

# A dinâmica entre peixes-predadores e invertebrados-presa em diferentes cenários ambientais



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**Programa de Pós-graduação em Ecologia de  
Ecossistemas Aquáticos Continentais**

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BRUNO RENALY SOUZA FIGUEIREDO

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## A dinâmica entre peixes-predadores e invertebrados-presa em diferentes cenários ambientais

### RESUMO

A sobrevivência de uma determinada presa depende de sua habilidade em reconhecer a presença do predador, e da eficiência das estratégias de anti-predação que ela emprega. Tais estratégias geralmente podem ser mais ou menos efetivas dependendo das condições de visibilidade, de temperatura e do período de coocorrência entre predadores e presas. Considerando que flutuações nas condições ambientais se tornarão eventos mais frequentes em virtude do aquecimento global, faz-se necessário investigar a influência de tais flutuações para o resultado da interação predador-presa. Neste estudo, utilizou-se uma abordagem experimental para observar a eficiência na captura de presas por peixes-predadores e o comportamento anti-predatório de invertebrados-presa (espécies de insetos, de anfípodas e de microcrustáceos) em cenários contrastantes de visibilidade subaquática, de temperatura da água e de disponibilidade de refúgio (provido por plantas aquáticas). Além disso, observou-se a implicação da invasão de espécies não-nativas para a interação entre predadores e presas sob duas perspectivas: após o estabelecimento de uma planta aquática não-nativa (*Hydrilla verticillata*), e após a introdução de um predador piscívoro não-nativo (*Astronotus crassipinnis*). De maneira geral, observou-se que a redução na transparência da água reduziu a eficiência na captura de invertebrados por peixes, e essa redução foi mais acentuada quando predadores piscívoros coocorreram com os peixes mesopredadores. A mera presença de predadores de topo aumenta a sobrevivência de invertebrados, mas a presença deles em condições de baixa visibilidade subaquática reduz sobremaneira o potencial predatório de peixes mesopredadores, os quais provavelmente utilizam mais tempo em comportamentos de vigilância, e reduzem a ingestão de presas. É predito que a elevação da temperatura aumente a frequência de eventos relacionados à busca e à captura de alimento por predadores, *Gymnogeophagus terrapurpura* apresentou similares taxas de consumo de anfípodas (*Hyaella curvispina*) em diferentes temperaturas da água (19,2; 22,2; 25,2 e 27°C). Apesar disso, na temperatura mais elevada anfípodas apresentaram um comportamento anti-predatório mais conspícuo. Verificou-se ainda que plantas aquáticas não-nativas são reconhecidas como *habitat* seguro, e são utilizadas como refúgio por presas na mesma proporção que plantas nativas. A pressão de predação exercida por *Astyanax altiparanae* sobre uma população de invertebrados pelágicos (*Daphnia magna*) foi extremamente intensa em tratamentos com o piscívoro nativo *Hoplias* aff. *malabaricus*, contudo, nos tratamentos com piscívoro não-nativo *A. crassipinnis* a sobrevivência de presas pelágicas foi proporcional a sobrevivência de presas bentônicas (*Chironomus sancticaroli*). A introdução de um piscívoro tem a capacidade de mudar a fonte do recurso alimentar de mesopredadores, com potenciais implicações para o fluxo de energia dentro do ecossistema. Simular, em microcosmo, cenários ambientais observados e preditos fornece subsídio para a determinação de tamanho de efeitos de variáveis isoladas, e de possíveis interações entre tais variáveis, que podem auxiliar para o manejo de espécies de peixes e invertebrados dependentes das condições ambientais para a manutenção de suas funções vitais, garantido a preservação da espécie e a conservação da vida.

**Palavras-chave:** Predação. Interação predador-presa. Comportamento animal. Insetos aquáticos. Heterogeneidade de *habitat*. Experimentação animal.



## Trophic interactions between predator-fishes and invertebrate-prey in different environmental scenarios

### ABSTRACT

The survival of a given prey is primarily determined by their capacity to recognize and respond to the current predation threat degree. Such prey responses could be more or less effective according to environmental characteristics, such as visibility conditions and temperature. However, the evolutionary time of co-occurrence between predators and prey could also be a crucial factor to shape the appropriated prey response to predator strike. Fluctuations in environmental conditions are expected to be more frequent as a result from global warming, which has been catalysed by human being's actions. Therefore, it is quite timely investigate the influence of environmental shifts on the outcomes of predator-prey encounters. Here, we used an experimental approach to observe the prey capture efficiency of fish-predators and anti-predator behaviour of some groups of invertebrates (aquatic insects and amphipods) in distinct ecological scenarios of underwater visibility, water temperature and presence/absence of refuge (provided by aquatic macrophyte). Also, we investigated the consequences of species invasions for the interaction between predators and preys on two perspectives: after the establishment of a non-native aquatic plant (*Hydrilla verticillata*), and after the introduction of a non-native piscivorous fish. In reduced water transparency, fish consumed fewer invertebrates than in clear water, but the efficiency in capturing invertebrates of mesopredators was strongly reduced when top-predator was present and water transparency low. Therefore, the mere presence of a top predator has potential to increase invertebrate survive, but underwater visibility is impaired mesopredator do not know the exact top predator location, then they tend to reduce their mobility to decrease the likelihood to be eaten, such response can concomitantly limits the mesopredator predatory potential. Despite of the literature supports the positive relation between water temperature and the distance travelled by predators, which often results in better feeding efficiency in warmer waters, *Gymnogeophagus terrapurpura* showed similar amount of amphipods (*Hyalella curvispina*) consumed in four different water temperature (19.2; 22.2; 25.2 and 27°C). In contrast, amphipods show more conspicuous anti-predator behaviour at 27°C. In addition, we found that non-native aquatic plants are recognized as a safe habitat and used by prey fish as refuge in a similar proportion than native plants are. We also found that the predation pressure performed by *Astyanax altiparanae* on one pelagic invertebrate population (*Daphnia magna*) was extremely high in treatments with the native piscivorous *Hoplias aff. malabaricus*. However, in treatments with the non-native *Astronotus crassipinnis*, the survival of pelagic invertebrates was proportional to benthic prey population (*Chironomus sancticaroli*). Therefore, the introduction of a piscivorous fish has potential to change the energy pathway in the entire ecosystem. Simulating observed and predicted ecological scenarios, even in microcosm, allow us to understand the main effect of a given variable on predation in the wild, and possible interactive effect among multiple variables. Knowing the factors that influence the predation can help ecologists to propose management project for fish and invertebrates species.

**Keywords:** Predation. Predator-prey interaction. Animal behaviour. Aquatic insects. Habitat heterogeneity. Animal experimentation.

## LISTA DE TRABALHOS CIENTÍFICOS ORIGINAIS

Essa tese é baseada nas seguintes publicações e manuscritos (Apêndice A), que são citadas ao longo do texto pelos números romanos atribuídos a elas:

- I Figueiredo, Bruno R. S.; Calvo, Clementina; López-Rodríguez, Anahí; Mormul, Roger P.; Benedito, Evanilde; Teixeira-de-Melo, Franco & Meerhoff, Mariana. Turbidity pulses have stronger effects than heat waves on fish-invertebrate trophic interactions. Submetido a *Canadian Journal of Fisheries and Aquatic Sciences* (em Agosto de 2016).
- II Figueiredo, Bruno R. S.; Alves, Gustavo H. Z.; Calvo, Clementina; López-Rodríguez, Anahí; Benedito, Evanilde & Meerhoff, Mariana. Consequências de variações na visibilidade subaquática e na temperatura sobre o comportamento de anfípodas. *Unless I see the predator I will not flee: influence of light and heat on anti-predator responses of amphipods*. Em preparação, periódico alvo: *Invertebrate Biology*.
- III Figueiredo, Bruno R. S.; Mormul, Roger P.; Chapman, Ben B.; Lolis, Lucas A.; Fiori, Leandro F & Benedito, Evanilde (2016) Turbidity amplifies the non-lethal effects of predation and affects the foraging success of characid fish shoals. *Freshwater Biology*, **61**, 293–300. doi: 10.1111/fwb.12703
- IV Figueiredo, Bruno R. S.; Fiori, Leandro F.; Mormul, Roger P. & Benedito, Evanilde. Non-lethal effects of an invasive piscivorous fish on invertebrates: the predator origin matters to trophic cascade. Submetido a *Biological Invasions* (em Julho de 2016).
- V Figueiredo, Bruno R. S.; Mormul, Roger P.; & Thomaz, Sidinei M. 2015. Swimming and hiding regardless of the habitat: prey fish do not choose between a native and a non-native macrophyte species as a refuge. *Hydrobiologia*, **746**, 285–290. doi: 10.1007/s10750-014-2096-x

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## 1 INTRODUÇÃO

De maneira simplificada, a sobrevivência de um animal depende de sua habilidade em capturar presas, enquanto evita ser consumido por predadores (Lima & Dill, 1990; Abrahams *et al.*, 2009). Esses atributos estão profundamente relacionados, pois enquanto caracteres que aperfeiçoam a habilidade de fuga de presas são favorecidos por seleção natural, caracteres que elevam a capacidade de captura da presa por predadores são também beneficiados, em um processo de coevolução (p. ex. Darwin, 1859; Dawkins & Krebs, 1979). Na prática, predadores podem afetar a estrutura e o tamanho de diferentes populações de presas através do consumo direto delas (Lima & Dill, 1990), mas a mera presença deles no ambiente pode também influenciar o comportamento adotado por suas potenciais vítimas (Abjörnsson *et al.*, 2002), as quais podem: (i) reduzir sua movimentação (Lehtiniemi, 2005), (ii) se esconder em *habitat* mais complexos (Padiál *et al.*, 2009), (iii) gastar mais tempo monitorando o ambiente (Brown & Kotler, 2004), ou mesmo iniciar uma resposta de fuga (Higham *et al.*, 2015). Qualquer que seja a estratégia adotada para evitar predadores, ela terá implicações para as atividades vitais das presas. Isto ocorre porque presas, sob ameaça de serem consumidas, tendem a reduzir a atividade natatória, o que influencia a procura por alimento (Pink & Abrahams, 2016), e ao mesmo tempo, reduz a probabilidade de encontro entre parceiros reprodutivos (Orrock *et al.*, 2013). Portanto, a estratégia de anti-predação adotada pode ter implicações para o *fitness* do organismo predador (Orrock *et al.*, 2013; Peacor *et al.*, 2013), mas também pode significar a extinção local da espécie de presa (Lima, 1998).

O resultado da interação entre um predador e uma presa, pode ter consequências para outros níveis tróficos, a ponto de influenciar, inclusive, propriedades e dinâmicas naturais dos ecossistemas (Carpenter *et al.*, 2001, Stief & Holker, 2006). Ao mesmo tempo, a condição ambiental pode favorecer o escape da presa ou a captura dela pelo predador, influenciando o resultado da interação predatória após um dado encontro entre predadores e presas (Smee *et al.*, 2010). Por exemplo, sabendo que detectar um potencial consumidor ou uma presa antes de ser percebido oferece uma clara vantagem (Lima & Dill, 1990), a predação pode aumentar quando o ambiente facilita a detecção de presas por predadores, mas ela pode diminuir quando as condições ambientais reduzem a habilidade de predadores detectarem suas presas (Robinson *et al.*, 2011). Portanto, um processo dinâmico entre predador e presa é estabelecido, e o resultado de

tal interação em um ambiente em mudança, será determinado por um aumento ou uma redução desproporcional da capacidade de fuga das presas e de captura de alimento dos predadores.

### 1.1 OSCILAÇÕES NO AMBIENTE AQUÁTICO E A INTERAÇÃO PREDADOR-PRESA

Os ecossistemas aquáticos estão sujeitos a flutuações em diversos parâmetros ambientais ao longo do tempo, tais como temperatura, transparência da água e oxigênio dissolvido (Winder & Schindler, 2004; Abrahams *et al.*, 2007). Algumas projeções estimam um aumento médio de 3°C na temperatura até 2100, considerando toda a superfície terrestre (IPCC, 2007; Marengo *et al.*, 2012). A elevação na temperatura atmosférica, também refletida na água, significará para a biota aquática, composta em sua maioria por organismos ectotérmicos, uma aceleração das reações bioquímicas, e consequente, aumento nas demandas energéticas dos organismos (Clarke & Johnston, 1999; Sheridan & Bickford, 2011). É observado, por exemplo, que em águas mais quentes, peixes predadores utilizam mais tempo em atividades relacionadas à ingestão de alimento, para tanto aumentam sua atividade natatória e a distância percorrida em busca da presa (Nowicki *et al.*, 2012; Como *et al.*, 2014). Tal aumento da temperatura da água pode ter consequências mais drásticas para indivíduos-presa, pois estes organismos necessitarão potencializar suas defesas, uma vez que o predador intensificará sua busca pelo alimento. Um maior tempo gasto para evitar predadores representa um menor tempo disponível para ingerir alimento. Além disso, em resposta a um período sem ingestão de alimento prolongado, organismos utilizam mais energia para encontrar comida, e tendem a reduzir a resposta a estímulos associados a presença de um predador (Dill & Fraser, 1984; Godin & Crossman 1994).

Da mesma forma que a temperatura da água, as mudanças na visibilidade subaquática (tanto de origem abiogênica quanto biogênica) afetam diretamente as interações tróficas nos ecossistemas aquáticos, porque uma grande quantidade de organismos aquáticos depende da visão para obter informações do ambiente circundante (Guthrie & Muntz, 1993), incluindo para a detecção de presas e fuga de predadores (Ranåker *et al.*, 2012). A visibilidade subaquática pode ser extremamente reduzida pelo aumento populacional de algas flutuantes (Kirk, 1986), pelo carreamento de partícula inorgânicas do ambiente terrestre para a água através da chuva (Davies-Colley *et al.*, 1993), e ainda pela ressuspensão de sedimento na coluna de água promovido tanto pelo

vento (Horppila & Nurminen, 2005) quanto por peixes bentônicos (Mormul *et al.*, 2012). A visibilidade subaquática pode ainda aumentar abruptamente em alguns ecossistemas aquáticos, em decorrência da ação de agentes bióticos, como os organismos zooplancctônicos, que podem deplecionar a população de algas flutuantes por predação, o que aumenta a penetração luminosa na coluna de água (Auer *et al.*, 1990). Há eventos que podem tornar a água mais transparente, como o barramento do curso natural dos rios para construção de reservatórios e/ou hidroelétricas, que retêm nutrientes e partículas em suspensão ao longo do tempo, promovendo a oligotrofização (Johnson *et al.*, 1995; Roberto *et al.*, 2009).

Aumento ou redução na visibilidade subaquática podem, respectivamente, facilitar ou impedir o uso sensorial da visão como meio de obter informações do ambiente. Na prática, qualquer variação na visibilidade através da coluna da água impacta (aumentando ou reduzindo) a distância de reação de presas, e a distância de ataque dos predadores. Portanto, a redução na transparência da água favorece os organismos capazes de obter informações do ambiente através de estímulos não visuais (Stankowich & Blumstein, 2005), ao passo que um aumento na transparência da água potencializa a habilidade predatória de espécies que caçam visualmente (Espínola *et al.*, 2010).

## 1.2 VARIAÇÕES INTRAPOPULACIONAIS NA RESPOSTA AO AMBIENTE EM MUDANÇA

Uma das estratégias comportamentais utilizadas por presas para reduzir a probabilidade do indivíduo ser predado é a formação de grupos (Krause & Ruxton, 2002). O mecanismo que promove o aumento de sobrevivência por meio da formação de grupos é simples: se um agrupamento de presas é atacado por um predador que tem a capacidade de capturar apenas um indivíduo, então a probabilidade de um indivíduo específico ser capturado é inversamente proporcional ao tamanho do grupo (efeito de diluição – Beauchamp, 2003). Além disso, animais que vivem em grupo têm a oportunidade de unir as capacidades sensoriais individuais para aumentar a probabilidade de detectar predadores, portanto o indivíduo pode reduzir o tempo gasto monitorando a possível presença de um predador, sem que haja aumento no risco de predação (Pulliam, 1973; Abrahams *et al.*, 2009). É inegável que viver em grupo está associado a determinados custos para todo o grupo, como a partilha dos recursos

disponíveis (em sua definição mais ampla), e o aumento da probabilidade do grupo ser detectado por um predador (Cresswell, 1994).

Para o indivíduo, os exatos benefícios e os custos de viver em grupo é dependente de uma série de fatores, como a posição ocupada pelo organismo dentro do grupo (Krause & Ruxton, 2002). Por exemplo, em um agrupamento de peixes-presa, os indivíduos que ocupam regiões mais periféricas do cardume estão mais susceptíveis a predação (Quinn & Cresswell, 2006), comparados a indivíduos que ocupam posições mais centrais dentro do cardume, e a permanência em uma dessas posições deve estar relacionado com aspectos comportamentais (Ward *et al.*, 2004; Jolles *et al.*, 2015). Isso exemplifica o porquê dos grupos não serem homogêneos, quando se trata do comportamento individual (Magurran, 1993).

Modelos matemáticos demonstram que indivíduos menos dependentes das condições ambientais para desempenhar suas funções vitais, ou aqueles que possuam maior plasticidade comportamental, devem apresentar maior capacidade adaptativa, e, portanto, sobreviverão (Öhlund *et al.*, 2015), principalmente nos atuais cenários de rápidas e profundas mudanças ambientais potencializadas pelo homem (Abrahams *et al.*, 2009). A plasticidade comportamental refere-se à capacidade de um animal de alterar seu comportamento de acordo com as diferentes condições encontradas (West-Eberhard, 1989) e pode ser considerada como a maneira mais rápida através da qual os animais se adequam as mudanças ambientais, uma vez que muito mais tempo é necessário para que ocorra evolução genética (Hendry *et al.*, 2008).

### 1.3 IMPLICAÇÕES DAS INVASÕES BIOLÓGICAS PARA A INTERAÇÃO PREDADOR-PRESA

Em geral, a coexistência entre predadores e presas só é possível porque presas reconhecem o predador, e reduzem a susceptibilidade dela aos ataques letais. Se presas forem incapazes de identificar e responder a presença do predador, elas sofrerão drástica mortalidade por predação (p.ex., Mathis & Smith, 1993), uma situação que tem sido chamada de “ingenuidade da presa” (*prey naiveté*). Geralmente, presas são ingênuas, ou incapazes de reconhecer seus predadores, quando não coocorreram com tais predadores (bem como com predadores filogeneticamente próximos) durante a história evolutiva delas (Cox & Lima, 2006). Como visto na seção anterior, alguns indivíduos possuem uma maior plasticidade quanto ao comportamento de anti-predação, e podem, por exemplo, permanecer tão distantes quanto for possível dessa nova ameaça de



predação em potencial (Sneddon *et al.*, 2003; Mesquita & Young, 2007), o que geralmente implica em um monitoramento excessivo dos movimentos da nova potencial ameaça, redução da mobilidade, e menor consumo de presas (Sih *et al.*, 2010). Portanto, se a mera presença de predadores nativos proporciona elevados custos para presas, estes custos devem ser ainda maiores quando os predadores são invasores (Pellicice & Agostinho, 2009), pois aliada à inadequada (ou inexistente) defesa da presa a ataques de predadores invasores, por vezes essas espécies invasoras apresentam maior eficiência no uso do recurso, em comparação às espécies nativas (p. ex. Vilà & Weiner, 2004; Van Kleuner *et al.*, 2010).

É inquestionável que a forma mais simples de presas evitarem serem detectadas e consumidas por predadores é ocupar *habitat* que eles simplesmente não podem acessar (Abrahams *et al.*, 2009). Se peixes-presa ocupam *habitat* vegetados, eles tendem a aumentar a sobrevivência (Padial *et al.*, 2009), pois a complexidade estrutural da vegetação limita a atividade natatória de predadores (Diehl, 1988). Portanto, a preferência da presa por um *habitat* vegetado específico aumenta a sobrevivência dela, e essa relação presa-planta seria favorecida por seleção natural (Goodman, 2009), e deve se intensificar ainda mais a cada geração. Se isso for verdade, o estabelecimento de uma espécie de planta exótica, com capacidade de excluir competitivamente as plantas nativas (Sousa, 2009), poderia elevar drasticamente a predação sobre os supracitados peixes-presa, porque estes não reconheceriam a espécie de planta recém-chegada como um *habitat* seguro. Se a escolha do *habitat* não for mediada pela coocorrência das espécies de presa e de planta, mas ao contrário, for determinada pela complexidade do *habitat* (Thomaz & Cunha, 2010; Figueiredo *et al.*, 2015), então o estabelecimento de uma planta exótica não afetaria o uso do *habitat* pelos peixes-presa. Compreender o que determina a escolha do *habitat*, na ausência de predadores, e sob ameaça de predação, pode auxiliar a proposição de medidas de manejo para espécies que ocupam *habitat* susceptíveis a invasão de plantas.

#### 1.4 CONTEXTUALIZANDO A INTERAÇÃO ENTRE PREDADORES E PRESAS

Considerando as teorias ecológicas apresentadas, este estudo investigará potenciais impactos de três variáveis-chave sobre a interação entre predadores e presas: a **temperatura da água**, a **visibilidade subaquática** e a **invasão de espécies**. Embora estas teorias ecológicas sejam exclusivamente testadas de forma experimental, os

delineamentos das experimentações simularam diferentes cenários ecológicos observados ou preditos para ocorrerem na planície de inundação do alto rio Paraná. O rio Paraná possui uma alta diversidade de peixes, que em grande parte é composta por organismos de pequeno porte (Agostinho *et al.*, 2007), que podem atuar ora como presa, ora como predador. Por esse motivo, nos estudos desta tese, para testar hipóteses ecológicas, peixes de pequeno porte atuaram como predadores (Artigos I e II), presas (Artigo V), ou ainda como mesopredadores (isto é, simultaneamente predadores e presas) (Artigo III e IV).

Estudos pre dizem maior incidência de chuvas em diversos ecossistemas ao longo do globo no futuro, o que tende a tornar mais frequentes os cenários sazonais de redução na visibilidade subaquática (IPCC, 2012). Esse aumento da frequência de chuvas também é esperado para ocorrer para a região do alto rio Paraná (Marengo *et al.*, 2012). Apesar disso, o padrão oposto, isto é elevação na visibilidade da água, é algo concretamente observável na planície de inundação do alto rio Paraná (Roberto *et al.*, 2009), em decorrência da construção de reservatórios em cascatas situados a montante da planície, os quais têm retido nutrientes e sólidos fundamentais para a reprodução de microalgas, tais como o fósforo, o que tem gerado um abrupto aumento da transparência da água. É razoável se questionar sobre os efeitos das observadas e preditas mudanças na transparência da água sobre a interação entre predadores e suas presas, como a relação entre peixes e invertebrados. As mesmas partículas que tendem a reduzir a transparência da água, também podem absorver calor e potencializar o aquecimento da água (Bohren & Huffman, 1983). Logo, é possível sugerir que a proporção de espécies menos sensíveis a alterações nas condições de turbidez e temperatura aumentará, em detrimento do declínio populacional de espécies mais sensíveis.

Na planície de inundação do rio Paraná, dois importantes eventos de invasões biológicas ocorreram: a introdução de *Astronotus crassipinnis*, um ciclídeo piscívoro, nativo da planície do rio Amazonas (Graça & Pavanelli, 2006), e o estabelecimento de *Hydrilla verticillata*, uma macrófita submersa originária da Ásia (Sousa, 2011). Ambas as espécies exóticas parecem ter encontrado na atual condição de transparência da água dos ambientes da planície de inundação do alto rio Paraná, *habitat* ideal para se estabelecer. *Astronotus crassipinnis* é um piscívoro ativo, bem adaptado a viver em lagos, que foi provavelmente introduzido no rio Paraná por aquarofilistas (Graça & Pavanelli, 2006), bem como por programas de estocagem de peixes (Alegretti *et al.*, 2016). Não há estudos sobre o tamanho do impacto causados pela introdução de A.

*crassipinnis* sobre a diversidade de peixes, entretanto, é possível estimar observando o estabelecimento de outro ciclídeo com similar comportamento de uso de *habitat* e caça: *Cichla kelberi*. Um ano após o primeiro registro de *C. kelberi* em um trecho de reservatório do rio Paraná (Rosana), observou-se redução de 95% na densidade de peixes nativos, e declínio em 80% na riqueza da ictiofauna (Pelicice & Agostinho, 2009; Pelicice *et al.*, 2015). Da mesma forma, o impacto negativo mais conspícuo atribuído a *H. verticillata* é a exclusão competitiva de outras espécies de macrófitas nativas (Sousa, 2011), com a qual espécies de predadores e de presas coocorreram ao longo de suas histórias de vida.

### 1.5 OBJETIVOS DA TESE

O propósito é investigar possíveis respostas de predadores (peixes), quanto ao consumo de presas, e de presas (espécies de insetos, de anfípodas e de microcrustáceos) quanto ao comportamento de anti-predação, para (i) a elevação na temperatura da água, (ii) a redução ou aumento na visibilidade subaquática, (iii) a introdução de predadores de topo exóticos, e (iv) o estabelecimento de plantas aquáticas exóticas. Variações nas condições ambientais vigentes podem ser positivas ou negativas para predadores e presas, e é evidente que qualquer alteração (positiva ou negativa) na capacidade de captura por predadores ou escape pela presa influenciará o equilíbrio dessa interação ecológica, com provável implicação sobre a sobrevivência das populações (Lima & Dill, 1990). Compreender o papel das características ambientais isoladas, e de seus potenciais efeitos sinérgicos sobre a eficiência de captura de presas, bem como sobre o comportamento de anti-predação, pode auxiliar na determinação das alterações na interação entre peixes e invertebrados em caso de mudanças na temperatura e na transparência da água, e em caso de estabelecimento de piscívoros invasores e de plantas exóticas. Os principais questionamentos específicos aqui abordados são:

- Como o aumento na transparência da água impactará a eficiência na captura de presas por predadores em elevadas temperaturas da água? (Artigo I). Da mesma forma, qual a resposta de invertebrados-presa para a elevada temperatura e reduzida visibilidade subaquática? (Artigo I e II);

- Como os efeitos não letais interagem com (i) a condição de visibilidade subaquática para afetar o comportamento de anti-predação de invertebrados (Artigo II), e (ii) a eficiência na captura de presas por peixes? (Artigo III);

- Os efeitos não letais podem interferir na partilha de alimento entre peixes predadores do mesmo cardume em ambientes transparentes ou túrbidos? (Artigo **III**);
- A sobrevivência de invertebrados é maior na presença de piscívoros exóticos que na presença de piscívoros nativos? (Artigo **IV**);
- Apenas a presença de sinais químicos de piscívoros é suficiente para estimular o uso de *habitat* vegetados por peixes mesopredadores? (Artigo **V**);
- Sob iminente risco de serem consumidos, peixes-presa escolhem um *habitat* que está mais familiarizado, ou nestas circunstâncias a escolha é aleatória? (Artigo **V**).

## 2 MÉTODOS

### 2.1 VARIÁVEIS E ORGANISMOS ESTUDADOS

O estudo **I** investigou a eficiência da captura de presas por um peixe ciclídeo (*Gymnogeophagus terrapurpura* Loureiro *et al.*, 2016), e também sobre o comportamento de anti-predação de anfípodes (*Hyaella curvispina* Shoemaker, 1942), em diferentes cenários de transparência e temperatura da água. O ciclídeo *G. terrapurpura* é um predador que utiliza a visão como principal fonte de informação do ambiente, portanto, deverá ser uma das espécies mais afetadas pelo predito aumento da frequência dos pulsos de turbidez (p. ex. Teixeira-de Mello *et al.*, 2009). O anfípode *Hyaella curvispina* ocorre nos mais diferentes tipos de ambientes aquáticos, incluindo locais com alta poluição, em quase toda a América do Sul (Doyle & Momo, 2009) e foi selecionado como organismo-modelo porque é, naturalmente, uma das principais presas de *G. terrapurpura*, e porque anfípodes, em geral, possuem amplo repertório de táticas para evitar predadores (Smith & Webster, 2015). Por esse mesmo motivo, o estudo **II** também verificou se a visibilidade subaquática e a temperatura interagem com a presença de uma ameaça de predação para moldar o comportamento de *H. curvispina*.

Nos estudos **III** e **IV** cadeias alimentares simplificadas (com poucas espécies por nível trófico) foram utilizadas para sugerir impactos de predadores de topo a elos de níveis tróficos inferiores. No estudo **III**, analisou-se o sucesso de forrageamento de um cardume (n=5) de *Moenkhausia forestii* (Benine, Mariguela & Oliveira, 2009) sobre larvas de *Chrinomus sancticaroli* (Strixino & Strixino, 1981) em diferentes cenários de transparência da água, e na ausência ou presença de um peixe piscívoro *Hoplias* aff. *malabaricus* (Bloch, 1794), observando ainda o padrão de partilha de alimento entre os membros do cardume, em cada um dos diferentes cenários supracitados. No estudo **IV**,

foi analisada a seletividade de presas por um cardume ( $n = 3$ ) de *Astyanax altiparanae* (Garutti & Britski, 2000), ao preda *C. sancticaroli* ou *Daphnia magna* (Straus, 1820), simulando o cenário que precede a invasão biológica pelo piscívoro *Astronotus crassipinnis* (Heckel, 1840), na presença de peixes nativos, tais como *H. aff. malabaricus*, os quais poderiam ocasionalmente estar ausentes (comparou-se a ausência com a presença de predador), e também simulando o cenário pós-invasão de *A. crassipinnis*, quando peixes-presa coocorreram com nova ameaça de predação (comparou-se a presença de peixes nativos com a presença de peixes invasores). Tanto *Chrinomus sancticaroli* quanto *Daphnia magna* são presas frequentes no espectro alimentar de diversas espécies de peixes de pequeno porte, tais como *Moenkhausia forestii* (Peretti & Andrian, 2004) e *Astyanax altiparanae* (Casatti et al. 2003). O corpo com pigmentação avermelhada dos *C. sancticaroli* e o tamanho de corpo de *D. magna* devem facilitar a detecção dessas presas para predadores visuais.

No estudo V, buscou-se compreender qual o mecanismo que primariamente influencia a escolha específica de plantas como *habitat* seguros, em condições de ausência de predador e em iminente ameaça de predação: a preferência por plantas que peixes-presa, como *Serrapinnus notomelas* (Eingenmann, 1915), coocorreram durante sua história de vida, tais como *Egeria najas* Planchon, ou alternativamente, a preferência não é espécie-específica, e, portanto, plantas com complexidades estruturais similares como *E. najas* e a exótica *Hydrilla verticillata* (L.F.) Royle possuem a mesma chance de serem selecionadas.

## 2.2 DELINEAMENTOS EXPERIMENTAIS

Para responder aos questionamentos supracitados, e sugerir relações de causa e efeito entre agentes abióticos, bióticos e a interação peixe-invertebrado, optou-se pela abordagem experimental em pequena escala, uma vez que na natureza diversos fatores podem influenciar (e mascarar) o efeito real da variável preditora, tornando complexa a identificação de relações de causa e efeito.

Para investigar possíveis efeitos da temperatura da água sobre o resultado da interação entre predador e presa (Artigos I e II), aquários foram inseridos dentro de câmaras térmicas, tornando possível controlar a temperatura do ar, e, por conseguinte também a temperatura da água. Os valores escolhidos entre 19 e 27°C encontram-se no intervalo natural de temperatura observado em ecossistemas aquáticos tropicais e subtropicais (p. ex. Teixeira-de Mello *et al.*, 2009; Gelós *et al.*, 2010). A visibilidade

subaquática foi manipulada nos estudos **I** e **III**, considerou-se as variações na visibilidade subaquática como uma consequência da presença/ausência de partícula sólidas em suspensão na coluna de água. Portanto, testou-se dois extremos: um tratamento de total visibilidade subaquática, com água transparente ( $\approx 3$  NTU), e um tratamento de visibilidade subaquática reduzida. Essa redução se deu pela adição de argila a água até obtenção de 100 NTU. A argila utilizada foi coletada no rio Paraná, seca ao sol e macerada antes de ser introduzida no aquário. O estudo **II** teve como objetivo simular cenários ainda mais contrastantes de visibilidade subaquática, por esse motivo, os tratamentos foram controlados com a disponibilidade e indisponibilidade de luz, gerando, respectivamente, os tratamentos de visibilidade subaquática total e visibilidade subaquática nula.

Para determinar a influência da simples presença de um predador de topo sobre (i) a eficiência de captura de presa e a partilha de alimento entre membros do cardume (Artigo **III**), (ii) sobrevivência de invertebrados (Artigo **IV**), (iii) preferência de *habitat* vegetados específicos (Artigo **V**), um peixe piscívoro alimentado foi introduzido aos aquários. Vale ressaltar que não houve consumo de peixes-presa, durante nenhuma das experimentações que utilizaram predador de topo.

