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Reconsiderando a tipologia das águas de Wallace: escolha de água de igarapé e maior sucesso de desova em um peixe amazônico

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Manaus, Amazonas
Março, 2018

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Sinopse: causas comportamental e reprodutiva de adaptação a tipos de água foram avaliadas. Para a causa comportamental, foram realizados testes de escolha de habitat em *Crenuchus spilurus*. Para a reprodutiva, estudamos o efeito do tipo de água no sucesso de desova.

Palavras-chave: Adaptação, tipo de habitat, isolamento reprodutivo.

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Resumo

Alfred Russel Wallace classificou as águas amazônicas em três: preta, branca e clara. Tem sido demonstrado que a adaptação a esses tipos d'água afeta o fluxo gênico, gerando uma importante fonte de diversificação para os peixes da Amazônia. Contudo, essa classificação é focada em grandes rios e não leva em consideração os pequenos cursos de água que permeiam a floresta de terra firme, conhecidos como igarapés. Comparados aos canais dos grandes rios, os igarapés cobrem uma área muito maior que a do Rio Amazonas e apresentam sua própria hidroquímica, principalmente devido à sua distinta composição florística. Nós avaliamos a importância da hidroquímica na escolha de habitat e na desova em *Crenuchus spilurus*, um pequeno peixe que habita igarapés inseridos em bacias de água preta e branca. Linhagens do Rio Negro (água preta) e Rio Amazonas (água branca) mostraram seleção ativa de água de igarapé e apresentaram sucesso de desova maior em água de igarapé quando comparado à água de floresta inundada. Devido ao papel das condições hidroquímicas de igarapé em moldar reprodução e comportamento, nossos resultados expandem as ideias de Wallace de que os tipos de água influenciam a diversidade, fornecendo evidência que igarapés representam um importante habitat mediando adaptação em peixes amazônicos.

Abstract

Following Alfred Russel Wallace, Amazonian freshwaters have been classified into three main types: black, white and clear. Adaptation to water types has been demonstrated to affect gene flow, making it an important source for diversification of the Amazonian fish fauna. However, this classification focuses on large rivers and fails to acknowledge the importance of small forest streams that flow through non-flooded forest, known as igarapés. As compared to large river channels, igarapés cover a much greater area than the Amazon River and have a distinct hydrochemistry, mostly due to soil and vegetation. We assessed the importance of water type on habitat choice and spawning in *Crenuchus spilurus*, a small fish that inhabits igarapés inserted within blackwater and whitewater river basins. Lineages from the Rio Negro (blackwater) and Amazon River (whitewater) basins showed active selection of igarapé water in habitat choice trials and had greater spawning success in igarapé water when compared to water from their respective flooded forest basin. Due to the hydrochemical conditions of igarapés acting to shape reproduction and behavior of *C. spilurus*, our results expand Wallace's intuition that water types influence diversity by providing evidence that igarapés represent an important habitat driving adaptation in Amazonian fishes.

Sumário

1. Introdução

Inspirado por um “desejo sincero de contemplar a exuberância da vida animal e vegetal” de uma terra tropical, o naturalista britânico Alfred Russel Wallace realizou expedições pelos rios do norte da América do Sul entre 1848 e 1852 (Wallace 1889; citação da página V). A partir da terminologia local, ele foi o primeiro a relatar os distintos tipos de água amazônica à comunidade científica, separando-as por sua cor: branca, clara e preta. Até hoje essa classificação tem seu valor, a despeito de simplificar a diversidade limnológica de um sistema tão grande e complexo (Fittkau 1967; Sioli 1984; Venticinque et al. 2016).

Rios de água branca são majoritariamente de origem Andina, obtendo sua coloração amarronzada da matéria orgânica dissolvida e partículas de argila em suspensão advindas do solo e degradação completa de material vegetal (Junk 1970), o que também resulta em pH aproximadamente neutro. Esses rios permeiam solo argiloso e são, na porção mais baixa, rodeados por floresta periodicamente inundada, a várzea (Sioli 1984). A vegetação de várzea é diversa e apresenta uma composição florística única (Wittmann et al. 2006), devido às adaptações necessárias para a vida parcialmente submersa durante a estação chuvosa (Wittmann et al. 2004). A alta riqueza de espécies vegetais em florestas de várzea em comparação a outras florestas alagadas vem da coexistência de espécies de áreas mais altas adaptadas à inundação e espécies exclusivas da várzea, resultando em águas com carbono orgânico dissolvido (COD) característico (Prance 1979).

Água clara é pobre em COD, permeando solo rochoso dos escudos antigos já fortemente erodidos (Sioli 1984). O pH de água clara varia de ácido a neutro, dependendo do solo, o que torna esse tipo de água um coletivo hidroquímico que compartilha pobreza de sólidos em suspensão como característica comum (Sioli 1984). Água preta é translúcida, correndo por solo rochoso ou arenoso, mas rica em COD da degradação incompleta de material vegetal da floresta, resultando em um tom avermelhado característico e pH ácido (Klinge 1967). Nas porções mais baixas, rios de água clara e preta são rodeados por outro tipo de floresta alagada, o igapó. Devido à falta de nutrientes, a riqueza e a abundância vegetal do igapó são muito menores do que na várzea (Junk et al. 2015).

