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Harnessing the potential for otolith microchemistry to foster the conservation of Amazonian fishes

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Abstract

1. Freshwater environments host roughly half of the world's fish diversity, much of which is concentrated in large, tropical river systems such as the Amazon. Fishes are critical to ecosystem functioning in the Amazon River basin but face increasing human threats. The basic biology of these species, and particularly migratory behaviour, remains poorly studied, in part owing to the difficulty associated with conducting tagging studies in remote tropical regions.

2. Otolith microchemistry can circumvent logistical issues and is an increasingly important tool for studying fish life histories. However, this approach is still new in the Amazon, and its potential and limitations to inform fish conservation strategies remain unclear.

3. Here, otolith microchemistry studies in the Amazon are reviewed, highlighting current possibilities, and several key factors that limit its use as a conservation tool in the Amazon are discussed. These include the dearth of spatiotemporal elemental data, poor understanding of environment–fish–otolith pathways, and insufficient funding, facilities, and equipment.

4. A research initiative is proposed to harness the potential of this technique to support conservation in the Amazon. Key aspects of the proposal include recommendations for internal and external funding, which are critical to acquiring and maintaining technical staff, cutting-edge equipment, and facilities, as well as fostering regular scientific meetings and working groups. Meetings can facilitate a systematic approach to investigating environment–otolith pathways, broadening the chemical baseline for most Amazonian tributaries, and exploring potential valuable elements.

5. These outcomes are urgently needed to conserve biodiversity and ecosystem functioning in the Amazon, especially given threats such as widespread hydroelectric damming. The initiative proposed here could make otolith microchemistry an important, cost-effective tool to inform and foster conservation in the Amazon, and act as a template for other imperilled tropical river basins, such as the Mekong and the Congo.

1 | INTRODUCTION

Freshwater ecosystems cover less than 1% of the Earth's surface but account for about half of the planet's 30,000-plus fish species (Arthington, Dulvy, Gladstone, & Winfield, 2016; Carrete Vega & Wiens, 2012; Dawson, 2012). The concentration of this remarkable biodiversity in environments of pivotal importance to humans makes freshwater fishes highly vulnerable to a range of threats (Arthington et al., 2016). Threats to freshwater fish diversity, and the many ecosystem services that those species provide, include fishing, pollution, habitat destruction, and damming of waterways, among others (Allan et al., 2005; Arthington et al., 2016; He et al., 2017). Tropical river basins, including the Congo, Mekong, Irrawaddy, and Amazon, contain most of this diversity but are poorly studied. South America alone contains more than 5,000 described freshwater fish species, of which approximately half are located in the Amazon Basin (Jézéquel et al., 2020; Reis et al., 2016). Total numbers of freshwater fishes in those regions are substantially higher, as 34–42% of the Neotropical fish fauna is estimated to remain undescribed (Reis et al., 2016).

Fishes are critical to ecosystem functioning in Neotropical freshwater ecosystems, as they, rather than invertebrates, are the dominant primary consumers (Lewis, Hamilton, Rodríguez, Saunders, & Lasi, 2001). In addition to feeding and migratory behaviours, the 'lower' trophic positions of these fishes means they play pivotal roles in terrestrial–aquatic carbon flows, seed dispersal, and food-web connectivity among disparate river habitats (Correa & Winemiller, 2018; Goulding, 1980; Lewis et al., 2001; Winemiller & Jepsen, 1998). Many of the larger-bodied species, which tend to be migratory, are also among the most commercially exploited fishes (Barthem & Goulding, 2007; Goulding et al., 2019), which makes them susceptible to a broad range of human impacts.

To date, the principal conservation tool for most Amazonian and Neotropical environments has been the implementation of protected areas that ignore the hydrological connectivity of freshwater systems because they were designed to preserve terrestrial ecosystems, thus failing to adequately protect fishes from 'upstream' human impacts (Castello et al., 2013; Frederico, Olden, & Zuanon, 2016; Frederico, Zuanon, & De Marco, 2018). The challenge of protecting Amazonian and Neotropical fishes within an ecosystem context (Goulding et al., 2019) is compounded by the difficulties of conducting basic research on fish ecology in such regions, as well as elsewhere in the tropical world, where field studies can be expensive and logistically difficult (Godoy, 1979).

As pressures on these fishes increase faster than our present capacity to understand and protect them, there is an unprecedented need to elucidate their life cycles. One analytical technique that has great potential for Neotropical and Amazonian migratory fishes is otolith microchemistry. Otoliths, which are small calcium carbonate structures in the inner ears of fishes, take up some elements and isotopes from the ambient environment (Figure 1). Trace elements may be transported across gill membranes into blood or ingested and then absorbed across the gut wall (Campana, 1999; Hüssy et al., 2020). Free or protein-bound ions can then be transported via ion channels into the otolithic chambers and the endolymph, and then either incorporated by enzymatic processes or trapped as free ions in the growing otolith surface (Hüssy et al., 2020; Thomas et al., 2019). Ions may also be mobilized from other parts of the body (Hüssy et al., 2020). These processes can alter the relative proportions of trace elements in otoliths compared with the external environment. Uncertainty regarding these processes can limit the interpretations of otolith microchemical patterns. Other elements and isotopes are affected by physiological processes and can provide useful information, particularly when
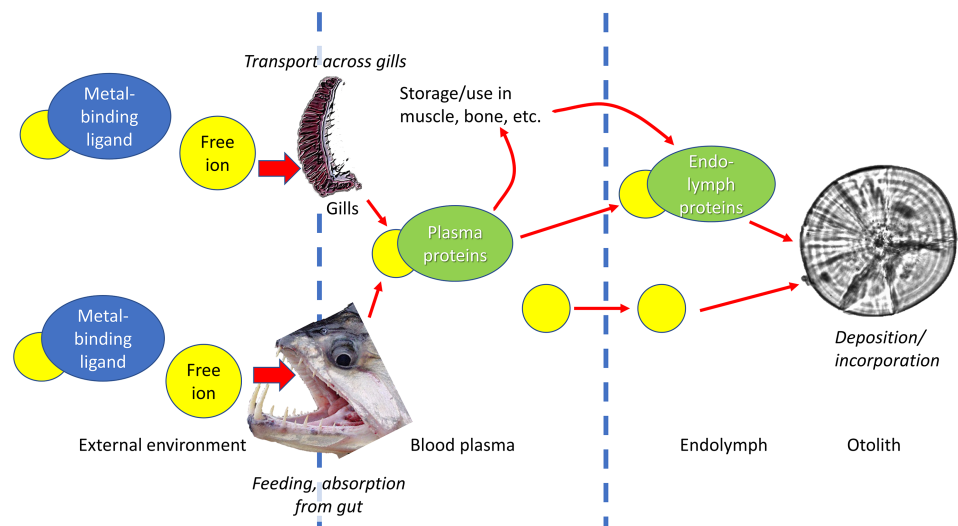
FIGURE 1 Schematic of ion transport from the environment into the otolith. Adapted from Hüssy et al. (2020)

interpreted together with environmentally informative otolith chemistry (cf. Limburg & Casini, 2019). As otoliths accrete daily, their lifetime activities are literally 'laid down in stone' as permanent environmental archives. Microchemical analysis thus enables the reconstruction of the life histories of migratory fishes without direct observation of their movements. Although the framework of this method is well established, its potential and limitations to inform fish conservation strategies remain unclear, in part because it is still a developing field of study in the Amazon.