### **3 RESULTADOS E DISCUSSÃO**

#### **3.1 O AMBIENTE EM MUDANÇA E A HABILIDADE DE CAPTURAR PRESAS**

Compreender o papel de distúrbios para as interações ecológicas é extremamente oportuno, principalmente em face do atual cenário de mudanças ambientais, aceleradas pelo homem. Para os organismos aquáticos, variações na visibilidade subaquática podem ser tão letais quanto o ataque de um predador. Por exemplo, é sabido que em ambientes túrbidos *Esox lucius* se alimenta menos (Engström-Öst *et al.*, 2006) e que isso resulta em menor crescimento (Engström-Öst & Mattila, 2008; Salonen *et al.*, 2009). Para algumas presas, turbidez pode atuar como um “refúgio visual” contra predadores (Pekcan-Hekim & Lappalainen, 2006), para outras, ela pode causar ‘um efeito de falsa segurança’ (Lehtiniemi *et al.*, 2005), em que presas reduzem o comportamento de vigilância, e tornam-se fácil refeição para predadores. O resultado do estudo **I** revela menor eficiência de captura de presas (em média 20%) por um peixe ciclídeo em água turbida (100 NTU), que em água transparente (3 NTU). Nos mesmos

níveis de turbidez, a eficiência de captura de presas de um cardume de peixes caracídeos foi levemente, mas não significativamente, menor (em média 3%) em água turbida que em água transparente (Artigo **III**). Em alta turbidez, predadores tornam-se incapazes de identificar a verdadeira posição de sua presa, reduzindo a distância de ataque, e até mesmo a taxa de encontro entre predadores e presa (Ranåker *et al.*, 2012). Porém, presas podem compensar a redução da visibilidade subaquática (Aksnes & Utne, 1997), aumentando a área percorrida para encontrar mais presas (Webster *et al.*, 2007; Wishingrad *et al.*, 2015), o que pode explicar a supracitada ausência de diferença estatística no estudo **III**, a qual pode ainda estar associada a presença de um cardume de peixes como predadores, o que aumenta a probabilidade de encontro entre peixe e invertebrados.

Tanto o estudo **I** quanto o estudo **III** realçam aspectos da plasticidade fenotípica dentro da população de peixes como crucial para determinar o tamanho do efeito das variações na transparência da água para o sucesso na captura de presas. No estudo **I**, observou-se que peixes com diferentes tamanhos corporais consomem quantidades similares de presas em águas com alta transparência, entretanto em águas túrbidas, peixes maiores foram mais eficientes na captura de presas do que peixes menores. Vale ressaltar que os peixes possuíam um tamanho corporal muito semelhante (entre 5,8 e 8,7 cm, valores médios de  $6,9 \pm 0,6$  cm). A análise dos resultados do estudo **III** mostra que os membros de cardumes partilham homoganeamente o alimento sob condição de água transparente. Entretanto, em ambientes túrbidos, apenas alguns indivíduos do cardume são capazes de consumir a presa, aumentando a dissimilaridade na proporção de alimento ingerido entre os membros do cardume. É evidente que em condições de reduzida visibilidade subaquática, indivíduos necessitam utilizar estímulos não-visuais (p. ex. de origem química ou mecânica) para identificar a real posição de sua presa (Åbjörsson *et al.*, 2002). Portanto, se apenas alguns indivíduos são capazes de ingerir alimento em uma dada situação, então esses indivíduos devem possuir uma maior capacidade de identificar sinais não-visuais (Chapman *et al.*, 2010). Os resultados do estudo **I** demonstram que peixes de maior tamanho corporal estão mais adaptados a ingerirem alimento em ambiente turbido. Porém, possuir tamanho corporal pode tanto ser uma causa da maior eficiência de captura de presas, quanto pode ser uma consequência da existência de caracteres melhor adaptados a condições de baixa visibilidade.

Embora seja predito que o aumento na temperatura média da água aumenta a demanda energética dos organismos ectotérmicos, resultando em um aumento no número de eventos relacionados à busca e à captura de alimento (Brown *et al.*, 2004; Pink & Abrahams, 2016), no estudo **I**, não se observou diferenças estatísticas na eficiência de captura de presas por um peixe ciclídeo em águas mais quente (foram comparadas quatro temperaturas da água: 19,2, 22,2, 25,2 e 27°C). Apesar disso, se for considerado que as presas escaparam em maior número no tratamento com água mais quente (ver seção seguinte), e, portanto, havia um menor número de presas disponíveis, então é simples propor que a ausência de diferença na eficiência de captura de presa em água a 27°C e em água a 19,2°C se deve a melhor eficiência de captura de presa em água mais quente, pois o ciclídeo manteve os níveis de predação estáveis, mesmo quando anfípodes-presa tiveram a capacidade de fuga aumentada. Esse resultado ilustra que o encontro entre predadores e presas pode depender de uma complexa interação de capacidade fisiológica e comportamental de ambos os organismos envolvidos (Öhlund *et al.*, 2015).

### 3.2 O AMBIENTE EM MUDANÇA E A CAPACIDADE DE EVITAR SER CONSUMIDO

Para reduzir os riscos de serem consumidos, animais geralmente adotam estratégias de anti-predação, por exemplo, eles podem escapar (Lima & Dill, 1990; Smith 200), se esconder (Padial *et al.* 2009; Figueiredo *et al.*, 2015) ou formar grupos (Jolles *et al.*, 2015). Tais estratégias reduzem os investimentos dos organismos em outros comportamentos como forrageamento e reprodução (Helfman, 1989), e possuem, portanto, custos elevados. Como predação é temporalmente e espacialmente muito flutuante, a plasticidade fenotípica que permite indivíduos alterarem o fenótipo de acordo com o risco de predação vigente é amplamente difundido na natureza, (Hoverman *et al.*, 2005; Meuthen *et al.*, 2016) e pode determinar a sobrevivência de um organismo. Contudo, os investimentos em estratégias de anti-predação dependem, fundamentalmente, da percepção do risco de predação, postulado como “hipótese da sensibilidade ao risco” por Helfman (1989). Nesse sentido, é simples associar variações na visibilidade subaquática à sobrevivência de presas, pelo simples mecanismo, de que quanto menor a visibilidade, menor o risco de predação percebido, e menos frequente é a resposta de anti-predação.

Os resultados dos estudos **I** e **III** suportam a hipótese de que mesmo que invertebrados tenham uma menor percepção do risco de predação e/ou reduzido



comportamento de predação em ambientes túrbidos, a eficiência da captura de presas por peixes invertívoros também é reduzida nessas condições, o que eleva a sobrevivência de invertebrados, comparando com a sobrevivência em água transparente. Os resultados do estudo **III** indicam ainda que quando peixes invertívoros coocorrem com seu próprio predador em um ambiente de baixa visibilidade subaquática, a sobrevivência dos invertebrados é aumentada em média 77%, comparando com um ambiente de total visibilidade subaquática e predadores de topo ausentes. Isto se deve porque peixes têm habilidade de reconhecer a presença de um predador por intermédio da sensibilidade química (Brown & Magnavacca, 2003), mas uma condição de baixa visibilidade torna improvável a determinação precisa posição do predador de topo no ambiente, o que leva mesopredadores a reduzir a sua atividade de busca por alimento e aumentar o comportamento de vigilância (Leahy *et al.*, 2011).

No estudo **II** investigou-se especificamente o comportamento de anfípodes para um ambiente no qual predação, visibilidade subaquática e temperatura flutuam (seja temporalmente ou espacialmente). Os resultados desse estudo também corroboraram a hipótese da sensibilidade ao risco, uma vez que anfípodes só foram capazes de responder a presença de predadores quando a visibilidade subaquática foi total, isto é, o comportamento de anti-predação de *H. curvispina* é desencadeado por estímulos visuais. Os resultados dos estudos **I** e **II** demonstram que o incremento na temperatura da água, tal como predito para ocorrer em todos os ecossistemas, pode intensificar o comportamento de anti-predação, mas isso não implica em significativa elevação, nem redução do número de anfípodes consumidos por peixes (resultado estudo **I**).

A presença de plantas aquáticas pode desempenhar papel fundamental para a sobrevivência de presas em ambientes aquáticos sujeitos a variações periódicas na condição ambiental (Orrock *et al.*, 2013). As plantas aquáticas limitam a movimentação e reduzem o campo visual do predador (Diehl 1988; Christensen & Persson, 1993). Os resultados do estudo **V** suportam as hipóteses de que, (i) peixes-presa utilizam preferencialmente *habitat* vegetados a *habitat* abertos, mesmo em condições experimentais e sem predadores, (ii) a presença de sinais químicos da presença de um predador eleva o número de peixes em *habitat* vegetados, e (iii) a real presença de um predador induz, praticamente todos os organismos, a ocuparem os *habitat* vegetados para se protegerem. Apesar disso, em todas as três condições de ameaça de predação, peixes-presa utilizaram *habitat* vegetados por plantas aquáticas nativas e exóticas indistintamente. Portanto, a escolha do *habitat* deve estar relacionada com a

complexidade estrutural do ambiente, e não é uma seleção baseada na história de vida entre o peixe e planta nativa. Nessa perspectiva, o estabelecimento de uma espécie de macrófita invasora pode ser “benéfico” para populações de peixes de pequeno porte, especialmente em áreas onde a presença de *habitat* complexos é limitada ou inexistente (Cunha *et al.*, 2011; Figueiredo *et al.*, 2015).

### 3.3 O AMBIENTE EM MUDANÇA E O EQUILÍBRIO ENTRE CONSUMIR E SER CONSUMIDO

Em geral, a predação é tida como processo importante para a comunidade ecológica na medida em que resulta na morte da presa e na remoção dela do sistema (Carpenter & Kitchell, 1993). Os aspectos dramáticos da letalidade da predação podem obscurecer os efeitos não letais provenientes da mera presença de predadores em um sistema ecológico (Lima, 1998). Isso é realmente importante quando considerado que (i) todos os animais são predadores (no sentido mais amplo) e potenciais presas para outros predadores, e (ii) um mesmo comportamento que torna um animal um eficiente predador (ou forrageador) geralmente aumenta o risco dele de ser a refeição de outro predador (Lima, 1998). A implicação dessa conjectura é que a seleção natural deve favorecer animais que balanceiam de forma mais apropriada o benefício da aquisição de energia e os custos de serem prematuramente mortos por predação. Os resultados dos estudos **III** e **IV** demonstram que a presença de um predador de topo pode ser tão impactante para o consumo de presas por mesopredadores quanto variações abióticas, embora tais efeitos possam sinergicamente reduzir sobremaneira o potencial predatório da espécie mesopredadora. Sinergismo entre presença de um predador de topo e a condição ambiental foi observada no estudo **III**, que apontou uma redução de 20% na eficiência na captura de presas por um cardume de peixes caracídeos sujeitos a predação por um piscívoro. Mas quando piscívoros estavam presentes e a visibilidade subaquática era reduzida, peixe-presa consumiram 77% menos invertebrados, o que pode estar associado ao aumento do comportamento de vigilância, uma vez que era improvável determinar a localização dos invertebrados e dos piscívoros (Lehtiniemi *et al.*, 2005; Leahy *et al.*, 2011).

O resultado estudo **IV** demonstra que predadores nativos e invasores reduzem proporcionalmente a quantidade total de presas ingeridas. Entretanto, a presença de piscívoros invasores leva peixes-presa a consumirem indistintamente presas pelágicas e bentônicas, enquanto em situação condições sem piscívoros, e na presença de

predadores nativos mesopredadores selecionaram significativamente presas bentônicas. Portanto, a introdução de um piscívoro tem a capacidade de mudar a fonte de recurso alimentar de mesopredadores, e pode ter implicações, inclusive, para a importância relativa dos compartimentos pelágicos e bentônicos para o fluxo de energia dentro do ecossistema (Schindler & Scheuerell 2002). Aliado a origem do predador de topo, o uso do *habitat* e o modo de caça desse predador pode também governar o padrão de seleção de presas por mesopredadores, pois quando predadores ocupam um dado *habitat*, eles criam uma pequena área de segurança, que é evitada por mesopredadores, gerando um refúgio para invertebrados que coabitam o micro-*habitat* com piscívoros. Quando é possível observar a posição dos predadores de topo, mesopredadores conseguem alterar o tipo de alimento selecionado, ao invés de diminuir a eficiência na captura de presas (Stuart-Smith *et al.*, 2007; Figueiredo *et al.*, 2015).

#### **4 CONCLUSÕES E PERSPECTIVAS FUTURAS**

Encontrou-se nos estudos que compõem esta tese grandes variações na habilidade de captura de presa, e no comportamento de fuga de predadores associadas a variações na visibilidade subaquática, na temperatura da água, e ao estabelecimento de espécies exóticas de piscívoros e de plantas. O que realça a dependência de tais características de predadores e presas às condições ambientais vigentes. Isso é ainda mais importante, quando as projeções estimam que os ambientes aquáticos serão mais turbidos, mais quentes (ou que esses cenários serão mais duradouros que em outrora) e que algumas espécies terão a capacidade de ampliar sua distribuição geográfica, aumentando a frequência de invasões biológicas nos ecossistemas aquáticos. Nessa perspectiva, organismos aquáticos precisam rapidamente alterar seu comportamento de acordo a condição ambiental circundante. Nesse cenário, a plasticidade fenotípica das populações poderia prover maior probabilidade de sucesso adaptativo em um ambiente em mudança (Hendry *et al.*, 2008).

Estudos recentes demonstram que organismos responderão ao aumento da temperatura, acelerando o metabolismo e o desenvolvimento de estágios larvais, o que tem como consequência redução no tamanho corpóreo dos animais (Sheridan & Bickford, 2011; Bestion *et al.*, 2015). Baseando-se nos resultados das experimentações dos estudos **I** e **II**, pode-se sugerir que se (i) os ambientes aquáticos, de fato, tornarem-se mais frequentemente turbidos, tal como predito por diversos estudos (Moss *et al.*,

2011; IPCC 2012), e (ii) os organismos-predadores possuem menor tamanho corporal, então (iii) a sobrevivência de pequenos indivíduos (como invertebrados aquáticos) aumentará. Isto porque predadores de menor tamanho corpóreo apresentaram menor taxa de consumo sob condições de limitada visibilidade subaquática. Como tal conclusão deriva da ocorrência de muitos acontecimentos, é importante ressaltar que se trata apenas de uma hipótese que ainda precisa ser testada por pesquisas futuras, e mais ainda que na natureza outros fatores interajam com as habilidades de predadores e presas. Por exemplo, essas preditas novas condições ambientais podem facilitar o aumento populacional de um peixe-predador com sofisticadas estratégias de caça, de tal modo que o resultado da interação entre peixe e invertebrado seja a redução da biomassa de invertebrados e não o aumento dela como sugerido pelo raciocínio simplificado da hipótese sugerida.

Os resultados também indicam que o estabelecimento de uma espécie de macrófita aquática invasora poderia ser benéfico para peixes de pequeno porte. Isto porque o estabelecimento da planta exótica pode aumentar a cobertura vegetal total do ecossistema, a qual geralmente dificulta a movimentação de predadores, facilitando as manobras evasivas de presas em uma perseguição (Abrahams *et al.*, 2009). O aumento da cobertura vegetal diminui ainda a capacidade de detecção de peixes-presa por piscívoros, pois a presa utiliza a vegetação para se esconder (Diehl, 1988). Portanto, poder-se-ia propor a adição de plantas aquáticas artificiais como medida de manejo, para a redução da pressão de predação sobre invertebrados aquáticos (tais como insetos, anfípodas e microcrustáceos), o que tem potencial para aumentar a abundância desses grupos animais, que em última análise, também beneficiaria a conservação das espécies de peixe de pequeno porte. Contudo, realça-se que nesta tese foi observado apenas o uso do *habitat* e seus potenciais benefícios para a sobrevivência de presas, e que podem existir efeitos negativos indiretos do estabelecimento de macrófitas aquáticas exóticas sobre outros aspectos vitais para a sobrevivência de presas, tais como crescimento, táticas reprodutivas e eficiência alimentar. Estas considerações são potencialmente importantes no desenvolvimento de abordagens em investigações futuras que tratem da complexa interação entre duas ou mais espécies exóticas.

## REFERÊNCIAS

- Åbjörnsson, K., C. Brönmark & L. A. Hansson, 2002. The relative importance of lethal and non-lethal effects of fish on insect colonisation of ponds. *Freshwater Biology* 47: 1489-1495.
- Abrahams, M. V., M. Mangel & K. Hedges, 2007. Predator-prey interactions and changing environments: who benefits? *Philosophical transactions of the Royal Society B* 362:2095-2104.
- Abrahams, M. V., M. Pink & C. Klassen, 2009. Predator avoidance. In *Encyclopedia of life sciences (ELS)*. John Wiley & Sons, Ltd: Chichester.
- Agostinho, A. A., S. M. Thomaz, L. C. Gomes & S. L. S. M. A. Baltar, 2007. Influence of the macrophytes *Eichhornia azurea* on fish assemblage of the upper Paraná River floodplain (Brazil). *Aquatic Ecology* 41:611–619.
- Alegretti, L., G. L. Ferreira, B. A. Burstin & M. N. Flynn. 2016. Composição específica e índices estruturais da ictiofauna coletada nos lagos da Estação Experimental da Syngenta no município de Holambra-SP. *Revista Intertox de Toxicologia Risco Ambiental e Sociedade*, 9:150-171.
- Aksnes, D. L. & A. C. W. Utne, 1997. A revised model of visual range in fish. *Sarsia* 82:137–147.
- Auer, M. T., M. L. Storey, S. W. Effler, N. A. Auer & P. Sze, 1990. Zooplankton impacts on chlorophyll and transparency in Onondaga Lake, New York, USA. *Hydrobiologia* 200/201:603–617.
- Beauchamp, G., 2003. Group-size effects on vigilance: a search for mechanisms. *Behavioural Processes* 63:111–121.
- Bestion, E., S. Teyssier, M. Richard, J. Clobert & J. Cote, 2015. Live fast, die young: experimental evidence of population extinction risk due to climate change. *Plos Biology* 13:e1002281.
- Bohren, C. F. & D. R. Huffman, 1983. Absorption and scattering of light by small particles. John Wiley & Sons, New York.
- Brown, G. E. & G. Magnavacca, 2003. Predator inspection behaviour in a characin fish: an interaction between chemical and visual information? *Ethology* 109:739–750.
- Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage & G. B. West, 2004. Toward a metabolic theory of ecology. *Ecology* 85:1771–1789.
- Brown, J. S. & B. P. Kotler, 2004. Hazardous duty pay and the foraging cost of predation. *Ecology Letters* 7:999–1014.
- Carpenter, S. R., J. J. Cole, J. R. Hodgson, J. F. Kitchell, M. L. Pace, D. Bade, K. L. Cottingham, T. E. Essington, J. N. Houser & D. E. Schindler, 2001. Trophic

- cascades, nutrients, and lake productivity: whole-lake experiments. *Ecological Monography* 71:163–186.
- Casatti, L. et al. 2003. Aquatic macrophytes as feeding site for small fishes in the Rosana reservoir, Paranapanema river, southeastern Brazil. *Brazilian Journal of Biology* 63: 213–222.
- Chapman B. B., L. J. Morrell, C. R. Tosh & J. Krause, 2010. Behavioural consequences of sensory plasticity in guppies. *Proceedings of the Royal Society B: Biological Sciences* 277:1395–1401.
- Christensen, B. & L. Persson, 1993. Species-specific antipredatory behaviours: effects on prey choice in different habitats. *Behavioral Ecology and Sociobiology* 32:1-9.
- Clarke, A. & N. M. Johnston, 1999. Scaling of metabolic rate with body mass and temperature in teleost fish. *Journal of Animal Ecology* 68:893–905.
- Como, S., C. Lefrancois, E. Maggi, F. Antognarelli & C. Dupuy, 2014. Behavioral responses of juvenile golden gray mullet *Liza aurata* to changes in coastal temperatures and consequences for benthic food resources. *Journal of Sea Research*, 92:66–73.
- Cox, J. G. & S. L. Lima, 2006. Naiveté and an aquatic-terrestrial dichotomy in the effects of introduced predators. *Trends Ecology Evolution* 21:674–80.
- Cresswell, W., 1994. Flocking is an effective anti-predation strategy in redshanks, *Tringa tetanus*. *Animal Behaviour* 47:433–442.
- Cunha, E. R., S. M. Thomaz, H. B. A. Evangelista, C. Carniato, C. F. Souza & R. Fugi, 2011. Small-sized fish assemblages do not differ between a native and a recently established non-indigenous macrophyte in a Neotropical ecosystem. *Natureza & Conservação* 9: 61–66.
- Darwin, C. R., 1859. *On the origin of species by means of natural selection*. Murray. London.
- Davies-Colley R. J., W. N. Vant & D. G. Smith, 1993. *Colour and clarity of natural waters: science and management of optical water quality*. The Blackburn Press, New Jersey.
- Dawkins, R. & J. R. Krebs, 1979. Arms races between and within species. *Proceedings of the Royal Society B: Biological Sciences* 205:489–511.
- Diehl, S., 1988. Foraging efficiency of three freshwater fishes: effects of structural complexity and light. *Oikos* 53:207–214.
- Dill, L. M. & A. G. H. Fraser, 1984. Risk of predation and the feeding behavior of juvenile coho salmon (*Oncorhynchus kisutch*). *Behavioral Ecology and Sociobiology* 16: 65–71.

- Doyle, S. R. & F. R. Momo, 2009. Effects of body weight and temperature on the metabolic rate of *Hyalella curvispina* Shoemaker, 1942 (Amphipoda). *Crustaceana* 82:1423–1439.
- Engström-Öst, J., M. Karjalainen & M. Viitasalo, 2006. Feeding and refuge use by small fish in the presence of cyanobacteria blooms. *Environmental Biology of Fishes* 76:109–117.
- Engström-Öst, J. & J. Mattila, 2008. Foraging, growth and habitat choice in turbid water: an experimental study with fish larvae in the Baltic Sea. *Marine Ecology Progress Series* 359:275–281.
- Espínola, L. A., C. V. Minte-Vera & H. F. Júlio, 2010. Invasibility of reservoirs in the Paraná Basin, Brazil, to *Cichla kelberi* Kullander and Ferreira, 2006. *Biological Invasions* 12:1873–1888.
- Figueiredo, B. R. S., R. P. Mormul & E. Benedito. 2015. Structural complexity and turbidity do not interact to influence predation rate and prey selectivity by a small visually feeding fish. *Marine and Freshwater Research* 66:170–176.
- Gelós, M., F. Teixeira-de Mello, G. Goyenola, C. Iglesias, C. Fosalba, F. García-Rodríguez, J. P. Pacheco, S. García & M. Meerhoff, 2010. Seasonal and diel changes in fish activity and potential cascading effects in subtropical shallow lakes with different water transparency. *Hydrobiologia* 646:173–185.
- Graça, W. J. & C. S. Pavanelli, 2007. Peixes da planície de inundação do alto rio Paraná e áreas adjacentes. Eduem: Maringá.
- Godin, J. G. J. & S. L. Crossman, 1994. Hunger-dependent predator inspection and foraging behaviours in the threespine stickleback (*Gasterosteus aculeatus*) under predation risk. *Behavioral Ecology and Sociobiology* 34:359–366.
- Goodman, B. A., 2009. Nowhere to run: the role of habitat openness and refuge use in defining patterns of morphological and performance evolution in tropical lizards. *Journal of Evolutionary Biology* 22:1535–1544.
- Guthrie, D. M. & W. R. A. Muntz, 1993. Role of vision in behaviour. In Pitcher T. J. (ed) *Behaviour of teleost Fishes*, 2nd ed. Chapman and Hall, London, pp 89–128.
- Helfman, G. S., 1989. Threat-sensitive predator avoidance in damselfish-trumpetfish interactions. *Behavioral Ecology and Sociobiology* 24:47–58.
- Hendry, A. P., T. J. Farrugia & M. T. Kinnison, 2008. Human influences on rates of phenotypic change in wild populations. *Molecular Ecology* 17:20–29.
- Higham, T. E., W. J. Stewart & P. C. Wainwright, 2015. Turbulence, temperature, and turbidity: the ecomechanics of predator–prey interactions in fishes. *Integrative and Comparative Biology* 55:6–20.

- Horppila, J. & L. Nurminen, 2005. Effects of different macrophyte growth forms on sediment and P resuspension in a shallow lake. *Hydrobiologia* 545:167–175.
- Hoverman, J. T., J. R. Auld & R. A. Relyea, 2005. Putting prey back together again: integrating predator-induced behavior, morphology, and life history. *Oecologia* 144:481–491.
- IPCC (2007) *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge.
- IPCC (2012) *IPCC special report: managing the risks of extreme events and disasters to advance climate change adaptation*. Cambridge University Press, Cambridge.
- Johnson, B. L., W. B. Richardson & T. J. Naimo, 1995. Past, present, and future concepts in large river ecology. *BioScience* 45:134–141.
- Jolles, J. W., A. Fleetwood-Wilson, S. Nakayama, M. C. Stumpe, R. A. Johnstone & A. Manica, 2015. The role of social attraction and its link with boldness in the collective movements of three-spined sticklebacks. *Animal Behaviour* 99:147–153.
- Krause J. & G. D. Ruxton, 2002. *Living in Groups*. Oxford University Press, Oxford.
- Krik, J. T. O., 1985. Effects of suspensoids (turbidity) on penetration of solar radiation in aquatic ecosystems. *Hydrobiologia* 125:195–208.
- Leahy, S. M., M. I. McCormick, M. D. Mitchell & M. C. O. Ferrari, 2011. To fear or to feed: the effects of turbidity on perception of risk by a marine fish. *Biology Letters* 7:811–813.
- Lehtiniemi, M., J. Engström-Öst & M. Viitasalo, 2005. Turbidity decreases anti-predator behaviour in pike larvae, *Esox lucius*. *Environmental Biology of Fishes* 73:1–8.
- Lima, S. L. & L. M. Dill, 1990. Behavioural decisions under the risk of predation: a review and prospectus. *Canadian Journal of Zoology* 68:619–640.
- Lima, S. L., 1998. Nonlethal effects in the ecology of predator-prey interactions. *Bioscience* 48:25–34.
- Magurran, A. E., 1993. Individual differences and alternative behaviors. In Pitcher, J. T. (ed), *Behaviour of teleost fishes*. London: Chapman & Hall, p. 440–477.
- Marengo, J. A., S. C. Chou, G. Kay, L. M. Alves, J. F. Pesquero, W.R. Soares, D. C. Santos, A. A. Lyra, G. Sueiro, R. Betts, D. J. Chagas, J. L. Gomes, J. F. Bustamante & P. Tavares, 2012. Development of regional future climate change scenarios in South America using the Eta CPTEC/HadCM3 climate change projections: climatology and regional analysis for the Amazon, São Francisco and the Paraná River basins. *Climate Dynamics* 38:1829–1848.



- Mathis, A. & R. J. F. Smith, 1993. Chemical alarm signals increase the survival time of fathead minnows (*Pimephales promelas*) during encounters with northern pike, *Esox lucius*. *Behavioral Ecology* 4:260–265.
- Mesquita, F. O. & R. J. Young, 2007. The behavioural responses of Nile tilapia (*Oreochromis niloticus*) to anti-predator training. *Applied Animal Behaviour Science* 106:144–154.
- Meuthen, D., S. A. Baldauf, T. C. Bakker & T. Thünken, 2016. Predator-induced neophobia in juvenile cichlids. *Oecologia* 181:947–958.
- Mormul, R. P., S. M. Thomaz, A. A. Agostinho, C. C. Bonecker & N. Mazzeo, 2012. Migratory benthic fishes may induce regime shifts in a tropical floodplain pond. *Freshwater Biology* 57:1592–1602.
- Moss, B., S. Kosten, M. Meerhoff, R. W. Battarbee, E. Jeppesen, N. Mazzeo, K. Havens, G. Lacerot, Z. Liu, L. De Meester, H. Paerl & M. Scheffer, 2011. Allied attack: climate change and eutrophication. *Inland Waters* 1:101–105.
- Nowicki, J. P., G. M. Miller & P. L. Munday, 2012. Interactive effects of elevated temperature and CO<sup>2</sup> on foraging behavior of juvenile coral reef fish. *Journal of Experimental Marine Biology and Ecology* 412:46–51.
- Öhlund, G., P. Hedström, S. Norman, C. L. Hein & G. Englund, 2015. Temperature dependence of predation depends on the relative performance of predators and prey. *Proceedings of the Royal Society B: Biological Sciences* 282:20142254.
- Orrock, J. L., E. L. Preisser, J. H. Grabowski & G. C. Trussell, 2013. The cost of safety: Refuges increase the impact of predation risk in aquatic systems. *Ecology* 94:573–579.
- Padial, A. A., S. M. Thomaz & A. A. Agostinho, 2009. Effects of structural heterogeneity provided by the floating macrophyte *Eichhornia azurea* on the predation efficiency and habitat use of the small Neotropical fish *Moenkhausia sanctaefilomena*. *Hydrobiologia* 624:161–170.
- Peacor, S. D., B. L. Peckarsky, G. C. Trussell & J. R. Vonesh, 2013. Costs of predator-induced phenotypic plasticity: a graphical model for predicting the contribution of nonconsumptive and consumptive effects of predators on prey. *Oecologia* 171:1–10.
- Pekcan-Hekim, Z. & J. Lappalainen, 2006. Effects of clay turbidity and density of pikeperch (*Sander lucioperca*) larvae on predation by perch (*Perca fluviatilis*). *Naturwissenschaften* 93:356–359.
- Pelicice, F. M. & A. A. Agostinho, 2009. Fish fauna destruction after the introduction of a non-native predator (*Cichla kelberi*) in a Neotropical reservoir. *Biological Invasions* 11:1789–1801.

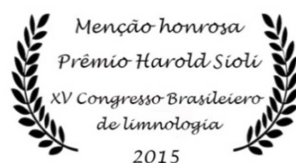
- Peretti, D. and Andrian, I. F. 2008. Feeding and morphological analysis of the digestive tract of four species of fish (*Astyanax altiparanae*, *Parauchenipterus galeatus*, *Serrasalmus marginatus* and *Hoplias* aff. *malabaricus*) from the upper Paraná River floodplain, Brazil. *Brazilian Journal of Biology* 68: 671–679.
- Pink, M. & M. V. Abrahams, 2016. Temperature and its impact on predation risk within aquatic ecosystems. *Canadian Journal of Fisheries and Aquatic Sciences* 73:1–8.
- Pulliam, H. R., 1973. On the advantages of flocking. *Journal of Theoretical Biology* 38: 419–422.
- Quinn, J. L. & W. Cresswell, 2006. Testing domains of danger in the selfish herd: sparrowhawks target widely spaced redshanks in flocks. *Proceedings of the Royal Society B: Biological Sciences* 273:2521–2526.
- Ranåker, L., M. Jönsson, P. A. Nilsson & C. Brönmark, 2012. Effects of brown and turbid water on piscivore-prey fish interactions along a visibility gradient. *Freshwater Biology* 57:1761–1768.
- Roberto, M. C., N. F. Santana & S. M. Thomaz, 2009. Limnology in the Upper Paraná River floodplain: large-scale spatial and temporal patterns, and the influence of reservoirs. *Brazilian Journal of Biology* 69:717-725.
- Robinson, E. M., D. L. Smee & G. C. Trussell, 2011. Green crab (*Carcinus maenas*) foraging efficiency reduced by fast flows. *PLoS ONE* 6:1–8.
- Salonen, M., L. Urho & J. Engström-Öst, 2009. Effects of turbidity and zooplankton availability on the condition and prey selection of pike larvae. *Boreal Environment Research* 14:981–989.
- Schindler, D. M. & M. Scheuerell, 2002. Habitat coupling in lake ecosystems. *Oikos* 98: 177–189.
- Sheridan, J. A. & D. Bickford, 2011. Shrinking body size as an ecological response to climate change. *Nature Climate Change* 1:401–406.
- Sih, A., D. I. Bolnick, B. Luttbeg, J. L. Orrock, S. D. Peacor, L. M. Pintor, E. Preisser, J. S. Rehage & J. R. Vonesh, 2010. Predator-prey naïveté, antipredatory behavior, and the ecology of predator invasions. *Oikos* 119:610–621.
- Smee, D. L., M. C. Ferner & M. J. Weissburg, 2010. Hydrodynamic sensory stressors produce nonlinear predation patterns. *Ecology* 91:1391–1400.
- Smith, M. E., 2000. Alarm response of *Arius felis* to chemical stimuli from injured conspecifics. *Journal of Chemical Ecology* 26:1635–1647.
- Smith, L. A. & M. M. Webster, 2015. *Gammarus pulex* show a grouping response to conspecific injury cues but not to predator kairomones. *Behavioral Ecology* 26:1188-1195.

