

## REVIEW

# Global change and physiological challenges for fish of the Amazon today and in the near future

Adalberto Luis Val<sup>1,\*</sup> and Chris M. Wood<sup>2,3</sup>

## ABSTRACT

Amazonia is home to 15% (>2700, in 18 orders) of all the freshwater fish species of the world, many endemic to the region, has 65 million years of evolutionary history and accounts for 20% of all freshwater discharge to the oceans. These characteristics make Amazonia a unique region in the world. We review the geological history of the environment, its current biogeochemistry and the evolutionary forces that led to the present endemic fish species that are distributed amongst three very different water types: black waters [acidic, ion-poor, rich in dissolved organic carbon (DOC)], white waters (circumneutral, particle-rich) and clear waters (circumneutral, ion-poor, DOC-poor). The annual flood pulse is the major ecological driver for fish, providing feeding, breeding and migration opportunities, and profoundly affecting O<sub>2</sub>, CO<sub>2</sub> and DOC regimes. Owing to climate change and other anthropogenic pressures such as deforestation, pollution and governmental mismanagement, Amazonia is now in crisis. The environment is becoming hotter and drier, and more intense and frequent flood pulses are now occurring, with greater variation between high and low water levels. Current projections are that Amazon waters of the near future will be even hotter, more acidic, darker (i.e. more DOC, more suspended particles), higher in ions, higher in CO<sub>2</sub> and lower in O<sub>2</sub>, with many synergistic effects. We review current physiological information on Amazon fish, focusing on temperature tolerance and ionoregulatory strategies for dealing with acidic and ion-poor environments. We also discuss the influences of DOC and particles on gill function, the effects of high dissolved CO<sub>2</sub> and low dissolved O<sub>2</sub>, with emphasis on water- versus air-breathing mechanisms, and strategies for pH compensation. We conclude that future elevations in water temperature will be the most critical factor, eliminating many species. Climate change will likely favour predominantly water-breathing species with low routine metabolic rates, low temperature sensitivity of routine metabolic rates, high anaerobic capacity, high hypoxia tolerance and high thermal tolerance.

**KEY WORDS:** Amazon formation, Fish diversity, Environmental adaptations, Hypoxia, DOC, Ionoregulation, Acidic environments, Temperature tolerance

## Introduction

Amazonia covers 7.76 million square kilometers, more than 5 million of which are in Brazil. The area encompasses rainforests (78%), Andean areas (1.5%), flooded areas (floodplains and igapós)

(5.83%), savannas (12.75%) and two tropical steppes (1.89%) (Hilty, 2012). Between these macro-zones, there are transition areas (ecotones) with mixed characteristics, such as the Amazon-Cerrado and the Amazon-Pantanal. Within the rainforest itself, there are 53 distinct environments, each with unique temperature and precipitation patterns that impose distinct selective pressures on resident organisms (Adeney et al., 2016; Hess et al., 2015). The biome is home to the longest river valley on Earth: the Amazon River (6992 km) arises in Peru and discharges 250,000 m<sup>3</sup> s<sup>-1</sup> into the Atlantic at Cabo do Norte, representing 20% of all of the freshwater entering the world's oceans (Sioli, 1984; Val et al., 2006; Val and Almeida-Val, 1995). Its tributaries include three more of the world's longest rivers (Rio Negro, Rio Madeira and Rio Japurá), and 20 more of the 34 largest tropical rivers (Latrubesse et al., 2005). These waters drain from diverse geologies, and the final discharge represents a mixture of three distinct water types characterized by their colour (Sioli, 1984) (Fig. 1). This is a result of 65 million years of geological rearrangements that include tectonism and changes in climate operating in a very singular environment.

All Amazon waters are ion-poor, but those originating from the Andes ('white waters') with a muddy, cafe au lait colour have the most ions and a pH close to neutrality. White waters carry suspended particulates from the mountains, down the Rio Solimões and its tributaries, all the way to the Atlantic mouth of the Amazon River. In contrast, the 'black waters' of the Rio Negro watershed are darkly coloured by their high content of dissolved organic carbon (DOC) (see Glossary) and have the lowest ion levels and pH (2.5–6.0), reflecting the mineral-poor soils of their jungle origin. The black (Rio Negro) and white waters (Rio Solimões) merge just east of Manaus. The third major water type, the 'clear waters', are also very dilute but low in DOC and particles, with a higher pH (6.0–6.8). These drain from ancient, eroded surfaces of the Guiana massif and central Brazil, the headwaters of certain Andean rivers, and clear-water streams of the central lowlands. These three different environments pose immense challenges to endemic fish species, some of them transiting between different water types, such as the freshwater sardine (Araújo et al., 2017).

The most important seasonal variation throughout the region is the annual hydrological cycle or 'flood pulse' (see Glossary), where the water levels vary dramatically throughout the year and rhythmically over the years (Junk et al., 1989; Schöngart and Junk, 2007) (Fig. 2). These flood and drought dynamics are the main ecological driving force shaping evolutionary and adaptive processes in Amazonia (Kramer et al., 1978; Sioli, 1984; Junk et al., 1989; Assahira et al., 2017). Water levels serve as a proxy for the profound environmental variations that demand continuous physiological adjustments by resident organisms (Kramer et al., 1978; Val and Almeida-Val, 1995). Rising waters reconnect water bodies, facilitating seasonal interchange of fish fauna (Val and Almeida-Val, 1995). Periodic inundation and desiccation of plains and rainforests change habitat size, opening up and removing feeding, breeding and migration

<sup>1</sup>Laboratory of Ecophysiology and Molecular Evolution, Brazilian National Institute for Research of the Amazon, Manaus, Brazil, 69080-971. <sup>2</sup>Department of Zoology, University of British Columbia, Vancouver, BC, Canada V6T 1Z4. <sup>3</sup>Department of Biology, McMaster University, Hamilton, ON, Canada L8S 4K1.

\*Author for correspondence (dalval@inpa.gov.br)

id A.L.V., 0000-0002-3823-3868; C.M.W., 0000-0002-9542-2219

**Glossary****Acid–base balance**

Balance between input and output of acid equivalents.

**Allochthonous**

Formed elsewhere; DOC of terrigenous origin (flooded forest).

**Autochthonous**

Originated locally, DOC from phytoplankton and bacteria.

**Dissolved organic carbon**

Organic carbon molecules that pass through a filter with a pore size of 0.45  $\mu\text{m}$ .

**Flood pulse**

Periodic inundation and drought.

**Hypoxic event**

Depletion of dissolved oxygen over a defined period.

**Ionoregulation**

Relative maintenance of the concentrations of the various ions in the body fluids.

 **$K_m$** 

Concentration of substrate at which the rate of transport is 50% of the maximum rate in a Michaelis–Menten relationship.

**Michaelis–Menten kinetics**

Kinetics models that explain how the rate of catalyzed reactions such as active transport depend on the concentration of the substrate.

**Stenohaline**

Able to withstand only a narrow range of salinity.

**Transepithelial potential**

Difference in the electrical charge across the gills between water and extracellular fluid.

**List of symbols and abbreviations**

|                   |   |
|-------------------|---|
| ASR               | aquatic surface respiration                               |
| $CL_{\text{max}}$ | chronic upper lethal temperature                          |
| $CO_2$            | carbon dioxide  |
| $CT_{\text{max}}$ | acute upper lethal temperature                            |
| IP2               | inositol diphosphate                                      |
| IPP               | inositol pentaphosphate                                   |
| $J_{\text{max}}$  | maximum transport rate in a Michaelis–Menten relationship |
| $K_m$             | Michaelis–Menten constant                                 |
| $O_2$             | molecular oxygen  |
| $P_{CO_2}$        | partial pressure of carbon dioxide                        |
| $P_{O_2}$         | partial pressure of oxygen                                |
| TEP               | transepithelial potential                                 |

opportunities for the fish (Cox-Fernandes, 1997; Moura and Val, 2019). It also mobilizes more or less DOC, particles and nutrients, and creates cycling  $CO_2$ , pH and  $O_2$  regimes through the respiration and photosynthesis of algae and vegetation (Val and Almeida-Val, 1995; Assis et al., 2019). Variations in  $O_2$ , which may range from virtual anoxia to several-fold supersaturation (hyperoxia), occur spatially, daily and seasonally, and are particularly important (Val and Almeida-Val, 1995).