In order to harness the potential of otolith microchemistry to inform the conservation of Amazonian fishes, this article assesses the potential and limitations of this method. After a brief description of Amazonian aquatic environments and the ecology of their migratory fishes, a synthesis is provided on how otolith microchemistry has been used in the Amazon, what has been discovered thus far, and how these findings might be used in the future. Then, key knowledge gaps, weaknesses, and assumptions in otolith microchemistry techniques are critically assessed. Finally, a research initiative is proposed for maximizing the potential of otolith microchemistry to inform conservation in the Amazon, which should be broadly applicable to other poorly studied tropical freshwater ecosystems. Our aim is to provide a resource synthesizing the existing published research on otolith microchemistry in the Amazon while also providing perspectives on its limitations and how these might be addressed in the future. We hope that this resource can act as a guide for conservation biologists to apply these methods more broadly, build collaborative networks, and foster the development of this high-potential field of research, and thereby acquire the baseline biological information required for the management of the Amazon's diverse fisheries resources.

2 | ENVIRONMENTS AND FISH MIGRATIONS OF THE AMAZON

The Amazon River basin covers nearly 7 million km^2 of South America (Figure 2a), bounded on the west by the Andes Mountains, to the north by the Guyana Shield, and to the south by the Brazilian Shield, and accounts for roughly 20% of global freshwater discharge to the oceans (Lundberg et al., 1998). The rivers originating in these different highland regions have distinct physicochemical properties. Wallace (1853) first described the diversity of Amazon rivers using three colour-based monikers: 'clearwater', 'whitewater', and 'blackwater' (Figure 2b–d). Sioli expanded upon this by demonstrating that these water-colour classifications also correspond to distinct water chemistries (for summary, see Sioli, 1984). Clearwater rivers similarly drain weathered areas, such as the Brazilian shield, resulting in a low suspended load, and have a slightly acidic to neutral pH and high transparency. Whitewater rivers originate in the Andes Mountains, from which they transport considerable volumes of organic and inorganic materials, causing them to have a near-neutral pH, high dissolved and suspended loads, and a 'café au lait' colour. These rivers are surrounded by diverse and productive floodplain forests (várzea)

(Junk et al., 2011). Blackwater rivers drain low-sediment, humic acid-rich areas such as the highly weathered Guyana shield, resulting in acidic, nutrient-poor water the colour of black tea. The streams feeding into blackwater rivers typically occur in areas with white sandy soils, low-growing vegetation, and stunted forests (caatinga) (Junk et al., 2011; Sioli, 1984). The lower reaches of the largest blackwater and clearwater rivers have also been described as 'river-lakes', because they widen dramatically with flow decreasing such that phytoplankton and zooplankton can proliferate (Sioli, 1984). These areas are thought to be drowned river valleys that were carved out during decreased sea level in the last glacial period (Wesselingh & Hoorn, 2011). At confluences of large rivers, water mixing may initially be incomplete when waters with very different physicochemical properties meet (Figure 2e). In addition, some streams may vary in type throughout the year, being clear when the flow is low and murky when high flows increase the sediment load (Sioli, 1984). The mouth of the Amazon (Figure 2f), which forms a vast deltaic region, has chemistry influenced by the seasonal flood of river waters, which seasonally shifts the relative influences of marine versus fresh waters.

The annual flood cycle of Amazonian rivers is a major driver of fish migration associated with the seasonal monsoon rainfall, with water levels rising 10 m or more in many areas (Irion, Junk, & de Mello, 1997; Trigg et al., 2009). Flooding connects main river channels with their adjacent floodplain ecosystems. The flooded forest (Figure 2g,h) is a critical habitat that links terrestrial and aquatic environments (Goulding, 1980). Generally, this flooding and interconnectivity drives fish migrations within river channels, or from floodplain lakes (Figure 2i) and river channels to the floodplain, as fish take advantage of access to new feeding and spawning areas, nursery habitats, and the avoidance of predators (Junk, Bayley, & Sparks, 1989). As flood waters recede, many species migrate back to lakes or river channels, or within the main river channels (Junk, Soares, & Carvalho, 1983). Such floodplain–river movements allow fish feeding on rich floodplains to subsidize oligotrophic river environments (Winemiller & Jepsen, 1998).

This cyclic regularity has resulted in a spectrum of migratory strategies, including 'lateral' and 'longitudinal' migrations (Duponchelle et al., this issue). Lateral migrants move short distances laterally from the river channel to adjacent floodplains, whereas longitudinal migrants move long or medium distances upstream and downstream within river channels. As they migrate only short distances, many lateral migrants have often been described as 'sedentary' or resident. Studies of migration in such species reveal complex migratory behaviours, however, as in the case of *Arapaima* spp., which migrate laterally from lakes into neighbouring floodplain environments during the seasonal flood (Castello, 2008). Some species, such as *Prochilodus nigricans*, have complex life histories involving lateral migrations into floodplain environments, migrations between whitewater and blackwater rivers, and longitudinal migrations along river channels (Araujo-Lima & Ruffino, 2003; Silva, 2000; Silva & Stewart, 2006, 2017; Winemiller & Jepsen, 1998). Thus, *P. nigricans* and similar species may be exposed to a variety of chemical conditions. Some

FIGURE 2 (a) Map showing the outline (teal) of the Amazon drainage basin. (b) The Xingu River, a large clearwater Amazon tributary. (c) The Madeira River, a large whitewater Amazon tributary. (d) The Nanay River, a large blackwater Amazon tributary. (e) Whitewater (left) from the Amazon mainstream meeting blackwater (right) from the Rio Negro. (f) The estuary at the mouth of the Amazon. (g, h) Forests flooded with whitewater (g) and blackwater (h). (i) A whitewater floodplain lake. Photo credits: (b, f) Michael Goulding (WCS); (c) Luis Claudio Marigo (WCS); (d, e) Fabrice Duponchelle; (g) Leandro Castello; and (h, i) Donald J. Stewart

longitudinal migrants undertake migrations of impressive scale in the Amazon mainstem and its major tributaries. These fishes are typified by the goliath catfish *Brachyplatystoma rousseauxii*, which performs the longest known freshwater fish migration, with a round trip of up to 12,000 km during its life that involves spawning in the headwaters of the Andean piedmont, the downstream drift of larvae to the estuary, and an upstream return by young adults (Barthem & Goulding, 1997; Barthem et al., 2017; Duponchelle et al., 2016).