- Sneddon, L. U., V. A. Braithwaite & M. J. Gentle, 2003. Novel Object Test: Examining Nociception and Fear in the Rainbow Trout. *The Journal of Pain* 4:431–440.
- Sousa, W. T. Z., 2011. *Hydrilla verticillata* (Hydrocharitaceae), a recent invader threatening Brazil's freshwater environments: a review of the extent of the problem. *Hydrobiologia* 669:1–20.
- Stankowich, T. & D. T. Blumstein, 2005. Fear in animals: a meta-analysis and review of risk assessment. *Proceedings of the Royal Society B: Biological Sciences* 272:2627-2634.
- Stief, P. & F. Holker, 2006. Trait-mediated indirect effects of predatory fish on microbial mineralization in aquatic sediments. *Ecology* 87:3152–3159.
- Stuart-Smith, R. D., J. F. Stuart-Smith, R. W. G. White & L. A. Barmuta, 2007. The effects of turbidity and complex habitats on the feeding of a galaxiid fish are clear and simple. *Marine and Freshwater Research* 58:429–435.
- Teixeira-de Mello, F., M. Meerhoff, Z. Pekcan-Hekim & E. Jeppesen, 2009. Substantial differences in littoral fish community structure and dynamics in subtropical and temperate shallow lakes. *Freshwater Biology* 54:1202–1215.
- Thomaz, S. M. & E. R. Cunha, 2010. O papel das macrófitas na estruturação de habitat em ambientes aquáticos: métodos de medida, causas e consequências para a composição das assembléias animais e biodiversidade. *Acta Limnologica Brasiliensia* 22: 218-236.
- van Kleunen, M., E. Weber & M. Fischer, 2010. A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecology Letters* 13:235–245.
- Vilà, M. & J. Weiner, 2004. Are invasive plant species better competitors than native plant species? - evidence from pair-wise experiments. *Oikos* 105:229–238
- Ward, A. J. W., P. Thomas, P. J. B. Hart & J. Krause, 2004. Correlates of boldness in three-spined sticklebacks (*Gasterosteus aculeatus*). *Behavioral Ecology and Sociobiology* 55:561–568.
- Webster M. M., N. Atton, A. J. W. Ward & P. J. B. Hart, 2007. Turbidity and foraging rate in threespine sticklebacks: the importance of visual and chemical prey cues. *Behaviour* 144:1347-1360.
- West-Eberhard, M. J., 1989. Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics* 20:249-278.
- Winder, M. & D. E. Schindler, 2004. Climate change uncouples trophic interactions in an aquatic ecosystem. *Ecology* 85:2100-2106.
- Wishingrad V., A. B. Musgrove, D. P. Chivers & M. C. O. Ferrari, 2015. Risk in a changing world: environmental cues drive anti-predator behaviour in lake sturgeon (*Acipenser fulvescens*) in the absence of predators. *Behaviour* 152:635-652.

APÊNDICE A - Artigos científicos publicados ou em preparação para serem publicados  
como produto final da tese

- I** Figueiredo, Bruno R. S.; Calvo, Clementina; López-Rodríguez, Anahí; Mormul, Roger P.; Benedito, Evanilde; Teixeira-de-Melo, Franco & Meerhoff, Mariana. Turbidity pulses have stronger effects than heat waves on fish-invertebrate trophic interactions. Submetido a *Canadian Journal of Fisheries and Aquatic Sciences* (em Setembro de 2016).....Página 35
- II** Figueiredo, Bruno R. S.; Alves, Gustavo H. Z.; Calvo, Clementina; López-Rodríguez, Anahí; Benedito, Evanilde & Meerhoff, Mariana. Consequências de variações na visibilidade subaquática e na temperatura sobre o comportamento de anfípodas. *Unless I see the predator I will not flee: influence of light and heat on anti-predator responses of amphipods*. Em preparação, periódico alvo: *Invertebrate Biology*.....Página 54
- III** Figueiredo, Bruno R. S.; Mormul, Roger P.; Chapman, Ben B.; Lolis, Lucas A.; Fiori, Leandro F & Benedito, Evanilde (2016) Turbidity amplifies the non-lethal effects of predation and affects the foraging success of characid fish shoals. *Freshwater Biology*, **61**, 293–300. doi: 10.1111/fwb.12703.....Página 71
- IV** Figueiredo, Bruno R. S.; Fiori, Leandro F.; Mormul, Roger P. & Benedito, Evanilde. Non-lethal effects of an invasive piscivorous fish on invertebrates: the predator origin matters to trophic cascade. Submetido a *Hydrobiologia* (em Setembro de 2016) .....Página 80
- V** Figueiredo, Bruno R. S.; Mormul, Roger P.; & Thomaz, Sidinei M. 2015. Swimming and hiding regardless of the habitat: prey fish do not choose between a native and a non-native macrophyte species as a refuge. *Hydrobiologia*, **746**, 285–290. doi: 10.1007/s10750-014-2096-x.....Página 99

I



## **Turbidity pulses have stronger effects than heat waves on fish-invertebrate trophic interactions**

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Running Headline: **Predator-prey interactions in future climates**

*Abstract* - Multiple environmental scenarios are predicted for different regions in the future. The increase of both average temperature and frequency of extreme rainfall events are expected to lead to warmer waters and higher turbidity in aquatic ecosystems. Such environmental changes might, respectively, increase the metabolism of ectothermic organisms like fishes and reduce the visual field of visually-oriented organisms. Here, we tested the effects of these expected environmental scenarios on the foraging success of a cichlid fish (*Gymnogeophagus terrapurpura*) and the biological response of its amphipod prey (*Hyalella curvispina*). We carried out a short-term laboratory experiment combining two treatments of turbidity (clear and turbid water – 3 and 100 NTU, respectively) under four water temperature treatments (19.2, 22.2, 25.2 and 27° C). In general, we found that changes in turbidity, but not in water temperature, had direct consequences on the number of invertebrates consumed by fish. Moreover, bigger fish had better feeding efficiency than smaller individuals in turbid waters. Turbidity, besides affecting the foraging success of the predator, seems to enhance individual differences in determining consumption rates. In contrast, more amphipods

showed anti predator behaviour (left the water in aquarium) under warmer temperatures. Based on our findings, we hypothesise that the tendency of prey consumption to decline in warmer waters could be a consequence of a better ability of the prey to avoid predators, rather than lower predator feeding efficiency. Our short-term experiments suggest that, if turbid and warmer water become lasting or more frequent events, both invertebrates and fish traits, such as body size and behaviour, could, at least partly, compensate for the negative effects of these environmental changes.

**Keywords:** Global warming; Water temperature; Prey consumption; Anti-predator behaviour; Cichlid fish; Amphipoda.

## **Introduction**

Climate change scenarios and models are varied and imply different predictions in terms of direction and magnitude for different locations around the world. The increase of average temperature and also higher frequency of extreme temperature and rainfall events are expected in many regions (IPCC, 2007; 2012), and particularly in the subtropics (Marengo *et al.*, 2012; Roland *et al.*, 2012). Such environmental changes may affect several ecological relationships among organisms (Estes *et al.*, 2011). For example, rises in ambient temperature increase energetic demands and feeding activities, especially of ectothermic organisms such as fishes (Brown *et al.*, 2004; McKenzie & Claireaux, 2010). Thus, in warmer waters, fish would likely spend more time in activities related to feeding, potentially increasing the number of feeding events (Nowicki *et al.*, 2012; Como *et al.*, 2014) and the area searched in pursuit of prey (Biro, Beckmann & Stamps, 2010).

Extreme rainfall events usually increase surface runoff from the surrounding land, causing pulses of turbidity in freshwater ecosystems (Krik, 1985). Then, increasing frequency of extreme rainfall events can lead to more frequent high turbidity levels, which, depending on local conditions, may become a quasi-permanent environmental condition. Turbidity reduces the visual field of visually-oriented organisms (Aksnes & Utne, 1997), and therefore changes in water turbidity may affect different stages of the foraging cycle (i.e. encounter, attack, capture and ingestion) (Horppila & Nurminen, 2003; Ranåker *et al.*, 2012). Moreover, rises in inorganic turbidity level may also increase water temperature, since suspended particles are able to absorb heat (Bohren & Huffman, 1983). Therefore, as higher temperature may

promote feeding activity and turbidity may decrease the predator ability to find its prey, the interaction of these environmental conditions may lead to unexpected effects on different stages of the foraging cycle, such as prey encounter and ingestion.

The consequences of rises in water temperature and turbidity may be as strong for prey as for predator performance, with important consequences for biological interactions (e.g. Mori & Burghardt, 2001). For instance, an increase in water temperature can increase the metabolic rate of invertebrates such as amphipods, resulting in an increase in their locomotion (Issartel *et al.*, 2005; Doyle & Momo, 2009). Specialized predators would need to improve their mobility in equivalent intensity for their chance to find prey remain stable. Similarly, an increase in suspended sediment strongly reduces the risk perception by prey, leading to a reduction in anti-predator responses (Lehtiniemi, Engström-Öst & Viitasalo, 2005; Leahy *et al.*, 2011). Depending on the relative magnitude of the changes in predator efficiency and in predation risk perception, greater oscillations in predator or prey populations can be expected with sudden changes in water temperature and water turbidity.

Here, we experimentally tested the effects of changes in water temperature and turbidity on the foraging success of a subtropical cichlid fish (*Gymnogeophagus terrapurpure* Loureiro *et al.*, 2016) and the response of its amphipod prey (*Hyalella curvispina* Shoemaker, 1942). We hypothesized that the effects of turbidity on prey consumption depend on water temperature. Thus, we expected the highest fish feeding efficiency in warmer and clear water.

## Methods

### Experimental design

We carried out a microcosm experiment, in which we tested the effects of predicted rises in both water temperature and turbidity level on a fish-invertebrate trophic interaction. We used rectangular aquaria as experimental units (width = 25 cm; length = 30 cm; height = 50 cm; water volume = 30 l; water depth = 40 cm; all sides being covered with black tissue to avoid mutual influence of fish). In heating rooms, we manipulated four water temperature treatments (19.2, 22.2, 25.2 and 27 °C), combined with clear and turbid water conditions (3 and 100 NTU, respectively), in a fully crossed factorial design. Treatments were assigned at random to the aquaria units using seven replicates per treatment (all aquaria were located at the same height inside the heating room to prevent temperature variations).



Each aquarium received 20 individuals of *Hyaella curvispina* (Crustacea: Amphipoda) as prey, which were subjected to predation by one small cichlid fish, *Gymnogeophagus terrapurpura* (Perciformes: Cichlidae). This small cichlid is considered representative of common Neotropical fishes, given that the fish assemblage in this region is primarily composed of small-sized species and individuals (Meerhoff *et al.*, 2007; Teixeira-de Mello *et al.*, 2009a). We chose the visually-oriented *G. terrapurpura* because of its wide distribution throughout the drainage basin of the Uruguay River (in south-eastern South America), and because it is one of the most abundant fish in Uruguayan aquatic systems (e.g. Teixeira-de Mello *et al.*, 2009b). *Hyaella curvispina* was used as prey due to its wide distribution across freshwater habitats of South America (Doyle & Momo, 2009), where it often reaches very high densities (Giorgi, Feijoó & Tell, 2005). Furthermore, *H. curvispina* may act as herbivorous on the phytobenthos and also process the organic matter deposited among plant (acting as detritivorous) (Galassi, Franceschini & Neiff, 2006), playing an important role subtropical in aquatic food-webs.

Specimens of *H. curvispina* were caught from aquatic plant roots in near-shore areas in a sub-urban stream (34°88'67"S, 54°95'24"W) near the laboratory facilities. Sampled amphipods (Length = 4.1 ± 0.7 mm) were kept in 600 ml bottles (20 individuals per bottle) containing stream water. Specimens of *G. terrapurpura* were caught in summer in Diario lake (34°90'41"S, 55°00'82"W, water temperature ≈ 21 °C) using electrofishing. We searched for fish with similar body size (body standard length SL = 69.1 ± 6.6 mm, and total fresh weight, FW = 13.7 ± 4.2 g). Differences in individual body size did not occur neither in the different water temperature (1-way ANOVA  $F_{3,48} = 0.79$ ;  $P = 0.51$ ) nor in the turbidity treatments (1-way ANOVA  $F_{1,48} = 0.17$ ;  $P = 0.68$ ). Despite fish body size (as standard length) was homogenised as much as possible among treatments, variations within treatments provided a gradient of body size (as fresh biomass), which was considered as a co-variable in our analyses to test for any interference with response variables (see more details in data analysis section).

Fish individuals were acclimated (without feeding) to the experimental water temperatures through gradual increase/decrease of room temperature along 72 h before the beginning of the experiment. Fish were not acclimated to water turbidity since we wanted to test the response to a sudden increase in inorganic turbidity following a pulse event in a river. Amphipods were acclimated to the room temperature just 1 h before the beginning of the experiment.

To create the turbidity treatment, we collected and macerated sun-dried clay. Each experimental unit was filled with 20 l of dechlorinated water (temperature  $\approx 25$  °C; Turbidity  $\approx 3$  NTU), and one hour before the beginning of the experiment, each received either 10 l from a 1000- NTU turbid water solution or 10 l from clear water, in order to: (i) achieve the treatment with the desired turbidity (i.e. 100 NTU), and (ii) standardize the disturbance, respectively.

The experimental units were aerated, but the aeration system was removed before the beginning of the experiment to prevent amphipods from hiding behind the air pump tubes. The experimental time of 1 hour was chosen to keep the turbidity level as homogenous as possible (i.e. varying no more than 10%) from the beginning to the end of the experiment.

After 1 h of experiment, fish were removed and killed with an overdoses of Eugenol (4 ml L<sup>-1</sup> of a solution of 1:10 Eugenol: 95% ethanol), weighed and measured, according to the guidelines of CHEA (Honorary Commission for Animal Research in Uruguay). The water from each aquaria was filtered through a plankton net (65  $\mu$ m), to count the number of surviving prey. Using filtration to count surviving prey allows recovering of more than 95% of the prey items (Figueiredo, Mormul & Benedito, 2013).

As the foraging success of *G. terrapurpura* could depend on the prey anti-predation response to our treatments, we also counted the number of *H. curvispina* that left the water and remained on the walls of the aquaria (hereafter “percentage of *H. curvispina* out of water”). Thus, “fish feeding efficiency” was considered as the percentage of *H. curvispina* eaten from those prey that remained inside the water and were therefore available.

### **Data analysis**

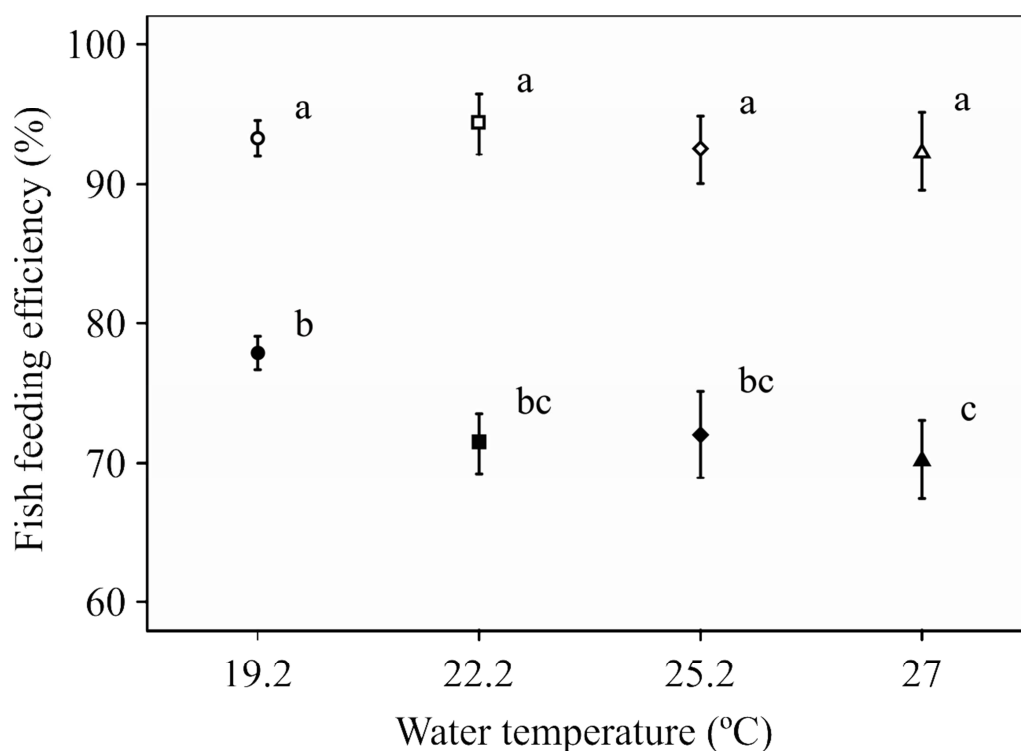
We applied a two-way ANOVA test, testing the main and interactive effects of water temperature and turbidity on fish feeding efficiency, and used Fisher's least significant difference (LSD) as a *post-hoc* test. We also evaluated with a two-way ANOVA whether the percentage of *H. curvispina* out of water (interpreted as anti-predator behaviour) varied between such treatments.

Additionally, we evaluated the correlations of fish body size and the intensity of amphipod anti-predation response with fish feeding efficiency, because such variables might act as confounding factors and mask the effects of water temperature and turbidity on predation. We therefore performed a two-way ANCOVA considering the individual fish body size (in g of fresh weight) as co-variable (continuous variable), and

temperature and turbidity as main factors. Thereafter we tested whether anti-predator behaviour of amphipods influenced fish feeding efficiency by applying ANCOVA, in this case considering the percentage of *H. curvispina* out of water in each unit as the co-variable. In both ANCOVAs Tukey's tests were used for the post hoc comparisons. The assumptions from each statistical test were previously tested, and data transformation was not required for either response variables. All tests were performed using the software Statistica (Statsoft, 2005).

## Results

Water turbidity negatively affected the feeding efficiency of *G. terrapurpura*. Higher predation occurred under clear water conditions, while in turbid waters prey consumption was reduced on average by a 20% (ANOVA  $F_{1, 48} = 151.92$ ;  $P < 0.01$ ). In contrast, temperature did not affect the feeding efficiency of *G. terrapurpura* ( $F_{3, 48} = 1.26$ ;  $P = 0.30$ ). We found no significant interaction between turbidity and water temperature on fish feeding efficiency (ANOVA  $F_{3, 48} = 1.06$ ;  $P = 0.37$ ). However, under turbid conditions, the lowest consumption of invertebrates occurred in the warmest treatment (Fisher's LSD post hoc test between treatments with extreme temperature values, i.e. 19.2 °C and 27 °C,  $P = 0.02$ ; Fig. 1).



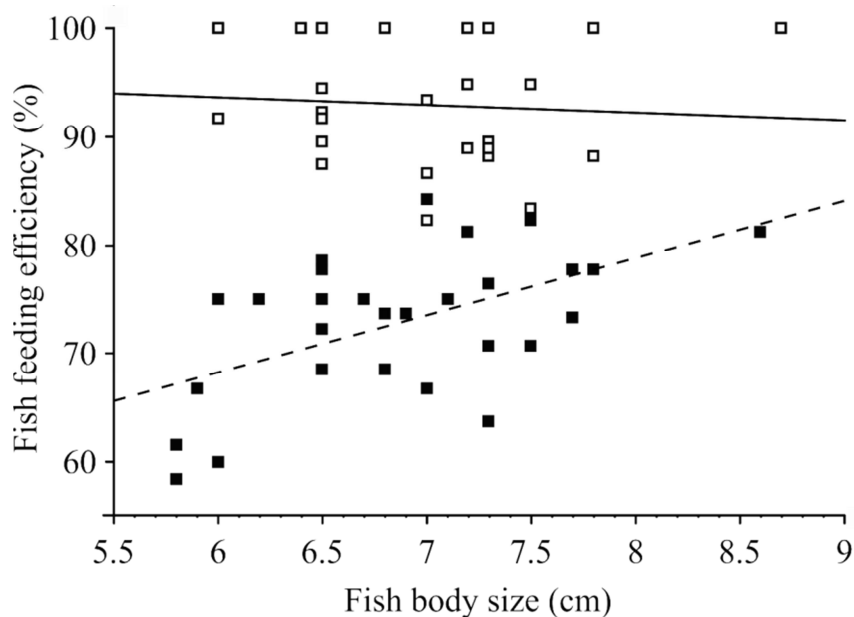
**Figure 1** – Mean and standard error of *Hyaella curvispina* (%) consumed at different water temperatures (°C) and transparency conditions: clear (3 NTU – white symbols) and turbid water

(100 NTU – black symbols). Letters indicate significant differences in Fisher's least significant difference (LSD) *post-hoc*.

Fish body size affected the feeding efficiency only under turbid conditions bigger fish had better feeding efficiency than smaller individuals (Interaction between Fish body size and Turbidity: ANCOVA  $F_{1, 40} = 6.25$ ;  $P = 0.02$ , Table 1, Fig. 2). Although no relationship between fish body size and fish feeding efficiency occurred in clear waters (Fig. 2). Also, the lack of a significant effect of temperature on fish feeding efficiency did not change even considering the potential effect of individual fish body size as a covariable (Interaction between Fish body size and Temperature: ANCOVA  $F_{3, 40} = 0.49$ ;  $P = 0.69$ , Table 1).

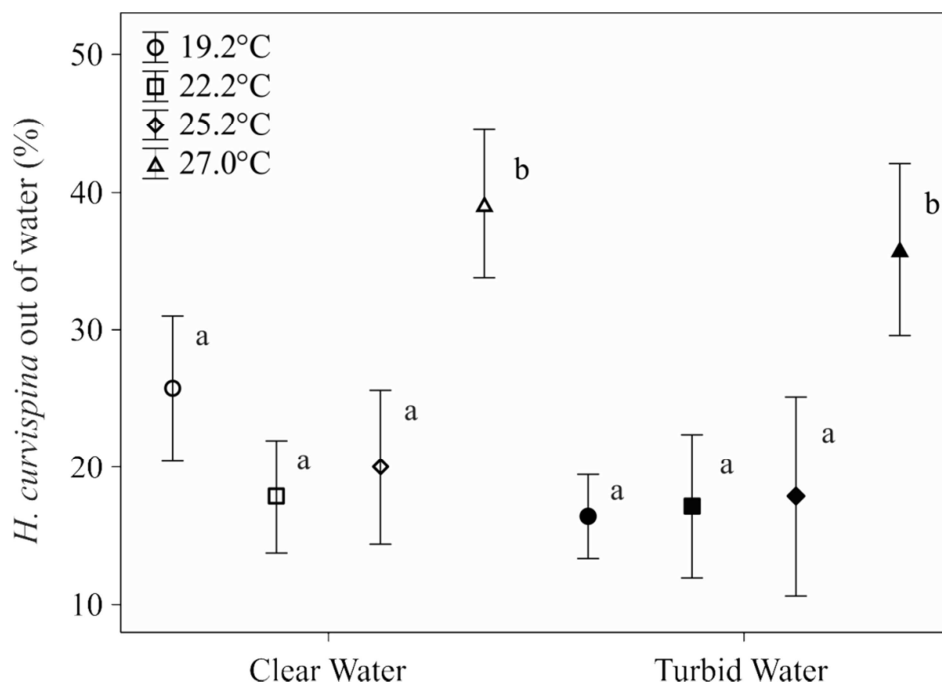
**Table 1** – Main and interactive effects of turbidity, water temperature and fish body size (a continuum of standard length) on the feeding efficiency of *Gymnogeophagus terrapurpura* (2-way ANCOVA). Statistically significant results are displayed in bold. Tukey's *post-hoc* showed that fish with different size consumed similar proportion of food in clear water, while bigger fish had better feeding efficiency in turbid water.

<i>Main effects</i>	Degrees of freedom	Mean of squares	<i>F</i>	<i>P</i>
<b>Turbidity (TU)</b>	<b>1</b>	<b>0.040</b>	<b>12.57</b>	<b>0.001</b>
Water temperature (WT)	3	0.005	1.56	0.214
Fish body size (FS)	1	0.005	1.69	0.201
Interaction TU * WT	3	0.002	0.49	0.691
<b>Interaction TU * FS</b>	<b>1</b>	<b>0.020</b>	<b>6.25</b>	<b>0.017</b>
Interaction WT * FS	3	0.005	1.61	0.203
Interaction TU * WT * FS	3	0.001	0.42	0.741
Error	40	0.003		



**Figure 2** – Relationship between fish body size (as standard length) and the feeding efficiency of *Gymnogeophagus terrapurpura*. White squares represent the data from clear waters, while black squares show the data from turbid waters. Full and dashed lines are the best models for the observed patterns of fish feeding performance in clear and turbid waters, respectively. All studied water temperatures (19.2, 22.2, 25.2 and 27° C) were pooled due to the absence of statistically significant differences among them. Observing the significance of the slopes resulting from the relationship between fish body size and turbidity on fish feeding efficiency, it is possible to note that fish size influence the consumption only in turbidity waters ( $t$ -test of the angular coefficient:  $t = 3.50$ ;  $P < 0.001$ ), not in clear water ( $t = -0.42$ ;  $P = 0.670$ ).

In contrast, prey anti-predator behaviour was affected by warming and not by turbidity. The percentage of *H. curvispina* out of water was significantly affected by temperature (ANOVA  $F_{3, 48} = 26.10$ ;  $P = 0.04$ ; Fig. 3), with the highest percentage of *H. curvispina* leaving the aquaria at 27.0°C. Despite less *H. curvispina* left the aquaria in turbid than in clear waters (Fig. 3), the effects of turbidity were not significant (ANOVA  $F_{1, 48} = 5.79$ ;  $P = 0.43$ ) and neither was the interaction between both factors ( $F_{3, 48} = 2.36$ ;  $P = 0.85$ ). The enhancement of the anti-predator response of amphipods thus reduced the food availability to fish in warmer conditions, independently of turbidity.



**Figure 3** – Mean and standard error of *Hyalella curvispina* (%) outside of water, in treatments with different conditions of water temperature and turbidity. *White symbols*: results from clear waters, *black symbols* results from turbid waters. Different letters indicate *post-hoc* test significant differences.

Despite more prey escaped under higher water temperature, such prey behavioural response did not affect fish feeding performance in any water temperature treatment (ANCOVA  $F_{3,40} = 1.86$ ;  $P = 0.15$ ). Moreover, the percentage of *H. curvispina* out of water did not shape the fish feeding efficiency in neither clear nor turbid water (ANCOVA  $F_{1,52} = 0.25$ ;  $P = 0.62$ ).

## Discussion

Simulating two environmental scenarios predicted as direct and indirect results of climate change, namely heating waves and turbidity pulses, we found that sudden increases in turbidity had negative consequences on the foraging success of a subtropical cichlid fish. This finding was expected due to a lower capacity of predator to find prey when visual detection is diminished or prevented (Schwalbe, Bassett & Webb, 2012; Figueiredo *et al.*, 2016). In contrast, and against our expectations, higher water temperature did not affect foraging success of this fish. Thermal generalist animals are expected to be less negatively impacted than animals that are physiologically specialized with respect to temperature (normally at both tropical and high latitude ecosystems, Pörtner & Knust, 2007) and have limited acclimation capacity (Stillman 2003). Lack of effects of rises in temperature in our experiment could reflect adaptation

of this typical subtropical fish to the often wide diel (besides seasonal) variations in water temperature (Roland *et al.*, 2012), characteristic of mid-latitudes, subtropical shallow water bodies. Fish populations less sensitive to regular changes in temperature (within the species-specific thermal range) would be favoured under such environmental conditions (Burkle & Alarcón, 2011; Fabina *et al.*, 2013). If so, the comparison of fish feeding efficiency between relatively close values of temperature would tend to not show differences.

Also in contrast to our hypothesis, the effects of turbidity on prey consumption did not depend on water temperature nor vice-versa. However, when comparing prey consumption in the more extreme temperatures (i.e. 19.2 °C and 27 °C), we observed that fish foraging success tended to be reduced in warmer and turbid water. A decrease in prey consumption in warmer waters was not expected since the predator would increase its activity under higher temperatures (Vucic-Pestic *et al.*, 2011, also seen in the field, Gelós *et al.*, 2010), often improving the attack success (Anderson *et al.*, 2001; Dell, Pawar & Savage, 2014). Moreover, in turbid waters visual predators are often more active than in clear waters (Wishingrad *et al.*, 2015, Teixeira de Mello *et al.*, 2016), due to difficulties in finding prey. Then, the combination of warming and increased turbidity would expectedly promote search and likely also prey consumption. However, prey consumption results from the combination of the thermal dependence of both predator and prey performance (Vucic-Pestic *et al.*, 2011; Öhlund *et al.*, 2015). In our experiments, there were substantially less invertebrates available for predation (i.e. less amphipods remained inside the water) in warmer waters, but the predators maintained the same consumption rates as in colder waters. Therefore, the slight trend of prey consumption to decline in warmer water seems the result of enhanced prey ability to avoid their predators, rather than lower predator feeding efficiency.

Since fish body size affects fish feeding efficiency, we controlled this factor by standardizing fish body size among treatments. Nevertheless, relatively small variations in fish standard length within treatments were enough to influence prey consumption rates (as in Scharf, Juanes & Rountree, 2000), but interestingly, only under turbid waters. Fish with different body size consumed similar amount of prey under clear waters, but in turbid waters bigger fish were more efficient than smaller individuals. This could be a consequence of either individual differences in behaviour and life history or ontogenetic variations (the latter disregarded in our set-up). Fish with larger body size could have higher behavioural flexibility, allowing them to adjust their

behaviour according to changes in environmental conditions (Lundvall *et al.*, 2000). Therefore, fish would have larger body size both as a consequence and as a cause of their better feeding efficiency.

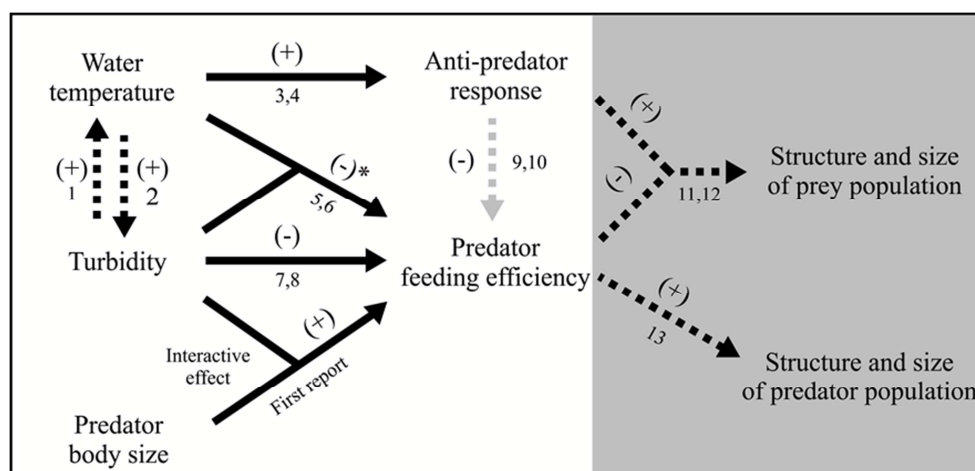
In a warmer and more turbid future, individual differences (e.g. larger body sizes) amongst fish populations could provide greater likelihood of survival and higher adaptive success, potentially leading to changes at the population and community levels. Alternatively, bigger individuals could see or detect their prey in slightly greater distances (Breck & Gitter *et al.*, 1983), or somehow more efficiently perceive the non-visual stimuli from prey, increasing the predation success (Kingsolver & Pfennig, 2004; Hone & Benton, 2005).

Besides behavioural traits and metabolic rates, physiology, body condition and the efficiency of the immune system of ectothermic animals can be substantially affected by heat waves (Dittmar *et al.*, 2014). In particular, recent studies have suggested that organisms respond to increasing temperatures with higher metabolism, quicker development and shrinking body sizes (Daufresne, Lengfellner & Sommer, 2009; Sheridan & Bickford, 2011; Bestion *et al.*, 2015). If (i) body size of predators (like fishes) declines with increasing water temperature (Desai & Singh, 2009), and (ii) small consumers have disproportionally lower predator feeding efficiency under low visibility conditions (as observed in *G. terrapurpura*), then it is likely that the survival of small prey individuals (like aquatic invertebrates) will increase (Peck *et al.*, 2009) in warmer and more turbid aquatic ecosystems. Allied to the predicted higher survival of small prey (like amphipods), their anti-predator behaviour seemed more pronounced in warmer water, likely yielding lower prey availability to a fish shoal under natural conditions.