This Review focuses on Amazon fish under climate change scenarios. Much remains to be known, but what we do know allows us to assess the potential effects of these changes. To this end, we present a rapid review of the biological diversity of Amazonian fish, the status and predictions of climate change in the Amazon, and what we know about the physiology of Amazonian fish that may be influenced by climate change (altered pH, DOC, suspended particles, temperature,  $P_{CO_2}$  and  $P_{O_2}$  levels). Finally, based on what we know, we suggest five classes of effects of global changes on Amazon fish.

**Current biodiversity of fish in Amazonia**

The Amazon basin hosts the richest ichthyofauna in the world – more than 2700 species representing approximately 15% of all freshwater species (Jézéquel et al., 2020; Tencatt et al., 2020). New species are still being discovered (Tencatt et al., 2020) or added by range extension, for example via the Casiquiare corridor to the Orinoco basin (Winemiller et al., 2008). Species diversification processes are still occurring (Albert et al., 2018; Dutra et al., 2020; Sá-Leitão et al., 2021). Other aquatic groups exhibit similar ongoing speciation, demonstrating the importance of Amazonia as a hotbed of neotropical biodiversity (Antonelli et al., 2018).

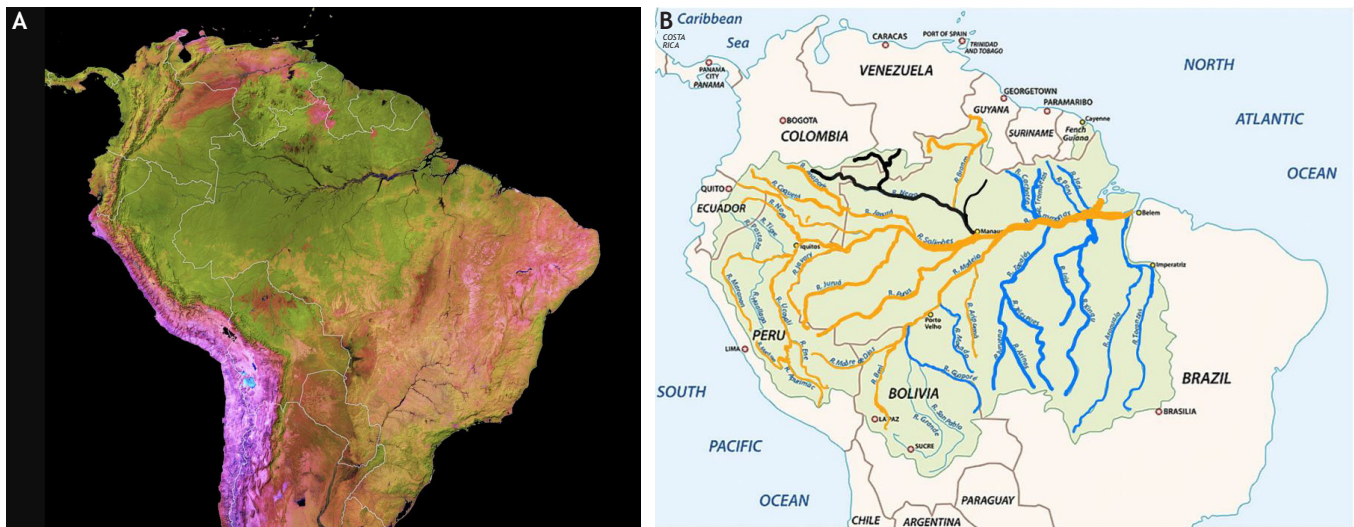
The diversity of fish encompasses an array of physiological and morphological specializations to cope with environmental challenges. Table 1 presents a list of the major families of the Neogene fish fauna of Amazonia (Lundberg et al., 2010), with

highlights of each group's physiology. This immense Amazonian ichthyofauna includes representatives from the most basal teleosts (e.g. Osteoglossiformes) to the most specialized groups (e.g. Cichliformes, until recently included among the Perciformes). Many peculiar species with unique characteristics are found here, such as the only stenohaline (see Glossary) freshwater elasmobranchs, the stingrays (Myliobatiformes, formerly Rajiformes) of the Rio Negro (Wood et al., 2003) and the giant osteoglossid pirarucu, among the largest freshwater fish in the world, with its red blood cells containing the potent haemoglobin allosteric effector inositol pentaphosphate (IPP) (Bartlett, 1978; Isaacks et al., 1977; Val et al., 1992). Amazonia also hosts an immense variety of catfish (Siluriformes), some of them armored, such as the acari-bodo that uses the stomach-intestine as an accessory organ for respiration (Cruz et al., 2008; Val et al., 1990), and others of large size, especially the piraiba (Petrere et al., 2004). There are also various electric fish (Gymnotiformes), notably the poraquê (electric eel), which can discharge up to 600 V (Catania, 2014; Val and Almeida-Val, 1995), and various serrasalmids (Characiformes), such as the legendary piranhas, and the tambaqui, which can expand its lower lips to breathe at the water surface (Saint-Paul, 1984; Val and Almeida-Val, 1995; Val and Oliveira, 2021). Some Amazonian fish species are already living at the limits of their biological abilities, such as the small characids inhabiting small forest streams. Thus, the rapid environmental transformations worsened by climate change can be devastating for native fish.

**Global change: current status and predictions for Amazonia**

$CO_2$  in the Earth's atmosphere has risen from approximately 280 ppm in 1850, when the industrial revolution was taking place mainly in Europe, to more than 411 ppm in 2019 (de la Vega et al., 2020). The greenhouse effect (which results also from emissions of  $CH_4$ ,  $N_2O$  and other anthropogenic gases), is compounded by other anthropogenic activities such as deforestation, wildfires, road and dam construction, and water harvesting and diversion. The Earth's surface has warmed by approximately  $0.8^\circ\text{C}$  in the last 40 years alone (Gross, 2018), with 2016 being the warmest year ( $+0.9^\circ\text{C}$ ) since 1950 (Marengo et al., 2018). Consequences include hotter and more widely fluctuating water temperatures, acidification and salinization of water bodies, increased mobilization of DOC, changes in DOC chemistry and decreases in dissolved oxygen (Lloyd and Shepherd, 2020; Scanes et al., 2020).

Amazonia is particularly vulnerable to the ecological impacts of predicted climate change (Castello and Macedo, 2016) and will face less rainfall, warmer and drier periods, and more extreme events, including more intense droughts and floods (Brodie et al., 2012;



**Fig. 1. The Amazon extends across all countries of the north of South America, representing unparalleled environmental and biological diversity.** (A) The topography of the region defines the flows of the main rivers in the region. (B) The major rivers of the Amazon with different types of water are depicted. Yellow indicates the main white-water rivers, black indicates the Rio Negro and affluents, and blue represents the clear-water rivers. Base map copyright [https://www.123rf.com/profile\\_lesniewski](https://www.123rf.com/profile_lesniewski) (Rainer Lesniewski© 123RF.com).

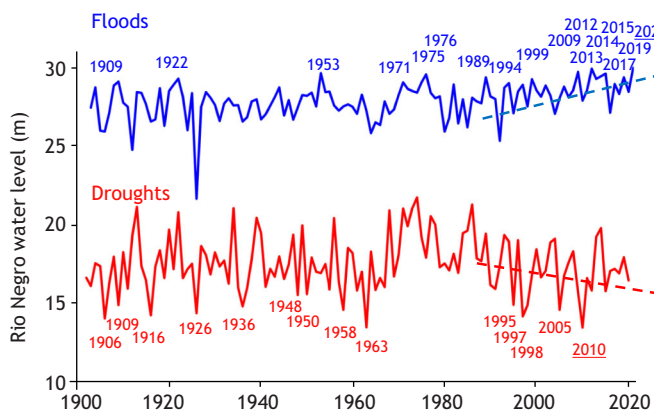
Dai, 2012), which could lead to the disappearance of the forest in eastern, southern and central Amazonia. This is being compounded by direct deforestation; 12% of the rainforest has already been lost to logging and agriculture (Stropp et al., 2020).

Decreasing deforestation rates in the earlier part of the 21st century were an encouraging trend (Nobre et al., 2016), but this has now been reversed owing to changes in government policy. Deforestation rates are now the highest of the past decade (Jaffé et al., 2021). At a threshold somewhere between 20 and 40% forest loss, often called a tipping point, it will be nearly impossible to recover the system: forest will be permanently replaced by savannah, and temperatures will further increase (Lovejoy and Nobre, 2018). These changes will reduce the carbon-removal capacity because photosynthetic CO<sub>2</sub> fixation will decrease, while more decaying organic matter will be available for respiratory CO<sub>2</sub>

production. Therefore, net CO<sub>2</sub> emissions will rise, further exacerbating the problem. Additionally, as forest land is converted to agriculture and savannah, major nutrients and ions are released into the water through the process of salinization (see ‘Increased ion levels’, below; Biggs et al. 2004; Chaussé et al. 2016; Markewitz et al. 2001). There is a clear connection between vegetation cover and moisture recycling, which in turn relates to the water, energy and carbon cycle in the region (Marengo et al., 2018).