Practically all migratory fishes of the Amazon are fished for subsistence and commercial purposes (Barthem & Goulding, 2007). Many such fishes already appear to be overexploited (Agudelo-Córdoba et al., 2013; Castello, McGrath, & Beck, 2011; Castello et al., 2013) and face threats, including: deforestation, which can change the flood regimes upon which migratory fishes rely (Coe, Costa, & Soares-Filho, 2009); widespread hydroelectric damming, which blocks

migration routes and disrupts flood pulses (Castello et al., 2013; Petrere, Barthem, Córdoba, & Gómez, 2004); and pollution from sources such as gold mining (Ashe, 2012), agriculture (Neill, Deegan, Thomas, & Cerri, 2001), and oil exploration (Finer, Jenkins, Pimm, Keane, & Ross, 2008). Addressing these threats to Amazon fishes requires an improved understanding of their migratory behaviour.

3 | APPLICATION OF OTOLITH MICROCHEMISTRY

Given the Amazon's diversity of fish species, its vast drainage area, and the remoteness of many locations, gaining the baseline biological information necessary for conservation is very difficult, because traditional tagging methods can be impractical if not impossible in the

Amazon (Godoy, 1979). Otolith microchemistry can circumvent many such logistical issues (Campana, 1999; Elsdon et al., 2008), because as otoliths grow in incremental rings proportional to body growth (Campana & Neilson, 1985; Mugiya et al., 1981; Panella, 1971), they preserve many rare-earth and other chemical elements (referred to as 'markers') at concentrations proportional to those found in the surrounding water (Campana, 1999; Campana & Thorrold, 2001; Elsdon et al., 2008), thereby providing records of growth, age, and environmental exposure. Otolith microchemistry has been used to deduce fish migrations in temperate-water diadromous fishes, such as salmoniforms (Kennedy, Folt, Blum, & Chamberlain, 1997) and freshwater eels *Anguilla* spp. (Tabouret et al., 2010), which experience changes in ambient water chemistry.

Assessing migratory behaviour in strictly freshwater fishes can be more difficult because differences in water chemistry between neighbouring freshwater bodies tend to be lower than those between marine and fresh waters. Otolith microchemistry can be useful in freshwater fishes when there are sufficient differences in water chemistry (Kennedy, Blum, Folt, & Nislow, 2000; Pouilly, Point, Sondag, Henry, & Santos, 2014; Walther, Dempster, Letnic, & McCulloch, 2011), but this requires an understanding of the eco-geochemical history of potential markers, which are influenced by the surrounding landscape, forest processes, weathering, hydrology, and chemistry (Figure 3). As a massive tropical ecosystem, the Amazon River basin is challenging in this regard. Elemental concentrations in tropical rivers can be highly influenced by flood pulses (Alibert et al., 2003), which are extreme in the Amazon (Irion et al., 1997; Trigg et al., 2009). Coupled with the enormous scale of the basin,

elucidating the spatiotemporal variability across the entire Amazon basin at both small and large scales remains a challenge.

Furthermore, the knowledge base on which to develop new studies in South America is small. Avigliano and Volpedo (2016) reviewed the application of otolith microchemistry on Latin American fishes and reported that, since the late 1990s, 45 otolith microchemistry articles focused on Latin American fishes had been published, of which only three were conducted on Amazon fish species; this number has improved only slightly since then. Despite these limitations, otolith microchemistry remains a promising method for studying migration in Amazon fishes (for further discussion on limitations and knowledge gaps, see Section 5).

4 | USEFUL MARKERS

4.1 | Strontium isotope ratios

Past studies conducted in the Amazon have identified several specific- and general-use chemical markers and techniques. To date, the most broadly used marker has been strontium (Sr) isotope ratios, specifically $^{87}Sr:^{86}Sr$ measured using laser ablation, multicollector, inductively coupled plasma mass spectrometry (LA-MC-ICPMS). This method is useful because there is no change in the ratio from the environment to the otolith, and the ratios are site specific, reflecting the underlying geology of a region (Kennedy et al., 1997; Kennedy, Klaue, Blum, Folt, & Nislow, 2002; Pouilly et al., 2014). Thus, with an adequate catalogue of values for a
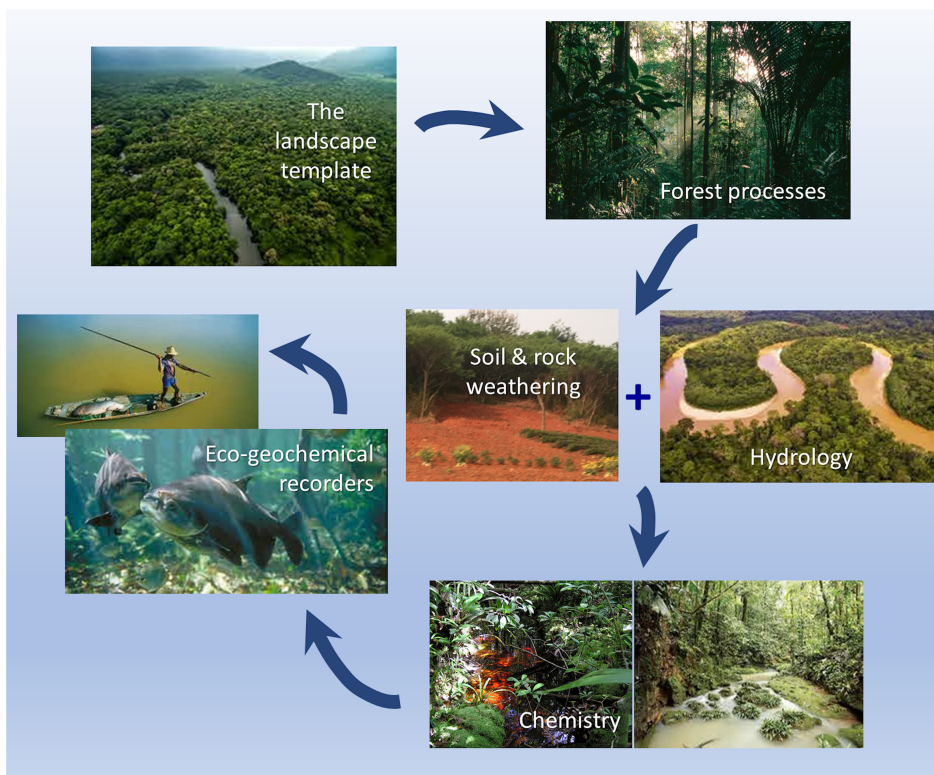
FIGURE 3 Conceptual diagram tracing the eco-geochemical processes that influence concentrations of trace elements in freshwater environments prior to being incorporated into structures such as otoliths. Photo credits (from upper left, moving clockwise): USA Today (stock image); A. Pave & G. Forner; Karin Limburg (left) and WWF (stock image, right); Fabrice Duponchelle (left) and Morley Read (right); Michael Goulding (WCS); Luis Claudio Marigo (WCS)