Tamanha variedade de condições físicoquímicas impõe múltiplos estressantes fisiológicos à comunidade aquática local (Junk et al. 1983; Wilson et al. 1999), e fornece uma fonte importante de pressões de seleção (Beheregaray et al. 2015). Assim, as vegetações distintas afetando o perfil hidroquímico dos tipos de água podem ser consideradas parcialmente responsáveis pela composição de espécies (Cooke et al. 2012a), adaptação local (Beheregaray et al. 2015) e especiação (Cooke et al. 2012b) da ictiofauna amazônica. Portanto, os tipos de água possivelmente afetam a diversidade biológica amazônica, como originalmente sugerido por Wallace (Pires et al., em revisão).

Não relatado por Wallace foi um quarto tipo de corpo d’água há muito reconhecido pelos indígenas sulamericanos: córregos da floresta, localmente conhecidos como igarapés (Tupi Antigo para “caminho da canoa”). Igarapés são pequenos riachos encontrados em toda bacia amazônica, independentemente da cor dos canais principais aos quais eles estão conectados (Sioli 1984). Esses córregos formam uma rede aquática densa, conectada a todos os grandes rios da região (Junk et al. 1983). Em termos de contribuição à bacia, o somatório da superfície de todos os igarapés é múltiplas vezes maior que a da superfície do próprio Rio Amazonas, e seu comprimento total resultaria em um corpo mais de mil vezes maior que o do grande rio (Fittkau 1967).

Igarapés são caracterizados por uma coloração translúcida e água variavelmente ácida, dependendo da profundidade, solo e composição florística da vegetação circundante (Junk e Furch 1980; Sioli 1984). Igarapés de região alta são cercados por floresta de terra firme, o ambiente de maior biodiversidade no mundo (Gentry 1988). Assim, a água drenando solos de terra firme incorpora compostos da decomposição vegetal, resultando em uma composição de COD diferente da área alagada, primordialmente advinda da dominância de espécies de terra firme (Pitman et al. 2001). Esses córregos não são afetados pelo pulso sazonal de inundação, sendo amplamente governados pelo regime de chuva local (Fittkau 1967) e apresentando fauna e flora marcadamente diferentes da encontrada na área alagada (Walker 1990; Crampton 2011). Apesar de suas características individuais, pouca ênfase tem sido dada a igarapés como um habitat distinto que contribui para originar e manter a diversidade aquática na Amazônia. Haja vista que igarapés não são apenas influenciados pela floresta adjacente mas também conectados a tributários maiores de diferente composição química, estes ambientes oferecem um grande leque de oportunidades ecológicas para a seleção natural.

Crenuchus spilurus Günther 1863 (Characiformes: Crenuchidae) é um peixe pequeno e sexualmente dimórfico e dicromático que habita igarapés de terra firme conectados a rios de água branca e preta em um área de mais de 3 milhões de km^2 (Pires et al. 2016). Os machos apresentam nadadeiras anal e dorsal hipertrofiadas, indicando que a seleção sexual provavelmente opera nessa espécie (Pires et al. 2016). Os adultos apresentam capacidade de dispersão limitada, comumente considerados territoriais (Planquette et al. 1996). Por ser tipicamente encontrado em igarapés de baixa correnteza, quase lênticos, *C. spilurus* é um exemplo perfeito de um peixe típico da terra firme, praticamente ausente de canais principais, resultando na distribuição fragmentada característica de organismos nesse ambiente (Crampton 2011).

Nesse trabalho nós estudamos as causas comportamental e reprodutiva de adaptação aos tipos de água (*sensu* Mayr 1963). A causa comportamental foi investigada em experimentos de escolha de habitat, nos quais indivíduos tiveram a escolha entre água de igarapé *versus* canal principal. Para investigar a reprodutiva, verificamos o efeito da hidroquímica na desova ao expor casais de *C. spilurus* à água de canais principais e à água simulada de igarapé de terra firme. Dessa maneira, nosso trabalho teve por objetivo responder o que desencadeia a desova em diferentes linhagens de *C. spilurus*, e como a escolha de tipos de água específicos pode mediar divergência nessa espécie.

2. Objetivos

Geral

Avaliar a influência do tipo de água no comportamento e na fisiologia de indivíduos de *Crenuchus spilurus* provenientes de linhagens de bacias de água branca e água preta na Amazônia.

Específicos

1. Verificar a escolha de água de igarapé em detrimento da água da respectiva bacia de drenagem (várzea para linhagem Amazonas, igapó para linhagem Negro).
2. Atestar a ocorrência de desovas entre casais de *C. spilurus* formados por indivíduos da mesma origem, em águas de igarapé e da respectiva bacia de drenagem.

