater, S. 2016. Bioaccumulation and trophic magnification of pharmaceuticals and endocrine disruptors in a Mediterranean river food web. Science of the Total Environment. 540: 250-259.

Sabr, Z. A. A. & Karim, A. P. Z. 2023. Effect of carbamazepine (Tegretol) on testicular parameters in adult male mice (*Mus musculus*). Annals of Forest Research. 66: 621-630.

Silva Santos, N.; Oliveira, R.; Lisboa, C. A.; Pinto, J. M.; Sousa-Moura, D.; Camargo, N. S.; Perillo, V.; Oliveira, M.; Grisolia, C. K. & Domingues, I. 2018. Chronic effects of carbamazepine on zebrafish: behavioral, reproductive and biochemical endpoints. Ecotoxicology and Environmental Safety. 164: 297-304.

Segner, H. 2011. Reproductive and developmental toxicity in fishes. In: Gupta, R. C. Reproductive and Developmental Toxicology. Academic Press.

Servili, A.; Canario, A. V.; Mouchel, O. & Muñoz-Cueto, J. A. 2020. Climate change impacts on fish reproduction are mediated at multiple levels of the brain-pituitary-gonad axis. General and Comparative Endocrinology. 291: 113439.

Shihomatsu, H.; Martins, E.; Cotrim, M.; Lebre, D.; Ortiz, N. & Pires, M. 2017. Guarapiranga Reservoir - pharmaceuticals and historical urban occupation in a water source. Journal of Geoscience and Environment Protection. 5: 1-17.

Soucek, D. J.; Linton, T. K.; Tarr, C. D.; Dickinson, A.; Wickramanayake, N.; Delos, C. G. & Cruz, L. A. 2011. Influence of water hardness and sulfate on the acute toxicity of chloride to sensitive freshwater invertebrates. Environmental Toxicology and Chemistry. 30: 930-938.

Sumpter, J. P. 1997. Environmental control of fish reproduction: a different perspective. Fish Physiology and Biochemistry. 17: 25-31.

Taslima, K.; Al-Emran, M.; Rahman, M. S.; Hasan, J.; Ferdous, Z.; Rohani, M. F. & Shahjahan, M. 2022. Impacts of heavy metals on early development, growth and reproduction of fish - a review. Toxicology Reports. 9: 858-868.

Thomas, K. V.; Silva, F. M. A.; Langford, K. H.; Souza, A. D. L.; Nizzeto, L. & Waichman, A. V. 2014. Screening for selected human pharmaceuticals and cocaine in the urban streams of Manaus, Amazonas, Brazil. Journal of the American Water Resources Association. 50: 302-308.

Treviño, C. L.; Santi, C. M.; Beltrán, C.; Hernández-Cruz, A.; Darszon, A. & Lomeli, H. 1998. Localization of inositol trisphosphate and ryano-dine receptors during mouse spermatogenesis: Possible functional implications. Zygote. 6: 159–172.

Valdebenito, I. I.; Gallegos, P. C. & Effer, B. R. 2013. Gamete quality in fish: evaluation parameters and determining factors. Zygote. 23: 177-197.

Valdebenito, I.; Gallegos, P. C. & Effer, B. R. 2015. Gamete quality in fish: evaluation parameters and determining factors. Zygote. 23: 177e97.

Wachten, D.; Jikeli, J. F. & Kaupp, U. B. 2017. Sperm sensory signaling. Cold Spring Harbor Perspectives in Biology. 9: a028225.

Windsor, F. M.; Pereira, M. G.; Morrissey, C. A.; Tyler, C. R. & Ormerod, S. J. 2020. Environment and food web structure interact to alter the trophic magnification of persistent chemicals across river ecosystems. Science of the Total Environment. 717: 137271.

Wirtz, S. & Steinmann, P. 2006. Sperm characteristics in perch *Perca fluviatilis* L. J. Journal of Fish Biology. 68: 1896e1902.

Wood, C. M.; McDonald, M. D.; Grosell, M.; Mount, D. R.; Adams, W. J.; Po, B. H. & Brix, K. V. 2020. The potential for salt toxicity: can the trans-epithelial potential (TEP) across the gills serve as a metric for major ion toxicity in fish? Aquatic Toxicology. 226: 105568.

Yan, S.; Wang, M.; Zha, J.; Zhu, L.; Li, W.; Luo, Q.; Sun, J. & Wang, Z. 2018. Environmentally relevant concentrations of carbamazepine caused endocrine-disrupting effects on nontarget organisms, Chinese rare minnows (*Gobiocypris rarus*). Environmental Science & Technology. 52: 886-894.