geographical region (assuming unique ratios for many sites within the region), it is possible to reconstruct fairly accurately the migratory life history of an individual. Isotope ratios for the geology of many drainages in the Amazon are available in the literature (Duponchelle et al., 2016; Hauser et al., 2019; for summaries, see Hegg, Giarrizzo, & Kennedy, 2015; Santos et al., 2015), which allows the study of species with very different migratory behaviours. For example, Sr isotope ratios have been used to study short-distance migrants such as *Arapaima* spp. (Pereira et al., 2019) and *Cichla* spp. (Garcez, Humston, Harbor, & Freitas, 2015; Sousa, Humston, & Freitas, 2016), as well as long-distance migrants such as *Brachyplatystoma platynemum* (Hauser et al., 2019) and *B. rousseauxii* (Duponchelle et al., 2016; Hauser et al., 2020; Hegg et al., 2015). The Sr isotope signatures of the Amazon mainstem and Madeira subbasin provided sufficient contrast to demonstrate the natal homing behaviour of *B. rousseauxii* in the Madeira (Duponchelle et al., 2016) (Figure 4a). In Figure 4a, low Sr isotope values (purple bracket) are consistent with values measured in the lower Amazon and estuary (purple ellipse), whereas higher values are (brown) were consistent with values measured in the Madeira tributary, from which this fish was caught (brown ellipse). The consistency between values at early and later life-history stages indicate that this fish homed back to its natal waters. This homing behaviour was also suspected in the upper Amazon but could not be demonstrated because of a lack of sufficient Sr isotopic contrasts along the Amazon mainstem. Strontium isotopic ratios in water depend almost exclusively on the age and bedrock geological composition of river basins, and thus they vary little across years and represent accurate habitat signatures (Kennedy et al., 1997, 2000; Walther & Limburg, 2012). Therefore, this can be an effective tool for studying migration in Amazonian species with diverse migratory strategies, provided the fish in question migrate among environments with sufficiently differing geology.

4.2 | Complementary elements

Additional markers have been used as 'helpers', providing complementary information in the form of other geochemical markers. For example, Hermann, Stewart, Limburg and Castello (2016) investigated Amazon fish otoliths using scanning X-ray fluorescence microscopy (SXFM) and found that the selenium (Se) to calcium (Ca) ratio in the otoliths of adult *B. rousseauxii* was elevated near the otolith core (i.e. during early life history) and the outer edge (i.e. when the fish was an adult) (Figure 4b). They noted that this was consistent with the fish returning as an adult to an environment that was chemically similar to where it was born, in this case an Andean tributary. Furthermore, Se:Ca had an inverse relationship with Sr:Ca, which led to the hypothesis that Se:Ca and Sr:Ca patterns in the goliath catfish otolith may indicate migrations into and out of Andean headwater rivers. Hauser et al. (2020) tested this idea by measuring Sr isotope ratios and Se:Ca and Sr:Ca in *B. rousseauxii* otoliths and found that both lines of evidence provided consistent life-history reconstructions, providing evidence of homing behaviour of *B. rousseauxii* in the upper Amazon.

Moreover, in some cases where Sr isotope ratio data were uninformative (e.g. along the Amazon mainstem), the Se:Ca and Sr:Ca data provided interpretable inferences regarding migration. Therefore, Se:Ca and Sr:Ca may be useful complementary tools when assessing migrations of catfishes into and out of the Andean headwater and estuary regions. In addition, Sr:Ca and Ba:Ca, together with Sr isotopes, have shown promise as markers of provenance for young *Cichla temensis* in a tributary network of the Negro basin (Garcez et al., 2015).

Barium (Ba) is an important tool for assessing fish migration using otolith microchemistry (Campana, 1999; Elsdon et al., 2008; Walther & Thorrold, 2006), but has been rarely used in the Amazon. Otoliths of diadromous fishes tend to show an inverse relationship between Sr:Ca and Ba:Ca deposited during migrations between fresh water and marine water (McCulloch, Cappo, Aumend, & Müller, 2005; Tabouret et al., 2010). Thus, Sr:Ca and Ba:Ca in combination may also be useful for assessing migrations into and out of the Amazon estuary. Indeed, this inverse relationship was found in the otoliths of *B. rousseauxii* (Hermann et al., 2016), which is known to inhabit the estuary during its juvenile life history. Using such markers, the relative proportions of this species both within and among populations that rely on the estuary could be estimated. This is especially useful given the difficulty in discriminating between residence in the lower Amazon versus the estuary using Sr isotope ratios (Duponchelle et al., 2016). Ba:Ca could also be used to study other species that use the Amazon estuary during their life history, including the closely related *Brachyplatystoma vaillantii*. In addition, Ba:Ca may provide evidence of exposure to certain pollutants, such as mining waste, as suggested by Hermann et al. (2016), but such uses will require further investigation.