Given the geographic and geological heterogeneity of Amazonia, climate change will differentially affect its various parts, with a mosaic of complex effects. If uncontrolled and unsustainable land exploitation continues, temperature projections will far exceed those experienced to date (~1°C). Predictions for the period 2041–2070 indicate that the northeastern region of the state of Amazonas could experience the greatest warming, with a further increase of up to 5°C. In contrast, the central region of the state of Amazonas and the upper Rio Negro will encounter the smallest thermal changes, with an increase of up to 3.5°C (Menezes et al., 2018). Regardless, both temperature elevations will have devastating effects on native fish. Unlike terrestrial and marine organisms (Jacox et al., 2020; Lenoir et al., 2008), there is little opportunity for Amazon fish to move up mountains or towards the poles to escape extreme temperatures and associated environmental changes. Some models indicate that one-third of the fish species in Amazonia, as well as in the neighbouring Tocantins and Araguaia watersheds, may face extinction if these changes persist in conjunction with the most extreme climate change scenarios (Xenopoulos et al., 2005).

Although the overall trend for Amazonia as a whole is desiccation, increases in precipitation and increased discharges of Amazon River tributaries are currently occurring in the Northern Andean region (Pabón-Cañedo et al., 2020). More intense and more frequent floods are now occurring, with a 5-fold increase after the year 2000, compared with the first half of the 20th century (Barichivich et al., 2018). There are two related implications. The first is that the amplitude between flood and ebb has increased significantly over the last 25 years (see Fig. 2) (Schöngart and Junk, 2020). Therefore, during extreme floods the fish have access to a greater extent of flooded forest, with impacts on their natural history, particularly regarding refuge, dispersal, feeding and reproduction.



**Fig. 2. Maximum (floods, blue line) and minimum (droughts, red line) annual water level of the Rio Negro at Manaus, Central Amazonia (1903–2021).** Underlined years indicate extreme flood (>29 m) and severe drought (<15.8 m). The dashed lines indicate trends for the last 30 years [adapted from Schöngart and Junk, 2020; data from the National Water Resources Information System (SNIRH) operated by the Brazilian National Water and Sanitation Agency (ANA) and the Geological Survey of Brazil (CPRM)]. Note the increase in the amplitude between flood and drought peaks over the last 25 years.

**Table 1. Major orders and number of fish species living in Amazonian waters according to Dagosta and De Pinna (2019) and their potential vulnerabilities to global change**

| Order              | Number of species | Vulnerabilities to global change   |
|--------------------|-------------------|--|
| Carcharhiniformes  | 1                 | Inhabits tropical marine waters but enters insular warming and acidifying environments, facing more extreme challenges   |
| Pristiiformes      | 2                 | Invades warming and acidifying Amazonian waters  |
| Myliobatiformes    | 27                | Includes species living in the waters of the Rio Negro, which can become more acidic than they already are   |
| Osteoglossiformes  | 5                 | Includes one obligate air- and one water-breathing species. They use different strategies to deal with high CO <sub>2</sub>  |
| Anguilliformes     | 1                 | Unknown vulnerabilities  |
| Clupeiformes       | 18                | Species experimented with habitat transitions and may have retained some resilience to environmental instabilities   |
| Characiformes      | 1063              | Includes the newly described Family Tarumaniidae and many species with an array of abilities to face environmental challenges, including aquatic surface respiration (ASR); several species live close to their upper critical temperature                                   |
| Siluriformes       | 956               | Includes species inhabiting extreme environmental conditions, species migrating long distances and species that use the stomach-intestine to uptake O <sub>2</sub> making several species vulnerable to environmental disturbances caused by climate changes                 |
| Gymnotiformes      | 164               | True electric eels; water quality changes (temperature, acidification and pollution) affect discharge patterns   |
| Batrachoidiformes  | 2                 | A marine invader, quite resilient; unknown vulnerabilities to global change  |
| Cyprinodontiformes | 166               | Some annual species (e.g. <i>Rivulus</i> spp.) vulnerable to extreme river water level oscillations  |
| Atheriniformes     | 1                 | Unknown vulnerabilities  |
| Beloniformes       | 9                 | Two families occur in the Amazon, one is endemic; unknown vulnerabilities  |
| Synbranchiformes   | 3                 | One family, three species, one endemic; their habitat can undergo significant seasonal changes, in particular swamp areas  |
| Perciformes        | 286               | A very specialized group, that formerly included the cichlids (Cichliformes now), showing a myriad of environmental adaptations at all levels of the biological organization; several species are resilient to increases in water temperature and decreases in oxygen and pH |
| Pleuronectiformes  | 9                 | A family distributed throughout the Amazon; these benthic flatfish face the dynamics of the water bottom that can undergo significant changes caused by global change (e.g. siltation)   |
| Tetraodontiformes  | 2                 | Known as pufferfish; only two species of one family of this large group of marine fish live in freshwater; vulnerability to climate change is unknown  |
| Ceratodontiformes  | 1                 | One family, one species: the well-known South American lungfish, <i>Lepidosiren paradoxa</i> ; deterioration of habitat (swamps and more intense drying river margins) and increased atmospheric CO <sub>2</sub> could represent new challenges for the species              |

According to the authors, these 18 orders represent 2716 analyzed species. Vulnerabilities to global change are generalizations based on very few species or extrapolations based on their life habits either presented in the text or based on our own experience.

The second is that increased discharge from tributaries puts a greater amount of suspended sediments into the central channel of the Amazon River, and will also elute more DOC from the submerged jungle soils and vegetation. Both higher suspended particles and higher DOC levels will have important effects on fish physiology. Estimates for the end of the 21st century suggest that the Andean region may become even warmer (Pabón-Caicedo et al., 2020). This additional warming may be locally amplified because of the reduction of vegetation cover, in a spiralling effect, because many tropical plant species will not germinate at higher temperatures (Sentinella et al., 2020), thereby contributing to environmental imbalances in both this region and the entire Amazon basin.

As temperatures rise and flooding events become more intense, hypoxic events are also becoming more intense and of longer duration, especially in floodplains and igapós (A.L.V., personal observation). Not only is the solubility of O<sub>2</sub> reduced, but the metabolic demand for O<sub>2</sub> is increased. Therefore, depletion of dissolved O<sub>2</sub> and build-up of dissolved CO<sub>2</sub> owing to respiration of greater amounts of organic matter happen more quickly, at a time when the fish's metabolism needs more O<sub>2</sub>. Associated increases in DOC and increases in P<sub>CO<sub>2</sub></sub> will further compound the problem by lowering pH in the poorly buffered Amazonian waters.

### Effects of global change on the physiology of Amazonian fishes

As previously discussed, in the future, Amazon waters will be more acidic, darker (i.e. more DOC and more suspended particles), hotter, higher in ions, higher in CO<sub>2</sub> and lower in O<sub>2</sub>. We will now use these forecasts to predict the resulting physiological effects on the native fishes of Amazonia.

### Acidification

Amazon waters are extremely dilute, low in alkalinity, poorly buffered and, therefore, easily acidified. However, many Amazon fishes, especially those native to the black waters of the upper Rio Negro watershed, are already adapted to acidic environments. Here, dissolved organic acids (humic substances) contribute approximately 85% of the acidity, and the high P<sub>CO<sub>2</sub></sub> levels contribute only approximately 15% (Leenheer, 1980). Waters with a pH ≤4.5 will suffer little further acidification, considering the local geochemical characteristics and the logarithmic nature of the pH scale, so the devastation seen with the acid rain crisis of the 1970s–1990s in many areas of the Northern Hemisphere (Schindler, 1988; Wood, 1989) will likely not occur in the black-water ecosystems. However, in the less acidic lower Rio Negro, as well as in white waters, clear waters and mixing zones, increases in environmental P<sub>CO<sub>2</sub></sub> and acidic precipitation may lower pH in waters currently in the pH 4.5–7.0 range, and thereby impact the organisms adapted to live there.