Capítulo 1

Stefanelli Silva, G., Pires, T. & Zuanon, J. 2018. Revisiting Wallace's water types: experimental evidence highlights the importance of stream water in shaping adaptation in an Amazonian fish. Submetido a *Hydrobiologia.*

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Revisiting Wallace's water types: experimental evidence highlights the importance of stream water in shaping adaptation in an Amazonian fish

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Revisiting Wallace's water types: experimental evidence highlights the importance of stream water in shaping adaptation in an Amazonian fish

Following Alfred Russel Wallace, Amazonian freshwaters have been classified into three main types: black, white and clear. Adaptation to water types has been demonstrated to affect gene flow, making it an important source for diversification of the Amazonian fish fauna. However, this classification focuses on large rivers and fails to acknowledge the importance of small forest streams that flow through non-flooded forest, known as igarapés. As compared to large river channels, igarapés cover a much greater area than the Amazon and have a distinct hydrochemistry, mostly due to soil and vegetation. We assessed the importance of water type on spawning and habitat choice in *Crenuchus spilurus*, a small fish that inhabits igarapés inserted within blackwater and whitewater river basins. Lineages from the Rio Negro (blackwater) and Amazon River (whitewater) showed active selection of igarapé water in habitat choice trials, and had greater spawning success in igarapé water when compared to water from their respective flooded forest basin. Due to the hydrochemical conditions of igarapés acting to shape reproduction and behavior of *C. spilurus*, our results expand Wallace's intuition that water types influence diversity by providing evidence that igarapés represent an important habitat driving adaptation in Amazonian fishes.

Keywords. Adaptation, habitat type, habitat choice, reproductive isolation.

Introduction

Inspired by an "earnest desire to behold the luxuriance of animal and vegetable life" of a tropical country, British naturalist Alfred Russel Wallace journeyed through the rivers of northern South America between 1848 and 1852 (Wallace, 1889; quote from page V). Borrowing from native terminology, he was the first to scientifically report the distinct types of Amazonian waters, separating them by their color: white, blue and black. Even to date, this classification still holds value despite simplifying the limnologic diversity of such a large and complex system (Fittkau, 1967; Sioli, 1984; Venticinque et al., 2016).

Whitewater rivers are mostly Andean in origin, having their distinct brownish color due to dissolved organic matter and suspended clay particles arising from soil composition and nearly complete degradation of plant material (Junk, 1970), which also result in approximately neutral pH. These rivers run through clayey soils and are on their lower portion surrounded by periodically inundated forests, the várzea (Junk, 1984). Várzea vegetation is diverse compared to other flooded forests and has a unique plant composition (Wittmann et al., 2006), given the necessary adaptations to living partially underwater during the high water season (Wittmann et al., 2004). High plant richness in várzea forests comes from coexistence between upland generalist species adapted to flooding and várzea-exclusive species, resulting in waters with characteristic dissolved organic carbon (DOC) (Prance, 1979).

Blue- or clearwater is poor on DOC, running through rocky soils of Archean shields which have been heavily eroded (Sioli, 1984). Clearwater pH ranges from acidic to neutral, depending on soil characteristics, making clearwater a collective of hydrochemistry that shares poorness of suspended solids as a common characteristic (Sioli, 1984). Blackwater is translucent, running through sandy or rocky soils, but rich in DOC from the incomplete degradation of forest plant material, giving it its characteristic reddish-black hue and strongly acidic pH (Klinge, 1967). On their lower portions, clearwater and blackwater rivers are surrounded by another type of flooded vegetation, the igapó. Given the lack of nutrients, igapó plant species richness and abundance is much lower than that of the várzea (Junk et al., 2015).

Such variety of physical and chemical conditions pose multiple physiological stressors upon the local aquatic life (Junk et al., 1983; Wilson et al., 1999), which may constitute an important source of selective pressures. As such, the distinct vegetation types may be considered partly responsible for the observed distinct species composition (Cooke et al., 2012a), local adaptation (Beheregaray et al., 2015) and speciation (Cooke et al., 2012b) of Amazonian fish fauna. Therefore, water types purportedly have important bearings on regional biological diversity, as originally suggested by Wallace.

Unreported by Wallace was a fourth type of water body long recognized by indigenous South Americans: forest streams, locally termed igarapés (Old Tupi for way of the canoe). Igarapés are small streams found across the Amazon basin, regardless of water color of the main channels they are connected to (Sioli, 1984). These streams form a dense aquatic network, connected to all the region's major rivers (Junk et al., 1983). In terms of contribution to the basin, the summed surface of igarapés are multiple times higher than the surface of the Amazon itself, and their combined lengths would result in a watercourse more than a thousand times longer than such mighty river (Fittkau, 1967). For a review on general terminology and stream importance see Biggs et al. (2017).

Igarapés are characterized by their translucent and variably acidic water, ranging from clear to dark-reddish, depending on depth, soil type and the floristic composition of the surrounding vegetation (Junk & Furch, 1980; Sioli, 1984). Upland igarapés are surrounded by terra firme forest, the most biodiverse environment in the world (Gentry, 1988). As such, waters draining terra firme soils incorporate compounds from decomposing plant material, resulting in a DOC composition which is different from that of the floodplains, arising from a dominance of typical terra firme plant species (Pitman et al., 2001). These streams are not affected by the seasonal flood pulse, being largely governed by local rainfall (Fittkau, 1967) and harboring a markedly different fauna and flora from that of the floodplains (Walker, 1990; Crampton, 2011). Despite their unique characteristics, little emphasis has been given to igarapés as a distinct habitat type that contributes in originating and maintaining freshwater diversity in Amazonia. Given that igarapés are not only influenced by adjacent forest but are also connected to larger tributaries of different chemical composition, these environments pose a great array of ecological opportunities for natural selection to act upon.