Capítulo 3

1. Discussão Geral e Conclusões

Diante do contexto problemático da presença de CBZ no ambiente aquático como um poluente de preocupação emergente, a presença de Ca na água e a possível interação entre ambos foi levantada a hipótese de que o íon poderia elevar a toxicidade da CBZ e afetar negativamente a qualidade seminal de *A. lacustris* (pH, morfologia, cinética e concentração espermática). No entanto, os resultados obtidos diferem da hipótese, pois de maneira geral, a CBZ sozinha foi capaz de alterar a qualidade seminal da espécie, e a presença do Ca na água não potencializou este efeito.

Apesar de refutada parte da hipótese inicial, ainda assim é claro o efeito negativo da CBZ na qualidade seminal de *A. lacustris* quando avaliado o resultado de morfologia e cinética espermática, mesmo que a concentração e o pH não tenham sido alterados. Considerando o processo reprodutivo da espécie e da maioria dos teleósteos, a fertilização acontece de forma externa, sendo assim dependente do encontro dos espermatozoides com os oócitos através da natação dos gametas masculinos pela movimentação do flagelo, que funciona como um motor de propulsão que comanda a trajetória através da cinética espermática (Inaba, 2003; Wachten et al., 2017; Dzyuba et al., 2017; Boryshpolets et al., 2018; Cosson, 2019). Este é o motivo pelo qual a má formação flagelar pode interferir nesse processo e ser prejudicial à fertilização e consequentemente à geração de descendentes.

Uma queda considerável na porcentagem de espermatozoides classificados com flagelo de morfologia normal foi notada por conta das concentrações de CBZ (250CBZ e 500CBZ), resultados semelhantes aos encontrados em diferentes gerações do teleósteo *D. rerio* (Fraz et al., 2019) e no rato-doméstico (*Mus musculus*) com relação à morfologia da cabeça dos espermatozoides (Sabr e Karim, 2023). Adicionalmente, nestes mesmos grupos a porcentagem de espermatozoides móveis diminuiu em 20,63% e 26,17%, respectivamente. Para afirmar mais precisamente que estas alterações são prejudiciais aos gametas masculinos, seria interessante que futuros estudos realizem a fertilização com os espermatozoides dos animais expostos e acompanhem a fertilização e o desenvolvimento embrionário das próximas gerações. Adicionalmente, o nosso grupo de pesquisa vem realizando distintos estudos com a exposição de *A. lacustris* ao fármaco CBZ, tanto fêmeas quanto machos, principalmente utilizando biomarcadores em diferentes níveis de organização biológica, com enfoque molecular, fisiológico e morfológico, inclusive avaliando diferentes eixos envolvidos no controle neuroendócrino do processo reprodutivo de *A. lacustris*. Os nossos resultados já estão sendo inseridos na literatura especializada como podemos constar nas recentes publicações em eventos científicos do nosso grupo (Assis et al., 2023a, b; Guerreiro et al., 2023; Moreira et al., 2023; Aguiar et al., 2023; Guerreiro et al., 2022)[1], assim como nas dissertações, teses e pós-doutoramentos em desenvolvimento (Verderame, 2023; Neto, 2023; Aguiar, 2023; Faria, 2023; Guerreiro, 2023; Vieira, 2023), incluindo também a presente dissertação.

2. Referências bibliográficas

Aguiar, G. 2023. Avaliação dos efeitos da carbamazepina em concentrações ambientalmente relevantes sobre a regulação neuroendócrina da reprodução em machos de *Astyanax lacustris*. Início: 2023 (em andamento). Dissertação (Mestrado em Ciências (Fisiologia Geral)) - Universidade de São Paulo.

Aguiar, G.; Assis, C. B.; Moreira, R. G. & Guerreiro, A. S. 2023. Biochemical Evaluation of the Pharmaceutical Carbamazepine in the Liver of *Astyanax lacustris* (Teleostei: Characidae). In: Society of environmental toxicology and chemistry (SETAC), Latin America 15th Biennial Meeting, Montevideo.

Assis, C. B.; Branco, G. S.; Guerreiro, A. S.; Honji, R. M.; Pinheiro, J. P. S. & Moreira, R. G. 2023a. The Influence of Environmental Concentrations of the Neuropharmaceutical Carbamazepine Associated With Calcium, on Sperm Morphology of *Astyanax lacustris*. In: Society of Environmental Toxicology and Chemistry (SETAC), Latin America 15th Biennial Meeting, 2023, Montevideo.

Assis, C. B.; Branco, G. S.; Guerreiro, A. S.; Honji, R. M.; Pinheiro, J. P. S. & Moreira, R. G. 2023b. Influence of Environmental Concentrations of the Neuropharmaceutical Carbamazepine Associated With Calcium, on Sperm Quality Parameters of *Astyanax lacustris*. In: Society of Environmental Toxicology and Chemistry (SETAC), Latin America 15th Biennial Meeting, 2023, Montevideo.

[1] Compilações das publicações em eventos nacionais e internacionais realizadas pelo nosso grupo de pesquisa até a data de 21 de fevereiro de 2024.