From studies on temperate species, we have learned that the damaging physiological effects of low pH are on ionoregulation (see Glossary), rather than on acid–base balance (see Glossary), as long as water calcium concentrations are relatively low (<10 mg l<sup>-1</sup> = <250 μmol l<sup>-1</sup>, ‘hardness’ <25 mg l<sup>-1</sup> as CaCO<sub>3</sub> equivalents; Wood, 1989). Virtually all Amazon waters fit this description, with calcium concentrations below 10 mg l<sup>-1</sup> (Val and Almeida-Val, 1995). The responses of native fish to low pH seem to fit this pattern, with little effect on acid–base regulation or the related function of ammonia excretion (Wilson et al., 1999; Wood et al., 2018, 1998). In contrast, many studies have documented disturbances of ionoregulation, as ionoregulatory

capacity is correlated with realized niche (see Zimmer et al., 2021). Nevertheless, Amazon fish are generally quite tolerant of low pH, with thresholds for serious effects being 1–3 pH units lower than in temperate species, though with considerable interspecific variation (e.g. Duarte et al., 2013; Gonzalez et al., 1998, 2002; Wilson et al., 1999). The disturbance invariably involves elevated rates of net  $\text{Na}^+$  and  $\text{Cl}^-$  loss at the gills. These losses provoke a fluid shift out of the blood plasma into the intracellular compartment and an accompanying concentration of red blood cells (Wood et al., 1998). The fish may eventually die of cardiovascular failure associated with this haemoconcentration (Milligan and Wood, 1982).

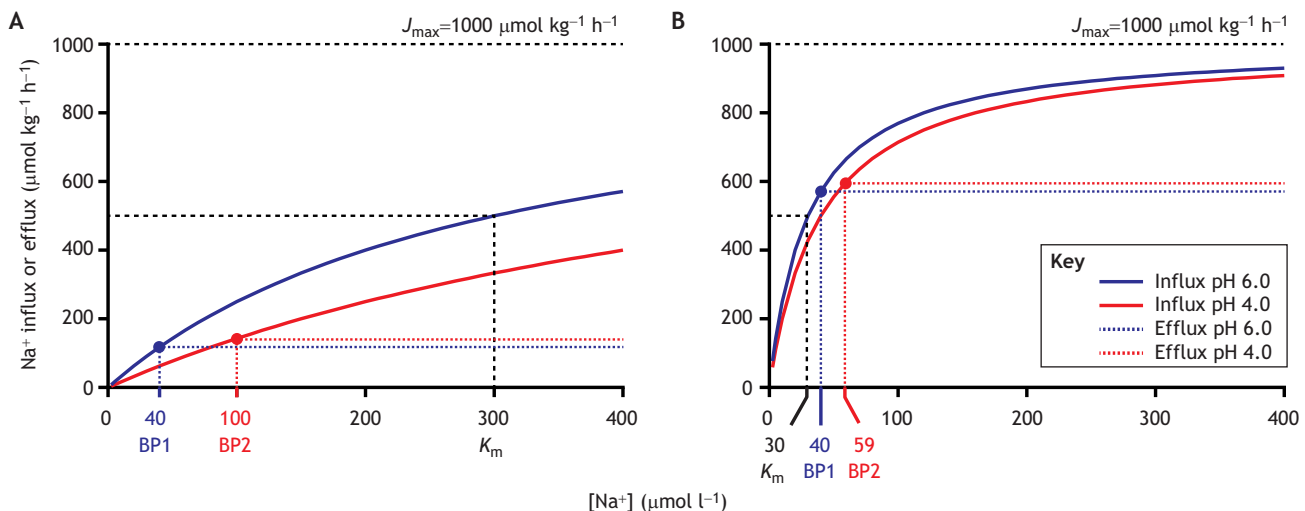
Most studies on low pH effects have focused on  $\text{Na}^+$  balance at the gills. The effects are two-fold: (i) inhibition of active  $\text{Na}^+$  uptake and (ii) stimulation of passive  $\text{Na}^+$  efflux (outflux), with the latter generally making the larger contribution to net  $\text{Na}^+$  loss. Using classic Michaelis–Menten kinetic analysis (see Glossary) and pharmacological tools, Gonzalez, Val and colleagues have identified two basic patterns of ionoregulation in fish adapted to these acidic, ion-poor waters (Duarte et al., 2013; Gonzalez et al., 2006, 1998, 2018, 2020, 2021, 2002; Gonzalez and Preest, 1999; Gonzalez and Wilson, 2001; Preest et al., 2005; Wood et al., 2003, 2002, 2014). There may be separate phylogenetic origins for these two strategies (Gonzalez et al., 2017, 2018, 2020). However, to date, only three of the 18 piscine orders endemic to the Amazon have been characterized as having low pH responses, so there may well be other response patterns.

In the first (Fig. 3A), typical of the Cichliformes, as well as freshwater stingrays (Myliobatiformes) endemic to the Amazon, the  $\text{Na}^+$  uptake system has a relatively low affinity (i.e. high  $K_m$ ), whereas transport capacity ( $J_{\max}$ ) is variable. Both unidirectional influx and efflux rates are low but in approximate balance at moderate pH values.  $\text{Na}^+$  uptake is strongly inhibited by low pH (3.0–4.5), and by standard pharmacological blockers commonly used to identify the  $\text{Na}^+/\text{H}^+$  exchange or coupled  $\text{Na}^+$  channel/ $\text{H}^+$

ATPase systems seen in most other fish, suggesting the presence of the same  $\text{Na}^+$  transporters as well documented in model species such as trout and zebrafish (Clifford et al., 2021; Dymowska et al., 2012). However, branchial efflux is extremely resistant to stimulation by low pH, so net loss rates are not high, and can probably be compensated by uptake from the diet. These species have a ‘wait it out’ strategy and may be the ones more affected by future acidification.

In the second (Fig. 3B), as seen in the Characiformes, the  $\text{Na}^+$  uptake system has a very high affinity (i.e. low  $K_m$ ) and high capacity (i.e. high  $J_{\max}$ ), so that at moderate pH values, unidirectional uptake rates are high, keeping balance with comparably high rates of efflux. The  $\text{Na}^+$  uptake system(s) are unusual and as yet poorly understood, being extremely resistant to inhibition by low pH (3.0–4.5) or any of the standard  $\text{Na}^+$ -transport blockers commonly effective in most other fish (Clifford et al., 2021). The diffusive efflux rates across the gills, while high, are again very resistant to the stimulatory effect of low pH. These fish have a ‘business as usual’ strategy and may be the ones more resistant to future acidification.

In temperate species, increased branchial efflux rates of major ions appear to be caused by the leaching of  $\text{Ca}^{2+}$  away from the tight junctions between branchial epithelial cells (McDonald et al., 1980; McWilliams, 1982), accompanied by a shift to less negative/more positive values of the transepithelial potential (TEP) across the gills (reviewed by Gonzalez et al., 2006). As the TEP at the freshwater gill is largely a diffusion potential (Potts, 1984), this suggests that the  $\text{Cl}^-/\text{Na}^+$  permeability ratio also increases. In several Amazon fish, 10-fold elevations in water calcium protected against the elevation of  $\text{Na}^+$  efflux at low pH (Gonzalez et al., 1998; Wood et al., 2003, 1998) and also against the rise in TEP (Wood et al., 1998). However, in several other black-water species, there was little or no protection provided by calcium (Gonzalez et al., 1998; Gonzalez and Preest, 1999). Other mechanisms may be involved, such as upregulation of tight junction genes in the gills, as reported



**Fig. 3. Michaelis–Menten analyses illustrating two strategies of  $\text{Na}^+$  balance in Amazon fish, and the effects of low pH.** Strategies typically seen in (A) Cichliformes and Myliobatiformes, and (B) Characiformes. The relationships of  $\text{Na}^+$  influx and efflux rates against ( $[\text{Na}^+]_{\text{ext}}$ ) are shown at pH 6.0 (blue) and when challenged at pH 4.0 (red). In A, absolute flux rates are low, reflecting low  $\text{Na}^+$  permeability and turnover. In B, absolute flux rates are much higher, reflecting higher  $\text{Na}^+$  permeability and turnover. In both A and B, the  $J_{\max}$  is the same ( $1000 \text{ nmol g}^{-1} \text{ h}^{-1}$ ) and is unaffected by low pH, while the  $\text{Na}^+$  efflux rate increases by  $24 \mu\text{mol kg}^{-1} \text{ h}^{-1}$  at pH 4.0. However, in A, the  $K_m$  for  $\text{Na}^+$  influx is high ( $[\text{Na}^+] = 300 \mu\text{mol l}^{-1}$ , i.e. low affinity) at pH 6.0, and is subject to strong competitive inhibition at pH 4.0, rising to  $600 \mu\text{mol l}^{-1}$ , whereas in B, the  $K_m$  is low ( $[\text{Na}^+] = 30 \mu\text{mol l}^{-1}$ , i.e. high affinity), and is very resistant to competitive inhibition at pH 4.0, so that the  $K_m$  is raised to only  $40 \mu\text{mol l}^{-1}$ . Therefore, at pH 6.0, the two strategies have the same balance point (BP1,  $40 \mu\text{mol l}^{-1}$ ). However, at pH 4.0, strategy B allows  $\text{Na}^+$  balance to be achieved at a much lower BP2 ( $59 \mu\text{mol l}^{-1}$ ) than does strategy A ( $100 \mu\text{mol l}^{-1}$ ).

for claudins in the sardine, a characiform that inhabits white, black and clear waters (Araújo et al., 2017). As discussed subsequently, the DOC in the black waters may also play a key role.