4.3 | Additional markers

Other isotopic and elemental markers show promise but require more study. The ratio of manganese (Mn) to Ca has been reported in the Amazonian drum *Plagioscion squamosissimus* and the characoid *P. nigricans* (Hermann et al., 2016) (Figure 4c). Both species are known to occur and/or migrate into acidic blackwater rivers, in addition to floodplain environments where oxygen levels may be low; therefore, it is hypothesized that Mn:Ca is a marker for one or both of these environments, given the tendency for Mn to increase in concentration in the water column under acidic, low-oxygen conditions (see Limburg et al., 2015). If this is the case, Mn:Ca may be a useful marker for studying the life histories of Amazonian fishes that migrate between blackwater and whitewater rivers and low-oxygen environments, such as floodplains. Sr and carbon (C) isotope ratios have been investigated preliminarily as tags for provenance of *Arapaima* spp. Given the importance of these fishes as a food fish and their threatened status, such methods could be crucial for demonstrating the origins of commercially available meat (i.e. farm raised versus wild caught, as well as differences within each category; Pereira et al., 2019).
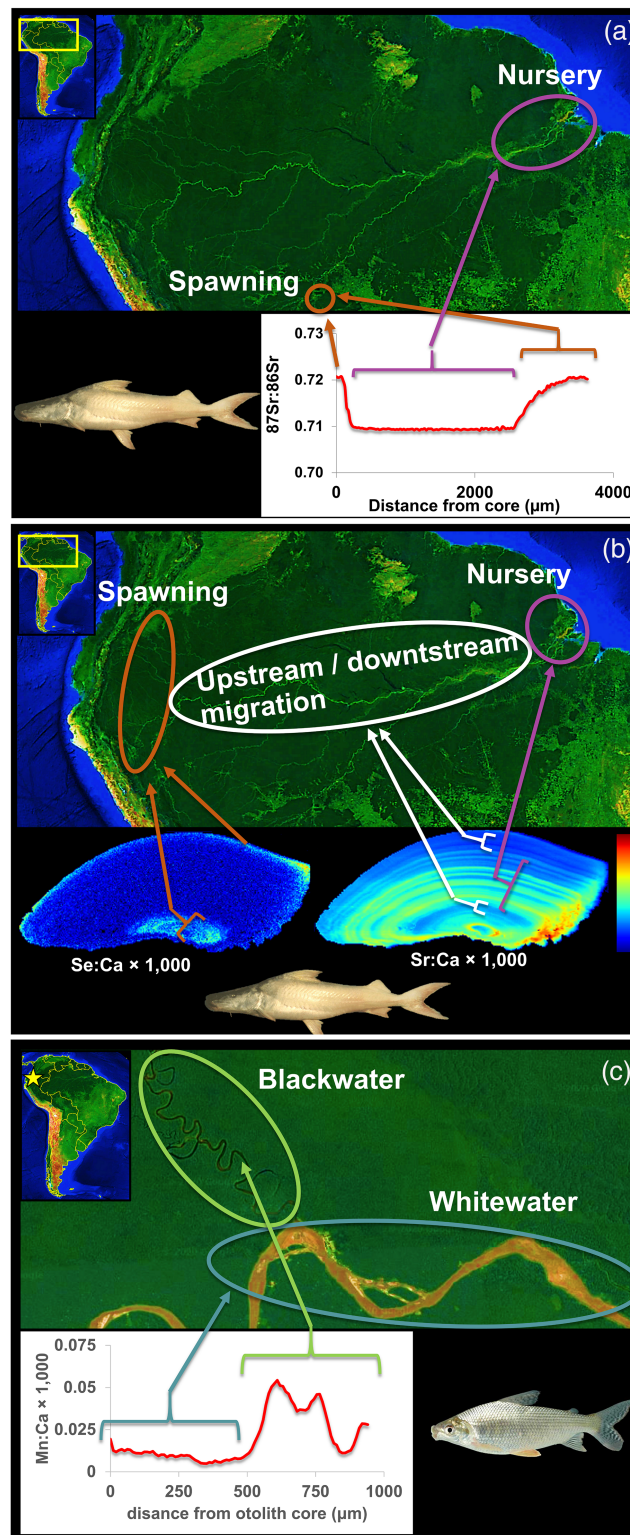
FIGURE 4 Reconstructions of migrations interpreted from otolith microchemistry studies in Amazonian fishes. (a) Strontium (Sr) isotope ratio data from the otolith of an adult *Brachyplatystoma rousseauxii* caught in the Madeira basin. (b) Sr and selenium (Se) to calcium (Ca) ratios from an adult *B. rousseauxii* caught in Colombia. The heat maps are scaled from low (blue) to high (red). (c) Manganese (Mn) to Ca ratio used to interpret movements in *Prochilodus nigricans* caught in a blackwater river (Cuyabeno) near its confluence with the whitewater Napo River in Ecuador. More details on these interpretations can be found in Duponchelle et al. (2016) and Hauser et al. (2020) (a, b) and Hermann, Stewart, Limburg, and Castello (2016) (b, c). All map imagery was provided by GOOGLE EARTH PRO 7.3.2.5491 (Google LLC) using Landsat/Copernicus images, and was edited using Microsoft POWERPOINT and Adobe PHOTOSHOP. Fish photo credit: Donald J. Stewart

4.4 | Case studies

4.4.1 | *Brachyplatystoma rousseauxii*

Brachyplatystoma rousseauxii is a piscivorous catfish that reaches 1.5 m in length and is heavily fished throughout the Amazon, including in the mainstem and estuary (Agudelo-Córdoba et al., 2013; Barthem & Goulding, 1997; García-Vásquez et al., 2009). The distribution of adults and juveniles led to the hypothesis in the 1990s that adults spawned in Andean whitewater tributaries of the Amazon, and then the eggs and larvae drifted downstream to the estuary, which acted as a nursery area where juveniles would feed and grow before returning upstream (Barthem & Goulding, 1997). Recent studies using otolith microchemistry have tested various aspects of this hypothesis, and have revealed chemical markers for assessing exposure to estuarine and headwater environments (Hauser et al., 2020; Hermann et al., 2016), have shown that *Brachyplatystoma* spp. have variable early life histories (Hegg et al., 2015), and have demonstrated that these fish may display homing behaviour similar to that of salmonids when returning to tributaries (Duponchelle et al., 2016; Hauser et al., 2020) (Figure 4a,b). Thus, otolith microchemistry has helped to elucidate a migratory life history spanning over 10,000 km and multiple countries without the need for extensive field studies.