Under this scenario, the proportion of species better adapted to sudden changes leading to turbid and warmer conditions would increase, in detriment of more sensitive species. Even short-term variations caused by climate change may promote a mismatch between predator requirements and prey availability (Durant *et al.*, 2007). With our findings, together with other published works, we propose a conceptual framework of the potential relationships and implications to predator-prey interactions considering increased turbidity level and water temperature as lasting or recurring events (Fig. 4). Our findings provide empirical evidence to the ideas that: (1) bigger fish individuals may be better prepared to withstand fluctuations in turbidity, and (2) some invertebrates



could respond to warmer temperatures with higher escape efficiency (or seeking refuge, not tested here).



**Figure 4** – Conceptual model on the effects of higher temperature, higher turbidity and greater body size on both predator and prey populations, highlighting our main findings (white region) and their potential implications (grey region). Arrows point to the direction of the effect, and symbols show whether the effects are positive (+) or negative (-). The resulting effects on predator and prey population can be determined by multiplying symbols along connecting pathways. *Full lines* suggest direct effects. *Black dashed lines* indicate theoretical effects (i.e. not directly tested in the present study); while *grey dashed lines* indicate effects supported by other works as individualised by numbers. \* = water temperature affected predator feeding efficiency only when the compared temperatures differed in more than 7 °C .References: (1) Bohrein and Huffman (1983); (2) Moss *et al.* (2011); (3) Weetman, Atkinson & Chubb (1999); (4) Grigaltchik, Ward & Seebacher (2012); (5) Brown *et al.* (2004); (6) Gelós *et al.* (2010); (7) Ranåker *et al.* (2012); (8) Figueiredo *et al.* (2016); (9) Lima and Dill (1990); (10) Sansom, Lind & Cresswell (2009); (11) Ives and Dobson (1987); (12) Öhlund *et al.* (2015); (13) Okuyama (2013). Note that the present study is the first, to our knowledge, to report the interaction between turbidity and predator body size affecting the feeding.

In a nutshell, our short-term experiments suggest that some traits of both invertebrate and fish, such as anti-predator behaviour and body size-dependent success, respectively, could at least partly compensate for the environmental changes promoted by sudden increases in temperature and water turbidity in subtropical systems.

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## References

- Aksnes D.L. & Utne A.C.W. (1997) A revised model of visual range in fish. *Sarsia*, **82**, 137–147. doi: 10.1080/00364827.1997.10413647
- Anderson M.T., Kiesecker J.M., Chivers D.P. & Blaustein A.R. (2001) The direct and indirect effects of temperature on a predator–prey relationship. *Canadian Journal of Zoology*, **79**, 1834–1841. doi: 10.1139/cjz-79-10-1834
- Bestion E., Teyssier S., Richard M., Clobert J. & Cote J. (2015) Live fast, die young: experimental evidence of population extinction risk due to climate change. *Plos Biology*, **13**, e1002281. doi: 10.1371/journal.pbio.1002281
- Biro P.A., Beckmann C. & Stamps J.A. (2010) Small within-day increases in temperature affects boldness and alters personality in coral reef fish. *Proceedings of the Royal Society B: Biological Sciences*, **277**, 71–77. doi: 10.1098/rspb.2009.1346
- Bohren C.F. & Huffman D.R. (1983) *Absorption and scattering of light by small particles*. John Wiley & Sons, New York.
- Breck J.E. & Gitter M.J. (1983) Effect of fish size on the reactive distance of bluegill (*Lepomis macrochirus*) sunfish. *Canadian Journal of Fisheries and Aquatic Sciences*, **40**, 162–167. doi: 10.1139/f83-026
- Brown J.H., Gillooly J.F., Allen A.P., Savage V.M. & West G.B. (2004) Toward a metabolic theory of ecology. *Ecology*, **85**, 1771–1789. doi: 10.1890/03-9000
- Burkle L.A. & Alarcón R. (2011) The future of plant-pollinator diversity: understanding interaction networks across time, space, and global change. *American Journal of Botany*, **98**, 528–538. doi: 10.3732/ajb.1000391
- Como S., Lefrancois C., Maggi E., Antognarelli F. & Dupuy C. (2014) Behavioral responses of juvenile golden gray mullet *Liza aurata* to changes in coastal

- temperatures and consequences for benthic food resources. *Journal of Sea Research*, **92**, 66–73. doi: 10.1016/j.seares.2013.10.004
- Daufresne M., Lengfellner K. & Sommer U. (2009) Global warming benefits the small in aquatic ecosystems. *Proceedings of National Academy of Sciences*, **106**, 12788–12793. doi: 10.1073/pnas.0902080106
- Dell A.I., Pawar S. & Savage V.M. (2014) Temperature dependence of trophic interactions are driven by asymmetry of species responses and foraging strategy. *Journal of Animal Ecology*, **83**, 70–84. doi: 10.1111/1365-2656.12081
- Desai A.S. & Singh R.K. (2009) The effects of water temperature and ration size on growth and body composition of fry of common carp, *Cyprinus carpio*. *Journal of Thermal Biology*, **34**, 276–280. doi: 10.1016/j.jtherbio.2009.03.005
- Dittmar J., Janssen H., Kuske A., Kurtz J. & Scharsack J.P. (2014) Heat and immunity: an experimental heat wave alters immune functions in three-spined sticklebacks (*Gasterosteus aculeatus*). *Journal of Animal Ecology*, **83**, 744–757. doi: 10.1111/1365-2656.12175
- Doyle S.R. & Momo F.R. (2009) Effects of body weight and temperature on the metabolic rate of *Hyaella curvispina* Shoemaker, 1942 (Amphipoda). *Crustaceana*, **82**, 1423–1439. doi: 10.1163/001121609X12475745628540
- Durant J.M., Hjermann D.Ø., Ottersen G. & Stenseth N.C. (2007) Climate and the match or mismatch between predator requirements and resource availability. *Climate Research*, **33**, 271–283.
- Estes J.A., Terborgh J., Brashares J.S., Power M.E., Berger J., Bond W.J. *et al.* (2011) Trophic Downgrading of Planet Earth. *Science*, **333**, 301–306.
- Fabina N.S., Putnam H.M., Franklin E.C., Stat M. & Gates R.D. (2013) Symbiotic specificity, association patterns, and function determine community responses to global changes: defining critical research areas for coral-*Symbiodinium* symbioses. *Global Change Biology*, **19**, 3306–3316. doi: 10.1111/gcb.12320
- Figueiredo B.R.S., Mormul R.P. & Benedito E. (2013) Non-additive effects of macrophyte cover and turbidity on predator-prey interactions involving an invertivorous fish and different prey types. *Hydrobiologia*, **713**, 23–30. doi: 10.1007/s10750-013-1540-7
- Figueiredo B.R.S., Mormul R.P., Chapman B.B., Lolis L.A., Fiori L.F. & Benedito E. (2016) Turbidity amplifies the non-lethal effects of predation and affects the foraging success of characid fish shoals. *Freshwater Biology*, **61**, 293–300. doi: 10.1111/fwb.12703

- Galassi M.E., Franceschini M.C. & Neiff A.P. (2006) Population Estimates of *Hyaella curvispina* Shoemaker (Amphipoda) in Aquatic Vegetation of Northeastern Argentinian Ponds. *Acta Limnologica Brasiliensia*, **18**, 101–108.
- Gelós M., Teixeira-de Mello F., Goyenola G., Iglesias C., Fosalba C., García-Rodríguez F., Pacheco J.P., García S. & Meerhoff M. (2010) Seasonal and diel changes in fish activity and potential cascading effects in subtropical shallow lakes with different water transparency. *Hydrobiologia*, **646**, 173–185. doi: 10.1007/s10750-010-0170-6
- Giorgi A., Feijoó C. & Tell G. (2005) Primary producers in a Pampean stream: temporal variation and structuring role. *Biodiversity and Conservation*, **14**, 1699–1718. doi: 10.1007/s10531-004-0694-z
- Grigaltchik V.S., Ward A.J.W. & Seebacher F. (2012) Thermal acclimation of interactions: differential responses to temperature change alter predator–prey relationship. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 4058–4064. doi: 10.1098/rspb.2012.1277
- Hone D.W.E. & Benton M.J. (2005) The evolution of large size: how does Cope’s Rule work? *Trends in Ecology and Evolution*, **20**, 4–6. doi: 10.1016/j.tree.2004.10.012
- Horppila J. & Nurminen L. (2003) Effects of submerged macrophytes on sediment resuspension and internal phosphorus loading in Lake Hiidenvesi (southern Finland). *Water Research*, **37**, 4468–4474. doi: 10.1016/S0043-1354(03)00405-6
- IPCC (2007) *Climate change 2007: The physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change*. Cambridge Univ. Press, Cambridge, UK.
- IPCC (2012) *IPCC special report: managing the risks of extreme events and disasters to advance climate change adaptation*. Cambridge Univ. Press, Cambridge.
- Issartel J., Hervant F., Voituron Y., Renault D. & Vernon P. (2005) Behavioural, ventilatory and respiratory responses of epigeal and hypogeal crustaceans to different temperatures. *Comparative Biochemistry and Physiology*, **141**, 1–7. doi: 10.1016/j.cbpb.2005.02.013
- Ives A.R. & Dobson A.P. (1987) Antipredator behavior and the population dynamics of simple predator-prey systems. *American Naturalist*, **130**, 431–447. doi: 10.1086/284719
- Kingsolver J.G. & Pfennig D.W. (2004) Individual-level selection as a cause of Cope’s Rule of phyletic size increase. *Evolution*, **58**, 608–612. doi: 10.1111/j.0014-3820.2004.tb01740.x

- Krik J.T.O. (1985) Effects of suspensoids (turbidity) on penetration of solar radiation in aquatic ecosystems. *Hydrobiologia*, **125**, 195–208. doi: 10.1007/BF00045935
- Leahy S.M., McCormick M.I., Mitchell M.D. & Ferrari M.C. (2011) To fear or to feed: the effects of turbidity on perception of risk by a marine fish. *Biology Letters*, **7**, 811–813. doi: 10.1098/rsbl.2011.064
- Lehtiniemi M., Engström-Öst J. & Viitasalo M. (2005) Turbidity decreases anti-predator behaviour in pike larvae, *Esox lucius*. *Environmental Biology of Fishes*, **73**, 1–8. doi: 10.1007/s10641-004-5568-4
- Lima S.L. & Dill L.M. (1990) Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology*, **68**, 619–640. doi: 10.1139/z90-092
- Lundvall D., Svanback R., Persson L. & Bystrom P. (2000) Size-dependent predation in piscivores: interactions between predator foraging and prey avoidance abilities. *Canadian Journal of Fisheries and Aquatic Sciences*, **56**, 1285–1292. doi: 10.1139/cjfas-56-7-1285
- Marengo J.A., Chou S.C., Kay G., Alves L.M., Pesquero, J.F., Soares, W.R. *et al.* (2012) Development of regional future climate change scenarios in South America using the Eta CPTEC/HadCM3 climate change projections: climatology and regional analysis for the Amazon, São Francisco and the Paraná River basins. *Climate Dynamics*, **38**, 1829–1848. doi: 10.1007/s00382-011-1155-5
- McKenzie D. & Claireaux G. (2010) The effects of environmental factors on the physiology of aerobic exercise. In: *Fish locomotion: an eco-ethological perspective* (Ed. P. Domenici & B. D. Kapoor), pp. 296–332. Science Publishers, Enfield, NH, USA.
- Meerhoff M., Iglesias C., Teixeira-de Mello F., Clemente J.M., Jensen E., Lauridsen T.L. & Jeppesen E. (2007) Effects of habitat complexity on community structure and predator avoidance behaviour of littoral zooplankton in temperate versus subtropical shallow lakes. *Freshwater Biology*, **52**, 1009–1021. doi: 10.1111/j.1365-2427.2007.01748.x
- Mori A. & Burghardt G.M. (2001) Temperature effects on anti-predator behaviour in *Rhabdophis tigrinus*, a snake with toxic nuchal glands. *Ethology*, **107**, 795–811. doi: 10.1046/j.1439-0310.2001.00706.x
- Moss B., Kosten S., Meerhoff M., Battarbee, R.W., Jeppesen, E., Mazzeo, N. *et al.* (2011) Allied attack: climate change and eutrophication. *Inland Waters*, **1**, 101–105. doi: 10.5268/IW-1.2.359

- Nowicki J.P., Miller G.M. & Munday P.L. (2012) Interactive effects of elevated temperature and CO<sub>2</sub> on foraging behavior of juvenile coral reef fish. *Journal of Experimental Marine Biology and Ecology*, **412**, 46–51. doi: 10.1016/j.jembe.2011.10.020
- Öhlund G., Hedström P., Norman S., Hein C.L. & Englund G. (2015) Temperature dependence of predation depends on the relative performance of predators and prey. *Proceedings of the Royal Society B: Biological Sciences*, **282**, 20142254. doi: 10.1098/rspb.2014.2254
- Okuyama T. (2013) Consequences of variation in foraging success among predators on numerical response. *Ecology and Evolution*, **3**, 4039–4043. doi: 10.1002/ece3.772
- Peck L.S., Clark M.S., Morley S.A., Massey A. & Rossetti H. (2009) Animal temperature limits and ecological relevance: effects of size, activity and rates of change. *Functional Ecology*, **23**, 248–256. doi: 10.1111/j.1365-2435.2008.01537.x
- Pörtner H.O. & Knust R. (2007) Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science*, **315**, 95–97. doi: 10.1126/science.1135471
- Ranåker L., Jönsson M., Nilsson P.A. & Brönmark C. (2012) Effects of brown and turbid water on piscivore-prey fish interactions along a visibility gradient. *Freshwater Biology*, **57**, 1761–1768. doi: 10.1111/j.1365-2427.2012.02836.x
- Roland F., Huszar V.L.M., Farjalla V.F., Enrich-Prast A., Amado A.M. & Ometto J.P.H.B. (2012) Climate change in Brazil: perspective on the biogeochemistry of inland waters. *Brazilian Journal of Biology*, **72**, 709–722. doi: 10.1590/S1519-69842012000400009
- Sansom A., Lind J. & Cresswell W. (2009) Individual behavior and survival: the roles of predator avoidance, foraging success, and vigilance. *Behavioral Ecology*, **20**, 1168–1174. (doi: 10.1093/beheco/arp110)
- Scharf, F.S., Juanes, F. & Rountree, R.A. 2000 Predator size - prey size relationships of marine fish predators: interspecific variation and effects of ontogeny and body size on trophic-niche breadth. *Marine Ecology Progress Series*, **208**, 229–248.
- Schwalbe, M.A.B., Bassett, D.K. & Webb, J.F. 2012 Feeding in the dark: lateral-line-mediated prey detection in the peacock cichlid *Aulonocara stuartgranti*. *Journal of Experimental Biology*, **215**, 2060–2071. (doi: 10.1242/jeb.065920)
- Sheridan, J.A. & Bickford, D. 2011 Shrinking body size as an ecological response to climate change. *Nature Climate Change*, **1**, 401–406. (doi: 10.1038/nclimate1259)

- Stillman, J.H. 2003 Acclimation capacity underlies susceptibility to climate change. *Science*, **30**, 65. (doi: 10.1126/science.1083073)
- Teixeira-de Mello, F., Meerhoff, M., Pekcan-Hekim, Z. & Jeppesen, E. (2009) Substantial differences in littoral fish community structure and dynamics in subtropical and temperate shallow lakes. *Freshwater Biology*, **54**, 1202–1215. doi: 10.1111/j.1365-2427.2009.02167.x
- Teixeira-de Mello, F., Oliveira, V.A., Loverde-Oliveira, S.M., Huszar, V.L.M., Barquín, J., Iglesias, C., Silva, T.S.F., Duque-Estrada, C.H., Silió-Calzada, A. & Mazzeo, N. (2016) The structuring role of free-floating plants on the fish community in a tropical shallow lake: an experimental approach with natural and artificial plants. *Hydrobiologia*. doi: 10.1007/s10750-015-2447-2
- Teixeira-de Mello, F., Vidal, N., Eguren, G. & Loureiro, M. 2009 Length–weight relationships of 21 fish species from the lower section of the Santa Lucía river basin (Canelones-Montevideo, Uruguay). *Journal of Applied Ichthyology*, **25**, 491–492. (doi: 10.1111/j.1439-0426.2009.01197.x)
- Vucic-Pestic, O., Ehnes, R.B., Rall, B.C. & Brose, U. (2011) Warming up the system: higher predator feeding rates but lower energetic efficiencies. *Global Change Biology*, **17**, 1301–1310. doi: 10.1111/j.1365-2486.2010.02329.x
- Weetman, D., Atkinson, D. & Chubb, J.C. (1999) Water temperature influences the shoaling decisions of guppies, *Poecilia reticulata*, under predation threat. *Animal Behaviour*, **58**, 735–741. doi: 0.1006/anbe.1999.1191
- Wishingrad, V., Musgrove, A.B., Chivers, D.P. & Ferrari, M.C.O. (2015) Risk in a changing world: environmental cues drive anti-predator behaviour in lake sturgeon (*Acipenser fulvescens*) in the absence of predators. *Behaviour*, **152**, 635–652. doi: 10.1163/1568539X-00003246

II



## Consequências de variações na visibilidade subaquática e na temperatura sobre o comportamento de anfípodas

### *Unless I see the predator I will not flee: influence of light and water temperature on biological responses of amphipods*

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Running Headline: **Amphipods' responses to predator, heat and light**

**Resumo:** Predadores moldam o ambiente ao seu redor, não apenas pela morte das presas, mas também por induzir alterações comportamentais em suas potenciais vítimas. No presente estudo, observou-se que, na natureza, espécimes de *Hyalella curvispina* são frequentemente encontrados associados a raízes de plantas terrestres, em uma região de alta umidade, ligeiramente acima da superfície da água. Da mesma maneira, em experimentações-piloto observou-se que esses anfípodas também migraram para fora da água do aquário. Portanto, hipotetizou-se que o uso do ecótono ar – água tanto na natureza quanto em condições laboratoriais é um comportamento de anti-predação de anfípodas. Para testar essa hipótese, e observar possíveis interações da presença de predadores com (i) a visibilidade subaquática, ou (ii) temperatura da água para moldar o comportamento de anfípodas, realizou-se duas experimentações, em microcosmo, cruzando: a presença/ausência de *Gymnogeophagus terrapurpura* e contrastantes condições de visibilidade subaquática (obstruída e total), e outra cruzando a presença de *G. terrapurpura* e duas temperaturas da água (19 e 27°C). Nas duas experimentações, a maior parte dos anfípodas ao coocorrer com um peixe ciclídeo migrou para a interface

água - ar, permanecendo fora do alcance de seus predadores. Essa migração de anfípodas para fora da água não foi observada na ausência de predadores, o que suporta a hipótese de que deixar a água é, de fato, um comportamento de anti-predação. Além disso, a maior parte dos anfípodas só respondeu a presença de um predador em situações em que a visibilidade subaquática era total, o que sugere que o comportamento de anti-predação de anfípodas é desencadeado a partir de estímulos visuais. Sugere-se que caso os ambientes tornem-se mais turbidos, tal como predito, os indivíduos com maior sensibilidade a estímulos não visuais poderiam ser favorecidos por seleção natural. E, se mais indivíduos-presa forem capazes de ocupar locais que predadores não possuem acesso, então tais predadores defrontarão um ambiente com baixa disponibilidade de presa, o que aumentará a competição.

**Palavras-chave:** Interação predador-presa, mudanças ambientais, aquecimento global, comportamento de presas, forrageamento.

## **Introdução**

A eficiência na habilidade de predadores e presas detectarem um ao outro é crucial para o resultado da interação predatória (Powers & Kittinger, 2002; Kobak & Ryńska, 2014). Enquanto o risco de falhar em perceber uma presa envolve relativamente baixos custos ao predador (ao menos quando o tempo sem alimentação é curto), os custos para o animal-presa, decorrentes da falha na percepção da presença do predador são altos, e geralmente acarretam na sua morte (Lima & Dill, 1990; Cronin, 2005). Portanto, para sobreviver, as presas necessitam reconhecer a presença do predador, detectar sua posição, para então iniciar uma manobra evasiva, ou alternativamente, monitorar os movimentos do predador visando maior sucesso na fuga em um eventual ataque (Abrahams et al., 2009; Higham et al., 2015). Um dilema para presas é que qualquer estratégia de anti-predação utilizada demandará uma quantidade de energia que poderia ser alocada para outros comportamentos relacionados ao *fitness*, como o forrageamento, ou táticas reprodutivas (Helfman, 1989; DeWitt & Langerhans, 2004). Considerando que na natureza o risco de predação varia ao longo do tempo e do espaço (Sih et al., 2010), presas devem ser capazes de alterar o fenótipo de acordo com o risco de predação vigente (Ghalambor et al., 2007), visando minimizar as perdas energéticas.

Embora as estratégias de anti-predação mais efetivas têm sido favorecidas por seleção natural ao longo do tempo (Darwin, 1859; Lima & Dill, 1990; Sih et al., 2011),

a real efetividade de um dado comportamento, para evitar predadores, pode ser modificada (tanto potencializada quanto reduzida) por vários fatores ambientais (Svensson et al., 2003; Smee et al., 2010), incluindo a visibilidade (Yorzinski et al., 2015) e a temperatura (Malavasi et al., 2013). A visibilidade do ambiente afeta as estratégias de anti-predação tão logo ela reduza a capacidade de presa em detectar alguma ameaça de predação. Se há risco de predação, mas ele é ignorado ou despercebido, presas são facilmente capturadas por predadores (Helfman, 1989). Da mesma forma, variações na temperatura resultam na aceleração de reações bioquímicas e, por conseguinte, aumentam as taxas metabólicas de animais ectotérmicos, os quais precisam adquirir mais comida para atender à maior demanda energética (Pink & Abrahams, 2016). Para tanto, em ambientes mais quentes, animais dedicam mais tempo em atividades relacionadas à ingestão de alimento (McKenzie & Claireaux, 2010). Portanto, em elevadas temperaturas, organismos-presa necessitam satisfazer suas próprias demandas metabólicas, aumentando a ingestão de alimento, e ao mesmo tempo, evitar os ataques mais frequentes dos predadores. Nesse cenário, mesmo que a temperatura afete levemente a atividade natatória das presas, ela influenciará drasticamente o equilíbrio da interação predatória, podendo conduzir a remoção do animal-presa do sistema, e por outro, pode elevar a sobrevivência. Em ambos os casos haverá consequências fisiológicas para o predador (Thomas & Kingsolver, 2002).

Para organismos aquáticos, mudanças na visibilidade subaquática podem ser mediadas pelo homem, por exemplo, o barramento do curso natural de rios que aumenta a retenção de nutrientes e de sólidos em suspensão na água, ocasionando um aumento na transparência da água, fenômeno conhecido como oligotrofização (Agostinho et al., 2007; Espínola et al., 2010). Por outro lado, o uso do solo (p. ex. agricultura, pastagem e urbanização) em regiões circundantes a ambientes aquáticos tende a facilitar processos de erosão, e a aumentar o carreamento de partículas orgânicas (p. ex. fertilizantes) e inorgânicas (p. ex. cargas de sedimento) do ambiente terrestre para o ambiente aquático, causando eutrofização e redução na transparência da água (Allan, 2004; Ajemian et al., 2015). Da mesma forma, aumento na temperatura média da água tem sido predito para ocorrer em toda a superfície da Terra, o que para peixes deve resultar em aumento no número de eventos relacionados à busca e à captura de alimento (Brown et al., 2004; Pink & Abrahams, 2016). É evidente que predadores e presas, em ambientes aquáticos, vivendo em simpatria estão sujeitos ao mesmo conjunto de condições ambientais. Portanto, o sucesso de presas em evitarem ser consumida ou de predadores em

capturarem a presa é um resultado de aumento ou diminuição desproporcional no desempenho de presas e predadores em resposta as variações do ambiente (Gerald, 2008; Öhlund et al., 2015).

O anfípode de água doce *Hyaella curvispina* Shoemaker, 1942 é amplamente distribuído em diferentes tipos de habitats aquáticos ao longo de toda a América do Sul (Doyle & Momo, 2009). A espécie atinge altas densidades populacionais, mesmo em sistemas próximos a regiões urbanizadas e é um importante item alimentar para muitos invertebrados, pássaros e peixes (Galassi et al., 2006). Em ecossistemas naturais, *H. curvispina* têm a capacidade de ocupar interstícios de plantas aquáticas (Galassi et al., 2006; Ferreira et al., 2014), mas eventualmente, eles também podem ser encontrados associados a raízes de plantas terrestres, em uma região de alta umidade, ligeiramente acima da superfície da água (Observação pessoal). Da mesma maneira, em experimentações-piloto para observar a relação entre *H. curvispina* e peixes, observou-se que esses anfípodas também migraram para fora da água do aquário. Portanto, hipotetizou-se que tanto o uso do ecótono ar – água, na natureza, quanto a migração para fora da água sob condições laboratoriais são comportamentos de anti-predação de anfípodas, os quais são primariamente mediados pela alta pressão predatória no meio aquático (p. ex. Camacho & Thacker, 2013). Para testar essa hipótese, e observar possíveis interações da presença de predadores com (i) a visibilidade subaquática, ou (ii) temperatura da água sobre o comportamento de anfípodas, realizou-se duas experimentações, em microcosmo, com delineamento de fatores totalmente cruzados: um experimento cruzando a presença de predadores e contrastantes condições de visibilidade subaquática, e outro cruzando a presença de predadores e duas temperaturas da água.

## **Métodos**

### **Amostragem e experimentações**

Para testar experimentalmente a hipótese supracitada, coletou-se a presa *Hyaella curvispina* (Crustacea: Amphipoda), e o predador *Gymnogeophagus terrapurpura* Loureiro et al., 2016 (Perciformes: Cichlidae). *Gymnogeophagus terrapurpura* foi utilizada como espécie predadora, porque a principal ameaça de predação para a sobrevivência de anfípodas são predadores que se orientam visualmente na busca de presas (Holmlund et al. 1990; Smith & Webster 2015), tais como *G. terrapurpura*, uma espécie recentemente descrita (anteriormente tratada como

*Gymnogeophagus meridionalis*). Tanto os peixes quanto os anfípodes foram coletados no verão de 2015, quando ambas as espécies coocorrem em regiões litorâneas de ecossistemas aquáticos (Ferreiro et al. 2014). Para a captura de espécimes de *H. curvispina* (comprimento =  $3,4 \pm 0,8$  mm), amostrou-se raízes de plantas aquáticas em um riacho próximo a área urbana de Maldonado, Uruguai ( $34^{\circ}88'67''S$ ,  $54^{\circ}95'24''O$ , temperatura da água  $\approx 21$  °C). Em laboratório, essas raízes foram lavadas e os anfípodes associados a elas foram estocados em recipientes de 600 ml (máximo de 20 indivíduos por recipiente) contendo água do riacho. Espécimes de *G. terrapurpura* (comprimento padrão =  $70,7 \pm 7,0$  mm) foram coletados na lagoa do Diário em Maldonado, Uruguai ( $34^{\circ}90'41''S$ ,  $55^{\circ}00'82''O$ , temperatura da água  $\approx 21$  °C) por meio do uso de pesca elétrica.

Aquários retangulares foram utilizados como unidades experimentais (largura = 25 cm; comprimento = 30 cm; altura = 50 cm; volume de água = 30 l; profundidade da água = 40 cm; todos os lados dos aquários foram recobertos com tecido negro, para impedir interação visual de organismos em aquários vizinhos). Todas as unidades experimentais foram inseridas dentro de câmaras térmicas à mesma altura para prevenir variações de temperatura e homogeneizar a quantidade de luz incidente. Em cada unidade experimental foi inserido um peixe ciclídeo, que foram aclimatados (sem alimentação) a temperatura da água através de um aumento (ou redução) gradual da temperatura das câmaras térmicas ao longo de 72 h. Os anfípodes foram aclimatados a temperatura experimental das câmaras térmicas uma hora antes do início das experimentações, a qual se iniciou com a inserção de 20 anfípodes por unidade experimental (ver descrição dos tratamentos e réplicas abaixo), que estavam expostas a predação por *G. terrapurpura*. Os aquários possuíam um sistema de aeração até imediatamente antes da introdução dos anfípodes aos aquários, entretanto devido à capacidade desses animais em utilizar qualquer objeto como refúgio (observação pessoal), optou-se pela remoção do sistema aerador imediatamente antes do início da experimentação.

Para observar possíveis interações da presença de predadores com a visibilidade subaquática ( $T_1$ ) para determinar o comportamento de *H. curvispina*, combinou-se visibilidade subaquática e presença do predador para criar quatro tratamentos: (i) controle – visibilidade subaquática obstruída e ausência de predadores; (ii) Tratamento de visibilidade – visibilidade subaquática total e ausência de predadores; (iii) Tratamento de predação – visibilidade subaquática obstruída e presença de um

predador; e (iv) Tratamento de interação – visibilidade subaquática total e presença de um predador (Fig. 1). Cada tratamento foi replicado sete vezes. Ambos os tratamentos de visibilidade subaquática total e obstruída foram obtidos através do controle de luz incidente posicionada de forma centralizada acima das unidades experimentais, o que permitia disponibilizar ou não luz dentro das câmaras térmicas. Quando as luzes eram acesas, devido à alta transparência da água, a visibilidade subaquática foi total, e quando as luzes eram apagadas não havia nenhuma fonte de luz incidente e, portanto, a visibilidade subaquática foi obstruída. Cruzar a visibilidade subaquática e a presença do predador esclareceria se a ação dos anfípodes é um comportamento de anti-predação ou é apenas um comportamento incitado pela luz, como um tipo *stress* do animal. Além disso, esta abordagem também permite reconhecer a real importância de estímulos visuais e não visuais da presença do peixe predador para o comportamento de anti-predação de anfípodes.

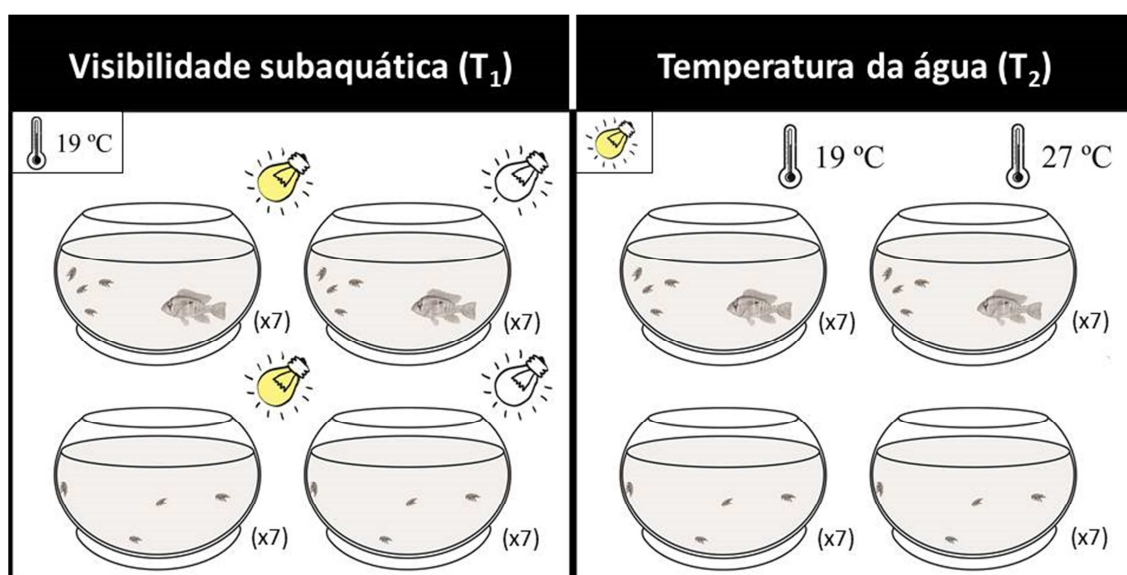


Figura 1 – Esquema dos delineamentos experimentais aplicados aos testes de hipótese do presente estudo. À esquerda, o esquema ilustra o primeiro experimento, no qual se observa a resposta de *Hyalella curvispina* à visibilidade subaquática e a presença de *Gymnogeophagus terrapurpura*, a temperatura da água é igual em todos os tratamentos. À direita, há uma ilustração dos tratamentos cruzados no segundo experimento (temperatura da água e presença de um predador), no qual também é observada a resposta de anfípodes, a visibilidade subaquática é total em todos os tratamentos.