The sailfin tetra *Crenuchus spilurus* Günther 1863 (Characiformes: Crenuchidae) is a small, sexually dimorphic and dichromatic fish inhabiting terra firme igarapés connected to both whitewater and blackwater rivers over a range of more than 3 million km^2 (Pires et al., 2016). Males possess hypertrophied dorsal and anal fins, indicating that sexual selection likely operates in this species (Pires et al., 2016). Adults have limited dispersal capability, being often considered territorial (Planquette et al., 1996). Because it is usually found in slow-flowing, almost lentic-like igarapés, *C. spilurus* is a perfect example of a typical terra firme fish, which is nearly absent from main channels, yielding a patchy distribution characteristic of organisms in this environment (Crampton, 2011).

In this study, we investigated reproductive and behavioral aspects of adaptation to water types (hydrochemical conditions). The effect of water types on reproduction was studied by exposing *C. spilurus* couples to water from main river channels and simulated terra firme igarapé water. The effect of water types on behavior was investigated in habitat choice experiments, where individuals were given the choice between igarapé versus main channel water types. As such, our work sought to answer what triggers reproduction on different lineages of *C. spilurus*, and how the choice of specific water types might mediate lineage divergence in this species.

Material and methods

Fish collection and husbandry

We collected *Crenuchus spilurus* individuals from two igarapés within the Amazon (Iquitos, 3°50'25"S; 73°22'51"W) and Negro (Manaus, 3°6'22.04"S; 59°57'60"W) basins. Fifty males and fifty females from each location were used, totaling 200 individuals. Fish were separated by origin and sex (based on secondary sexual characteristics) and kept isolated for at least one month prior to experimentation in filtered water. No fish pair was used more than once and no fish was used in two consecutive experiments. Light/darkness regime consisted of 12:12 h, with a constant temperature of 24 °C. Fish were fed daily high quality commercial fish food. This work complied with INPA's Ethics Committee on Animal Use (CEUA) regulations, process nº 046/2016.

Water collection and preparation

To reproduce the two main types of Amazonian flooded forest environments (várzea and igapó) in the laboratory, we collected water via a tank truck. Whitewater was obtained from the Amazon River (upriver from the Rio Negro confluence, 3°17'24"S; 60°11'20"W), and blackwater was obtained from the Tarumã-Mirim River, a Rio Negro tributary (2°58'57"S; 60°06'16"W). We checked pH (7.1 and 5.8, respectively) and electrical conductivity (120 and 30 µS*cm^{-1}, respectively) before and after experiments to guarantee the original chemical condition of whitewater and blackwater was maintained.

We reconstituted igarapé water by immersing decomposing plant material from a terra firme forest (Adolfo Ducke Reserve, 2°57’42’’S; 59°55’40’’W) into groundwater (pH = 7; conductivity of 9 µS*cm^{-1}), since the majority of stream DOC stems from decomposing leaves (McDowell & Fisher, 1976). The plant material was left to leach for 10 days, and 1 kg of plant material was used per 100 l. After 10 days, the reconstituted igarapé water was stained in a brownish coloration similar to that found in the natural environment.

Water type choice

To assess whether *C. spilurus* immediately chose a specific water type, we carried out a dichotomous choice experiment using 30x20x15 cm aquariums divided into three equal parts.

One of the sides was filled with igarapé water and the other was filled with water from the floodplain native to the individual (várzea water for fish from the Amazon lineage and igapó water for the Negro lineage, conductivity values of 120 and 30 µS*cm^{-1}, respectively). The central partition was filled with DOC-free water (filtered groundwater, conductivity of 9 µS*cm^{-1}). To remove the influence of acidity/alkalinity, all waters were buffered for neutral pH. Water types were randomly allocated to each side, and all aquariums were visually isolated with opaque dark plates on all sides but the top.

Once all partitions were filled, fish were carefully introduced in the central partition and left for one hour before dripping DOC-free water slowly started to flow into the middle partition (1.2 ml*s^{-1}). Water level was eventually high enough to allow for free movement between compartments (~20 min after flow start). After one hour, we took note of where each fish had chosen to settle (figure 1). Experimentation was carried out during daytime. To ascertain the appropriate evaluation time window before complete water mixing, we conducted a pilot experiment using dyes. Within 70 minutes, each compartment still had its original properties (see supplementary material). 30 replicates were carried out for each *C. spilurus* lineage, totaling 60 samples. Individuals that remained in the middle section of the aquariums were considered as showing uncertain preference and were excluded from the analysis.