4.4.2 | *Prochilodus nigricans*

Prochilodus nigricans is a schooling detritivore growing to around 40 cm in length (Silva & Stewart, 2017). *Prochilodus* spp. often dominate ecosystem biomass (Flecker, 1996) and, through migration, create critical ecosystem linkages that affect the structure and function of food webs (Winemiller & Jepsen, 1998). The migratory life histories of *Prochilodus* species and populations tend to vary, however (e.g. in timing or direction) (Winemiller & Jepsen, 1998), and many populations in the Amazon remain poorly studied. Otolith microchemistry analysis indicated that young *P. nigricans* in Ecuador used downstream whitewater habitats as nurseries and then migrated upstream to blackwater rivers before 1 year of age (Hermann et al., 2016) (see Figure 4c). These results were consistent with the conclusions of a field study conducted in the same region (Silva & Stewart, 2017). *Prochilodus nigricans* is also an important species in subsistence and commercial fisheries (Castello et al., 2011, 2013). As migratory detritivores, *Prochilodus* spp. may consume and bioaccumulate anthropogenic pollutants from one region and transport them to another, affecting food quality for predators and humans alike (Speranza & Colombo, 2009; Speranza, Colombo, Heguilor, Tatone, & Colombo, 2020). Anomalously high Ba:Ca values in otoliths from Ecuador were suggested as signs of barite contamination from oil-drilling sites (Hermann et al., 2016), as this region has a record of spills and leaks from oil exploration (San Sebastián & Hurtig, 2004). Otolith microchemistry may thus provide insights both on fish migration and the movement of pollutants in the Amazon.

5 | CURRENT LIMITATIONS

5.1 | Environment–fish–otolith pathways

The use of otolith microchemistry in an enormous, hyperdiverse, and poorly studied system like the Amazon, where there are more 'unknowns' than 'knowns', faces a few limitations that require consideration to avoid misleading conclusions. A fundamental but often overlooked consideration is the environment–fish–otolith pathway (Figure 1): that is, by which process do elements in the environment enter a fish, and from there how do they become incorporated into an otolith? And does this vary among species? For example, $Sr^{87}:Sr^{86}$ in fish otoliths reflects the ratio found in the surrounding water (Kennedy et al., 1997); thus, this information could be used to reconstruct migratory details confidently given sufficient data about the isotope ratios in the environment. Incorporation of otolith magnesium (Mg), however, appears to be influenced by fish growth and metabolism (Limburg, Wuenschel, Hüssy, Heimbrand, & Samson, 2018), so that attempts to reconstruct migratory life history using, for example, Mg:Ca may lead to the wrong conclusions.

In the Amazon, Mn and Se have been found in non-random patterns in the otoliths of several species, suggesting that these elements reflect migration patterns (Hauser et al., 2020; Hermann et al., 2016); however, the uptake of these elements by fish and potential filters that may influence their incorporation into fish otoliths remains poorly understood (Hüssy et al., 2020; cf. Thomas, Ganio, Roberts, & Swearer, 2017). Also, these elements have not been found universally in Amazon fish species. Mn has been found in *P. nigricans* and *P. squamosissimus*, but not in pimelodid catfishes, whereas Se has been found in pimelodid catfishes but not in *P. nigricans* or *P. squamosissimus*, despite the potential for environmental overlap among these species (Hermann et al., 2016). Therefore, there may be biotic processes (physiological or dietary dynamics) or abiotic factors (temperature) that affect the environment–otolith pathways of these elements, which has been observed for other elemental ratios (Elsdon & Gillanders, 2003).

The type of otolith analysed also plays a role. To date, lapilli have yielded promising results in ostariophysine fishes, such as Siluriformes (e.g. *Brachyplatystoma* spp.) and Characiformes (*P. nigricans*), whereas the sagittae have yielded the best results in Perciformes (*P. squamosissimus*), Cichliformes (*Cichla* spp.), and Osteoglossiformes (*Arapaima* sp.) (T. Hermann and K. Limburg, pers. obs.). Although the lapilli of pimelodid catfishes and the sagittae of drums such as *Plagioscion* tend to be quite large, such results are not a function of otolith size. For example, Hermann et al. (2016) reported data from the small lapilli of *Prochilodus* rather than the large, distinctly shaped asterisci, because the asterisci yielded poor results, with elements frequently found at concentrations below the detection levels of analytical equipment. This may be a result of the vateritic, as opposed to aragonitic, minerality of the asteriscus (Campana, 1999).

5.2 | Water chemistry data

A major limitation of otolith microchemistry in the Amazon is the current dearth of environmental data on dissolved elements and isotopes. Given the sheer scale of the Amazon River basin, capturing the spatial variability in dissolved elements and isotopes across the entire basin is a considerable task. Furthermore, the Amazon River basin is driven by powerful floods that affect water chemistry, meaning that seasonal variability in dissolved elements and isotopes should also be considered (for processes comprising eco-geochemical history, see Figure 3). Strontium isotope ratios probably have the best spatiotemporal database in the Amazon basin (Gaillardet, Dupré, Allegre, & Négrel, 1997; Palmer & Edmond, 1992; Queiroz, Horbe, Seyler, & Moura, 2009; Santos et al., 2015; Viers et al., 2005). To date, the available information allows a clear distinction between the Sr isotopic signatures of the main tributaries of the Amazon basin: the Amazon mainstem, the Madeira and Negro basins, and the granitic shield tributaries of the lower Amazon (for a synthesis, see Duponchelle et al., 2016). This database was therefore critical to several recent studies in the Amazon using otolith microchemistry to track the long-distance, sometimes trans-Amazonian, movements of goliath catfishes (Duponchelle et al., 2016; Hauser, 2018; Hauser et al., 2019, 2020), or to determine the provenance of marketed *Arapaima* spp. (Pereira et al., 2019). Some of these studies have also provided additional Sr isotope data, thereby increasing the database of environmental data available to future otolith microchemistry studies (Hauser, 2018; Hauser et al., 2019). Sr isotopes would also be perfectly suited to track the mid-distance movements of many characiforms and siluriforms between, for instance, the Amazon–Solimões and the granitic tributaries of the lower (e.g. Xingu, Tapajos, Trombetas, Urucara, and Itenez-Guaporé basins) and middle (Negro basin) Amazon, or between the Amazon mainstem and the Madeira. Expanding the spatial coverage of the database would increase the discriminatory power of Sr isotopes at finer scales, as exemplified by the unexpectedly high variations observed within tributaries of the poorly documented Negro (Garcez et al., 2015; Sousa et al., 2016) or Madre de Dios basins (Hauser et al., 2019).