Para observar possíveis interações da presença de predadores com a temperatura da água ( $T_2$ ) para moldar o comportamento de *H. curvispina* combinou-se duas temperaturas da água e a presença de *G. terrapurpura* produzindo quatro tratamentos:

(i) controle – ausência de predadores a 19°C; (ii) tratamento quente – ausência de predadores a 27°C; (iii) tratamento de predação – presença de um predador a 19°C, e (iv) tratamento de interação – presença de um predador a 27°C (Fig. 1). Cada um dos tratamentos foi replicado sete vezes. Ambos os tratamentos de temperatura da água foram obtidos aumentando a temperatura do ar dentro das câmaras térmicas até que a água alcançasse as temperaturas desejáveis (19 e 27°C). As temperaturas da água de 19°C e de 27°C foram escolhidas devido ao intervalo natural de temperatura observado em sistemas aquáticos uruguaios durante o verão, e considerando um aumento médio de 3°C previstos para ocorrer (p. ex. Teixeira-de Mello et al., 2009, Gelós et al., 2010). Cruzar experimentalmente a temperatura da água e a presença do predador pode experimentalmente prever possíveis diferenças no resultado da interação entre peixes e anfípodes em cenários de elevação na temperatura da água.

Após 1 h de experimento, os peixes foram removidos dos aquários, eutanasiados com overdose de Eugenol (4 ml L<sup>-1</sup> de uma solução de 1:10 Eugenol: 95% etanol), pesados e medidos, de acordo com as orientações da CHEA (Comissão Honorária de Estudos com Animais no Uruguai). Foi também quantificado o número de anfípodes que deixaram a água do aquário em resposta a cada tratamento aplicado, o qual foi utilizado como variável resposta. Tal protocolo foi aplicado em ambas as experimentações. Além disso, nos tratamentos que anfípodes poderiam ser consumidos, a quantidade de anfípodes sobreviventes ao final da experimentação foi também mensurada.

### **Análise de dados**

Uma análise de variância permutacional (perANOVA) foi conduzida para testar os efeitos isolados e a interação entre visibilidade subaquática (total e obstruída) e a presença de predador (presença/ ausência de um peixe ciclídeo) sobre a porcentagem de *H. curvispina* fora da água. Da mesma forma, uma segunda perANOVA foi realizada para avaliar os efeitos isolados e a interação entre temperatura da água (19 e 27°C) e a presença de predador (presença/ ausência de um peixe ciclídeo) sobre a porcentagem de *H. curvispina* fora da água. Para identificar quais pares de médias de tratamentos diferem significativamente entre si, utilizou-se o teste de Tukey nas duas perANOVA como *post-hoc*. O nível de significância estatística adotado foi de  $\alpha < 0,05$ .

Em ambos os testes estatísticos optou-se por uma abordagem permutacional porque a variável resposta (porcentagem de *H. curvispina* fora água) não atendeu aos pressupostos de igualdade de variâncias, e as transformações de dados mais comuns não

foram suficientes para homogeneizar as variâncias. A abordagem permutacional é tão robusta quanto a ANOVA tradicional, mas ela não exige o pressuposto de homocedasticidade da variável resposta (Anderson, 2001; McArdle & Anderson, 2001). Todas as análises estatísticas foram realizadas utilizando o *software* R (R development Core Team 2008).

## Resultados

Anfípodes se deslocaram em massa para fora da água da unidade experimental apenas nos tratamentos com total visibilidade subaquática e presença do predador (Pseudo  $F_{1,24} = 97,40$ ;  $P < 0,001$  – Fig. 2). Uma quantidade significativamente menor de anfípodes se deslocou para fora da água, na presença de predador, mas com a visibilidade subaquática nula obstruída devido à ausência de luz, o que realça o papel crucial de estímulos visuais para as estratégias de anti-predação de anfípodes. Na ausência do peixe predador, a porcentagem de anfípodes fora da água não diferiu entre os tratamentos de visibilidade subaquática (Pseudo  $F_{1,24} = 0,71$ ;  $P = 0,53$  – Fig. 2).

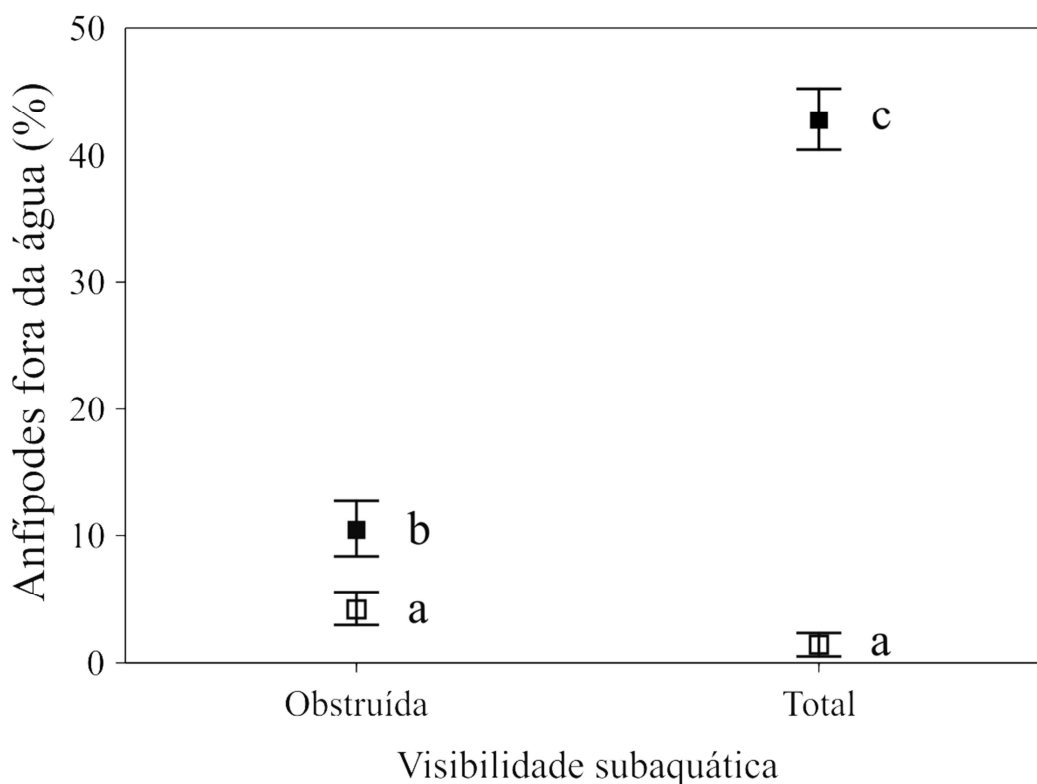


Figura 2 – Média e erro padrão de *Hyalella curvispina* (%) fora da água dos aquários, em tratamentos com diferentes condições de visibilidade subaquática e presença/ausência de *Gymnogeophagus terrapurpura*. **Quadrados brancos:** resultado da ausência de predadores, **quadrados negros:** resultado da presença de predadores. Letras distintas indicam diferenças no teste *post-hoc* entre os tratamentos.



A presença de um peixe ciclídeo *per se* aumentou a quantidade de anfípodes que deixou a água do aquário independentemente da temperatura da água. Entretanto, o deslocamento em massa de anfípodes para fora da água ocorreu quando um predador esteve presente e a água estava a 27°C (perANOVA Pseudo  $F_{1,24} = 15,45$ ;  $P < 0,001$  – Fig. 1). Na ausência de predadores, o número de anfípodes fora da água foi levemente maior a 27°C que em 19°C (mas essas médias não são estatisticamente distintas: perANOVA Pseudo  $F_{1,24} = 2,12$ ;  $P = 0,38$  – Fig. 3).

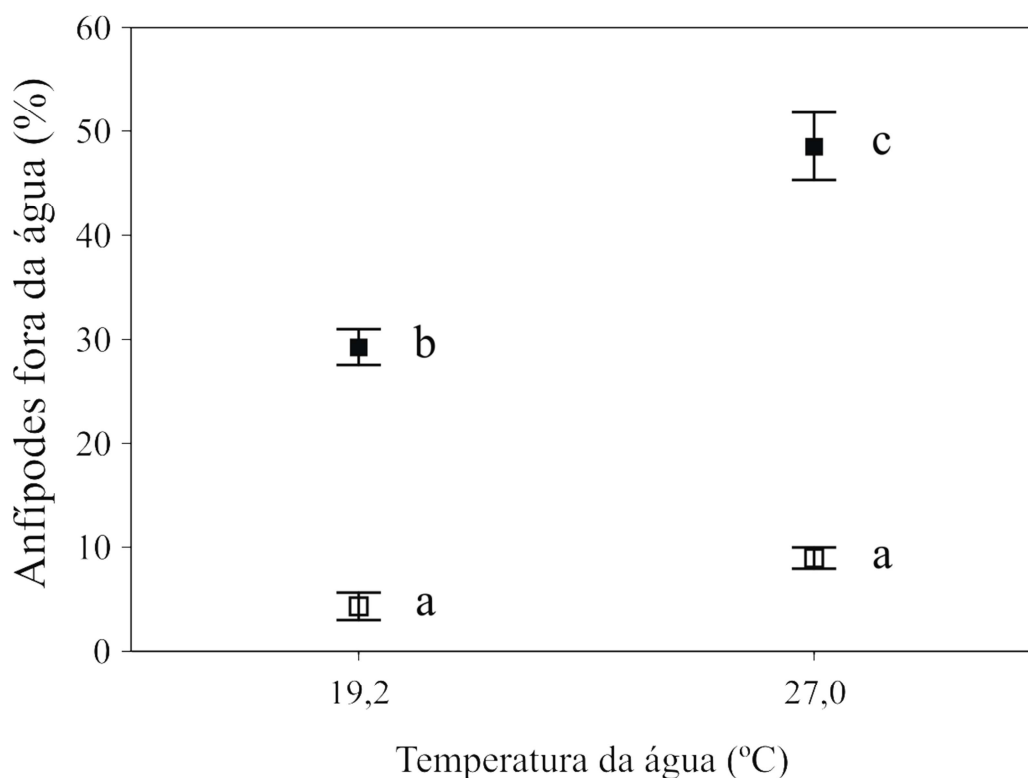


Figura 3 – Média e erro padrão de *Hyalella curvispina* (%) fora da água dos aquários, em tratamentos com diferentes condições de temperatura da água e presença/ausência de *Gymnogeophagus terrapurpura*. **Quadrados brancos:** resultado da ausência de predadores, **quadrados negros:** resultado da presença de predadores. Letras distintas indicam diferenças no teste *post-hoc* entre os tratamentos.

### Discussão

Em comum, as duas experimentações conduzidas mostraram que a maior parte dos anfípodes ao coocorrer com um peixe ciclídeo migrou para a interface água - ar, permanecendo fora do alcance de seus predadores. Essa migração de anfípodes para fora da água não foi observada na ausência de predadores, o que suporta a hipótese de que deixar a água é, de fato, um comportamento de anti-predação. Embora a saída do meio aquático possa ser interpretada como um comportamento incomum, ela pode

também ser considerada como a única estratégia que anfípodas possuíam para evitar serem consumidos, em um ambiente desprovido de refúgio subaquático. Da mesma forma, o uso de raízes de plantas terrestres por anfípodas, em regiões úmidas acima da superfície da água, deve estar associado à alta pressão predatória no meio aquático exercida tanto por peixes, quanto por larvas de anfíbios (Camacho & Thacker, 2013). De fato, a forma mais simples de presas evitarem ser detectadas e consumidas é ocupar habitats que os predadores não conseguem acessar (Lima & Dill, 1990; Abrahams et al., 2009), geralmente este habitat seguro é encontrado em áreas vegetadas (Diehl 1988), mas virtualmente qualquer objeto que confira complexidade espacial ao ambiente pode ser utilizado como refúgio (Kovalenko et al., 2012; Orrock et al., 2013; Jermacz et al., 2015). Na ausência de refúgio subaquático, se *H. curvispina* possui a capacidade de evitar espacialmente o encontro com predadores, isso pode garantir maior chance de sobrevivência, especialmente quando a ameaça de predação no ambiente aquático é alta (p. ex. Pulliam & Danielson, 1991; Figueiredo et al., 2013).

A maior parte dos anfípodas somente exibiu o comportamento de anti-predação nos tratamentos em que havia predadores e a visibilidade subaquática foi total, o que indica que estímulos visuais desencadeiam respostas de anti-predação em *H. curvispina*. De fato, a visão é responsável por uma rápida detecção das condições do ambiente circundante (Cronin, 2005), e respostas fenotípicas fisiológicas e comportamentais de presas associadas à percepção de estímulos visuais são frequentes em diversos grupos animais (Helfman, 1989; Dionne & Dodson, 2002). Neste estudo, alguns poucos anfípodas apresentaram o comportamento de anti-predação no tratamento de visibilidade obstruída, o que indica que dentro da mesma população de anfípodas pode haver indivíduos com maior plasticidade fenotípica quanto à sensibilidade a estímulos não visuais que outros (assim como há em peixes-presa, Chapman et al., 2010). Reconhecer e responder a estímulos não visuais da presença de predadores pode aumentar a probabilidade de sobrevivência de um animal, em ecossistemas, nos quais a transparência da água varia com frequência ao longo do tempo (p. ex. Wudkevich et al., 1997; Kim et al., 2009).

A temperatura afeta todos os aspectos da biologia de um organismo, desde a velocidade de reações biogeoquímicas até componentes importantes do *fitness* do indivíduo e, portanto, é uma variável com alto poder de seleção na natureza (Polčák & Gvoždík, 2014). Constatou-se, no presente estudo, que a temperatura da água pode intensificar a fuga de predadores por anfípodas. Isto porque a quantidade de anfípodas

fora da água, induzida pela presença de um predador, foi muito maior em tratamentos em que a temperatura da água a 27°C, que a 19°C. É importante notar que os mesmos comportamentos que tornam um animal menos vulnerável a predação, frequentemente reduzem a sua chance de encontrar o próprio alimento (Lima, 1998; Orrock et al., 2013). Neste estudo, se de alguma forma o aumento de temperatura da água aperfeiçoa a habilidade de fuga de *H. curvispina*, isso resultou, por um lado, na redução da taxa de consumo de presas em águas mais quentes (Abrahams et al., 2009; Pink & Abrahams, 2016), mas por outro lado, anfípodas refugiados em habitats semi-terrestres intensificam a competição por espaço e alimento, o que também poderia provocar o retorno de muitos organismos ao ambiente aquático.

Embora seja importante reconhecer que na natureza vários fatores podem interagir e moldar o comportamento de anti-predação de presas, os resultados indicam que se o comportamento de anti-predação de *H. curvispina* está intimamente relacionado com a disponibilidade de luz, estes anfípodas podem não possuir uma apropriada estratégia de defesa em condições de águas estavelmente turbidas, o que geralmente resulta em alta mortalidade de presas (Sih et al., 2010). Por outro lado, reconhecendo que alguns anfípodas foram capazes de fugir do ambiente aquático responder a presença do predador na ausência de luz, sugere-se que caso os ambientes tornem-se mais turbidos, tal como predito (IPCC, 2012), os indivíduos com maior sensibilidade a estímulos não visuais poderiam ser favorecidos por seleção natural (Thomas & Kingsolver, 2002) e tendem a prevalecer. Neste estudo, investigou-se apenas um aspecto do comportamento de anti-predação de invertebrados, e para uma total compreensão de como o resultado da interação predador-presa será afetado por variações na visibilidade subaquática e na temperatura da água, estudos adicionais ainda são necessário, inclusive para testar a hipótese supracitada, e incorporando outros mecanismos de defesa exibidos por organismos-presa. De todo modo, essa hipótese é suportada por evidências encontrada no presente estudo, mas também por resultados já reportados na literatura de inapropriadas estratégias de anti-predação levando a intensa redução do tamanho populacional de presas (Pelicice & Agostinho, 2009; Sih et al., 2010; Martin, 2014).

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## Referências

- Abrahams MV, Pink M & Klassen C 2009. Predator avoidance. In: Encyclopedia of life sciences (ELS). John Wiley & Sons Ltd, Chichester.
- Abrahams MV 2006. The physiology of antipredator behaviour: what you do with what you've got. In: Behaviour and Physiology of Fish, Fish physiology, Vol. 24. Sloman K.A, Wilson RW & Balshine S, eds., pp.79-108. Elsevier, San Diego.
- Agostinho AA, Thomaz SM, Gomes LC & Baltar SLSMA 2007. Influence of the macrophytes *Eichhornia azurea* on fish assemblage of the upper Paraná River floodplain (Brazil). *Aquat. Ecol.* 41:611–619.
- Ajemian MJ, Sohel S & Mattila J 2015. Effects of turbidity and habitat complexity on antipredator behavior of three-spined sticklebacks (*Gasterosteus aculeatus*). *Environ. Biol. Fish.* 98: 45–55.
- Allan, JD 2004. Landscapes and riverscapes: the influence of land use on stream ecosystems. *Annu. Rev. Ecol. Evol. Syst.* 35: 257-84.
- Anderson MJ 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26: 32–46.
- Brown JH, Gillooly JF, Allen AP, Savage VM & West GB 2004. Toward a metabolic theory of ecology. *Ecology* 85: 1771–1789.
- Camacho FA & Thacker RW 2013. Predator cues alter habitat use by the amphipod *Hyalella azteca* (Saussure). *Freshwater Sci.* 32: 1148-1154.
- Cerri RD 1983. The effect of light intensity on predator and prey behaviour in cyprinid fish: factors that influence prey risk. *Anim. Behav.* 31: 736–42.
- Chapman BB, Morrell LJ, Tosh CR & Krause J 2010. Behavioural consequences of sensory plasticity in guppies. *Proc. R. Soc. Lond. B Biol. Sci.* 277: 1395–1401.

- Cronin TW 2005. The role of vision in predator–prey interactions. In: Ecology of Predator–Prey Interactions. Barbosa P & Castellanos I, eds., pp. 105–138. Oxford University Press, New York.
- Darwin CR 1859. On the origin of species by means of natural selection. Murray, London. 502 pp.
- DeWitt TJ & Langerhans RB 2004. Integrated solutions to environmental heterogeneity: theory of multimoment reaction norms. In: Phenotypic Plasticity: Functional and Conceptual Approaches. DeWitt TJ & Scheiner SM, eds., pp. 98–111, Oxford University Press, New York.
- Diehl S 1988. Foraging efficiency of three freshwater fishes: effects of structural complexity and light. *Oikos* 53: 207–214.
- Dionne M & Dodson JJ 2002. Impact of exposure to a simulated predator (*Mergus merganser*) on the activity of juvenile Atlantic salmon (*Salmo salar*) in a natural environment. *Can. J. Zool.* 80: 2006–2013.
- Doyle SR & Momo FR 2009. Effects of body weight and temperature on the metabolic rate of *Hyaella curvispina* Shoemaker, 1942 (Amphipoda). *Crustaceana* 82: 1423–1439.
- Effertz C & von Elert E 2014. Light intensity controls anti-predator defences in *Daphnia*: the suppression of life-history changes. *Proc. R. Soc. Lond. B Biol. Sci* 281: 20133250.
- Espínola LA, Minte-Vera CV & Júlio HF 2010. Invasibility of reservoirs in the Paraná Basin, Brazil, to *Cichla kelberi* Kullander and Ferreira, 2006. *Biol. Invasions* 12: 1873–1888.
- Ferreiro N, Feijoó C, Giorgi A & Rosso J 2014. Macroinvertebrates select complex macrophytes independently of their body size and fish predation risk in a Pampean stream. *Hydrobiologia* 740: 191–205.
- Galassi ME, Franceschini MC & Neiff AP 2006. Population Estimates of *Hyaella curvispina* Shoemaker (Amphipoda) in Aquatic Vegetation of Northeastern Argentinian Ponds. *Acta Limnologica Brasiliensia* 18: 101–108.
- Gerald GW 2008. Feign versus flight: influences of temperature, body size and locomotor abilities on death feigning in neonate snakes. *Anim. Behav.* 75: 647–654.
- Ghalambor CK, McKay JK, Carroll SP & Reznick DN 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Funct. Ecol.* 21: 394–407.

- Godin JGJ & Crossman SL 1994. Hunger-dependent predator inspection and foraging behaviours in the threespine stickleback (*Gasterosteus aculeatus*) under predation risk. *Behav. Ecol. Sociobiol.* 34: 359-366.
- Helfman GS 1989. Threat-sensitive predator avoidance in damselfish-trumpetfish interactions. *Behav. Ecol. Sociobiol.* 24: 47–58.
- Helfman GS, Collette BB, Facey DE & Bowen BW 2009. *The diversity of fishes: biology, evolution, and ecology*. Wiley-Blackwell, Oxford. 720 pp.
- Higham TE, Stewart WJ & Wainwright PC 2015. Turbulence, temperature, and turbidity: The ecomechanics of predator–prey interactions in fishes. *Integr. Comp. Biol.* 55: 6–20.
- Holmlund MB, Peterson CH & Hay ME 1990. Does algal morphology affect amphipod susceptibility to fish predation? *J Exp Mar Biol Ecol* 139: 65–83.
- Jermacz Ł, Dzierżyńska A, Poznańska A & Kobak J 2015. Experimental evaluation of preferences of an invasive Ponto-Caspian gammarid *Pontogammarus robustoides* (Amphipoda, Gammaroidea) for mineral and plant substrata. *Hydrobiologia* 746: 209–221.
- Jokela A, Arnott SE, Beisner BE. 2013. Influence of light on the foraging impact of an introduced predatory cladoceran, *Bythotrephes longimanus*. *Freshwater Biol.* 58: 1946-1957.
- Kim J-W, Brown GE, Dolinsek IJ, Brodeur NN, Leduc AOHC & Grant JWA 2009. Combined effects of chemical and visual information in eliciting antipredator behaviour in juvenile Atlantic salmon *Salmo salar*. *J. Fish Biol.* 74: 1280–1290.
- Kobak J & Ryńska A. 2014. Environmental factors affecting behavioural responses of an invasive bivalve to conspecific alarm cues. *Anim. Behav.* 96: 177-186.
- Kovalenko KE, Thomaz SM & Warfe DM 2012. Habitat complexity: approaches and future directions. *Hydrobiologia* 685: 1–17.
- Lampert W 1993. Ultimate causes of diel vertical migration of zooplankton: new evidence for the predatoravoidance hypothesis. *Archiv für Hydrobiologie* 39: 79–88.
- Lima SL & Dill LM 1990. Behavioral decisions made under the risk of predation: a review and prospectus. *Can. J. Zool.* 68: 619–640.
- Loureiro M, Zarucki M, Malabarba LR & González-Bergonzoni I 2016. A new species of *Gymnogeophagus* Miranda Ribeiro from Uruguay (Teleostei: Cichliformes). *Neotrop. Ichthyology* 14: e150082.
- Malavasi S, Cipolato G, Cioni C, Torricelli P, Alleva E, Manciocco A & Toni M. 2013. Effects of temperature on the antipredator behaviour and on the cholinergic

- expression in the European sea bass (*Dicentrarchus labrax* L.) juveniles. *Ethology* 119: 592-604.
- Martin CW 2014. Naïve prey exhibit reduced antipredator behavior and survivorship. *PeerJ* 2: e665.
- McArdle BH & Anderson MJ 2001. Fitting multivariate models to community data: a comment on distance-based redundancy analysis. *Ecology* 82: 290–297.
- McKenzie D & Claireaux G 2010. The effects of environmental factors on the physiology of aerobic exercise. In: *Fish locomotion: an eco-ethological perspective*. Domenici P & Kapoor BD, eds., pp. 296–332. Science Publishers, Enfield, USA.
- Mori A & Burghardt GM 2001. Temperature effects on anti-predator behaviour in *Rhabdophis tigrinus*, a snake with toxic nuchal glands. *Ethology* 107: 795–811.
- Moss B, Kosten S, Meerhoff M, Battarbee RW, Jeppesen E, Mazzeo N, Havens K, Lacerot G, Liu Z, De Meester L, Paerl H & Scheffer M 2011. Allied attack: climate change and eutrophication. *Inland Waters* 1:101–105.
- Öhlund G, Hedström P, Norman S, Hein CL & Englund G 2015. Temperature dependence of predation depends on the relative performance of predators and prey. *Proc. R. Soc. Lond. B Biol. Sci.* 282: 20142254.
- Orrock JL, Preisser EL, Grabowski JH & Trussell GC 2013. The cost of safety: Refuges increase the impact of predation risk in aquatic systems. *Ecology* 94: 573–579.
- Pangle KL & Peacor SD 2009. Light-dependent predation by the invertebrate planktivore *Bythotrephes longimanus*. *Can. J. Fish. Aquat. Sci.* 66: 1748–1757.
- Pelicice FM & Agostinho AA 2009. Fish fauna destruction after the introduction of a non-native predator (*Cichla kelberi*) in a Neotropical reservoir. *Biol. Invasions* 11: 1789–1801.
- Pink M & Abrahams MV. 2016. Temperature and its impact on predation risk within aquatic ecosystems. *Can. J. Fish. Aquat. Sci.* 73:1–8.
- Polčák D & Gvoždík L 2014. Should I stay or should I go? The influence of temperature and sex on predator-induced responses in newts. *Anim. Behav.* 89: 79-84.
- Powers SP & Kittinger JN 2002. Hydrodynamic mediation of predator–prey interactions: Differential patterns of prey susceptibility and predator success explained by variation in water flow. *J. Exp. Mar. Bio. Ecol.* 273: 171–187.
- Pulliam HR & Danielson BJ 1991. Sources, sinks, and habitat selection: a landscape perspective on population dynamics. *Am Nat* 137: 50–66.

- Sih A, Bolnick DI, Luttbeg B, Orrock JL, Peacor SD, Pintor LM, Preisser E., Rehage JS & Vonesh JR 2010. Predator-prey naïveté, antipredatory behavior, and the ecology of predator invasions. *Oikos* 119: 610–621.
- Sih A, Ferrari MCO & Harris DJ 2011. Evolution and behavioural responses to human-induced rapid environment change. *Evolutionary Applications* 4: 367-387.
- Smee DL, Ferner MC & Weissburg MJ 2010. Hydrodynamic sensory stressors produce nonlinear predation patterns. *Ecology* 91: 1391-1400.
- Smith LA & Webster MM 2015. *Gammarus pulex* show a grouping response to conspecific injury cues but not to predator kairomones. *Behav. Ecol.* 26: 1188-1195.
- Svensson AM, Eklöf J, Skals N & Rydell J 2003. Light dependent shift in the anti-predator response of a pyralid moth. *Oikos* 101: 239–246.
- Tátrai I, Boros G, György ÁI, Mátyás K, Korponai J, Pomogyi P, Havasi M & Kucserka T 2009. Abrupt shift from clear to turbid state in a shallow eutrophic, biomanipulated lake. *Hydrobiologia* 620: 149-161.
- Teixeira-de Mello F, Meerhoff M, Pekcan-Hekim Z & Jeppesen E 2009. Substantial differences in littoral fish community structure and dynamics in subtropical and temperate shallow lakes. *Freshw. Biol.* 54: 1202–1215.
- Thomas SC & Kingsolver JG 2002. Natural selection: responses to current (anthropogenic) environmental changes. In: *Encyclopedia of life sciences*. John Wiley & Sons, Chichester.
- van der Sluijs IS, Gray M, Amorim MCP, Barber I, Candolin U, Hendry AP, Krahe R, Maan ME, Utne-Palm AC, Wagner HJ & Wong BBM 2011. Communication in troubled waters response of fish communication systems to changing environments. *Evol. Ecol.* 25: 623–640.
- van Uitregt VO, Alton LA, Heiniger J & Wilson RS 2016. Warmer temperatures reduce the costs of inducible defenses in the marine toad, *Rhinella marinus*. *J Comp Physiol B* 186: 123-130.
- Wudkevich K, Wisenden BD, Chivers DP & Smith RJF. 1997. Reactions of *Gammarus lacustris* to chemical stimuli from natural predators and injured conspecifics. *J Chem Ecol.* 23: 1163–1173.
- Yorzinski JL, Chisholm S, Byerley SD, Coy JR, Aziz A, Wolf JA & Gnerlich AC (2015) Artificial light pollution increases nocturnal vigilance in peahens. *PeerJ* 3: e1174



III

# Turbidity amplifies the non-lethal effects of predation and affects the foraging success of characid fish shoals

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## SUMMARY

1. In aquatic systems, many species rely primarily on visual cues to choose optimal foraging sites, capture prey and avoid potential threats. Increases in the turbidity of water reduce visibility and impede animals in determining the precise location of both predators and food. How individuals balance foraging decisions with anti-predator behaviour in turbid environments is not well understood.
2. We tested the effects of turbidity and predation risk on the foraging behaviour and feeding of an invertivorous fish, *Moenkhausia forestii* (Characidae), using a mesocosm experiment with a 2 × 2 design, crossing water clarity (clear versus turbid) with predation risk as reflected by the presence or absence of the piscivorous wolf-fish *Hoplias aff. malabaricus* (Erythrinidae). We predicted that turbidity and predator presence would interact additively to reduce foraging rates, and that increased turbidity or predator presence would result in disproportionate food partitioning among shoal members.
3. The combination of high turbidity and predator presence resulted in a significant reduction in prey consumption. Foraging success exhibited a skewed distribution in the turbid treatment, i.e. there was a decreased evenness of food partitioning within shoals. Hence, both turbidity and predator presence affect the prey consumption and foraging behaviour of invertivorous fish, with turbidity amplifying the non-lethal effects of predation risk on foraging success.
4. Our results imply that turbidity-induced visual obstruction amplifies the negative effects of predator presence on invertivorous fish feeding behaviour, resulting in higher prey survival. Also, our finding that food intake by an intermediate consumer decreased in turbid water with a top predator lends no support to the hypothesis that intermediate consumer fish reduce their anti-predator behaviour in turbid water.
5. From a management perspective, our findings suggest that the oligotrophication of aquatic systems could dramatically increase predation on basal prey organisms naturally adapted to turbid waters, and reduce within-shoal differences in feeding behaviour.

**Keywords:** anti-predator behaviour, foraging behaviour, individual specialisation, predator–prey interaction, shoaling, turbidity, visually oriented predation

## Introduction

Predators can affect population and community processes directly through the consumption of prey or indirectly by inducing changes in prey behaviour in

response to predation risk (Estes *et al.*, 2011; Forbes & Hammill, 2013). The total impact of predators (e.g. piscivorous fish) can be partitioned into the lethal effects of predation and predator-induced avoidance by prey (non-lethal) (Åbjörnsson, Brönmark & Hansson, 2002). In

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both cases, impacts propagate to different trophic levels of food webs, and hence have ecological consequences (Cresswell & Quinn, 2013; Des Roches *et al.*, 2013). Among potential cascading effects, the most unclear are non-lethal, behaviourally mediated effects, such as avoidance of areas of perceived risk. In addition, non-lethal effects might influence the ability of prey to acquire food (Ahlgren, Åbjörnsson & Brönmark, 2011), the quality and quantity of resources consumed (Stuart-Smith *et al.*, 2007) and prey habitat use (Figueiredo, Mormul & Thomaz, 2015a), in a way analogous to direct predation (e.g. Turner, 1997).

In aquatic ecosystems, turbidity has been identified as a key factor regulating interactions between and within species, and in shaping community structure (Horppila & Liljendahl-Nurminen, 2005; Pangle *et al.*, 2012; Figueiredo, Mormul & Benedito, 2015b). Turbidity directly affects fitness via trade-offs between acquiring food and avoiding predators (Lima & Dill, 1990; Leahy *et al.*, 2011), and by disrupting the quality and amount of visual information available to animals. For example, when suspended sediment enters an aquatic system from the catchment or tributaries, it reduces underwater visibility (De Robertis *et al.*, 2003) and impedes visual foragers from locating food and predators (Ferrari, Lysak & Chivers, 2010a). The importance of turbidity for predator-prey interactions has increased and will likely continue to increase due to climatic changes (Trenberth *et al.*, 2007; Thompson *et al.*, 2013). By contrast, the construction of cascading reservoirs might lead to higher retention of suspended solids and nutrients resulting in increased water transparency (Roberto, Santana & Thomaz, 2009). Such oligotrophication could facilitate prey detection by visual predators, intensifying predator-prey interactions.

Under turbid conditions, visually feeding organisms such as fish may compensate for the reduction in visual information by utilising alternative sensory modalities and recognising predation threats through non-visual cues from predators (Chapman *et al.*, 2010; Leahy *et al.*, 2011). Prey organisms can detect predator alarm cues even at great distances (Ferrari, Wisenden & Chivers, 2010b), thus decreasing the efficacy of non-visual cues in accurately locating a predator (Brown & Magnavacca, 2003). Uncertainty about the precise location of a predation threat could increase a predator's non-lethal effect, resulting in increased immobility and lower food intake by prey (Pangle *et al.*, 2012; Swanbrow Becker & Gabor, 2012). However, as predicted by the 'contrast degradation' model, high turbidity may result in the reduced visual range of a predator that can identify prey at greater distances (e.g. piscivorous fish) compared to a predator

that can only identify food at short distances (e.g. invertivorous fish) (Duntley, 1962). Thus, turbid water may homogenise the distance of prey detection regardless of prey size (Utne-Palm, 2002; De Robertis *et al.*, 2003). Accordingly, the visual refuge provided by suspended particles may lead to a reduction in anti-predator behaviour in prey fish (Ajemian, Sohel & Mattila, 2015), leading to an increase in their rate of food consumption.