### Increased ion levels

Increases in major ion levels are directly associated with deforestation and agriculturalization (Biggs et al., 2004; Chaussé et al., 2016; Markewitz et al., 2001). Overall, these may be beneficial in helping the uptake of  $\text{Na}^+$ ,  $\text{Cl}^-$  and  $\text{Ca}^{2+}$  by fish in the ion-poor waters, by shifting the uptake and balance points on the Michaelis–Menten relationships (Fig. 3) to a higher level, and possibly also by lowering the efflux rates by the ‘tightening’ action of  $\text{Ca}^{2+}$  on gill permeability. However, they may change species assemblages and facilitate invasions by exotic species less tolerant of ion-poor waters.

### Increased DOC

DOC encompasses a variety of large, heterogeneous molecules originating from the physical breakdown or microbial processing of formerly living materials (Leenheer, 1980; Thurman, 1985). Carbon makes up approximately 50% by mass of these molecules. DOC concentrations as high as  $30 \text{ mg l}^{-1}$  have been recorded, but more typical levels are  $5\text{--}15 \text{ mg l}^{-1}$  in black waters,  $2\text{--}5 \text{ mg l}^{-1}$  in white waters and  $1\text{--}5 \text{ mg l}^{-1}$  in clear waters. Amazon DOCs are chemically diverse among the three water types (Gonsior et al., 2016; Leenheer, 1980), but in all, allochthonous (see Glossary) sources dominate – i.e. of terrigenous origin, derived largely from plant molecules such as lignins. Humic substances are defined as those organic compounds that cannot be classified as any other chemical class of compounds (e.g. polysaccharides, proteins, etc.) (Gaffney et al., 1996). They are often highly coloured owing to the presence of aromatic rings (phenolic groups) and constitute >50% of most Amazon DOCs. A variety of smaller, less coloured, more hydrophilic molecules (proteins, amino acids, carbohydrates, fatty acids, small organic acids, etc.) comprise the remainder. However, in black waters, violacein, a purple pigment with anticancer, antimicrobial and antiparasitic properties produced by the abundant microbe *Chromobacterium violaceum*, contributes colour to the latter fraction (Caldas, 1990; Duran et al., 2007). Its effects on fish remain unknown. Within the humic substances, the proportions of humic acids (higher molecular weight, defined by precipitation at pH 1.0–2.0; Gaffney et al., 1996; Perry et al., 2021) and fulvic acids (lower molecular weight, soluble at all pH values) vary amongst sites. Upstream waters have the most recently formed DOC with the highest humic acid content, and as the water flows downstream, the molecules become older, more degraded by bacterial action and photo-oxidation, and therefore smaller, and there is the addition of more autochthonous (see Glossary) molecules from phytoplankton and bacteria.

There is now considerable evidence (reviewed by Gonzalez et al., 2006; Wood et al., 2011; Nelson, 2015; Morris et al., 2021) that DOC has positive effects on ionoregulation in fish, especially at low pH. Indeed, the presence of the very molecules that cause the acidity of black waters (Kullberg et al., 1993) may be a key factor also protecting endemic fish against the negative effects of low pH (Gonzalez et al., 1998, 2002; Matsuo and Val, 2007; Wood et al., 2003). Herein lies an as yet unsolved experimental conundrum – how can we separate the effects of low pH from those of high DOC? The use of strong mineral acids (e.g.  $\text{HNO}_3$ ,  $\text{HCl}$ ,  $\text{H}_2\text{SO}_4$ ) to experimentally lower pH is clearly not the same as adding natural DOC, and creates an unnatural water chemistry with elevated concentrations of strong anions, while  $P_{\text{CO}_2}$  levels

needed to reach pH 4.0 would be both unnatural and likely fatal to most species.

Although protection by DOC against low pH pathology was first shown with Amazon DOC and species, it is now clear that the phenomenon extends to species and DOC from other parts of the world (Al-Reasi et al., 2016; Duarte et al., 2016, 2018; Galvez et al., 2008; Matsuo et al., 2004). DOC also protects against metal toxicity by binding and removing the free cationic forms of metal from solution, thereby decreasing their bioavailability (Niyogi and Wood, 2004; Al-Reasi et al., 2011; Morris et al., 2021). This is undoubtedly the case in Amazon waters, where substantial concentrations of naturally occurring Fe, Al, Mn and Hg are complexed by DOC (Aucour et al., 2003; Furch, 1984; Holland et al., 2017; Silva et al., 2009; Wood et al., 2003), as well as contaminant metals such as Cu (Rocha et al., 1999). Cu is an ionoregulatory toxicant and experimental additions of Amazonia DOC reduced Cu toxicity to fish (Barbosa et al., 2019; Crémazy et al., 2016; Duarte et al., 2009). Crémazy et al. (2016) demonstrated through geochemical modelling that only part of the toxicity reduction could be explained by Cu–DOC complexation and attributed the other portion to the supportive physiological effects of DOC on ionoregulatory homeostasis. Future environmental acidification may release metal cations from natural DOC, causing toxicity to native organisms (Tipping, 1998).

Morris et al. (2021) have reviewed the possible mechanisms by which natural DOCs may promote ionoregulatory homeostasis and protect against acid toxicity. These include: (i) a  $\text{Ca}^{2+}$ -like action to ‘tighten’ gill membrane permeability (Wood et al., 2003); (ii) making the gill TEP more negative, thereby opposing the depolarizing effect of low pH and favouring net  $\text{Na}^+$  uptake (Galvez et al., 2008; Sadauskas-Henrique et al., 2019); (iii) maintaining the coupling of  $\text{Na}^+$  uptake to ammonia excretion (Duarte et al., 2016; Wood et al., 2003); (iv) buffering the external micro-environment of the gills so as to minimize the inhibitory effects of low pH on apical  $\text{Na}^+$  uptake transporters (Al-Reasi et al., 2016; Wood et al., 2003); and (v) increasing the  $J_{\text{max}}$  values of the branchial ion uptake systems (Al-Reasi et al., 2016; Glover et al., 2005; Glover and Wood, 2005; Matsuo et al., 2004; Matsuo and Val, 2007; Wood et al., 2003), perhaps owing to increased  $\text{Na}^+, \text{K}^+$ -ATPase activity in the gills (McGeer et al., 2002). These actions may be both acute and chronic, and DOC may bind directly to gill surfaces (Campbell et al., 1997). Indeed, after 7 days of pre-exposure to DOC, the protective effects against acute low pH persisted even when the DOC was removed from the water (Duarte et al., 2016, 2018).

The properties of DOC that exert these physiological actions appear to be the same as those protecting against metal toxicity (Wood et al., 2011). Al-Reasi et al. (2013) have related both phenomena to an optical property, the specific absorbance coefficient at 340 nm (SAC340, an indicator of aromaticity) and a chemical property, the proton binding index (PBI, an indicator of reactivity). These are greatest for large, optically dark humic acid molecules with abundant phenolic groups, of allochthonous origin. Therefore, as DOC levels increase with climate change, whether the effects are beneficial to fish will depend not only on concentration, but also on the chemical structure of the DOCs. The DOCs mobilized from deforested areas will probably be composed of older, smaller molecules that are less aromatic and more labile to microbial degradation than DOC exported from forested areas (Drake et al., 2019). Therefore, we predict that DOC from deforested areas will be less beneficial to fish. Furthermore, not all DOC is protective or benign; some, such as commercially available humic acid from peat or coal (Wood et al., 2003), as well as natural

allochthonous DOC from the upper Rio Negro that has degraded during storage (Sadauskas-Henrique et al., 2019), may actually increase ionoregulatory disturbance at low pH.