In some cases, an approach that uses a suite of elements may be more beneficial: for example, for fish that move among environments with similar Sr isotope ratios. To achieve this, there is a need to improve not only our understanding of the spatial characteristics of dissolved elements, dictated by the underlying geology of a region, but also the temporal characteristics, possibly at a microenvironmental scale. For example, there is strong evidence that Mn:Ca increases in hypoxic and acidic conditions (Limburg et al., 2015; Siskey, Frisk, Cerrato, & Limburg, 2020), and it has been hypothesized to indicate exposure to such environments in *P. nigricans* and *P. squamosissimus* (Hermann et al., 2016). These conditions may appear seasonally within microenvironments (Anjos, De Oliveira, & Zuanon, 2008) rather than, for example, across a floodplain as a whole. Given the importance of microenvironments to Amazon fish assemblages (Petry, Bayley, & Markle, 2003), understanding fine-scaled environmental conditions may be essential to interpreting otolith microchemistry in some

species. The spatiotemporal environmental Mn data required to test these hypotheses in Amazon fishes, however, are lacking, so the ability of Mn:Ca to inform the reconstruction of fish movements among microenvironments is at present limited.

5.3 | Useful elemental and isotopic markers

Another limitation is the selection of elemental or isotopic markers. Owing to the diversity of Amazon fish life histories and our poor understanding of the topics outlined above, it may be inadequate to rely only on a single, well-established approach, such as Sr isotope ratios, for some or many species. Therefore, it is important to first use an open-ended, exploratory approach to identify which elements or isotopes are both present and useful, as undertaken by Hermann et al. (2016), before attempting to answer very specific questions about poorly understood species using inadequate otolith markers. A multi-elemental approach could be used first, to discover which elements may be valuable and those elements that probably are not. Methods such as laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) and SXFM allow the simultaneous sampling of many elements. Although some elements are not likely to be informative, there is no added cost (monetary or otherwise) associated with using a nonselective approach. Thus, elements that are not heavily represented in the Amazon literature but are possibly still valuable, such as Mn and Se (Hermann et al., 2016), can be identified.

5.4 | Access to equipment and funding

A final major limitation is access to equipment and funds to collect and analyse otolith samples. Although otolith microchemistry facilitates studies of fish migration by avoiding the logistical complexity of fieldwork, the necessary equipment is highly specialized, expensive, and relatively uncommon. We also note that no single instrument will perform well on all elements and isotopes. This is because of differences in instrument sensitivities as well as interferences within the material (in this case, otoliths are dominated by the high levels of Ca) that can obscure the measurement of some analytes. At present, most studies involving otolith microchemistry use one or a combination of LA-ICPMS, LA-MC-ICPMS, and SXFM. The LA-ICPMS and LA-MC-ICPMS methods are the most common, and even small collections of samples will cost hundreds to thousands of US dollars to analyse once the issue of access is addressed. SXFM is even more difficult in terms of access because it often requires a sports-stadium-sized facility, although some facilities are smaller (e.g. at Nagoya University, Japan). The difficulties of access to such equipment are exacerbated by the general lack of availability of research funds to collect otolith samples and analyse them. Unfortunately, the government research funds available in most Amazonian countries have decreased in recent decades despite the increasing degradation of fish resources.

6 | HARNESSING OTOLITH MICROCHEMISTRY FOR CONSERVATION

Otolith microchemistry has great potential to improve our understanding of the life histories of Amazonian migratory fishes, particularly those that are harvested by fisheries and migrate among environments with different water chemistries. Recent advances in understanding the interplay of physiology and environment in what is deposited in an otolith can shed light not only on where in the aquatic environment a fish has been, but also how the environment(s) may have affected the condition of the fish throughout its lifetime, as different analytes may serve as independent proxies for cause and effect (cf. Limburg & Casini, 2019). However, a lack of environmental data, a poor understanding of useful markers, uncertainties regarding how environmental conditions, fish physiology, and otoliths interact, and a lack of funding, facilities, and research coordination currently limit the application of this approach in the Amazon. Here, we provide a conceptual framework of priority actions for a novel initiative that would harness the potential of otolith microchemistry to foster the conservation of Amazonian fishes (Figure 5).

6.1 | Coordinating research efforts

The study, assessment, and conservation of Amazon migratory fishes needs infrastructure and a coordinated approach (the orange boxes in Figure 5). Funding for appropriate facilities (i.e. LA-ICPMS and/or MC-LA-ICPMS) in the Amazon – for example, in Manaus, the largest regional city and main research centre – could jump-start new regional research efforts. Brazil's national research foundation (i.e. CNPq) and Ministry of Science and Technology (i.e. MCT) have the financial ability to invest in such infrastructure; the budget of CNPq for 2018 was approximately $320 million. International cooperation among Amazonian and other countries could alleviate the cost. Dedicated facilities and continuous and sufficient research funding could allow regular working-group meetings that are specific to otolith microchemistry in the Amazon. Coordinating research efforts would bring additional exposure to the cutting-edge research being carried out in this field and broaden the scientific user base. Without a coordinated approach to acquiring fundamental

information, otolith microchemistry in the Amazon may remain a peripheral sub-field, limited by the available literature from other domains (such as geochemistry). Scheduled meetings among Amazonian fish ecologists could provide excellent opportunities for setting research agendas, forming working groups, and coordinating regional, national, and international collaborations, which would facilitate access to additional analytical equipment, such as SXFM, and engage scientists working in other tropical systems, such as the Mekong River. Recent initiatives in this direction include the Interdisciplinary Latin American Otolith network (RIO-LA), the 6th International Otolith Symposium 2018 (IOS2018), the inclusion of otolith science as a featured research topic in *Frontiers in Marine Science* (Avigliano, Volpedo, & Walther, 2020), and the emergence of the field of sclerochronology, which includes the study of otoliths as well as other calcified chronometric structures, such as bivalve shells, corals, and teeth (Peharda, Schöne, & Limburg, 2020).

6.2 | Critical areas of research

The agendas of working groups could focus on addressing three key areas (the yellow boxes in Figure 5), including: (i) improving our understanding of water geochemistry; (ii) expanding otolith sampling for more genera; and (iii) elucidating environment–fish–otolith pathways.