A common behavioural strategy to reduce individual predation risk is group formation (Krause & Ruxton, 2002). Although group formation has anti-predator benefits, it also increases competition (Pitcher, 1986). The precise costs and benefits of group membership depend on a number of factors, including position in the shoal (Krause & Ruxton, 2002). Fish swimming at the front of the shoal are at greater risk of predation, and behaviourally bold, risk-prone individuals tend to occupy these positions (Ward *et al.*, 2004). Due to these kinds of trade-offs, groups are rarely homogenous in composition with respect to individual behaviour. Individual behavioural variation such as differences in boldness can have differential fitness consequences depending on environmental conditions such as water transparency and predator regime. For example, given the potential for an interaction between individual behavioural type and the response to altered sensory conditions such as turbidity, we may expect shifts in the distribution of foraging success within groups, as individuals vary in their behavioural responses to turbid conditions.

Using a mesocosm experiment manipulating clear and turbid water conditions combined with the presence and absence of a top predator (*Hoplias aff. malabaricus* Bloch, 1794), we evaluated the foraging success of shoals of characid fish (*Moenkhausia forestii* Benine, Mariguela & Oliveira, 2009). Our main focus was on investigating trade-offs between prey consumption of chironomid (Diptera) (larvae of *Chironomus sancticaroli* Strixino & Strixino, 1981) and predator avoidance. We also evaluated the effects of turbidity and predator presence on food partitioning (evenness) within the shoal. We hypothesised that (i) both turbidity and predator presence reduce the prey consumption of shoals, and interact additively, and that (ii) increased turbidity or predator presence results in uneven food partitioning among shoal members.

## Methods

### *Experimental design*

We conducted a microcosm experiment with invertebrate midges (larval *C. sancticaroli*) as basal prey,

subjected to predation by a shoal of the small fish *M. forestii*. We combined turbidity and predator presence (*H. aff. malabaricus*) to create four treatments: (i) control – clear water and absence of predator; (ii) increased turbidity treatment – turbid water and absence of predator; (iii) predator treatment – clear water and presence of predator and (iv) interactive treatment – turbid water and presence of predator. Each treatment was replicated six times in rectangular aquaria (width = 25 cm, length = 30 cm, water depth = 40 cm, water volume = 30 L), totalling 24 experimental units. The experiment was conducted in the field station at the Centre for Research in Limnology, Ichthyology, and Aquaculture (Paraná, Brazil, 22°45'S; 53°15'W).

We chose *M. forestii* as an intermediate consumer because it represents a typical Neotropical fish. The ichthyofauna in this region is primarily composed of small-sized fishes, which use vision as their primary source of environmental information (e.g. Agostinho *et al.*, 2007). In each experimental unit, we added four individuals of *M. forestii*, based on the schooling behaviour of this fish under natural conditions. Twenty *C. sancticaroli* larvae, common components in the diet of *M. forestii* (e.g. Peretti & Andrian, 2004) and abundant in the Paraná River floodplain (Thomaz *et al.*, 2008), were used as basal prey. A native piscivorous *H. aff. malabaricus* was used in the predator presence treatments. *H. aff. malabaricus* is a sit-and-wait predator, most active during twilight hours (e.g. Fraser & Gilliam, 1992; Botham & Krause, 2005), the period when *M. forestii* is also most active (Pelicice & Agostinho, 2006). Moreover, *M. forestii* and *H. aff. malabaricus* often co-occur in Paraná River floodplain environments (e.g. Oliveira *et al.*, 2001).

Sediment-dwelling *C. sancticaroli* individuals were cultivated in laboratory tanks in the State University of Maringá. *M. forestii* were collected from floodplain lakes using Plexiglas traps (minnow trap type, 30 × 30 cm wide, 30 cm tall; with a 10 mm wide, 220 mm tall funnel slot on each of four sides), light traps and cast nets in near-shore areas with littoral vegetation. *H. aff. malabaricus* were collected by angling in the river and shallow lakes of the Paraná River floodplain. Sediment clay was also collected at the same location for use in the treatments. The clay was sun dried and macerated to a fine powder. Predators [standard length (SL) = 175.0 ± 27.0 mm] and intermediate consumer fish (SL = 30.9 ± 1.9 mm) were kept separately for 6 days in two 250 L tanks with controlled temperature (23 ± 1 °C), and treated with fungicide and bactericide solution to prevent infections. Both fish species were acclimated for 72 h to the experimental aquaria and water temperature before

the experiment. During this period, *M. forestii* was not fed, while *H. aff. malabaricus* was regularly fed to satiation with live food. Feeding to satiation was carried out to avoid the consumption of *M. forestii* by *H. aff. malabaricus* during the experimental period, because we did not include refuges or divided enclosures to avoid predation. Invertebrates (SL = 6.2 ± 0.8 mm) were kept in 600 mL bottles (20 individuals per bottle) containing river water. The experimental period lasted 4 hours.

Each experimental aquarium received water (temperature ~ 26 °C; turbidity ~ 3 NTU) from the Paraná River filtered through a plankton net (0.02 mm mesh). Control treatments received filtered clear water treatment (3 NTU). Turbid water treatments received 30 g of clay, resulting in an average turbidity of 80 NTU, to simulate conditions of turbidity during the rainy season in the Paraná River floodplain (Rocha *et al.*, 2009). On the basis of a previous study (Figueiredo *et al.*, 2015b), we estimated the distance that visual predators could see at both turbidity levels, converting turbidity to Secchi disk depth according to Padial & Thomaz (2008). After conversion, three and 80 NTU corresponded to Secchi disk depths of 1.56 and 0.15 m, respectively. Four hours pilot trials showed that turbidity variation from the start to the end of the experimental period was less than 10% over 4 h, a turbidity decay that is considered experimentally acceptable.

At the end of the experiment, all *M. forestii* were removed, anaesthetised, and fixed in formaldehyde solution for subsequent stomach analysis, following the 'Ethical Principles in Animal Research' guidelines adopted by the Brazilian College of Animal Experimentation (COBEA). Predators were returned to the wild after being held in aquaria. The surviving *C. sancticaroli* larvae were returned to the culture tanks.

#### Data analysis

To test our first hypothesis that turbidity and predator presence interact additively to reduce foraging rate, we performed a two-way ANOVA with the percentage of *C. sancticaroli* consumed by the shoal as the response variable. The response variable was arcsine transformed to comply with the assumptions of normality and homoscedasticity (Zar, 2010). To test our second hypothesis that increased turbidity or predator presence results in disproportionate food partitioning by the shoal, we carried out a two-way ANOVA with evenness of individual feeding as the response variable. Feeding evenness was based on the Pielou's evenness index, where the amount of *C. sancticaroli* in each invertivorous fish

stomach was used to calculate the proportion of prey consumed by each fish within the shoal. Evenness values range between 0 and 1, with higher values indicating a lower degree of inter-individual variation. Turbidity (clear water/turbid water) and predator presence (presence/absence) treatments were considered as independent variables. When significant main effects or interactions were revealed in ANOVA, Tukey's *post hoc* comparisons were done. All tests were performed using the software *Statistica* (Statsoft, 2005).

## Results

The presence of a predator decreased prey consumption by the shoal ( $F_{1, 20} = 113.81$ ;  $P < 0.01$ : Fig. 1), as did increased turbidity ( $F_{1, 20} = 47.50$ ;  $P < 0.01$ : Fig. 1). However, the strongest reduction in prey consumption by the shoal was found in the interaction between turbidity and predator presence ( $F_{1, 20} = 37.65$ ;  $P < 0.01$ : Fig. 1). These results indicate that although prey consumption by shoals was reduced when a predator was present (on average 20%), the combined effect of increased turbidity and predator presence resulted in a 77% decrease of prey consumption by the shoal. Indeed, in the absence of a predator, prey consumption did not differ between clear and turbid water. It is worth noting that during our experiments predators remained immobile 95% of the time, similar to their behaviour in the field, and did not consume *M. forestii* in any trial. Additionally, in the clear water treatment, *M. forestii* shoals were always situated on the opposite side of the aquarium from the predator.

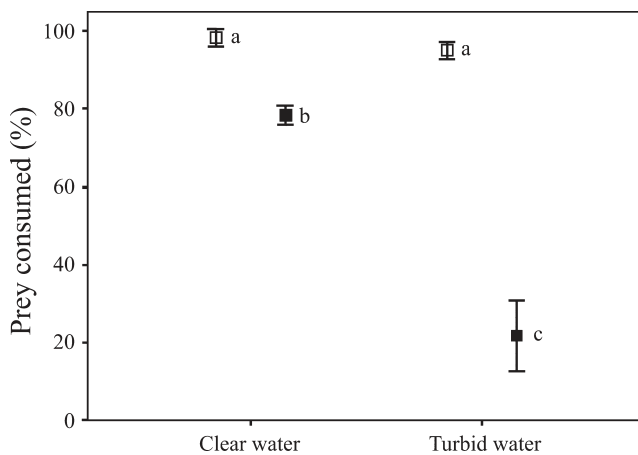


Fig. 1 Mean percentage of *Chironomus sancticarloi* larvae consumption ( $\pm$  standard error) in different turbidity conditions, with (dark squares) and without (clear squares) predators. Differences in Tukey's *post hoc* tests are presented with different letters.

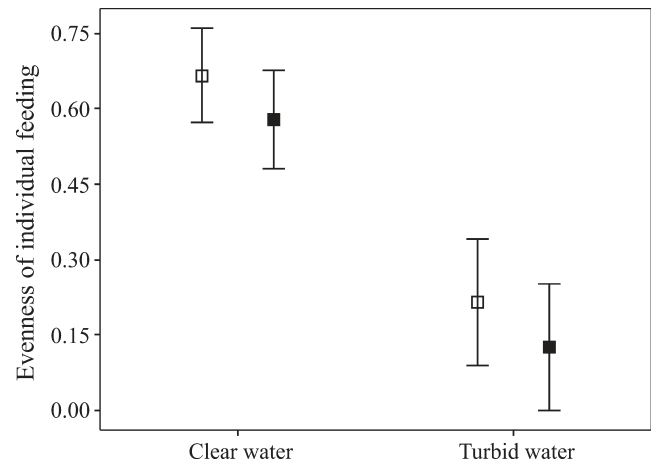


Fig. 2 Mean evenness of individual feeding ( $\pm$  standard error) in different turbidity conditions, with (dark squares) and without (clear squares) top predators. Evenness index values range between 0 and 1, with higher values indicating a lower degree of inter-individual variation.

Turbidity decreased the evenness of food partitioning within shoals ( $F_{1, 20} = 9.88$ ;  $P < 0.01$ : Fig. 2), implying that increased turbidity amplifies within-shoal differences. In clear water, invertebrates were consumed in a similar proportion among individuals, but in turbid water only a few individuals (usually one) consumed prey. Predator presence and the interaction between turbidity and predator presence did not change the evenness of individual feeding ( $F_{1, 20} = 1.59$ ;  $P = 0.22$ ;  $F_{1, 20} = 0.29$ ;  $P = 0.59$ , respectively). Therefore, food partitioning within fish shoal shifted from feeding evenness in clear water to a pattern of feeding unevenness with increasing turbidity, independent of predator presence.

## Discussion

Using an experimental setup to test four different ecological scenarios that occur in floodplains (e.g. in the Paraná River), we found that turbidity and the presence of a top predator interact to amplify basal prey survival, due to a strong decrease in the foraging success of an intermediate predator (*M. forestii*). Moreover, turbidity, but not predator presence, decreased the evenness of food partitioning within *M. forestii* shoals.

Combinations between clear/turbid water and presence/absence of a top predator were expected to shift the perception of the predation-growth trade-off for prey fish, influencing their foraging intake and feeding behaviour. In the clear water and predator absent treatments, our findings showed that invertebrate populations are strongly affected by predation pressure by the shoals, probably due to the absence of both environmental



(turbidity) and biological (predation risk) factors to limit the predatory potential of *M. forestii*. Perceiving that no threat is present, in clear water these fish can easily find and feed on *C. sancti-caroli* larvae. Thus, we suggest that in a scenario where the water is transparent and piscivores are absent, prey population size may undergo a catastrophic reduction.

In turbid water conditions without predators, prey consumption did not differ from the clear water/no predator control, although consumption was slightly lower in the turbid treatment. However, the lack of a significant turbidity effect should be interpreted with caution. The 4 h experimental period resulted in prey depletion greater than 50%, which might have had knock-on effects on foraging behaviour (Båmstedt *et al.*, 2000). Alternatively, it is possible that invertivorous fish, compensating for the decrease in visual detection by predators, increased foraging activity and prey consumption in the turbidity treatments (Webster *et al.*, 2007; Wishingrad *et al.*, 2015). Other alternative explanations are that *M. forestii* found invertebrates prey using non-visual cues (Chapman *et al.*, 2010; Schwalbe, Bassett & Webb, 2012), or that under turbid conditions *C. sancti-caroli* has no efficient anti-predator behaviour.

Interestingly, we found lower prey consumption by the shoal when the predator was present in clear water than in the absence of predator in turbid water, highlighting the role of indirect effects of predation on foraging dynamics. This pattern may be driven by anti-predator behaviour. Under perceived predation risk, intermediate consumer fish need to divide their attention between locating food and avoiding predator encounters (e.g. Ferrari *et al.*, 2014). Moreover, our observations revealed that after visual perception of the predator, the *M. forestii* shoal remained on the opposite side of the aquarium from the predator (at least in clear water). This avoidance behaviour might create a refuge zone for invertebrate prey. A similar anti-predator behaviour has been reported for another characid species (Kovalenko *et al.*, 2010).

In the combined high turbidity and predator present treatment, we recorded a substantial reduction in invertebrate consumption compared to all other treatments. This pattern was most likely driven by an increased degree of uncertainty of predator location (Higham, Stewart & Wainwright, 2015), combined with a reduction in foraging efficiency due to altered sensory conditions. An increased frequency or duration of encountering turbid conditions in high predation environments might also affect the fitness of *M. forestii*. To address this conjecture, future work should focus on

long-term responses in foraging performance of aquatic animals to turbidity pulses (Chapman *et al.*, 2010).

Our findings that food intake by an intermediate consumer decreased in turbid water with a top predator lends no support to the hypothesis that intermediate consumer fish reduce their anti-predator behaviour in turbid water (Abrahams & Kattenfeld, 1997; Pangle *et al.*, 2012; Ajemian *et al.*, 2015). This hypothesis assumes that reduced anti-predator behaviour and visual risk perception should result in higher or similar ingestion of invertebrates in turbid compared to clear waters. Conversely, our results indicated that fish shoals exhibited stronger predator avoidance behaviour in turbid than in clear water (Golub, Vermette & Brown, 2005; Swanbrow Becker & Gabor, 2012). We suggest that turbidity-induced visual obstruction amplifies the negative effects of predator presence on intermediate consumer fish feeding behaviour, ultimately resulting in higher prey survival. From a management perspective, this finding implies that oligotrophication of aquatic systems (like observed in the Paraná River) could dramatically increase predation on basal prey organisms naturally adapted to turbid waters (e.g. Pelicice & Agostinho, 2009).

In clear water, invertebrates were consumed equally among individuals within shoals, whereas only a few fish from each shoal consumed invertebrates in turbid water. There are two potential explanations for this finding: (i) individuals differed behaviourally in response to turbid conditions or (ii) the capacity to switch between alternative sensory modalities varied between individuals (Carter *et al.*, 2010). Individual behavioural differences may impact foraging efficiency under conditions of uncertainty, as movement under these conditions is risky (Leahy *et al.*, 2011). This explanation would be better supported if we had found an effect of predator presence on feeding evenness within shoals. Therefore, we suggest that exposure to a turbid environment potentially led individuals to switch to an alternative sensory modality. However, individuals may differ in their foraging performance under these conditions due to variation in their capacity to effectively utilise alternative sensory modalities to locate food. In this case, individual variation may have affected feeding evenness. Notwithstanding, oligotrophication could increase feeding evenness, homogenising individual variation. Future work could test these hypotheses by individually assaying fish for risk-taking behaviour and performance under altered sensory conditions prior to the shoaling trials.

In conclusion, we show that both variation in environmental (turbidity) and ecological conditions (predation)

have consequences for the foraging behaviour and feeding outcomes of invertivorous fish. Given that many future scenarios of environmental change predict increased turbidity in freshwater habitats, and that massive constructions of cascading reservoirs have driven oligotrophication in rivers, understanding the behavioural responses of major players in aquatic ecosystems and their concomitant fitness implications will provide important information about the potential ecological consequences of environmental changes.

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### References

- Åbjörnsson K., Brönmark C. & Hansson L.A. (2002) The relative importance of lethal and non-lethal effects of fish on insect colonisation of ponds. *Freshwater Biology*, **47**, 1489–1495.
- Abrahams M. & Kattenfeld M. (1997) The role of turbidity as a constraint on predator-prey interactions in aquatic environments. *Behavioral Ecology and Sociobiology*, **40**, 169–174.
- Agostinho A.A., Thomaz S.M., Gomes L.C. & Baltar S.L.S.M.A. (2007) Influence of the macrophytes *Eichhornia azurea* on fish assemblage of the upper Paraná River floodplain (Brazil). *Aquatic Ecology*, **41**, 611–619.
- Ahlgren J., Åbjörnsson K. & Brönmark C. (2011) The influence of predator regime on the behaviour and mortality of a freshwater amphipod, *Gammarus pulex*. *Hydrobiologia*, **67**, 139–149.
- Ajemian M.J., Sohel S. & Mattila J. (2015) Effects of turbidity and habitat complexity on antipredator behavior of three-spined sticklebacks (*Gasterosteus aculeatus*). *Environmental Biology of Fishes*, **98**, 45–55.
- Båmstedt U., Gifford D.J., Irigoien X., Atkinson A. & Roman M. (2000) Feeding. In: *ICES Zooplankton Methodology Manual*. (Eds R.P. Harris, P.H. Wiebe, J. Lenz, H.R. Skjoldal & M. Huntley), pp. 297–400. Academic Press, New York.
- Botham M.S. & Krause J. (2005) Shoals receive more attacks from the wolf-fish (*Hoplias malabaricus* Bloch, 1974). *Ethology*, **111**, 881–890.
- Brown G.E. & Magnavacca G. (2003) Predator inspection behaviour in a characin fish: an interaction between chemical and visual information? *Ethology*, **109**, 739–750.
- Carter M.W., Shoup D.E., Dettmers J.M. & Wahl D.H. (2010) Effects of turbidity and cover on prey selectivity of adult smallmouth bass. *Transactions of the American Fisheries Society*, **139**, 353–361.
- Chapman B.B., Morrell L.J., Tosh C.R. & Krause J. (2010) Behavioural consequences of sensory plasticity in guppies. *Proceedings of the Royal Society B: Biological Sciences*, **277**, 1395–1401.
- Cresswell W. & Quinn J.L. (2013) Contrasting risks from different predators change the overall non-lethal effects of predation risk. *Behavioral Ecology*, **24**, 871–876.
- De Robertis A., Ryer C.H., Veloza A. & Brodeur R.D. (2003) Differential effects of turbidity on prey consumption of piscivorous and planktivorous fish. *Canadian Journal of Fisheries and Aquatic Sciences*, **60**, 1517–1526.
- Des Roches S., Shurin J.B., Schluter D. & Harmon L.J. (2013) Ecological and evolutionary effects of stickleback on community structure. *PLoS ONE*, **8**, e59644.
- Duntley S.Q. (1962) Underwater visibility. In: *The sea. Vol. 1. Physical Oceanography*. (Ed M.N. Hill), 452–455. John Wiley and Sons, London.
- Estes J.A., Terborgh J., Brashares J.S., Power M.E., Berger J., Bond W.J. *et al.* (2011) Trophic Downgrading of Planet Earth. *Science*, **333**, 301–306.
- Ferrari M.C.O., Lysak K.R. & Chivers D.P. (2010a) Turbidity as an ecological constraint on learned predator recognition and generalization in a prey fish. *Animal Behaviour*, **79**, 515–519.
- Ferrari M.C.O., Ranåker L., Weinersmith K.L., Young M.J., Sih A. & Conrad J.L. (2014) Effects of turbidity and an invasive waterweed on predation by introduced largemouth bass. *Environmental Biology of Fishes*, **97**, 79–90.
- Ferrari M.C.O., Wisenden B.D. & Chivers D.P. (2010b) Chemical ecology of predator-prey interactions in aquatic ecosystems: a review and prospectus. *Canadian Journal of Zoology*, **88**, 698–724.
- Figueiredo B.R.S., Mormul R.P. & Thomaz S.M. (2015a) Swimming and hiding regardless of the habitat: prey fish do not choose between a native and a non-native macrophyte species as a refuge. *Hydrobiologia*, **746**, 285–290.
- Figueiredo B.R.S., Mormul R.P. & Benedito E. (2015b) Structural complexity and turbidity do not interact to influence predation rate and prey selectivity by a small visually feeding fish. *Marine and Freshwater Research*, **66**, 170–176.

- Forbes C. & Hammill E. (2013) Fear in the dark? Community-level effects of non-lethal predators change with light regime. *Oikos*, **122**, 1662–1668.
- Fraser D.F. & Gilliam J.F. (1992) Nonlethal impacts of predator invasion: facultative suppression of growth and reproduction. *Ecology*, **73**, 959–970.
- Golub J.L., Vermette V. & Brown G.E. (2005) Response to conspecific and heterospecific alarm cues by pumpkinseeds in simple and complex habitats: field verification of an ontogenetic shift. *Journal of Fish Biology*, **66**, 1073–1081.
- Higham T.E., Stewart W.J. & Wainwright P.C. (2015) Turbulence, temperature, and turbidity: the ecomechanics of predator–prey interactions in fishes. *Integrative and Comparative Biology*, **55**, 6–20.
- Horppila J. & Liljendahl-Nurminen A. (2005) Clay-turbid interactions may not cascade – a reminder for lake managers. *Restoration Ecology*, **13**, 242–246.
- Kovalenko K.E., Dibble E.D., Agostinho A.A., Catanhede G. & Fugi R. (2010) Direct and indirect effects of an introduced piscivore, *Cichla kelberi* and their modification by aquatic plants. *Hydrobiologia*, **638**, 245–253.
- Krause J. & Ruxton G.D. (2002) *Living in Groups*. Oxford University Press, Oxford.
- Leahy S.M., McCormick M.I., Mitchell M.D. & Ferrari M.C.O. (2011) To fear or to feed: the effects of turbidity on perception of risk by a marine fish. *Biology Letters*, **7**, 811–813.
- Lima S.L. & Dill L.M. (1990) Behavioural decisions under the risk of predation: a review and prospectus. *Canadian Journal of Zoology*, **68**, 619–640.
- Oliveira E.F., Luiz E.A., Agostinho A.A. & Benedito E. (2001) Fish assemblages in littoral areas of the upper Paraná river floodplain Brazil. *Acta Scientiarum. Biological Sciences*, **23**, 369–376.
- Padiá A.A. & Thomaz S.M. (2008) Prediction of the light attenuation coefficient through the Secchi disk depth: empirical modeling in two large Neotropical ecosystems. *Limnology*, **9**, 143–151.
- Pangle K.L., Malinich T.D., Bunnell D.B., DeVries D.R. & Ludsins S.A. (2012) Context-dependent planktivory: interacting effects of turbidity and predation risk on adaptive foraging. *Ecosphere*, **3**, art114.
- Pelicice F.M. & Agostinho A.A. (2006) Feeding ecology of fishes associated with *Egeria* spp. patches in a tropical reservoir, Brazil. *Ecology of Freshwater Fish*, **15**, 10–19.
- Pelicice F.M. & Agostinho A.A. (2009) Fish fauna destruction after the introduction of a non-native predator (*Cichla kelberi*) in a Neotropical reservoir. *Biological Invasions*, **11**, 1789–1801.
- Peretti D. & Andrian I.F. (2004) Trophic structure of fish assemblages in five permanent lagoons of the high Paraná River floodplain, Brazil. *Environmental Biology of Fishes*, **71**, 95–103.
- Pitcher T.J. (1986) Functions of shoaling behaviour in teleosts. In: *The Behaviour of Teleost Fishes*. (Ed T.J. Pitcher), pp. 294–337. Croom Helm, London.
- Roberto M.C., Santana N.F. & Thomaz S.M. (2009) Limnology in the Upper Paraná River floodplain: large-scale spatial and temporal patterns, and the influence of reservoirs. *Brazilian Journal of Biology*, **69**, 717–725.
- Rocha R.R.A., Thomaz S.M., Carvalho P. & Gomes L.C. (2009) Modeling chlorophyll-a and dissolved oxygen concentration in tropical floodplain lakes (Paraná River, Brazil). *Brazilian Journal of Biology*, **69**, 491–500.
- Schwalbe M.A.B., Bassett D.K. & Webb J.F. (2012) Feeding in the dark: lateral-line-mediated prey detection in the peacock cichlid *Aulonocara stuartgranti*. *The Journal of Experimental Biology*, **215**, 2060–2071.
- Stuart-Smith R.D., Stuart-Smith J.F., White R.W.G. & Bar-muta L.A. (2007) The effects of turbidity and complex habitats on the feeding of a galaxiid fish are clear and simple. *Marine and Freshwater Research*, **58**, 429–435.
- Swanbrow Becker L. J. & Gabor C. R. (2012) Effects of turbidity and visual vs. chemical cues on anti-predator response in the endangered fountain darter (*Etheostoma fonticola*). *Ethology*, **118**, 994–1000.
- Thomaz S.M., Dibble E.D., Evangelista L.R., Higuti J. & Bini L.M. (2008) Influence of aquatic macrophyte habitat complexity on invertebrate abundance and richness in tropical lagoons. *Freshwater Biology*, **53**, 358–367.
- Thompson R.M., Beardall J., Beringer J., Grace M. & Sardina P. (2013) Means and extremes: building variability into community-level climate change experiments. *Ecology Letters*, **16**, 799–806.
- Trenberth K.E., Jones P.D., Ambenje P., Bojariu R., Easterling D., Klein Tank A. *et al.* (2007) Observations: atmospheric surface and climate change. In: *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. (Eds S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor & H.L. Miller), pp. 235–336. Cambridge University Press, Cambridge.
- Turner A.M. (1997) Contrasting short-term and longterm effects of predation risk on consumer habitat use and resources. *Behavioral Ecology*, **8**, 120–125.
- Utne-Palm A.C. (2002) Visual feeding of fish in a turbid environment: physical and behavioural aspects. *Marine and Freshwater Behaviour and Physiology*, **35**, 111–128.
- Ward A.J.W., Thomas P., Hart P.J.B. & Krause J. (2004) Correlates of boldness in three-spined sticklebacks (*Gasterosteus aculeatus*). *Behavioral Ecology and Sociobiology*, **55**, 561–568.
- Webster M.M., Atton N., Ward A.J.W. & Hart P.J.B. (2007) Turbidity and foraging rate in threespine sticklebacks: the importance of visual and chemical prey cues. *Behaviour*, **144**, 1347–1360.



- Wishingrad V., Musgrove A.B., Chivers D.P. & Ferrari M.C.O. (2015) Risk in a changing world: environmental cues drive anti-predator behaviour in lake sturgeon (*Acipenser fulvescens*) in the absence of predators. *Behaviour*, **152**, 635–652.
- Zar J.H. (2010) *Biostatistical Analysis*. Pearson Prentice-Hall, Upper Saddle River.
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IV

**Non-lethal effects of an invasive piscivorous fish on invertebrates: the predator origin matters to trophic cascade**

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Running Headline: **Non-lethal impacts of an invasive predator**

*Abstract* - Top predator presence may cause non-lethal costs for prey, and these costs could be more pronounced if such predator and prey did not naturally co-occur, which usually happened after a species introduction. Here, we experimentally evaluated the survival of two aquatic invertebrate (*Chironomus sancticaroli*–benthic and *Daphnia magna*–pelagic), exposed to a mesopredator shoal (*Astyanax altiparanae*) in two ecological scenarios: (i) a “pre-invasion”, comparing presence/absence of native piscivorous (*Hoplias* aff. *malabaricus*); and (ii) a “post-invasion”, comparing the non-lethal effects from piscivorous fish with different origins (native vs. invasive–*Astronotus crassipinnis*). In both ecological scenarios vegetated habitats could concomitantly be available or not. In pre-invasion scenario, we hypothesized that non-lethal cues from a native piscivorous and vegetated habitat increases the invertebrate survival. In post-invasion scenario, we hypothesized that non-lethal cues from an unfamiliar top predator and vegetated habitats propitiate an addictive effect on invertebrate survival. We found that (i) although separately vegetated habitat and native piscivorous fish presence increased the invertebrate survival, together these variables did not promote an addictive increase in invertebrate survival; (ii) with invasive piscivorous fish more invertebrates survived without vegetated habitats, while with native fish more invertebrates survived in the presence of vegetated habitats; and (iii) top predator origin may also determine the predation pressure intensity on the pelagic and benthic preys. We concluded that the top predator origin and their hunting mode

matter to drive trophic cascade interactions, especially in vegetated habitats, and the invasive top predators might change the main food resource of mesopredators.

**Keywords:** Anti-predator behavior; Evolutionary history; Predation threat; Predator-prey interaction

## **Introduction**

Top predators perform a key role in community structuring, because the presence/absence of a top predator species may affect entirely a food web (Paine 1963; Estes et al. 2011), determining trophic links and population survival in lower trophic levels, in a process called trophic cascade (Carpenter et al. 1985; Pace et al. 1999). For aquatic ecosystems, it can be predicted that increasing piscivorous biomass will reduce both zooplanktivorous and benthivorous fish biomass, leading to enhanced abundance of aquatic invertebrates (Benndorf et al. 2002; Jeppesen et al. 2003). The main limitation from this traditional concept of trophic cascade is considering only the top predators foraging success (i.e. the lethal effects). However, the simple presence of a top predator may also provide an indirect or non-lethal cost for prey, which often spends more time in vigilance state after perceiving the predation threat (Lima 1998; Werner and Peacor 2006; Hoeinghaus and Pelicice 2010; Ajemian et al. 2015). When prey plays the anti-predation behavior, it concomitantly reduces its own feed rate as result from the reduction in its activities related to foraging (Ahlgren et al. 2011; Leahy et al. 2011; Pangle et al. 2012). So, behavioral changes of prey generated from the presence of top predators can be decisive for the habitat use patterns in natural systems, and may be more pronounced than the effects observed resulting from direct predation (Romare and Hansson 2003).

Non-lethal effects of predator on prey behavior could be more pronounced if the prey is not accordingly prepared to avoid be eaten by a new top predator, which usually arrive at a given ecosystem by species introduction. Allied to this, introduced predators might be able to decrease prey populations, of course not by a xenophobic effect (Simberloff 2003), but because invaders usually present higher resource use efficiency than native species and have superior competitive ability (e.g. Vilà and Weiner 2004; Van Kleuner et al. 2010). On the other hand, species with higher plasticity in anti-predator behavior could avoid new object in the water, then keeping as far as possible from any new predator (Sneddon et al. 2003; Mesquita and Young 2007), but it usually

decreases the exploited area, reducing their consumption, which may benefit a lower trophic level. Given those evidences, the evolutionary history between a piscivorous and prey-fish could drive the predation pressure intensity on invertebrates (Schmitz et al. 2007; Lazzaro et al. 2009). Although species origin do matter (Paolucci et al. 2013) differences in hunting modes of predators may also have different impacts on their prey and consequently to the lower trophic levels (Guariento et al. 2014). Therefore, it can be predicted that the interaction between predator species origin and hunting mode may elicit deeper changes in prey-fish behavior, which might either decrease invertebrate consumption or influence mesopredator shoal to eat different food items (Persson and Eklöv 1995).