### Increased particles

Deforestation and agriculturalization will be accompanied by increased erosion, siltation and turbidity (Figueiredo et al., 2010; Leitão et al., 2018). The concentration of suspended particles in white waters is much higher (>10-fold) than in black or clear waters (Crémazy et al., 2019; Holland et al., 2017), so the species native to white waters will likely be more resistant. Because particles tend to settle, benthic species appear to be most affected, and there is evidence of associated shifts in species assemblages in silt-impacted areas of the Amazon, opening up opportunities for exotic invaders (Leitão et al., 2018). In temperate species, a variety of lethal and sublethal effects of increased particles have been reported, including behavioural (e.g. avoidance, disruption of predator–prey activities and mating), respiratory (e.g. suffocation, increased O<sub>2</sub> consumption, decreased swimming performance), reproductive (impacts on spawning and early life stages) and generalized stress responses (Kjelland et al., 2015). Ionoregulatory impacts have been reported in black-water species exposed to particle-rich white water, perhaps associated with physical damage to the gills (Crémazy et al., 2019). However, on the positive side, particles can complex cationic free Cu<sup>2+</sup> out of solution with great efficiency, thereby reducing toxicity (Crémazy et al., 2019; Dal Pont et al., 2017). The same is probably true for other metals (Seyler and Boaventura, 2003).

### Increased temperature

In the next century, water temperatures (currently 28–32°C) in Amazonia are predicted to increase by 2.2 to 7.0°C (IPCC, 2014) and this may be exacerbated by shade removal by deforestation (Ilha et al., 2018; Leitão et al., 2018). Unless there is rapid evolutionary change, the consequences for fish appear dire. A recent study (Jung et al., 2020) on 37 Amazonian species found that the acute upper lethal temperatures (CT<sub>max</sub>) ranged from 36.1 to 42.8°C. Acclimation to higher temperature caused modest increases in CT<sub>max</sub> but decreases in hypoxia tolerance. In a subset of 13 species, the chronic (4 weeks) upper lethal temperatures (CL<sub>max</sub>) were typically 6–9°C below the CT<sub>max</sub> values. This means that long-term increases in temperature only slightly above current river temperatures (28–31°C) may eliminate a large number of Amazonian fish species. Overall, there was a positive interspecific correlation between CT<sub>max</sub> and acute hypoxia tolerance, and on an intraspecific basis, small acute increases in temperature resulted in decreases in hypoxia tolerance, whereas acute hyperoxia exposure caused small increases in CT<sub>max</sub> (Jung et al., 2020). Furthermore, low factorial aerobic scope (associated with high routine metabolic rate) was correlated with low CT<sub>max</sub> on an interspecific basis (Campos et al., 2017). In a comparison of two closely related Characiform species, greater thermal tolerance was associated with lesser temperature sensitivity of routine metabolic rate, lower critical oxygen tension (Campos et al., 2017) and greater capacity for anaerobic metabolism (Fé-Gonçalves et al., 2018). From these and other studies (e.g. LaPointe et al., 2018), it is clear that there is great inter-species variability. Nevertheless, in total, these observations suggest an interaction between thermal tolerance and the O<sub>2</sub> delivery cascade, such that climate warming will favour species with low routine metabolic rates, low temperature sensitivity of routine metabolic rates, high anaerobic capacity and high hypoxia tolerance.

Thermal effects on growth rate in the laboratory are variable (e.g. Oliveira and Val, 2017; LaPointe et al., 2018). However, in the field, there is a general trend for smaller mean body size of fish at the population level in streams that have recently warmed owing to deforestation (Ilha et al., 2018), a manifestation of Bergmann's rule (Bergmann, 1847) over very short temporal and spatial scales. In the Amazon context, the causative factors for smaller body size are likely multifactorial, as with many of the other effects of increased temperature (Ilha et al., 2018).

Indeed, as with hypoxia, the effects of increased temperature will likely be additive or synergistic with a variety of other stressors – for example, the effect of both low pH and elevated temperature in decreasing blood oxygen affinity (Val et al., 2016). The pathological effects of combined, realistic elevations in both atmospheric P<sub>CO<sub>2</sub></sub> and temperature, equilibrated with the water holding the fish in special climatic chambers, have now been demonstrated for many parameters. These include Cu-induced oxidative stress (Braz-Mota et al., 2017), the intensity of parasitic infections (Costa and Val, 2020) and the prevalence of skeletal deformities (Lopes et al., 2018).

### Higher P<sub>CO<sub>2</sub></sub> levels

Most physiologists would consider a water P<sub>CO<sub>2</sub></sub> of approximately 0.04 kPa (0.3 Torr) to represent the normal situation. However, for Amazon fishes, well-aerated laboratory 'control' conditions may be unrealistic as their natural waters are strongly supersaturated with CO<sub>2</sub>, owing to the respiratory breakdown of organic materials. Reported values include a yearly mainstream average of 0.44 kPa (3.3 Torr) in major rivers, 0.51–2.03 kPa (3.8–15.2 Torr) in forest streams, 0.17–0.48 kPa (1.3–3.6 Torr) in the black-water Rio Negro, 0.35–0.72 kPa (2.6–5.4 Torr) in the white-water Rio Solimões, 0.03–0.36 kPa (0.2–2.7 Torr) in several clear-water rivers and >8 kPa (>60 Torr) in floodplains (Furch and Junk, 1997; Rasera et al., 2013; Richey et al., 2002). The worst-case climate change scenarios (0.13 kPa (≤1 Torr) elevation in atmospheric P<sub>CO<sub>2</sub></sub>), while significant, will be superimposed on natural aquatic P<sub>CO<sub>2</sub></sub> levels that are already much higher.

Therefore, many Amazon fish are pre-adapted for high P<sub>CO<sub>2</sub></sub> tolerance. Equilibration of environmental CO<sub>2</sub> across the gills is unavoidable, so blood levels are always above water levels. However, air-breathers such as the South American lungfish may be able to counteract CO<sub>2</sub> entry from the water by excretion through the air-breathing organ, thereby controlling the rise in blood P<sub>CO<sub>2</sub></sub> levels (which are already high because of air-breathing; Dejours, 1975), so as to keep them below waterborne levels (Sanchez et al., 2005).

Many Amazon fish prioritize the regulation of intracellular over extracellular pH (Brauner et al., 2004b; Harter et al., 2014; Heisler, 1982). When experimentally challenged with high waterborne P<sub>CO<sub>2</sub></sub>, blood pH drops immediately, with little subsequent compensation by extracellular HCO<sub>3</sub><sup>−</sup> accumulation, whereas tissue pH is regulated rapidly and precisely. This is very different from the classic 'coupled regulation' seen in model temperate species, where extracellular and intracellular pH are regulated in tandem (Brauner and Baker, 2009). Possible explanations for this strategy include: (i) the dilute, acidic, weakly buffered Amazon waters are a poor source of HCO<sub>3</sub><sup>−</sup>, and a difficult medium into which to excrete H<sup>+</sup>, compounded by low Na<sup>+</sup> and Cl<sup>−</sup> as counterions for branchial acid–base transporters (Larsen and Jensen, 1997); (ii) the intracellular compartment is much better buffered than the extracellular compartment, so intracellular pH correction requires far less HCO<sub>3</sub><sup>−</sup>; (iii) this small amount of HCO<sub>3</sub><sup>−</sup> can be moved easily from plasma to tissues; and (iv) the HCO<sub>3</sub><sup>−</sup> threshold argument that it is impossible to raise plasma [HCO<sub>3</sub><sup>−</sup>]

above approximately 30 mmol l<sup>-1</sup>, because it will drive plasma [Cl<sup>-</sup>] too low (Heisler, 1986). The absolute limit is debatable, but it certainly would prevent complete compensation at water P<sub>CO<sub>2</sub></sub> ≥2.67 kPa (≥20 Torr), which occurs frequently in Amazonia. Shartau et al. (2016) have argued that preferential regulation of intracellular pH is an exaptation for air-breathing, which is accompanied by large elevations in blood P<sub>CO<sub>2</sub></sub> (Dejours, 1975). Indeed, the Amazonian species exhibiting preferential intracellular pH regulation all have some air-breathing capacity (Brauner et al., 2004b; Harter et al., 2014; Heisler, 1982; Shartau et al., 2016).