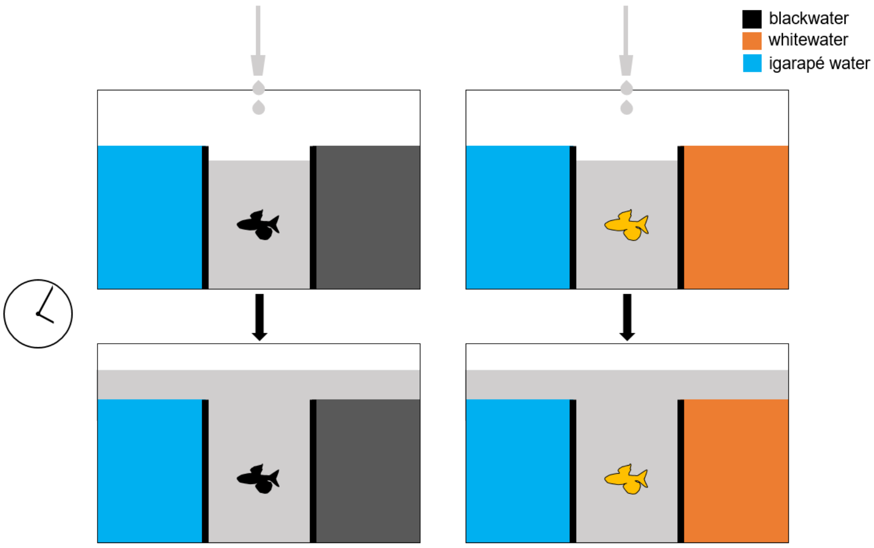

Fig. 1 Choice aquariums before and after compartment connection via water. Blue color represents igarapé water, while dark grey and orange represent blackwater and whitewater, respectively. Light gray represents filtered water. Fish from Rio Negro basin are represented in black, whereas fish from the Amazon River basin are yellow. Thick black bars inside the aquariums are glass panes and the light gray arrow-like shape at the top represents the water dripping mechanism. The clock symbol and black arrows denote passage of time.

Spawning success

We used 40x30x30 (36 l) aquariums, each of them containing substrate composed of a fine layer of sand and pebbles, two PVC tubes of 20 mm in diameter and 150 mm in length (used as shelter and spawning site) and artificial plants (for habitat enrichment, aimed at reducing stress from the captivity condition). A flow-through system assured that each aquarium had running water at a flow of 5 ml*s^{-1}, which allowed for circulation and complete water renewal every 12 hours. Each aquarium housed one male and one female *C. spilurus*, both sexually mature, which were fed once a day.

To reduce the influence of body size on mate choice (Olsson, 1993; Werner & Lotem, 2003; Wada et al., 2011), couples were size-matched for this experiment. Individuals were measured (standard length, in mm) before the experiment and couples were paired within a maximum of 3 mm size asymmetry margin. Visual contact between aquariums was avoided by opaque dark plates on all sides but one. To allow inspection, the front panel was blocked by a dark cloth that was only removed during feeding and inspection of nesting sites (PVC pipes). Couples were maintained for 20 days and monitored every morning for spawning inside the tubes (figure 2). Three experimental batches were executed in random, following the three treatments (whitewater from várzea, blackwater from igapó, and prepared igarapé water), totaling 108 samples (Table 1).

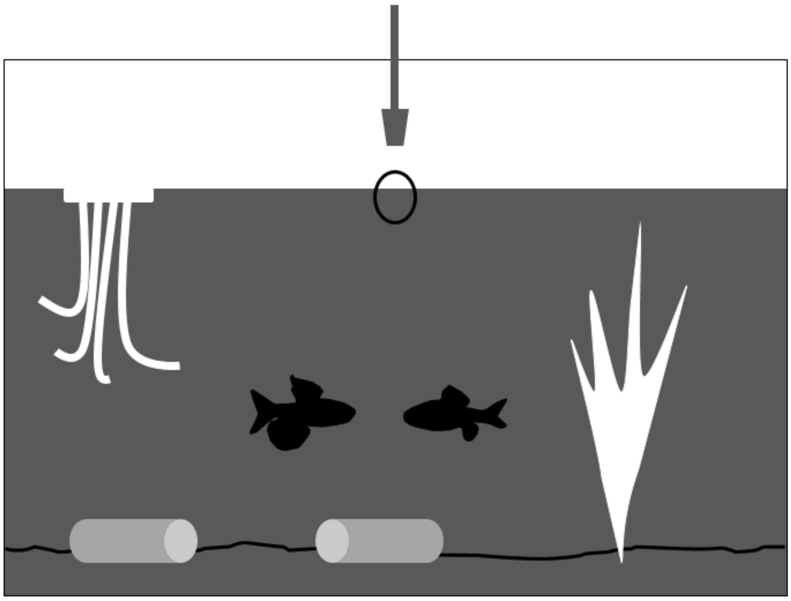

Fig. 2 Schematic view of the experimental aquariums. White shapes are artificial vegetation and light gray tubes are PVC pipes for shelter/spawning. The black outline on the bottom denotes fine sand/pebbles soil. The dark gray arrow-like shape at the top represents water flow entrance and the black circle below it represents the water drainage system.