*Hoplias* aff. *malabaricus* (Osteichthyes: Erythrinidae) is a native sit-and-wait predator (Montenegro et al. 2013), in lakes of the upper Paraná River floodplain (Brazil), where it is one of the most abundant and important piscivorous fish, and feeds upon almost all fish species (Peretti and Andrian 2008; Montenegro et al. 2013). However, around the year 2000 another piscivorous fish, *Astronotus crassipinnis* (Perciformes: Cichlidae), was introduced in the upper Paraná River by aquarists. It is an invasive fish well-adapted to live in lakes (Abujanra et al. 2009; Horácio-Junior et al. 2009), that actively pursuit their prey. There are no reports regarding the extent of environmental damage caused by *A. crassipinnis*. However, it is possible to have an estimate of the *A. crassipinnis* potential damage observing the responses in native fish populations to the establishment of *Cichla kelberi*, another invasive cichlid fish with similar hunting mode. *Cichla kelberi* was responsible for a 95% decline in native fish density, and 80% decline in richness recorded in a reservoir (Pelicice and Agostinho 2009; Pelicice et al. 2015). Such relation between the arrival of a new predator and native fish population decline may be due to absence of specific anti-predator traits, which should have been naturally selected during evolutionary time. Hence, prey fish would not be prepared to recognize and avoid such new threat (naiveté hypothesis, Cox and Lima 2006), generating a higher amount of preyed fish compared to a condition without exotic predator (Sih et al. 2010). Therefore, *A. crassipinnis* can be a potential danger to native fish populations in lentic environments, as *C. kelberi* was in lotic ones.

Both *H. aff. malabaricus* and *A. crassipinnis* inhabit lakes with large littoral areas, where vegetated habitats are abundant. Vegetated habitats are recognized as crucial providers of refuge for prey, because the structural complexity of aquatic macrophyte can limits the movement of large predators and reduces the likelihood of an

encounter between predators and prey (Diehl 1988; Padial et al. 2009). The presence of vegetated habitats might reduce the chances of invertebrates be eaten by mesopredator. However, if a top predator is present, mesopredators could also occupy the vegetated habitats to decrease their susceptible to top predator strikes. Such co-occurrence between invertebrate and mesopredators amid the interstices of the vegetation may improve the mesopredator feeding rate, decreasing the importance of vegetated habitats as refuge for invertebrates. Hence, the presence of aquatic plants could either amplify or attenuate the non-lethal effect of piscivorous on survival of different aquatic invertebrates.

Firstly, we experimentally simulated a scenario that precedes the invasion process by *A. crassipinnis*, to test the hypothesis that non-lethal cues from a native piscivorous (*H. aff. malabaricus*) and vegetated habitat increases the survival of invertebrate populations (*Chironomus sancticarloi* – benthic and *Daphnia magna* – pelagic). Because invertebrate populations can hide in aquatic plants, and invertivorous fish may reduce their locomotion in response to the presence of a native predator, we expected higher invertebrate survival in vegetated habitat as well as in the presence of a native piscivorous fish. Secondly, we resembled a scenario that succeeds the invasion by *A. crassipinnis*. In this second scenario, we tested the hypothesis that non-lethal cues from piscivorous fish in vegetated habitats propitiate an additive effect on the invertebrate survival. We expected higher invertebrate survival in vegetated habitats with presence of an invasive piscivorous fish compared to vegetated habitats with presence of a native piscivorous fish. This expectation is related to the mesopredator shoal behavior, which may have no appropriate anti-predator strategies to avoid a recently introduced predator (e.g. Cox and Lima 2006), and to the use of vegetation as refuge against predation. Lastly, we evaluated whether predation pressure of *Astyanax altiparanae* on benthic and pelagic preys in presence of a native sit-and-wait predator differs from predation pressure pattern observed in the presence of an invasive active predator. The same approach was used to compare prey selection in presence/absence of vegetated habitats.

## Methods

### Experimental design

We conducted an experiment in mesocosm, using rectangular aquaria as experimental units (water volume 35 l; width = 25 cm; length = 45 cm; height = 35 cm).

In the experiment, it was evaluated the survival of two populations of aquatic invertebrates: larval stages of *Chironomus sancticaroli* Strixino and Strixino (1981), and adults of *Daphnia magna* Straus (1820), simultaneously exposed to a mesopredator shoal ( $n = 3$ ), the invertivorous fish *Astyanax altiparanae* Garutti and Britski (2000). Such relation between invertebrates and a mesopredator was evaluated in three predation treatments: the presence of a native piscivorous fish (*Hoplias* aff. *malabaricus* Bloch, 1794), the presence of an invasive piscivorous fish (*Astronotus crassipinnis* Heckel, 1840) and a control treatment without top predators. All the three predation treatments were crossed with absence and presence of vegetated habitats, totalizing six treatments. Vegetated habitats were simulated with sixteen artificial fragments of submersed aquatic plants (30 cm long), which have architecture similar to the submersed macrophyte *Egeria najas*. All treatments were replicated five times and received seven *C. sancticaroli* ( $7 \pm 1.6$  mm) and fifteen *D. magna* ( $2 \pm 0.6$  mm).

Fish were collected in summer 2014. Piscivorous fish (*H. aff. malabaricus*, Total Length - TL =  $27.2 \pm 0.35$  cm; *A. crassipinnis*, TL =  $25.6 \pm 0.20$  cm) were collected through angling, while the mesopredator individuals (TL =  $8.6 \pm 1.1$ cm) was collected with trawl (long: 20 m; net mesh: 5 mm), in lakes connected to the Paraná River, Brazil. Then, fishes were transported to the laboratory, where they were kept in aquaria acclimating to the experimental water conditions. Aquatic invertebrates were cultivated in tanks at laboratory located in State University of Maringá, Brazil. We used different densities of invertebrates to simulate the real proportions of these organisms found in the natural environments (e.g. Thomaz et al. 2008). *Astyanax altiparanae* was used due to its wide distribution in the upper Paraná River floodplain and obtain food in several compartments of the water column (Hahn et al. 2004; Peretti and Andrian 2008), influencing several populations of aquatic invertebrates. It is worth mentioning that Chironomidae and Daphniidae are important food items of *A. altiparanae* (Casatti et al. 2003; Gomiero and Braga 2008).

To prevent the consumption of mesopredator shoal during the experiment, native and invasive piscivorous fish were fed before the beginning of the experiment. However, with this procedure, piscivorous could not release any cues related to hungry in the water (Åbjörnsson et al. 1997), which could be identified as a predation threat by the mesopredator shoal (Figueiredo et al. 2015a). Then, we resembled more realistic conditions of predation threat inserting chemical cues from a hungry predator (hereafter “chemical cues”) to the treatments with predators. The chemical cue was obtained days

prior to the experiment, by keeping the piscivorous in tanks of 100 liters without food for 48 hours according to Figueiredo et al. (2015a). After this period, water samples having predator secretions were collected with a syringe and frozen for later use (e.g. Ferrari et al. 2008; Epp and Gabor 2008; Kovalenko et al. 2010; Becker and Gabor 2012).

The experiment began with piscivorous fish introduction to the experimental units together with their respective chemical cues (150 ml). The next step was the addition of invertebrates followed by the mesopredators. After one hour of experiment all fish were removed, water was filtered and final amount of survival invertebrates were counted. Due to the scarce number of piscivorous fish we carried out the experiment in two blocks. However, the block effect was not significant (ANOVA:  $F_{1,30} = 2.2$ ;  $P = 0.13$ ) and we disregarded this effect in the posterior analyses.

### **Data analysis**

The first hypothesis that non-lethal cues from a native piscivorous fish (*H. aff. malabaricus*) and vegetated habitats increases the survival of invertebrates was investigated applying a two-way analysis of variance. In this analysis we used the number of prey survived at the end of the experimental period as response variable. Presence/absence of a native piscivorous fish and presence/absence of vegetated habitat were considered as independent variables. Multiple comparisons among treatments were performed with Fisher LSD.

The second hypothesis that non-lethal cues from an invasive piscivorous fish (*A. crassipinnis*) in vegetated habitats propitiates an additive effect on the invertebrate survival, was evaluated performing a two-way analysis of variance. The number of prey survived at the end of the experimental period was used as response variable. Predator origin (native or invasive piscivorous) and presence/absence of vegetated habitat were considered as independent variables. Comparisons among treatments were performed with Fisher LSD as a post-hoc test.

To evaluate whether predation pressure of the mesopredator on benthic and pelagic preys follows the same pattern in all treatments, we used the electivity index (Ivlev 1961) as surrogate of the predation pressure. The electivity index was calculated using the initial and final amount of each type of prey in the experimental units. The values of this index range from -1.0 to +1.0, values between 0 to +1.0 indicate preference, while values between -1.0 to 0 indicate rejection of prey (Krebs 1989). Considering that 0 (zero) represents randomness of prey selection, we performed a *t*-test



to compare electivity index values of *C. sancticaroli* and *D. magna* in each treatment to the average 0. Significant results would indicate preference for one prey type, while non-significant results indicate randomness in prey selection. All tests were performed using the software *Statistica 7.1*® (Statsoft 2005), considering  $P < 0.05$  for significant results.

## Results

Feeding the piscivorous fish before the experiment was enough to avoid the consumption of mesopredators during the experiment. In the scenario that precedes the invasion process by *A. crassipinnis*, the non-lethal cues from a native piscivorous fish significantly increased 57% the survival of aquatic invertebrates ( $F_{1,20} = 29.9$ ;  $P < 0.001$  - Fig. 1). Similarly, the presence of a vegetated habitat significantly increased 29% the survival of invertebrates ( $F_{1,20} = 4.18$ ;  $P = 0.05$  - Fig. 1). Also, there was no interaction between the non-lethal cues from a native piscivorous fish and the presence of vegetated habitat to shape the invertebrate survival ( $F_{1,20} = 2.23$ ;  $P = 0.154$ ), indicating no additive effect between the native piscivorous fish and the vegetated habitat.

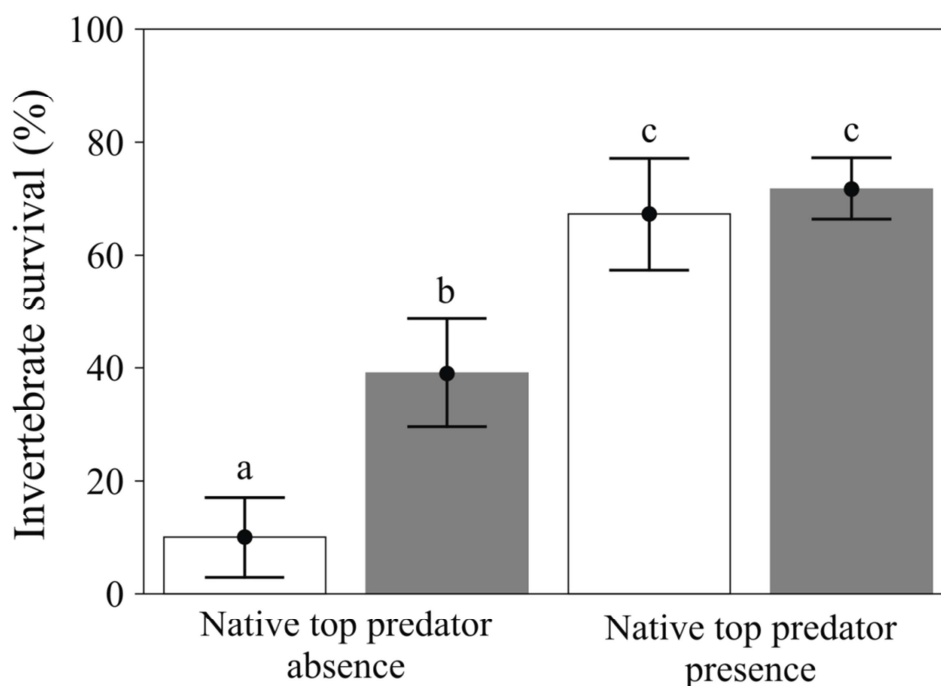


Figure 1 - Mean and standard error of aquatic invertebrates survival (%), in presence/absence of a native piscivorous fish and presence/absence of vegetated habitat. *White column*: absence of vegetated habitat, *Grey column*: presence of vegetated habitats. Different letters indicate post-hoc test significant differences.

Evaluating the scenario that succeeded the invasion process by *A. crassipinnis*, we found that the effect of piscivorous fish origin on the aquatic invertebrates survival depends on the vegetated habitat ( $F_{1,20} = 6.36$ ;  $P = 0.02$ ). The highest survival of aquatic invertebrates was recorded in the presence of non-lethal cues of the invasive piscivorous fish and absence of vegetated habitat, while the lowest survival of aquatic invertebrates was recorded in the presence of non-lethal cues from an invasive piscivorous fish and presence of vegetated habitat (Fig. 2).

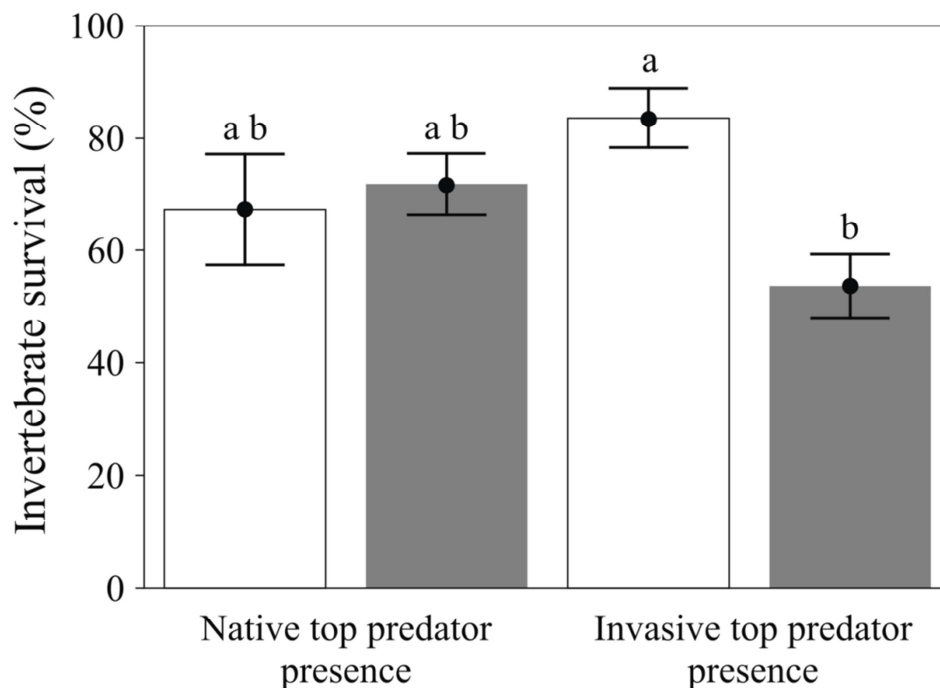


Figure 2 - Mean and standard error of aquatic invertebrates survival (%), in presence of a native and an invasive piscivorous fish. *White column*: absence of vegetated habitat, *Grey column*: presence of vegetated habitats. Different letters indicate post-hoc test significant differences.

Mesopredators significantly select the pelagic prey *D. magna*, instead of the benthic prey *C. sancticaroli* in the presence of non-lethal cues from a native piscivorous fish (Table 1, Fig. 3). The survival of *C. sancticaroli* reached 91.4%, while the survival of *D. magna* reached a maximum of 59.3%. Such prey selection pattern was the same in presence/absence of vegetated habitats. In contrast, mesopredators randomly selected both pelagic and benthic aquatic invertebrates (Table 1, Fig. 3) under influence of non-lethal cues from an invasive piscivorous fish. The maximum survival recorded for *C. sancticaroli* was 67.5%, while for *D. magna* was 70.3%. The presence of vegetated

habitat did not affect this random pattern of prey selection. Therefore, the predation pressure on pelagic and benthic prey shifted according to predator origin.

*Table 1* Significance of Student's *t*-tests comparing the consumption on both aquatic invertebrates types (benthic *C. sancticaroli* and pelagic *D. magna*) by mesopredator shoal against the constant zero mean. Significant results indicate preference for one prey type (positive selection), while non-significant results indicate randomness in prey selection (negative selection). Bold values indicate significant differences.

Treatments	df	N	<i>t</i>	<i>P</i>
<i>H. aff. malabaricus</i>				
Absence of vegetated habitats				
<i>Chironomus sancticaroli</i>	4	5	-3.01	<b>0.041</b>
<i>Daphnia magna</i>	4	5	6.74	<b>0.002</b>
Presence of vegetated habitats				
<i>Chironomus sancticaroli</i>	4	5	-6.82	<b>0.002</b>
<i>Daphnia magna</i>	4	5	28.82	<b>0.001</b>
<i>A. crassipinnis</i>				
Absence of vegetated habitats				
<i>Chironomus sancticaroli</i>	4	5	-1.48	0.212
<i>Daphnia magna</i>	4	5	-0.52	0.637
Presence of vegetated habitats				
<i>Chironomus sancticaroli</i>	4	5	-0.871	0.448
<i>Daphnia magna</i>	4	5	0.19	0.860

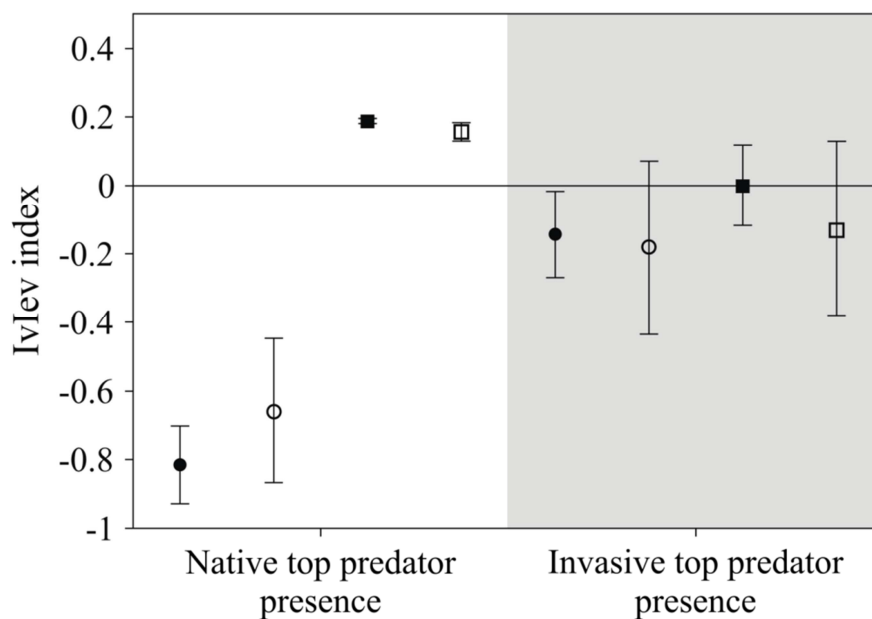


Figure 3 – Mean and standard error of the Ivlev electivity index, related to prey selection by *A. altiparanae* on benthic *C. sancticaroli* (circles) and pelagic *D. magna* (squares), in treatments with a native piscivorous fish (white side) and with an invasive piscivorous fish (grey side). *Clear symbols*: absence of vegetated habitats, *Black symbols*: presence of vegetated habitats.

## Discussion

Our experimental setup resembled two ecological scenarios: (i) a scenario that precedes the invasion process by *A. crassipinnis* (hereafter, pre-invasion), and (ii) a scenario that succeeds the invasion by *A. crassipinnis* (hereafter, post-invasion). In the pre-invasion scenario, combining presence/absence of a native piscivorous and presence/absence of vegetated habitats, we expected higher invertebrate survival in vegetated habitat as well as in the presence of a native piscivorous fish. This expectation was corroborated. Although vegetated habitat and native piscivorous fish presence separately increased the invertebrate survival, we did not observe an additive increase in invertebrate survival in presence of vegetated habitat with a native piscivorous fish. The lack of additive effects of top predator presence and vegetation on invertebrate survival is likely related to the feeding efficiency of mesopredator shoal members. The feeding efficiency could remain high even in the presence of top predators, if mesopredators are able to cautiously eat preys that are farther from the predator location (e.g., Figueiredo et al. 2016). In this case, even if benthic and pelagic invertebrate species used the vegetated habitat as a hiding place from mesopredators (Santos et al.

2013; Grutters et al. 2015), this not guaranteed an improvement in their survival. An alternative explanation to the lack of interaction between native piscivorous fish presence and vegetation on invertebrate survival is that the presence of native piscivorous reduced mesopredator feeding efficiency so much that the addition of new feeding-constraint factor (such as a heterogeneous habitat) could be attenuated.

In the post-invasion scenario, combining presence of native/ invasive piscivorous fish and presence/absence of vegetated habitats, we expected higher invertebrate survival in vegetated habitats with presence of an invasive piscivorous fish compared to vegetated habitats with presence of a native piscivorous fish. This second expectation was not corroborated. However, we have found that piscivorous fish origin interact with vegetation to determine the invertebrate survival, suggesting that trophic cascade interactions may be shaped by the top predator origin depending on the vegetation presence. In the treatment with invasive fish more invertebrates survived in the absence of plants, while in the treatment with native fish more invertebrates survived in the presence of plant. The lowest invertebrate survival in presence of vegetated habitat with an invasive piscivorous fish could be attributed to the mesopredator inability to properly respond to a new predator (Kovalenko et al. 2010). For instance, if mesopredator shoal members increased their swimming activity to avoid be eaten by an active new predator (McPeck et al. 1996), then they also would enhance the size of explored area and improve the encounter rate with a potential prey. Additionally, vegetated habitats provide a safety refuge for invertebrates (Meerhoff et al. 2007) and the active behavior of the invasive piscivorous fish could lead the mesopredator to enter more frequently in the vegetated habitat to escape from the piscivorous strike (e.g. Brendonck et al. 2003). If so, the mesopredator could easier find and eat the hidden invertebrates. It is important to note that the artificial stand of submerged macrophyte used in our experiment may be considered as stands of low complexity (Figueiredo et al. 2015b), and some studies have shown that low complexity of submerged macrophytes are less efficient refuge for large pelagic invertebrates like *Daphnia* sp. (Meerhoff et al. 2006; Tavşanoğlu et al. 2012) and for benthic invertebrates like *Chironomus* sp. (Figueiredo et al. 2015b).

In addition to interact with vegetation to determine invertebrate survival, top predator origin may also determine the predation pressure on the pelagic and benthic preys. The mesopredator preferentially selected the pelagic prey in presence of a native piscivorous fish, while randomly ingested pelagic and benthic preys in the presence of

an invasive piscivorous fish. Thus, the introduction of an invasive piscivorous fish might change the main food resource of mesopredators in a food chain, what could have implications to the relative importance of the pelagic and benthic compartments as the main pathway of energy flow in aquatic ecosystems (Fig. 4). Additionally to the predator origin, the hunting mode of piscivorous fish may also drive the prey selection by the mesopredator (Guariento et al. 2014). In general, when a predator occupies a given area, it creates around it a small safety area, which might be used as a refuge area by invertebrates. In this condition, mesopredator shoal members could alter the selected food item instead of decrease their foraging success (Stuart-Smith et al. 2007; Figueiredo et al. 2015b). On one hand, the sit-and-wait strategy from native piscivorous fish allows its co-occurrence with the benthic prey, forcing the mesopredator to take less predation risk and spend more time capturing pelagic preys instead of benthic ones. On the other hand, the constant swimming activity of the invasive piscivorous fish could generate an intense movement-response by the mesopredator shoal, leading the shoal members to indistinctly ingest both available preys.

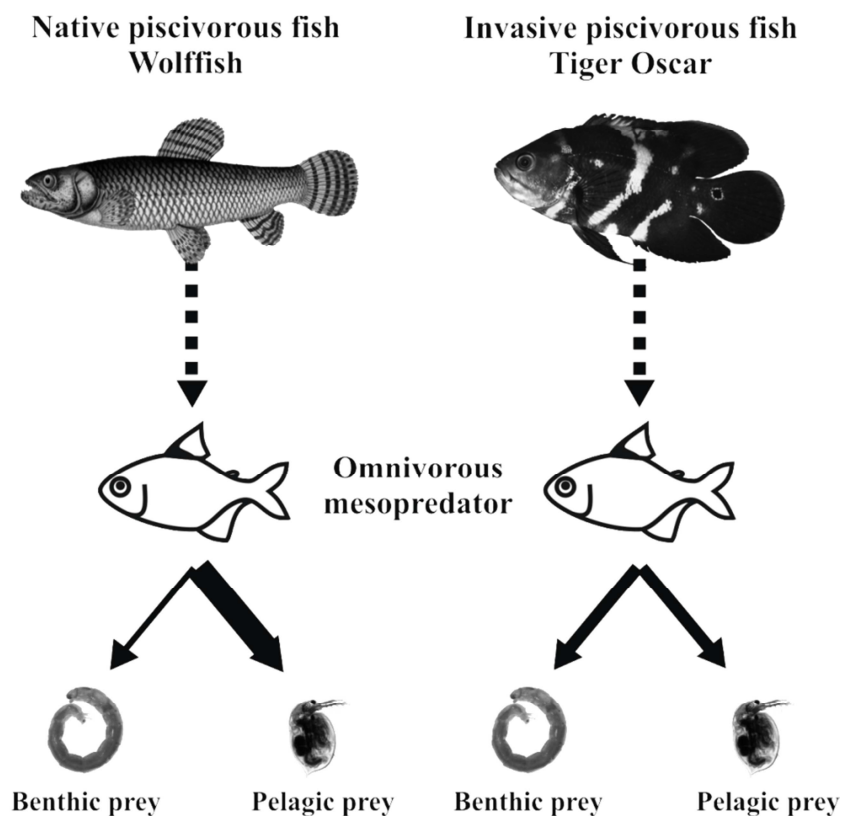


Figure 4 – Conceptual model of trophic cascades, highlighting the non-lethal effects from native and invasive piscivorous fish presence on prey selection by omnivorous mesopredator and survival of aquatic invertebrates. *Full lines* indicate lethal effects, while *dashed lines* indicate

non-lethal effects. Line widths designate predation pressure intensity (supported by our data) on aquatic invertebrate.

The increase in invertebrate survival is directly linked to a reduction in mesopredators feeding efficiency. Unbalanced response between feed and hide from the piscivorous fish might represent a decrease in mesopredator feeding activity (Lehtiniemi 2005). If this response last long, then it will lead to a loss on fitness of the mesopredator population (Lima and Dill 1990; Chapman et al. 2010). Our findings indicate that *A. altiparanae* is able to maintain their feeding rates even in presence of an invasive piscivorous fish, indistinctly eating pelagic and benthic prey. Therefore, the flexibility in feeding behavior observed in tropical omnivorous mesopredators like *A. altiparanae*, together with the predator avoidance behavior might attenuate the food chain effects caused by the introduction of an invasive top predator fish. Similar behavioural adjustments to a novel ecological scenario should not be expected to all mesopredators species, especially to the specialist ones (like major of temperate fishes), which has narrow niche breadth (González-Bergonzoni et al. 2012), and therefore, specialist species could have their survival more threatened.

Our results demonstrate that the invasion of *A. crassipinnis* in vegetated environments where *H. aff. malabaricus* was the main top predator, may have negatively impacted the invertebrate survival. Moreover, *A. crassipinnis* invasion may have changed the mesopredator prey selectivity from the selection of pelagic preys to a random consumption of pelagic and benthic preys. Then, we suggest that the predator origin and their hunting mode matter to drive trophic cascade interactions, especially in vegetated habitats. Furthermore, our findings are based on non-lethal effects of top predators through the food chain and lethal effects should be more aggressive and deserve attention in further studies. We suggest that future studies resembling the co-occurrence of native and invasive piscivorous fish could find additive or synergic effects of predator presence on the invertebrate survival. Finally, we encourage the carrying out of experiments when verifying lethal effects of top predators on invertebrates also consider predator origin with omnivorous and specialist mesopredators should be also carried out.

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## References

- Åbjörnsson, K. et al. 1997. Responses of *Acilius sulcatus* (Coleoptera: Dytiscidae) to chemical cues from perch (*Perca fluviatilis*). *Oecologia* 111: 166–171.
- Abujanra, F. et al. 2009. Effects of the flood regime on the body condition of fish of different trophic guilds in the Upper Paraná River floodplain, Brazil. *Brazilian Journal of Biology* 69: 469–479.
- Ahlgren, J. et al. 2011. The influence of predator regime on the behaviour and mortality of a freshwater amphipod, *Gammarus pulex*. *Hydrobiologia* 67:139–49.
- Ajemian, M. J. et al. 2015. Effects of turbidity and habitat complexity on antipredator behavior of three-spined sticklebacks (*Gasterosteus aculeatus*). *Environmental Biology of Fishes* 98: 45–55.
- Becker, L. J. S. et al. 2012. Effects of turbidity and visual vs. chemical cues on anti-predator response in the endangered fountain darter (*Etheostoma fonticola*). *Ethology* 118: 994–1000.
- Benndorf, J. et al. 2002 Top-down control of phytoplankton: the role of time scale, lake depth and trophic state. *Freshwater Biology* 47: 2282–2295.
- Brendonck, L. et al. 2003. The impact of water hyacinth (*Eichhornia crassipes*) in a eutrophic subtropical impoundment (Lake Chivero, Zimbabwe). *Archiv fur Hydrobiologie* 158: 389–405.
- Carpenter, S. R. et al. 1985. Cascading trophic interactions and lake productivity. *BioScience* 35: 634–639.
- Casatti, L. et al. 2003. Aquatic macrophytes as feeding site for small fishes in the Rosana reservoir, Paranapanema river, southeastern Brazil. *Brazilian Journal of Biology* 63: 213–222.
- Chapman, B. B., Morrell, L. J., Tosh, C. R., Krause, J. 2010 Behavioural consequences of sensory plasticity in guppies. *Proceedings of the Royal Society B* 277:1395–1401
- Cox, J. G. and Lima, S. L. 2006. Naiveté and an aquatic-terrestrial dichotomy in the effects of introduced predators. *Trends Ecology Evolution* 21: 674–80.



- Estes J.A., Terborgh J., Brashares J.S., Power M.E., Berger J., Bond W.J. et al. (2011) Trophic Downgrading of Planet Earth. *Science*, 333, 301–306.
- Epp, K. J. and Gabor, C. R. 2008. Innate and learned predator recognition mediated by chemical signals in *Eurycea nana*. *Ethology* 114: 607–615.
- Ferrari, M. C. O. et al. 2008. Can prey exhibit threat-sensitive generalization of predator recognition? Extending the predator recognition continuum hypothesis. *Proceedings of the Royal Society B* 275: 1811–1816.
- Figueiredo, B. R. S. et al. 2015a. Swimming and hiding regardless of the habitat: prey fish do not choose between a native and a non-native macrophyte species as a refuge. *Hydrobiologia* 746: 285–290.
- Figueiredo, B. R. S. et al. 2015b. Structural complexity and turbidity do not interact to influence predation rate and prey selectivity by a small visually feeding fish. *Marine & Freshwater Research* 66: 170–176.
- Figueiredo, B. R. S. et al. 2016. Turbidity amplifies the non-lethal effects of predation and affects the foraging success of characid fish shoals. *Freshwater Biology* 61: 293–300.
- González-Bergonzoni, I. et al. 2012. Meta-analysis shows a consistent and strong latitudinal pattern in fish herbivory across ecosystems. *Ecosystems* 15: 492–503.
- Gomiero, L. M. and Braga, F. M. S. 2008. Feeding habits of the ichthyofauna in a protected area in the state of São Paulo, southeastern Brazil. *Biota Neotropica* 8: 41–47.
- Guariento, R. D. et al. 2014. The effect of predation pressure and predator adaptive foraging on the relative importance of consumptive and non-consumptive predator net effects in a freshwater model system. *Oikos* 123: 705–713.
- Grutters, B. M. C., Pollux, B. J. A., Verberk, W. C. E. P., Bakker, E. S. 2015. Native and Non-Native Plants Provide Similar Refuge to Invertebrate Prey, but Less than Artificial Plants. *PLoS ONE* 10(4): e0124455. doi: 10.1371/journal.pone.0124455
- Hahn, N. S. et al. 2004. Trophic ecology of the fish assemblages. In Thomaz S. M. et al. (ed.) *The upper Paraná river and its floodplain: physical aspects, ecology and conservation*. Leiden: Backhuys Publishers, pp. 247–269.
- Hoeinghaus, D. J. and Pelicice, F. M. 2010. Lethal and nonlethal effects of predators on stream fish species and assemblages: A synthesis of predation experiments. In Gido K. B. and Jackson, D. A. (ed) *Community ecology of stream fishes: concepts, approaches, and techniques*. American Fisheries Society, Bethesda, pp. 619–648.