**Lower oxygen levels**

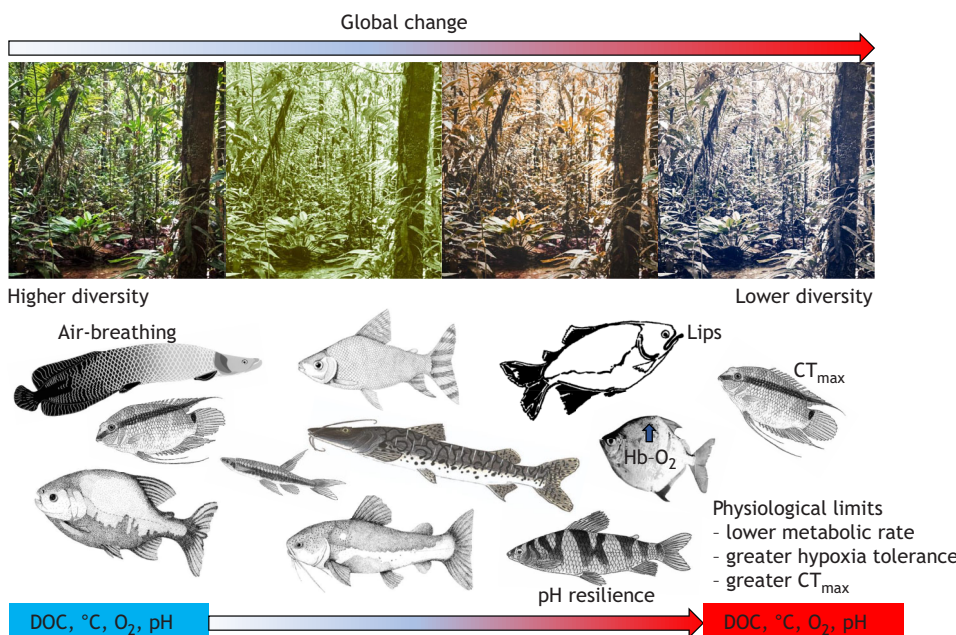
Intermittent aquatic hypoxia is one of the most important evolutionary and ecological drivers in Amazonia and will become more intense with global change. Most Amazon fish are already adapted to some degree to cope with hypoxia at the levels of the tissues (e.g. high glycolytic capacity and/or ability to turn down metabolic rate; Muusze et al., 1998; Almeida-Val et al., 2000) and blood O<sub>2</sub> transport (e.g. blood–O<sub>2</sub> affinities which can be quickly increased; Val et al., 2015; Scott et al., 2017), and many have a capacity for aquatic surface respiration and/or air-breathing (Graham, 1997; Randall et al., 1981; Val and Almeida-Val, 1995). Organs that have been adapted for these purposes include the swim bladder, gills, lips, buccal cavity, skin, fins and gastrointestinal tract. In most Amazon fish, regulation of haemoglobin–O<sub>2</sub> affinity is accomplished by a routinely higher concentration of GTP, which is a stronger modulator than ATP, commonly found in temperate zone fishes. However, several other organic phosphates (2,3DPG, IP2, IPP) are also found, and are regulated according to environmental challenges (Marcon et al., 1999; Val, 2000; Weber et al., 2000). At first glance, those species that have the greatest capacity for O<sub>2</sub> uptake from air, such as the obligate air-breathing pirarucu (Pelster et al., 2020a), would seem best-equipped to cope. However, the early life stages are obligate water-breathers (Brauner et al., 2004a). Furthermore, throughout life they must continue to breathe water for CO<sub>2</sub> and ammonia excretion, ionoregulation and acid–base regulation (Frommel et al., 2021; Gonzalez et al., 2010). Therefore, gill function will continue to be impacted by aquatic hypoxia. Indeed, the pirarucu (Wood et al., 2020), like several other hypoxia-

tolerant Amazonian species (Robertson et al., 2015), displays the exact opposite of the standard osmorepiratory compromise (Randall et al., 1972; Wood and Eom, 2021). Gill ion fluxes quickly decrease rather than increase during hypoxia. In the oscar (De Boeck et al., 2013; Wood et al., 2007, 2009), this is achieved by rapid extension of pavement cells over gill ionocytes (Matey et al., 2011), thereby reducing ion and water permeability without impeding O<sub>2</sub> transfer (Scott et al., 2008). The short-term benefits for osmoregulation are obvious, but longer-term impacts (impeded ammonia and acid–base regulation) may be costly. Additional costs associated with increased reliance on air-breathing include the potential for aerially sourced O<sub>2</sub> to be lost at the gills during aquatic hypoxia (Scott et al., 2017; Aaskov et al., 2022), greater oxidative stress in the air-breathing tissues (Pelster et al., 2016, 2018, 2020b; Pelster and Wood, 2018), increased risk from avian predation when surfacing (Sloman et al., 2009, 2006) and increased reliance on the diet for ion acquisition (Pelster and Wood, 2018; Wood et al., 2016).

Given these costs, it may well be that some predominantly water-breathing species will be favoured in a future more hypoxic Amazon, especially those with high glycolytic capacity and/or great ability to reduce metabolic rate during hypoxia, such as the oscar (Almeida-Val et al., 2000; Chippari-Gomes et al., 2005; Muusze et al., 1998). Additional specific survival mechanisms may include skimming with a protruded lip as seen in matrincha and tambaqui (Anjos et al., 2008; Rantin and Kalinin, 1996; Val and Almeida-Val, 1995; Val et al., 1998), an ability to oxygenate the nests as in angelfish (Korzelecka-Orkisz et al., 2012), the ability to rapidly find normoxic refugia as in the lateral migrations (Cox-Fernandes, 1997; Silva and Stewart, 2017; Val and Almeida-Val, 1995) and the school migrations near the surface that help oxygenate the water (A.L.V., personal observation).

**Predicting the impact of climate change on Amazon fishes**

The fish of Amazonia evolved under continuous environmental change. Throughout the evolutionary process, climate change has acted additively, causing the emergence of adaptations, speciation and extinctions, already well documented among fish (Graham, 1997; Somero, 2010; Val et al., 1998). Therefore, many native species can live at the extreme limits of environmental challenges,



**Fig. 4. Predicting the impact of climate changes on the physiology of Amazonian fishes.** Note the degradation of the forest, reduction of fish diversity and physiological responses of fishes to challenging environmental conditions as climate change progresses.



**Table 2. Types of effects, definitions and examples of global change impacts on Amazonian fish**

| Effect         | Definition   | Amazonian fishes   |
|----------------|--|--|
| Direct         | Direct effect on biological processes (e.g. warmer and acidic waters)  | Thermo-tolerance as in forest stream fishes such as <i>Apistogramma agassizii</i> , <i>Pyrrhulina brevis</i> and <i>Hyphessobrychon melazonatus</i> (Campos et al., 2018, 2019), and acidic pH vulnerability as in <i>Corydoras julii</i> and <i>Hemigrammus</i> sp. (Gonzalez et al., 2002) |
| Indirect       | Biological changes occurring as a consequence of primary environmental changes (e.g. deforestation)                                    | Increased UV exposure potentially causing DNA changes as in <i>Colossoma macropomum</i> and <i>Arapaima gigas</i> (Groff et al., 2010)   |
| Interactive    | Effects caused by one global change stressor augmenting toxicity of one or more pollutants (water acidification and transition metals) | Increased toxicity of copper on fish exposed to acidic water as in many ornamental fish (Duarte et al., 2009) and nickel in <i>Paracheirodon axelrodi</i> (Holland et al., 2017)   |
| Additive       | Additional effect on an already challenging environmental condition (local extinctions and range contractions)                         | Contraction of environmental species distribution as for Andean Amazon fish species (Herrera-R et al., 2020)   |
| Non-predictive | Atypical biological effects occurring in a non-predictive way (occurrence of diseases in climate-stressed organisms)                   | Parasitism in fish exposed to extreme climate scenarios, as in <i>Colossoma macropomum</i> (Costa and Val, 2020)   |
| Sequential     | Biological impacts of events of similar nature caused by global change occurring sequentially (extreme droughts and floods)            | Extreme droughts and floods affecting fish populations as in the Central Amazon (Röpke et al., 2017)   |

particularly with respect to water temperature, low pH, hypoxia and even anoxic conditions. Climate change is now pushing many fish species to almost physiologically unsustainable conditions. This situation has different contours in different ecosystems, such as in the estuaries of Australia (Scanes et al., 2020). Furthermore, these synergistic interactions of climate change with other environmental features have specific effects on local fauna (Pörtner et al., 2021), including ichthyofauna. As already mentioned, the responses of different fish species and fish from different regions to environmental variations are not homogeneous (there is a vast literature on this; see Brauner et al., 2019; Farrell and Richards, 2009; Lefreve et al., 2021). Besides direct effects such as temperature and  $P_{CO_2}$  increase, associated specific factors also affect Amazonian fishes: increased amplitudes of flood pulses, and changes in vegetation cover, siltation and DOC characteristics (Fig. 4).