Boryshpolets, S.; Kholodnyy, V.; Cosson, J. & Dzyuba, B. 2018. Fish sperm motility analysis: the central role of the flagellum. Reproduction, Fertility and Development. 30: 833-841.

Cosson, J. 2019. Fish sperm physiology: structure, factors regulating motility, and motility evaluation. Biological Research in Aquatic Science. 1: 1-26.

Dzyuba, B.; Bondarenko, O.; Fedorov, P.; Gazo, I.; Prokopchuk, G. & Cosson, J. 2017. Energetics of fish spermatozoa: the proven and the possible. Aquaculture. 472: 60-72.

Faria, N. P. V. M. 2023. Efeitos da carbamazepina, em concentrações ambientalmente relevantes, sobre o eixo neuroendócrino de *Astyanax lacustris*. Início: 2024 (Doutorado em andamento). Tese (Doutorado em Ciências (Fisiologia Geral)) - Universidade de São Paulo.

Fraz, S.; Lee, A. H.; Pollard, S.; Srinivasan, K.; Vermani, A.; David, E. & Wilson, J. Y. 2019. Paternal exposure to carbamazepine impacts zebrafish offspring reproduction over multiple generations. Environmental Science & Technology. 53: 12734-12743.

Guerreiro, A. S. 2023. Efeitos da carbamazepina, um Contaminante de Preocupação Emergente, sobre a fisiologia de fêmeas de *Astyanax lacustris*. Bolsista de Pós-Doutoramento da FAPESP (Processo 2020/11583-0). Disponível em: https://bv.fapesp.br/pt/bolsas/199430/efeitos-da-carbamazepina-um-contaminante-de-preocupacao-emergente-sobre-a-fisiologia-de-femeas-de-as/. Acessado no dia 21 de fevereiro de 2024.

Guerreiro, A. S.; Aguiar, G.; Godoi, F. G. A. & Moreira, R. M. 2022. Is carbamazepine toxic for *Astyanax lacustris*? Analysis of the reproductive physiology of females. In: 9th International Conference of Fish Endocrinology, Faro.

Guerreiro, A. S.; Godoi, F. G. A.; Branco, G. S. & Moreira, R. G. 2023. Influence of carbamazepine, a contaminant of emerging concern, on the reproductive axis of female lambari (*Astyanax lacustris*). In: 12th International Symposium on Reproductive Physiology of Fish, Hersonissos. Abstract.

Inaba, K. 2003. Molecular architecture of the sperm flagella: molecules for motility and signaling. Zoological Science. 20: 1043-1056.

Moreira, R. G.; Assis, C. B.; Guerreiro, A. S.; Aguiar, G. 2023. Biochemical Evaluation of the Pharmaceutical Carbamazepine in the Liver of *Astyanax lacustris*

(Teleostei: Characidae). In: Society of environmental toxicology and chemistry (SETAC), Latin America 15th Biennial Meeting, Montevideo.

Neto, D. J. A. 2023. Avaliação dos efeitos da carbamazepina, em concentrações ambientalmente relevantes, sobre a regulação neuroendócrina em machos de *Astyanax lacustris* (Teleostei: Characiformes). Bolsista de Mestrado da FAPESP (Processo 2023/03227-8). Disponível em: https://bv.fapesp.br/pt/bolsas/212641/avaliacao-dos-efeitos-da-carbamazepina-em-concentracoes-ambientalmente-relevantes-sobre-a-regulacao-/. Acessado no dia 21 de fevereiro de 2024.

Sabr, Z. A. A. & Karim, A. P. Z. 2023. Effect of carbamazepine (Tegretol) on testicular parameters in adult male mice (*Mus musculus*). Annals of Forest Research. 66: 621-630.

Verderame, M. C. 2023. Avaliação dos efeitos do neurofármaco carbamazepina na via esteroidogênica e na morfologia hepática e gonadal de *Astyanax lacustris*. Bolsista de Mestrado da FAPESP (Processo 2023/13281-0). Disponível em: https://bv.fapesp.br/pt/bolsas/214446/avaliacao-dos-efeitos-do-neurofarmaco-carbamazepina-na-via-esteroidogenica-e-na-morfologia-hepatica-/. Acessado no dia 21 de fevereiro de 2024.

Vieira, C. E. D. 2023. Fármacos como desreguladores endócrinos em teleósteos neotropicais: uma abordagem integrativa. Bolsista de Pós-Doutoramento da FAPESP (Processo 2023/09837-2). Disponível em: https://bv.fapesp.br/pt/bolsas/213569/farmacos-como-desreguladores-endocrinos-em-teleosteos-neotropicais-uma-abordagem-integrativa/. Acessado no dia 21 de fevereiro de 2024.

Wachten, D.; Jikeli, J. F. & Kaupp, U. B. 2017. Sperm sensory signaling. Cold Spring Harbor Perspectives in Biology. 9: a028225.