- Horácio, Jr. H. F. J. et al. 2009. A massive invasion of fish species after eliminating a natural barrier in the upper rio Paraná basin. *Neotropical Ichthyology* 7: 709–718.
- Ivlev, V. S. 1961. *Experimental Ecology of Feeding Fishes*. Yale University Press: New Haven, CT.
- Jeppesen, E. et al. 2003. The impact of nutrient state and lake depth on top-down control in the pelagic zone of lakes: A study of 466 lakes from the Temperate Zone to the Arctic. *Ecosystems* 6: 313–325.
- Kovalenko, K. E. et al. 2010. Recognition of non-native peacock bass, *Cichla kelberi* by native prey: testing the naiveté hypothesis. *Biological Invasions* 12: 3071–3080.
- Lazzaro, X et al. 2009. Predator foraging behaviour drives food-web topological structure. *Journal of Animal Ecology* 78: 1307–1317.
- Leahy, S. M. et al. 2011. To fear or to feed: the effects of turbidity on perception of risk by a marine fish. *Biology Letters* 7: 811–813.
- Lehtiniemi, M. 2005. Swim or hide: predator cues cause species specific reactions in young fish larvae. *Journal of Fish Biology* 66: 1285–1299.
- Lima, S. L. 1998. Nonlethal effects in the ecology of predator-prey interactions. *Bioscience* 48: 25–34.
- Lima, S. L. and Dill, L. M. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology* 68: 619–640.
- McPeck, M. A. et al. 1996. Adaptation to predators in a new community: swimming performance and predator avoidance in damselflies. *Ecology* 77: 617–629.
- Meerhoff, M. et al. 2006. An experimental study of habitat choice by *Daphnia*: plants signal danger more than refuge in subtropical lakes. *Freshwater Biology* 51: 1320–1330.
- Meerhoff, M. et al. 2007. Effects of habitat complexity on community structure and predator avoidance behaviour of littoral zooplankton in temperate versus subtropical shallow lakes. *Freshwater Biology* 52: 1009–1021.
- Mesquita, F. O. and Young, R. J. 2007. The behavioural responses of Nile tilapia (*Oreochromis niloticus*) to anti-predator training. *Applied Animal Behaviour Science* 106: 144–154.
- Montenegro, A. K. A. et al. 2013. Piscivory by *Hoplias* aff. *malabaricus* (Bloch, 1794): A question of prey availability? *Acta Limnologica Brasiliensia* 25: 68–78.
- Pace, M. L. et al. 1999. Trophic cascades revealed in diverse ecosystems. *Trends Ecology Evolution* 14: 483–488.

- Padial, A. A. et al. 2009. Effects of structural heterogeneity provided by the floating macrophyte *Eichhornia azurea* on the predation efficiency and habitat use of the small Neotropical fish *Moenkhausia sanctaefilomena*. *Hydrobiologia* 624: 161–170.
- Paine, R. T. (1963). Food recognition and predation on opisthobranchs by *Navanax inermis* (Gastropoda: Opisthobranchia).
- Pangle, K. L. et al. 2012. Context-dependent planktivory: interacting effects of turbidity and predation risk on adaptive foraging. *Ecosphere* 3: art114.
- Pelicice, F. M. et al. 2015. Fish fauna disassembly after the introduction of a voracious predator: main drivers and the role of the invader's demography. *Hydrobiologia* 746: 271–283.
- Pelicice, F. M. and Agostinho, A. A. 2009. Fish fauna destruction after the introduction of a non-native predator (*Cichla kelberi*) in a Neotropical reservoir. *Biological Invasions* 11:1789–1801.
- Peretti, D. and Andrian, I. F. 2008. Feeding and morphological analysis of the digestive tract of four species of fish (*Astyanax altiparanae*, *Parauchenipterus galeatus*, *Serrasalmus marginatus* and *Hoplias aff. malabaricus*) from the upper Paraná River floodplain, Brazil. *Brazilian Journal of Biology* 68: 671–679.
- Persson, L. and Eklöv, P. 1995. Prey refuges affecting interactions between piscivorous perch and juvenile perch and roach. *Ecology* 76: 70–81.
- Paolucci, E. M. et al. 2013 Origin matters: alien consumers inflict greater damage on prey populations than do native consumers. *Diversity and Distributions* 19: 988–995.
- Romare, P. and Hansson, L. A. 2003. A behavioral cascade: Top-predator induced behavioral shifts in planktivorous fish and zooplankton. *Limnology Oceanography* 48: 1956–1964.
- Santos, A. F. G. N. et al. 2013. When habitat complexity increases predation risk: experiments with invasive and neotropical native fishes. *Marine & Freshwater Research*, 64: 752–760.
- Schmitz, O. J. 2007. Predator diversity and trophic interactions. *Ecology* 88: 2415–2426.
- Sih, A. et al. 2010. Predator-prey naivete, antipredator behavior, and the ecology of predator invasions. *Oikos* 119: 610–621.
- Simberloff, D. 2003. Confronting introduced species: a form of xenophobia? *Biological Invasions* 5:179–192.

- Stuart-Smith, R. D., Stuart-Smith, J. F., White, R. W. G., Barmuta, L. A. 2007. The effects of turbidity and complex habitats on the feeding of a galaxiid fish are clear and simple. *Marine & Freshwater Research* 58: 429–435.
- Sneddon, L. U. et al. 2003. Novel Object Test: Examining Nociception and Fear in the Rainbow Trout. *The Journal of Pain* 4: 431–440.
- Tavşanoğlu, Ü. N. et al. 2012. Sediments, not plants, offer the preferred refuge for *Daphnia* against fish predation in Mediterranean shallow lakes: an experimental demonstration. *Freshwater Biology* 57: 795–802.
- Thomaz, S. M. et al. 2008. Influence of aquatic macrophyte habitat complexity on invertebrate abundance and richness in tropical lagoons. *Freshwater Biology* 53: 358–367.
- Van Kleunen, M. et al. 2010. A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecology Letters* 13: 235–245.
- Vilà, M. and Weiner, J. 2004. Are invasive plant species better competitors than native plant species? - evidence from pair-wise experiments. *Oikos* 105: 229–238.
- Werner, E. E. and Peacor, S. D. 2006. Lethal and nonlethal predator effects on an herbivore guild mediated by system productivity. *Ecology* 87: 347–361.



# Swimming and hiding regardless of the habitat: prey fish do not choose between a native and a non-native macrophyte species as a refuge

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**Abstract** The ability to respond to a predation threat may be the key factor influencing prey survival. Thus, small-sized fish may adapt to use macrophyte patches as refugia in ecosystems where they face predators. We evaluated the habitat choices of a small fish species (*Serrapinnus notomelas*) to determine whether these fish prefer native versus recently introduced submerged macrophyte stands in the context of predator avoidance. Specifically, we applied three predator cue treatments: no cue, chemical cue from a hungry predator and presence of a satiated predator. First, we empirically tested the theoretical assumption that the prey fish use vegetated habitats and that the presence of an actual predator has a stronger effect on the choice of habitat than simply a chemical cue. Then we tested the hypothesis that prey do not choose a habitat according to macrophyte species and whether this pattern changed as a result of increasing predation risk. We found that the prey fish preferred vegetated habitats; however, they did not appear to distinguish native from invasive macrophytes. Our results support the hypothesis that the physical structure of

macrophytes is more important in determining habitat choice than the evolutionary relationship between the fish and the native macrophyte species.

**Keywords** Antipredator behaviour · Evolutionary history · Predation threat · Predator–prey interaction

## Introduction

The choice of a specific habitat requires that prey identify the predation risk in available habitats (Hossie & Murray, 2010). In aquatic systems, prey fish may avoid predator-rich or refuge-poor habitats, such as the pelagic zone, to reduce the risk of encountering predators (Dupuch et al., 2009). An increase in predation risk reduces prey survival and often results in the use of lower risk habitats (Orrock et al., 2013). For example, physically complex habitats, such as submerged macrophyte patches, can reduce predator encounters by prey species in aquatic ecosystems (Figueiredo et al., 2013). Hence, habitat complexity may be a determinant factor in prey habitat choice (Savino & Stein, 1989; Pappal et al., 2012). If so, then we expect that prey fish use macrophyte patches as refuge sites in aquatic ecosystems where they face predators, because these more complex habitats offer more protection from predators than is offered by simpler habitats.

Recognising and responding to potential predation risk may depend on the type of alarm cues sensed by prey and their ability to assess predation risk based on

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these cues. Prey may recognise predation threats from both chemical and visual cues (Kovalenko et al., 2010a). Chemical cues are often emitted by a predator before any visual encounter with its prey (Holmes & McCormick, 2011), which could give prey greater amount of time to choose a specific habitat. Alternatively, once a top predator sees and pursues its prey fish, the role of chemical cues in predator detection may decrease (Brown & Magnavacca, 2003). As a result of this imminent predation threat, prey fish would have less time to choose between available habitats and might use any available physically complex habitat as refuge. The predator satiation condition (i.e. whether the predator has recently fed or not) may also affect the habitat choice of prey fish because alarm cues from a hungry predator have a stronger effect on prey behaviour than those of a satiated predator (Appelberg et al., 1993; Abjornsson et al., 1997). These cues released with the excrement give the prey information about whether the predator is hungry (Abjornsson et al., 1997). Similar studies seldom compare the relative influence of chemical cues from a hungry predator to the presence of an actual predator, despite these scenarios being routinely observed in nature and often inducing anti-predator responses of prey fish (e.g. Appelberg et al., 1993; Bell et al., 2006).

Habitat choice may result from a species-specific evolutionary relationship between the prey species and its refuge, because the repeated use of a habitat type could result in the selection of traits that favour predator avoidance in that specific habitat type (Goodman, 2009). However, the introduction of invasive species can alter these predator–prey relationships (Carlsson & Strayer, 2009; Carlsson et al., 2009). In 1985, the highly efficient predator *Cichla kelberi* Kullander & Ferreira, 2006 (peacock bass; Kovalenko et al., 2010b) was introduced into the Paraná River floodplain (Agostinho et al., 1997). Following *C. kelberi* establishment, a 95% decline in the native fish density and an 80% decline in richness were recorded in a reservoir in the Upper Paraná river basin, indicating the great impact of this predator on native fish (Pelicice & Agostinho, 2009). Then in 2005, a submerged invasive macrophyte species (*Hydrilla verticillata* (L.F.) Royle) was first recorded in the Parana River floodplain (Sousa, 2011), which colonised areas devoid of a native submerged macrophyte, *Egeria najas* Planchon. This second invasion potentially increased the amount of refuge available

for native prey fish, because they were found to use both the native and invasive macrophytes (Cunha et al., 2011). The use of *H. verticillata* for refuge suggests that the habitat choice made by prey fish could be driven by the presence of physically complex structure alone, rather than by the evolutionary history of the prey fish and native macrophyte.

We first experimentally tested the assumption that the small-sized fish use structured littoral habitats, as shown in the field (Cunha et al., 2011). Then, we tested whether the presence of a predator has a stronger effect on the choice of structured habitat than simply a chemical cue. Having confirmed these assumptions, we tested the hypothesis that prey do not preferentially choose a specific habitat, regardless of the level of predation risk. Because both the native and the invasive macrophytes share similar morphology and architecture (Cunha et al., 2011), we expected no difference in habitat use by the prey fish. Alternatively, if the evolutionary history of the prey fish and native macrophyte is more important to habitat selection by the prey fish, we would observe a greater proportion of fish using the native macrophyte compared to the invasive macrophyte.

## Methods

### Experimental design

The experiment was conducted using 200-l (width = 40 cm; length = 100 cm; height = 50 cm) aquaria in November 2012. The habitat choice of six small-sized fish (*Serrapinnus notomelas* Eingenmann, 1915) was recorded on video. All sides of the aquaria were covered with black tissue, and a camera was positioned above the centre of the aquaria to record fish movements. In addition, a silicon tube was added at the centre of the aquaria to introduce chemical cues described below. River water was stored for four hours to degrade any residual fish chemical cues (Ferrari et al., 2008) then used to fill each of the aquaria halfway. Furthermore, the significant response of prey to the presence of chemical cues (see Fig. 1) provides support for the assumption that there was little to no interference of cues present in river water. Thus, we concluded that river water could be used to simulate field conditions with minimal interference from residual chemical cues.

Habitat conditions were prepared with two species of submerged macrophytes: the native *E. najas* and invasive *H. verticillata*. Macrophytes were collected from the Paraná River, washed, and the apical portions of the plants (30 cm long) were used to create a patch for each species comprising 32 plant fragments each. Patches of both species were added to the same side of the aquaria and at similar densities to those found in natural conditions. Around each macrophyte patch, we marked a radius of 12 cm to facilitate the quantification of our response variable. At three times the total length of prey fish, this radius size was considered to be a safe distance from the refuge. An additional eight centimetres of space was left between the 12-cm radii associated with each macrophyte patch.

Six *S. notomelas* (~4 cm total length) caught in the same region where the macrophytes occur were included in the aquarium. The six *S. notomelas* used in each experimental unit came from different natural populations and were used only once. This prey species was chosen because it occurs in association with submerged macrophytes, including *E. najas* and *H. verticillata* (Cunha et al., 2011), and we added six individuals because *S. notomelas* is a schooling species (Figueiredo et al., 2014).

Our experimental design incorporated three predator cue treatments: no cue, hungry predator chemical cue (hereafter “chemical cue”) and satiated predator presence (hereafter “predator presence”). In the predator presence treatment, we used a live predator, thus we did not isolate the chemical from the visual and others cues to better resemble what is found in nature. This procedure is considered a more realistic approach and has been employed in other investigations (e.g. Kovalenko et al., 2010a; Gonzalo et al., 2012). However, to minimise the effect of chemical cues regarding predator satiation condition, we used a satiated predator. The experiment was randomised with treatments replicated 14 times, for a total of 42 experimental units. The chemical cue was prepared using six hungry individuals of the invasive piscivorous fish *C. kelberi* (22–29 cm total length). The piscivorous fish were kept in a tank of 100 l of river water without feeding for 24 h, which stimulates the release of chemical compounds related to hunger that indicate predation risk to prey (e.g. Kovalenko et al., 2010a). To remove the solid excrement and concentrate chemical cues, the water volume was reduced to 30 l by opening a valve located in the bottom of the

tank. Two hours later, all of the piscivorous fish were removed from the tank. Water samples (50 ml each) were collected with a syringe and frozen at  $-20^{\circ}\text{C}$ . Predators were caught in the field, collectively kept in a tank at a temperature of  $26^{\circ}\text{C}$  and were fed regularly until they were used in the experiment. The predator presence treatment involved simply adding *C. kelberi* to the aquarium. The prey fish response in “no cue” treatment was considered as a control to compare with the chemical cue treatment, and the chemical cue treatment was considered as a control to compare with the predator presence treatment. Differences in the first comparison indicated the effect of chemical cues, while differences in the second comparison indicated an effect of intimidation caused by actual predator presence.

Two hours after adding *S. notomelas* to the aquaria, the fish were recorded for 1 min before and after the addition of the treatment, because most chemical cues can degrade in as little as 5 min (Ferrari et al., 2008). The chemical cue water sample was defrosted to reach the temperature of the aquaria ( $26^{\circ}\text{C}$ ), and 10 ml of the sample was added slowly through a silicon tube positioned close to the water surface in the centre of the aquaria. *C. kelberi* was added on the opposite side from the macrophytes, thus simulating the oncoming predator in the field.

During video analysis, the prey were considered to be within a macrophyte patch if they remained inside the patch radius at least 70% (42 s) of the recorded time after the addition of the treatment. This length of time was used because it was observed to be enough time to measure consistent behavioural responses in all treatments, for all 14 replicates (see “Results” section). The first 15 s of video were discarded due to possible disturbance linked to the physical addition of cues.

#### Data analysis

We first tested our assumption of whether *S. notomelas* preferred to use the macrophytes as refuge through a pairwise *t* test between the percentages of fish that remained in vegetated habitat (in both native and non-native submerged macrophyte patches) and open water for each cue treatment. Then, we tested the difference of the percentages of prey inside the macrophyte patches among cue treatments via a one-way analysis of variance. In addition, we applied *Tukey* test as a post hoc analysis.

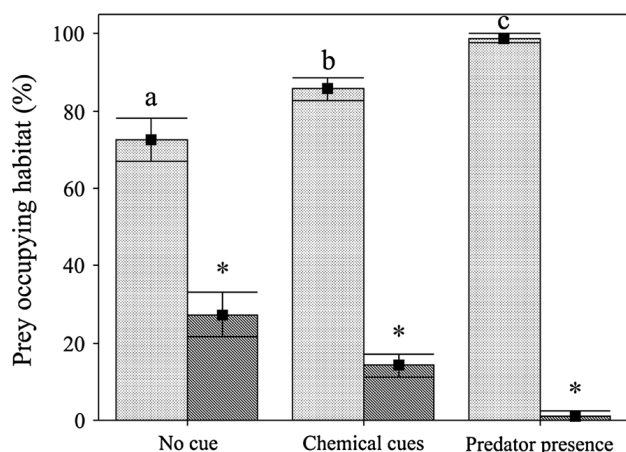


Finally, our main hypothesis was tested using a pairwise *t* test to compare the percentage of prey inside the native and invasive macrophyte patches. In this test, we used only the number of prey that remained inside the macrophyte patches to calculate the percentage of prey in each macrophyte patch, to each predator cue treatment. Then, after applying a one-way analysis of variance, we compared the difference of percentages of prey inside invasive macrophyte patches among cue treatments using a *Tukey* test as a post hoc.

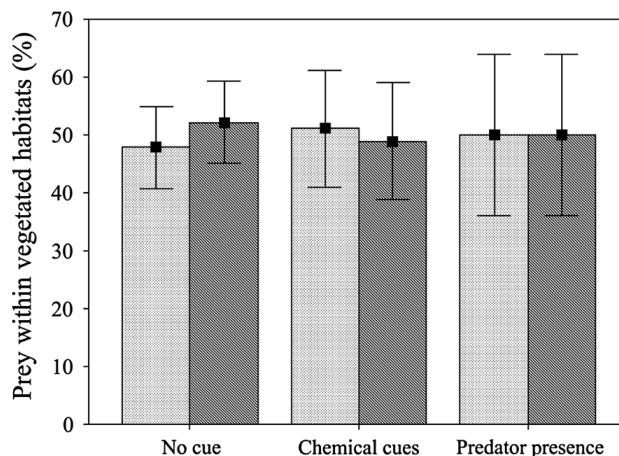
## Results

At the beginning of the experiment, all of the prey were active, whereas at the end, they spent most of their time in the vegetated habitats ( $t_{13} = 3.98$ ;  $P = 0.001$ ; Fig. 1), and no predation occurred in the predator presence treatment. This result corroborates our assumption that small-sized fishes are adapted to structured littoral habitats, even under experimental conditions.

The use of vegetated habitats (considering both native and non-native submerged macrophyte patches) increased when predator cues were present ( $F_{2,39} = 12.1$ ;  $P < 0.001$ —Fig. 1), which suggests these treatments elicited a predator-avoidance response. The percentage of fish in macrophyte patches rose when



**Fig. 1** Mean and standard error of the percentage of prey fish using either native or non-native submerged macrophyte patches (dotted column) and open habitat (dashed column) regarding absence of cues (no cue), chemical cue from a hungry predator and the presence of a satiated predator. \*Significant difference in *t* test between macrophyte patches and open habitat. Different letters indicate difference in *F* test among macrophyte patches



**Fig. 2** Mean and standard error of the percentage of the prey fish that remained hidden in native *Egeria najas* (dotted column) and invasive *Hydrilla verticillata* (dashed column) submerged macrophyte species according to the absence of cues (no cue), chemical cue from a hungry predator and the presence of a satiated predator

the chemical cue was released into the water column, but a greater increase occurred when the actual predator was present. Thus, this difference in magnitude can be attributed to other kind of cues, such as visual contact, that were accompanied by chemical cue in the predator presence treatment. In this treatment, almost all of the prey used the macrophytes instead of open water where the predator was present (Fig. 1).

Despite choosing vegetated habitats, the prey did not use the native macrophyte more than the invasive macrophyte for refuge ( $t_{13} = -0.30$ ;  $P = 0.77$ —Fig. 2). This pattern did not change when the chemical cue was released or when a predator was present, which represented a notable predation risk ( $F_{2,39} = 0.03$ ;  $P = 0.97$ —Fig. 2).

## Discussion

We observed that most of the prey remained in vegetated habitats, even when there was no predation threat. The choice of macrophytes may reflect a strong selective pressure on *S. notomelas*, which could have selected this habitat type during its evolutionary history to avoid predators that exist within *S. notomelas* native range (Luz-Agostinho et al., 2009). In this scenario, natural selection would favour individuals that hide according to the magnitude of the predation threat (Helfman, 1989). The chemical cue intensified the dependence on the macrophytes by the prey,

evidenced by increased use of macrophytes with the addition of the chemical cue. The presence of a predator increased the prey response even more, leading prey to avoid this habitat so intensely that the number of prey fish in open habitat decreased to nearly zero (Fig. 1). This pattern suggests a remarkable spatial segregation in response to predators (Lautala & Hirvonen, 2008). These results suggest that the prey could reduce the likelihood of an encounter with their predators by assessing the local predation risk with a higher accuracy when simultaneous predator cues (including visual and chemical) were present.

Although *S. notomelas* were found in vegetated habitats the majority of the time, we did not observe preferential selection for either the native macrophyte or the invasive macrophyte. This pattern was observed even with no predation cue where a longer time was available to choose a specific habitat. This finding suggests that small-sized fishes depend more on physical structure than species of macrophyte vegetation. A possible explanation is that small-sized fish perceive the two macrophyte species similarly, as *E. najas* and *H. verticillata* share the same morphology and show a similar architecture (Cunha et al., 2011). Even with a possible replacement of the native species by a non-native one, the non-native species would not alter predator–prey relationships. Thus, we found no evidence to reject our hypothesis, and suggest that physical structure provided by macrophytes could be more important than the evolutionary history between *S. notomelas* and *E. najas* in determining habitat choice.

Our results should be interpreted carefully, because we combined the effects of different cues and predator satiation condition (fed versus hungry predator). In addition, we evaluated only the short-term response of prey fish to different degrees of predation risk. Finally, we tested only one predator feeding strategy (active predator). Despite these potential shortcomings, our results suggested that the satiation condition of a predator could be less important than the imminent predation threat for the prey anti-predation response.

In summary, our findings indicate that the establishment of an invasive submerged macrophyte species could be beneficial for small-sized fishes, especially in areas where refuge is limited or absent. This direct benefit to prey fishes results from an overall increase in macrophyte cover, in turn providing more available habitat to avoid a voracious, invasive

predator, which could mitigate declines in densities of small native fish. However, we highlight that our experiment measured only habitat choice and that indirect negative effects of the invasive macrophytes on small-sized fish (such as on fish feeding or survival, which were not evaluated in our experiment) could still exist. These considerations are potentially important in developing approaches for future investigations addressing complex invasive–invasive interactions.

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## References

- Abjornsson, K., B. Wagner & A. Axelsson, 1997. Responses to *Acilius sulcatus* (Coleoptera: Dytiscidae) to chemical cues from perch (*Perca fluviatilis*). *Oecologia* 111: 166–171.
- Agostinho, A. A., H. F. Julio-Junior, L. C. Gomes & L. M. Bini, 1997. Composição, abundância e distribuição espaço-temporal da ictiofauna. In Vazzoler, A. E. A. M., A. A. Agostinho & N. S. Hahn (eds), *A Planície de inundação do alto rio Paraná: aspectos físicos, biológicos e socio-econômico*. EDUEM, Maringá PR: 177–205.
- Appelberg, M., B. Soderback & T. Odelstronl, 1993. Predator detection and perception of predation risk in the crayfish *Astacus astacus* L. *Nordic Journal of Freshwater Research* 68: 55–62.
- Bell, R., A. L. Rypstra & M. H. Persons, 2006. The effect of predator hunger on chemically-mediated antipredator responses and survival in the wolf spider *Pardosa milvina*. *Ethology* 112: 903–910.
- Brown, G. E. & G. Magnavacca, 2003. Predator inspection behaviour in a characin fish: an interaction between chemical and visual information? *Ethology* 109: 739–750.
- Carlsson, N. O. L. & D. L. Strayer, 2009. Intraspecific variation in the consumption of exotic prey—a mechanism that increases biotic resistance against invasive species? *Freshwater Biology* 54: 2315–2319.
- Carlsson, N. O. L., O. Sarnelle & D. L. Strayer, 2009. Native predators and exotic prey—an acquired taste? *Frontiers in Ecology and the Environment* 7: 525–532.
- Cunha, E. R., S. M. Thomaz, H. B. A. Evangelista, C. Carniato, C. F. Souza & R. Fugi, 2011. Small-sized fish assemblages do not differ between a native and a recently established

- non-indigenous macrophyte in a Neotropical ecosystem. *Natureza & Conservação* 9: 61–66.
- Dupuch, A., P. Magnan, A. Bertolo, L. M. Dill & M. Proulx, 2009. Does predation risk influence habitat use by northern redbelly dace *Phoxinus eos* at different spatial scales? *Journal of Fish Biology* 74: 1371–1382.
- Ferrari, M. C. O., F. Messier & D. P. Chivers, 2008. Degradation of chemical alarm cues in the natural conditions: risk assessment by larval woodfrogs. *Chemoecology* 17: 263–266.
- Figueiredo, B. R. S., R. P. Mormul & E. Benedito, 2013. Non-additive effects of macrophyte cover and turbidity on predator-prey interactions involving an invertivorous fish and different prey types. *Hydrobiologia* 716: 21–28.
- Figueiredo, B. R. S., R. P. Mormul & E. Benedito, 2014. Structural complexity and turbidity do not interact to influence predation rate and prey selectivity by a small visually feeding fish. *Marine and Freshwater Research*. doi:10.1071/MF14030.
- Gonzalo, A., C. Cabido, P. López & J. Martín, 2012. Conspecific alarm cues, but not predator cues alone, determine anti-predator behavior of larval southern marbled newts, *Triturus pygmaeus*. *Acta Ethologica* 15: 211–216.
- Goodman, B. A., 2009. Nowhere to run: the role of habitat openness and refuge use in defining patterns of morphological and performance evolution in tropical lizards. *Journal of Evolutionary Biology* 22: 1535–1544.
- Helfman, G. S., 1989. Threat-sensitive predator avoidance in damselfish-trumpetfish interactions. *Behavioral Ecology and Sociobiology* 24: 47–58.
- Holmes, T. H. & M. I. McCormick, 2011. Response across a gradient: behavioural reactions of newly settled fish to predation cues. *Animal Behaviour* 81: 543–550.
- Hossie, T. J. & D. L. Murray, 2010. You can't run but you can hide: refuge use in frog tadpoles elicits density-dependent predation by dragonfly larvae. *Oecologia* 163: 395–404.
- Kovalenko, K. E., A. A. Agostinho, E. D. Dibble & F. M. Pelicice, 2010a. Recognition of non-native peacock bass, *Cichla kelberi* by native prey: testing the naïveté hypothesis. *Biological Invasions* 12: 3071–3080.
- Kovalenko, K. E., E. D. Dibble, A. A. Agostinho, G. Catanhede & R. Fugli, 2010b. Direct and indirect effects of an introduced piscivore, *Cichla kelberi* and their modification by aquatic plants. *Hydrobiologia* 638: 245–253.
- Lautala, T. & H. Hirvonen, 2008. Antipredator behavior of naïve Arctic charr young in the presence of predator odours and conspecific alarm cues. *Ecology of Freshwater Fish* 17: 78–85.
- Luz-Agostinho, K. D. G., A. A. Agostinho, L. C. Gomes, H. F. Júlio-Jr. & R. Fugli, 2009. Effects of flooding regime on the feeding activity and body condition of piscivorous fish in the Upper Paraná River floodplain. *Brazilian Journal of Biology* 69: 481–490.
- Orrock, J. L., E. L. Preisser, J. H. Grabowski & G. C. Trussell, 2013. The cost of safety: refuges increase the impact of predation risk in aquatic systems. *Ecology* 94: 573–579.
- Pappal, A. L., R. A. Rountree & D. G. MacDonald, 2012. Relationship between body size and habitat complexity preference in age –0 and –1 year winter flounder *Pseudopleuronectes americanus*. *Journal of Fish Biology* 81: 220–229.
- Pelicice, F. M. & A. A. Agostinho, 2009. Fish fauna destruction after the introduction of a non-native predator (*Cichla kelberi*) in a neotropical reservoir. *Biological Invasions* 11: 1789–1801.
- Savino, J. F. & R. A. Stein, 1989. Behavior of fish predators and their prey: habitat choice between open water and dense vegetation. *Environmental Biology of Fishes* 24: 287–293.
- Sousa, W. T. Z., 2011. *Hydrilla verticillata* (Hydrocharitaceae), a recent invader threatening Brazil's freshwater environments: a review of the extent of the problem. *Hydrobiologia* 669: 1–20.

## APÊNDICE B - Artigos publicados ou preparados durante o período do doutorado

- Figueiredo, Bruno R. S., Mormul, Roger P. & Benedito, Evanilde. Structural complexity and turbidity do not interact to influence predation rate and prey selectivity by a small visually feeding fish. *Marine and Freshwater Research*, v. 66, p. 170-176, 2015.
- Figueiredo, Bruno R. S., Mormul, Roger P. & Benedito, Evanilde. Non-additive effects of macrophyte cover and turbidity on predator-prey interactions involving an invertivorous fish and different prey types. *Hydrobiologia*, v. 713, p. 23-30, 2013.
- Figueiredo, Bruno R. S., Araújo, Gabrielle J. M., Silva, Márcio J. da & Medeiros, Elvio S. F. Implications of low food availability on resource partitioning among three species of Cichlidae (Pisces: Perciformes) in a Brazilian semi-arid reservoir. *Acta Limnologica Brasiliensia (Online)*, v. 27, p. 93-104, 2015.
- Bando, Fabielle M., Michelan, Thaisa S., Cunha, Eduardo R., Figueiredo, Bruno R. S. & Thomaz, Sidinei M. Macrophyte species richness and composition are correlated with canopy openness and water depth in tropical floodplain lakes. *Revista Brasileira de Botânica (Impresso)*, v. 38, p. 289-294, 2015.
- Braghin, Louizi S. M., Figueiredo, Bruno R. S., Meurer, Thamis, Michelan, Thaisa S., Bonecker, Cláudia C. & Simões, Nadson R. Zooplankton diversity in a dammed river basin is maintained by preserved tributaries in a tropical floodplain. *Aquatic Ecology (Print)*, v. 49, p. 175-187, 2015.
- Lopes, Célia A., Manetta, Gislaine I., Figueiredo, Bruno R. S., Martinelli, Luiz A. & Benedito, Evanilde. Carbon from littoral producers is the major source of energy for bottom-feeding fish in a tropical floodplain. *Environmental Biology of Fishes*, v. 98, p. 1081-1088, 2015.

**[Submetidos]**

- Alves, Gustavo H. Z., Figueiredo, Bruno R. S., Manetta, Gislaine I., Sacramento, Patrícia A., Tófoli, Raffael M. & Benedito, Evanilde. Trophic segregation underlies the coexistence of two piranha species after the removal of a geographic barrier. *Biological Invasions* (submetido em Julho 2016).
- Calvo, Clementina, Figueiredo, Bruno R. S., Cunha, Eduardo R., Mormul, Roger P. & Meerhoff, Mariana. Temperature, herbivory and spatial arrange can act as factor controlling the invasive potential of *Hydrilla verticillata*. *Biological Invasions* (submetido em Agosto 2016).

**Esta tese é baseada nos seguintes manuscritos**

- I. Figueiredo, Bruno R. S.; Calvo, Clementina; López-Rodríguez, Anahí; Mormul, Roger P.; Benedito, Evanilde; Teixeira-de Melo, Franco & Meerhoff, Mariana. Turbidity pulses have stronger effects than heat waves on fish-invertebrate trophic interactions. Submetido a Canadian Journal of Fisheries and Aquatic Sciences (em Agosto de 2016).
- II. Figueiredo, Bruno R. S.; Alves, Gustavo H. Z.; Calvo, Clementina; López-Rodríguez, Anahí; Benedito, Evanilde & Meerhoff, Mariana. Consequências de variações na visibilidade subaquática e na temperatura sobre o comportamento de anfípodes. *Unless I see the predator I will not flee: influence of light and water temperature on anti-predator responses of amphipods*. Em preparação, periódico alvo: Invertebrate Biology.
- III. Figueiredo, Bruno. R. S.; Mormul, Roger P.; Chapman, Ben B.; Lolis, Lucas A.; Fiori, Leandro F & Benedito, Evanilde 2016. Turbidity amplifies the non-lethal effects of predation and affects the foraging success of characid fish shoals. *Freshwater Biology*, 61, 293–300. doi: 10.1111/fwb.12703
- IV. Figueiredo, Bruno. R. S.; Fiori, Leandro F.; Mormul, Roger P. & Benedito, Evanilde. Non-lethal effects of an invasive piscivorous fish on invertebrates: the predator origin matters to trophic cascade. Submetido a Biological Invasions (em Julho de 2016).
- V. Figueiredo, Bruno R. S.; Mormul, Roger P & Thomaz, Sidinei M. 2015. Swimming and hiding regardless of the habitat: prey fish do not choose between a native and a non-native macrophyte species as a refuge. *Hydrobiologia*, 746, 285–290. doi: 10.1007/s10750-014-2096-x