6.2.1 | Improved spatiotemporal geochemical data

The most pressing knowledge gap for otolith microchemistry in the Amazon concerns geochemical data. Water sampling at various times throughout the year is clearly one direct approach, and will no doubt be necessary to address this problem. A promising alternative or complementary pathway is the use of bivalve shells (Gaillard et al., 2019) or even plants (Krems, Rajfur, Waclawek, & Klos, 2013) as sessile data loggers. Although calibration through measurements of direct water samples would also be necessary with bivalves, the sessile nature of these organisms means that separating potential metabolic influences on elemental concentrations would be far simpler than in mobile organisms. Thus, spatiotemporal patterns of elements could be
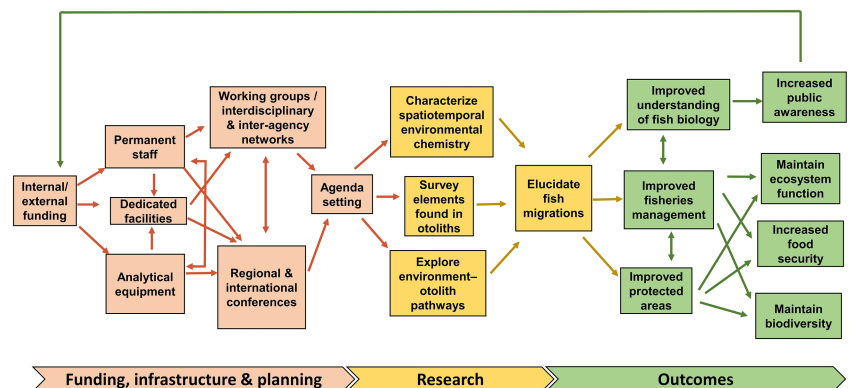
FIGURE 5 Conceptual framework outlining the priority actions for developing a novel initiative to harness the potential of otolith microchemistry to foster conservation

assessed quickly across many study sites to build a geochemical database for the Amazon. The multielement approach for otoliths described previously (i.e. using LA-ICPMS or SXFM) could also be applied here.

6.2.2 | Expanded otolith microchemistry sampling

The literature on Amazonian otolith microchemistry consists of studies on only five genera (*Arapaima*, *Brachyplatystoma*, *Cichla*, *Plagioscion*, and *Prochilodus*; but for *Hoplias* and *Schizodon*, also see Pouilly et al., 2014); for comparison, at least 529 fish genera (Dagosta & Pinna, 2019) have been recorded in the Amazon. Sr isotope ratios are relatively well established in the study of Amazonian fishes. In addition, C isotope ratios have been used for provenance identification in *Arapaima* spp. in the Amazon (Pereira et al., 2019), and for the assessment of population structure in *Prochilodus mariae* in the Orinoco River basin (Collins et al., 2013). Ba, Mn, and Se also appear to be useful markers in several of these species; however, these elements may not be detectable among all species (Hermann et al., 2016). The complexity of potential markers found already in so few fish species highlights the need for an exploratory approach to identify markers that may be generally useful or useful within groups or species.

One potential strategy for rapidly assessing the otolith microchemistry of many species is to re-use otoliths collected for previous fisheries studies. Such materials may be stored in museums and laboratories across South America, and beyond, and could provide valuable baseline information for more focused studies in the future. This would also reduce the impacts on populations of imperilled species, as otolith sampling is necessarily lethal. Although samples from collections may lack associated spatiotemporal data on environmental chemistry, and thus require caution when interpreting their otolith microchemistry, they can still provide valuable insights and new, testable hypotheses for future studies (Hermann et al., 2016).

It is worth noting that scales and fin spines represent viable, non-lethal, alternatives to otoliths (Avigliano et al., 2019; Pouilly et al., 2014), which could be beneficial for studying endangered or rare species such as *Arapaima* spp. However, the broad use of these structures is limited by a number of factors. First, scales and spines are not metabolically inert like otoliths, meaning that they can be partially resorbed, such as during periods of starvation or migration (Campana & Neilson, 1985; Tzadik et al., 2017); therefore, their records of environmental exposure may be less complete than those of otoliths. Furthermore, processes such as deposition of new material over old growth rings can affect elemental concentrations in scales, thereby making elemental markers more difficult to interpret (Wells, Bath, Thorrold, & Jones, 2000; Wells, Rieman, Clayton, Horan, & Jones, 2003). Many fish species simply do not have scales, particularly the catfish species that have been the focus of recent studies using otolith microchemistry. Therefore, otoliths still represent the most complete and broadly applicable hard structures for microchemistry analyses.

6.2.3 | Understanding environment–fish–otolith pathways

As new elements and isotopes are identified as markers, research could also focus on elucidating environment–fish–otolith pathways. New research demonstrates the complexity of the protein matrix of otoliths and the processes by which elements might become entrained (Hüssy et al., 2020; Thomas et al., 2017; Thomas & Swearer, 2019). Understanding the influences of environment versus biology on elemental and isotopic ratios in otoliths is critical when using these elements as markers. Such research would benefit not only otolith science in the Amazon but the field as a whole.

6.3 | Future perspectives

The benefits of addressing these three proposed research areas have been partially demonstrated in the study of *Brachyplatystoma*, whereby: (i) substantial spatiotemporal data for Sr isotopes existed in the literature, but more were collected during the course of otolith microchemistry studies; (ii) the collection of additional elements (i.e. Se and Ba) allowed inferences not provided by Sr isotope data; and (iii) the environment–otolith pathways of Sr isotopes were already understood, allowing clear interpretations of the isotopic data. The spatial scales and migratory behaviour of *B. rousseauxii* revealed by these studies showed that this species performs natal homing behaviour (Duponchelle et al., 2016; Hauser et al., 2020), and that protected areas and fisheries management need to account for its entire home range in order to be effective. The impressive scale of these migrations has been featured in the media worldwide, increasing public awareness of their importance in the Amazon. Thus, this research approach can provide the basic data needed to improve fisheries management and protected areas, which maintain biodiversity and ecosystem functioning and increase food security (the green boxes in Figure 5). Furthermore, increased research into the life histories of migratory fishes can lead to exciting discoveries of interest to the general public, thereby raising awareness of Amazonian fishes and the threats they face, and potentially increasing the likelihood of new funding (green boxes in Figure 5).

In the Amazon basin, human threats to aquatic environments and fishes are becoming increasingly alarming, particularly hydropower development and overfishing (Castello et al., 2013; Winemiller et al., 2016). There is an urgent need to improve our knowledge of fish life-history strategies and migration patterns to develop adequate conservation actions. The conservation of Amazonian fishes is vital not only to aquatic ecosystems but to the Amazon as a whole, given the importance of fishes to food webs and the strong aquatic–terrestrial linkages across the basin (Correa & Winemiller, 2018; Goulding, 1980; Lewis et al., 2001; Winemiller & Jepsen, 1998). The initiative outlined here to harness the potential of otolith microchemistry could turn this analytical method into a major, cost-effective tool to better inform and hence foster conservation. Such an

initiative could build the foundation for otolith science in the Amazon and act as a model for other imperilled freshwater systems worldwide, such as the Congo and the Mekong, which face similar threats (Winemiller et al., 2016).

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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