Table 1. Replicates for each water treatment

Lineage	Amazon	Negro
Water type		
Whitewater	27	-
Blackwater	-	27
Igarapé water	27	27

Statistical analyses

Success and failure to spawn in our aquariums were scored respectively as one or zero for each couple. Comparison of the success between lineages (Negro and Amazon) and treatments (water types: whitewater from várzea, blackwater from igapó and prepared igarapé water) was conducted using a binomial model that included the interaction between these two factors. Since the interaction term indicated that the relationship between spawning success and water types depended on the lineages, we conducted two separated binomial models for each lineage to assess whether the treatment had effect within each lineage.

Habitat choice was measured as the proportion of fish that chose igarapé or floodplain (igapó and várzea) water, and such proportions were compared using two-tailed binomial exact tests, one for each lineage. All statistical analyses were conducted in R v. 3.4.1 (R Development Core Team, 2017).

Results

Water type trials (figure 3) indicated a marked habitat choice favoring igarapé water by the Rio Negro (76%, 19 of 25, binomial test $P < 0.05$) and Amazon lineages (75%, 18 of 24, binomial test $P < 0.05$).

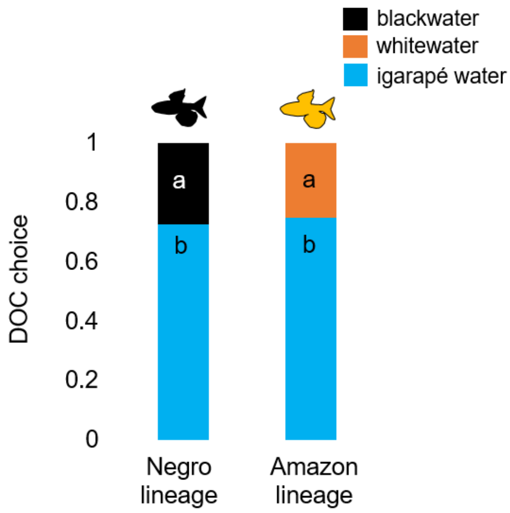

Fig. 3 Water type choice (%) in the Negro (black fish, left) and Amazon (yellow fish, right) lineages of *Crenuchus spilurus*. Letters denote significant differences between water types for each fish lineage ($P < 0.05$)

There was a significant interaction between water type and fish origin ($F = 4.995$, $P < 0.05$). *Crenuchus spilurus* from the Rio Negro lineage attained greater spawning success in igarapé water as opposed to blackwater ($F = 11.37$, $P < 0.001$) (spawning success of 81.48%, 22 of 27, *versus* spawning success of 33.33%, 9 of 27); *C. spilurus* from the Amazon lineage similarly attained higher success in igarapé water when compared to whitewater ($F = 4.24$, $P < 0.05$) (spawning success of 24%, 6 of 25 *versus* spawning success of 3.7%, 1 of 27) (figure 4).

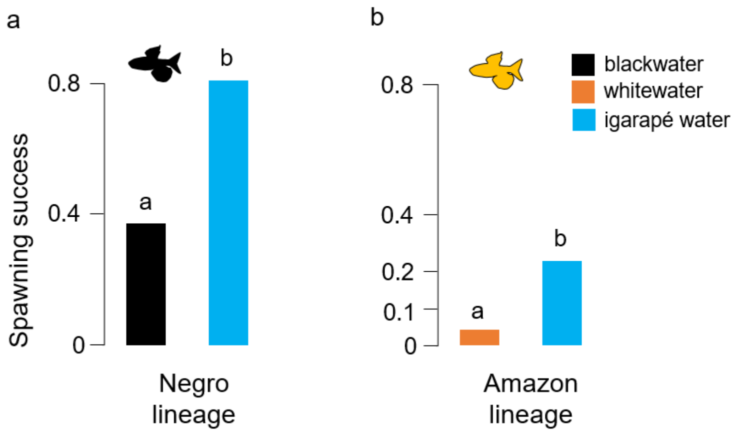

Fig. 4 Spawning success (%) between couples in the (a) Negro (black fish) and (b) Amazon (yellow fish) lineages. Lowercase letters denote significant differences between water types ($P < 0.05$)

Discussion

Adaptations of the aquatic biota to different Amazonian water types is a topic that has gained importance in recent years (see Val & De Boeck, 2017). Here we investigated spawning success in the nominal species *Crenuchus spilurus*, which comprises two reproductively isolated lineages occurring in small forest streams (igarapés) inserted in basins of different Amazonian water types: black- and whitewater. Our results show that both lineages attain higher relative spawning success in water similar to igarapés, regardless of the water type of their basins (*sensu* Wallace, 1889; Sioli, 1984). In a follow-up experiment, fish presented active habitat choice, with individuals of both lineages moving towards igarapé water. Because of the observed importance of forest stream water in mediating reproductive output and habitat choice, we propose that igarapé water is an important source of selective pressure that drives diversity within Amazonian freshwaters. Here, we show that these two lineages presented a diminished tendency to reproduce in the floodplain water of their respective basins.

Changes in environment characteristics are often detected by sensorial systems, which act as information to regulatory mechanisms, rendering possible changes in physiology (Endler, 1992; Boughman, 2002). As such, when presented with a choice, individuals may move towards environments where life and reproduction can be facilitated (Barton & Hewitt, 1989; Schluter, 2000; Nosil et al., 2005). Habitat choice in alignment with higher spawning success in a given environment indicate that natural selection acted to tune the sensory system to ideal conditions for spawning (Berner & Thibert-Plante, 2015). As such, the results presented here indicate that the sailfin tetra can discriminate among habitat/water types and is able to detect and move toward the environment where they achieve higher spawning success.

Our habitat choice trial showed that, when given the opportunity, both lineages chose igarapé water over that of their respective floodplain basin. Coupled with the spawning success results, these indicate that *C. spilurus* not only shows environmental-dependent behavior and physiological responses, but actively distinguishes between different waters. Such choices were probably based on olfactory cues related to DOC, the most noticeable difference among tested water due to the steep difference in floristic composition among várzea, igapó and terra firme environments (Campbell et al., 1986; Junk et al., 2015). Therefore, we suggest that DOC cues likely constituted an indicative of overall habitat type and quality for *C. spilurus*, and probably for other fish in the Amazon basin as well.

Natural selection drives organisms to adjust their reproductive investment to match the best environmental conditions for offspring survival (Stearns, 1989; Lindström, 1999). Likewise, ultimate causation (*sensu* Mayr, 1963) for active choice and higher spawning success in igarapés in both lineages can be related to elevated parental and offspring fitness in this environment. Igarapés may represent ideal conditions for *C. spilurus* through a combination of relatively still waters (Fink & Fink, 1979), refuge from predator pressure (Bührnheim & Fernandes, 2003), and availability of allochthonous plant material for shelter (e.g. fallen palm tree leaves) (Walker, 1987). *Crenuchus spilurus* deposits its eggs onto hard substrates, which are then cared for by the males for up to eight days (Pires et al., 2016), a strategy that might face diminished success in a highly seasonal environment such as the igapó or the várzea floodplains (Henderson, 1990; Petry et al., 2003).

The observed water type/habitat choice in our trials suggests that populations were exposed to natural selection against inhabiting or reproducing in floodplain waters. Indeed, *C. spilurus* is only found in igarapés with specific habitat characteristics, despite its huge geographical range of over 3 million km^2 (Pires et al., 2016). In combination, these facts suggest that the sailfin tetra probably experienced episodes of unsuccessful floodplain occupation, likely via passive dispersal, as individuals are not good swimmers (Pires et al., 2016). Thus, past contact with floodplain waters could have shaped habitat choice via low offspring survival in this environment.

Traits responsible for habitat choice affecting both reproductive isolation and local adaptation can be considered multiple-effect traits, or magic traits (*sensu* Gavrilets, 2004). The joint effects may increase odds of reproductive isolation when compared to cases where single-effect traits are predominant (Webster et al., 2012, but see Nosil et al., 2009). Our results imply that active habitat choice and local adaptation contributes to limiting interbreeding between lineages by making among-population contact improbable, as individuals would avoid the regions where contact with individuals of the other lineage is higher (i.e. the flooded forest downstream). In addition, the fitness reduction of individuals when in contact with the drainage systems which igarapés are connected may also limit the odds of interbreeding between lineages of *C. spilurus*.

As such, reduced sexual interactions outside of igarapés coupled with low tolerance for floodplain water within one's own basin should pave the way for reproductive isolation and ecological speciation in *C. spilurus*. Berner & Thibert-Plante (2015) argue that empirical examples of how habitat preference induces speciation in non-host-parasite scenarios are still rare (see MacCallum et al., 1998; Cruz et al., 2004; Grant & Grant, 2008; Bolnick et al., 2009; Eroukhmanoff et al., 2011), but *C. spilurus* and igarapés may be an example of one such case.

Because the studied individuals were captured from natural environments, habitat choice of igarapé water is in alignment with natal habitat imprinting, where experience in early life history stages at a given habitat shapes preference for that specific environment when individuals are presented with other opportunities (Immelmann, 1975; Davis & Stamps, 2004). Interestingly, and contrary to this theoretical model (Stamps et al., 2009), natal habitat preference would increase the likelihood of interbreeding when igarapés from distinct basins are directly connected, as in the case in headwater capture events, a process put forth as an important component shaping Amazonian freshwater diversity (Waters et al., 2001; Albert & Carvalho, 2011; Bloom et al., 2013).

Conclusions

During his travels in South America, A. R. Wallace investigated major tributaries of the Amazon River and established a simple yet efficient classification of water types; however, there was still another water type already acknowledged by the natives that he failed to recognize. Travelling mostly on boats through large rivers, he apparently neglected the distinctiveness of upland streams inside deep forests, aggregating those water bodies into his three optical categories (Wallace, 1889). Although previous studies have established the beneficial effects of blackwater DOC on fish physiology (Steinberg et al., 2006; Duarte et al., 2016), our study is, to the best of our knowledge, the first to put forward the importance of igarapé water in driving habitat choice in an Amazonian fish. This work provides evidence as to how habitat cuing might contribute to the evolution of two distinct lineages separated by a non-physical barrier, corroborating and improving upon Wallace's intuition that water (or habitat) types have a part in promoting diversification in Amazonian fishes.

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Supplementary material

We used dyes to assess the optimal time window for recording the position of the fish in the water type trials (figure 1). Through the Gpick color picker tool we selected 6 random green values from each compartment, for 13 pictures representing 2 hours' worth of experimentation (0 to 120 min, pictures taken every 10 min). These values were plotted against picture number (time) and the maximum time in which no mixing was visible was obtained (~70 min, photo 8) (figure 2).

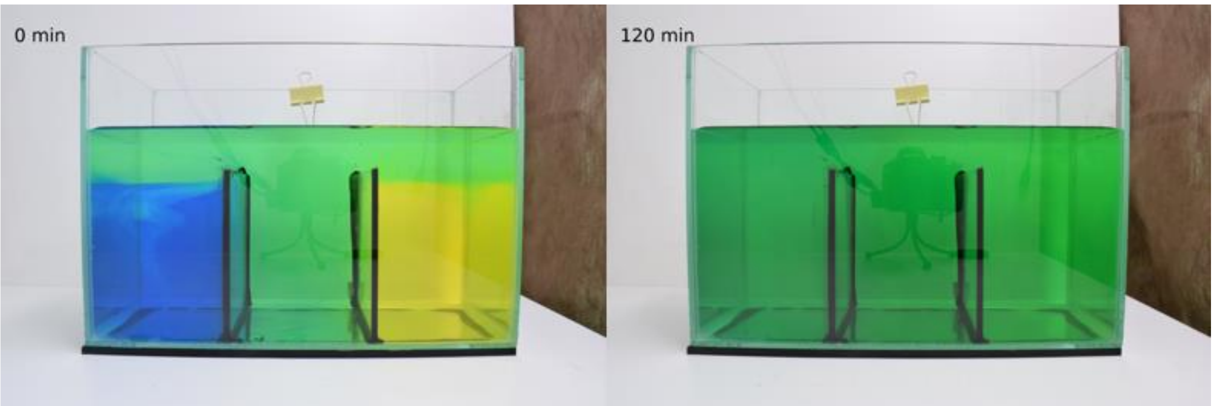

Figure 1. Water choice aquarium containing blue and yellow dyes in each lateral compartment at 0 (left) and 120 min (right). Green color indicates water mixing.

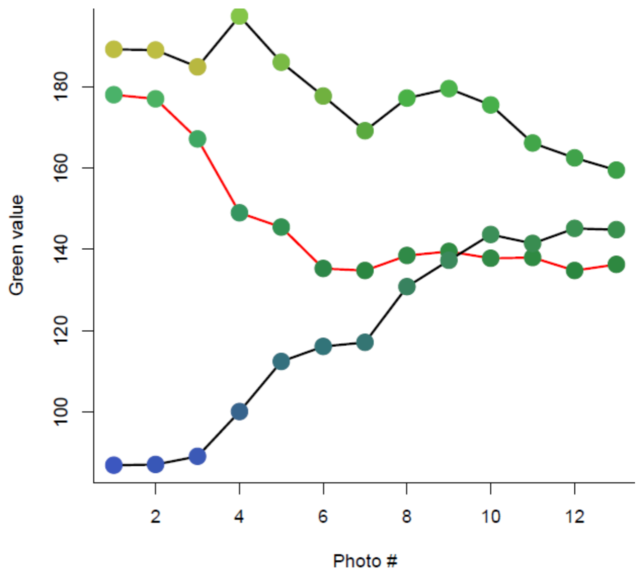

Figure 2. Green values plotted against photo number. Upper curve represents average green values for the right compartment, with the bottom curve for the left compartment. Middle red curve represents those values for the central compartment. Juxtaposition indicates total mixing between compartment waters.

3. Conclusões

O presente estudo avaliou a influência do tipo de água sobre o sucesso de desova e a escolha de ambiente em indivíduos de *Crenuchus spilurus* provenientes de linhagens de bacias de água branca e água preta na Amazônia. A preferência clara por água de igarapé, tanto pela maior ocorrência de desovas (objetivo específico 1) quanto pela escolha ativa desse habitat (objetivo específico 2) evidencia um processo de adaptação local a esse tipo de ambiente em detrimento das regiões alagadas de várzea e igapó. Tais escolhas provavelmente se baseiam em pistas olfatórias relacionadas ao perfil de carbono orgânico dissolvido (COD) de cada ambiente. Florestas de várzea, igapó e terra firme apresentam composição florística distinta, levando a uma variação de COD ambiental que influencia diretamente os respectivos ambientes aquáticos e, por consequência, a fauna aquática. Dada a relevância dos igarapés em mediar preferência nessa espécie, faz-se necessária uma nova consideração da classificação de Wallace levando em conta o potencial evolutivo propiciado por esses corpos d'água. Este trabalho fornece evidência de como a percepção ambiental pode contribuir para a evolução de duas linhagens distintas separadas por uma barreira não física, contribuindo para a intuição de Wallace a respeito do papel da água (ou ambiente) em promover diversificação de peixes na Amazônia.