At least five classes of climate change effects on fish can be recognized (Table 2). These may occur simultaneously and amplify the effects of a particular parameter. Note that synergistic effects of climate change on fish can be either additive or interactive. Controlled laboratory simulations of future climate conditions indicate that synergistic effects will impose new challenges to Amazon fish, different from the effects of a given single environmental condition. Some species such as tambaqui seem to be resilient (Val and Oliveira, 2021), while others are very sensitive. It is worth noting that in their natural environments, although physiological effects can be attenuated or amplified, animals have a range of possibilities to defend themselves biologically, which is not the case under experimental conditions. Thus, long-term studies in the natural environment involving life history, predation and other ecological factors are needed to predict the future resilience of different species.

### Conclusions and perspectives

The major fish groups of Amazonia appeared during geological periods that had very high  $CO_2$  and low  $O_2$  relative to current levels. Will present and future populations be able to use information hidden in their genome that allowed them to survive these conditions in the past? Conceivably this evolutionary history could favour their persistence in the face of current and future climate change scenarios. Our Review suggests that climate change will favour predominantly water-breathing species with low routine metabolic rates, low temperature sensitivity of routine metabolic rates, high anaerobic capacity, and high thermal and hypoxia tolerance. Water temperature may be a particularly decisive factor.

Amazon fish appear to have reached an evolutionary point of very high sensitivity to temperature, as they now live very close to their critical thermal limits. This may be a turning point for the whole fish diversity of Amazonia, resulting in many local extinctions and yet through intense selection pressures, the appearance of new adaptations to extreme synergistic challenges. For now, so few species have been studied out of the immense diversity of fishes in the region that any generalizations will probably overlook other important biological parameters. Over the next few decades, the many unanswered questions will call for integrated analysis, and physiologists and experimental biologists, among others, will be able to study evolutionary change in real time in Amazonia.

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The authors declare no competing or financial interests.

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### References

- Aaskov, M. L., Jensen, R. J., Skov, P. V., Wood, C. M., Wang, T., Malte, H. and Bayley, M. (2022). *Arapaima gigas* maintains gas exchange separation in severe aquatic hypoxia but does not suffer branchial oxygen loss. *J. Exp. Biol.* 225, jeb243672. <https://doi.org/10.1242/jeb.243672>
- Adeney, J. M., Christensen, N. I., Vicentini, A. and Cohn-Haft, M. (2016). White-sand ecosystems in Amazonia. *Biotropica* 48, 7-23. doi:10.1111/btp.12293
- Albert, J., Val, P. and Hoorn, C. (2018). The changing course of the Amazon River in the Neogene: center stage for neotropical diversification. *Neotrop. Ichthyol.* 16, e180033. doi:10.1590/1982-0224-20180033
- Al-Reasi, H. A., Wood, C. M. and Smith, D. S. (2011). Physicochemical and spectroscopic properties of natural organic matter (NOM) from various sources and implications for ameliorative effects on metal toxicity to aquatic biota. *Aquat. Toxicol.* 103, 179-190. doi:10.1016/j.aquatox.2011.02.015
- Al-Reasi, H. A., Wood, C. M. and Smith, D. S. (2013). Characterization of freshwater natural dissolved organic matter (DOM): mechanistic explanations for protective effects against metal toxicity and direct effects on organisms. *Envir. Int.* 59, 201-207. doi:10.1016/j.envint.2013.06.005
- Al-Reasi, H. A., Smith, D. S. and Wood, C. M. (2016). The influence of dissolved organic matter (DOM) on sodium regulation and nitrogenous waste excretion

- in the zebrafish (*Danio rerio*). *J. Exp. Biol.* **219**, 2289–2299. doi:10.1242/jeb.139444
- Almeida-Val, V. M. F., Val, A. L., Duncan, W. P., Souza, F. C. A., Paula-Silva, M. N. and Land, S. (2000). Scaling effects on hypoxia tolerance in the Amazon fish *Astronotus ocellatus* (Perciformes, Cichlidae): contribution of tissue enzyme levels. *Comp. Biochem. Physiol.* **125B**, 219–226. doi:10.1016/S0305-0491(99)00172-8
- Anjos, M. B., Oliveira, R. R. and Zuanon, J. S. (2008). Hypoxic environments as refuge against predatory fish in the Amazonian floodplains. *Braz. J. Biol.* **68**, 45–50. doi:10.1590/S1519-69842008000100007
- Antonelli, A., Zizka, A., Carvalho, F., Scharn, R., Bacon, C., Silvestro, D. and Dondamine, F. (2018). Amazonia is the primary source of neotropical biodiversity. *Proc. Natl. Acad. Sci. USA* **115**, 6034–6039. doi:10.1073/pnas.1713819115
- Araújo, J. D., Ghelfi, A. and Val, A. L. (2017). *Triplotheus albus* Cope, 1872 in the blackwater, clearwater, and whitewater of the Amazon: a case of phenotypic plasticity? *Front. Genet.* **8**, 114. doi:10.3389/fgene.2017.00114
- Assahira, C., Piedade, M. T. F., Trumbore, S. E., Wittmann, F., Cintra, B. B. L., Batista, E. S. and Schöngart, J. (2017). Tree mortality of a flood-adapted species in response of hydrographic changes caused by an Amazonian river dam. *For. Ecol. Manag.* **396**, 113–123. doi:10.1016/j.foreco.2017.04.016
- Assis, R. A., Wittmann, F., Bredin, Y. K., Schöngart, J., Quesada, C. A. N., Piedade, M. T. F. and Haugsaen, T. (2019). Above-ground woody biomass distribution in Amazonian floodplain forests: effects of hydroperiod and substrate properties. *For. Ecol. Manag.* **432**, 365–375. doi:10.1016/j.foreco.2018.09.031
- Aucour, A. M., Tao, F. X., Moreira-Turcq, P., Seyler, P., Sheppard, S. and Benedetti, M. F. (2003). The Amazon River: behaviour of metals (Fe, Al, Mn) and dissolved organic matter in the initial mixing at the Rio Negro/Solimões confluence. *Chem. Geol.* **197**, 271–285. doi:10.1016/S0009-2541(02)00398-4
- Barbosa, D. S., Barbosa, D. S., Espíndola, E. L. and Daam, M. A. (2019). The direct effects of a tropical natural humic substance to three aquatic species and its influence on their sensitivity to copper. *Ecotoxicology* **28**, 550–558. doi:10.1007/s10646-019-02031-3
- Barichivich, J., Gloor, E., Peylin, P., Brienen, R. J. W., Schöngart, J., Espinoza, J. C. and Pattnayak, K. C. (2018). Recent intensification of Amazon flooding extremes driven by strengthened Walker circulation. *Sci. Adv.* **4**, eaat8785. doi:10.1126/sciadv.aat8785
- Bartlett, G. R. (1978). Phosphates in red cells of two South American osteoglossids: *Arapaima gigas* and *Osteoglossum bicirrhosum*. *Can. J. Zool.* **56**, 878–881. doi:10.1139/z78-121
- Bergmann, K. G. L. C. (1847). Über die Verhältnisse der wärmeökonomie der Thiere zu ihrer Grösse. *Göttinger Studien* **3**, 595–708.
- Biggs, T. W., Dunne, T. and Martinelli, L. A. (2004). Natural controls and human impacts on stream nutrient concentrations in a deforested region of the Brazilian Amazon basin. *Biogeochemistry* **68**, 227–257. doi:10.1023/B:BI0G.0000025744.78309.2e
- Brauner, C. J. and Baker, D. W. (2009). Patterns of acid-base regulation during exposure to hypercarbia in fishes. In *Cardio-Respiratory Control in Vertebrates* (ed. M. L. Glass and S. C. Wood), pp. 43–63. Berlin: Springer.
- Brauner, C. J., Matey, V., Wilson, J. M., Bernier, N. J. and Val, A. L. (2004a). Transition in organ function during the evolution of air-breathing: insights from *Arapaima gigas*, an obligate air-breathing teleost from the Amazon. *J. Exp. Biol.* **207**, 1433–1438. doi:10.1242/jeb.00887
- Brauner, C. J., Wang, T., Wang, Y., Richards, J. G., Gonzalez, R. J., Bernier, N. J., Wang, X., Patrick, M. L. and Val, A. L. (2004b). Limited extracellular but complete intracellular acid-base regulation during short-term environmental hypercapnia in the armoured catfish, *Liposarcus pardalis*. *J. Exp. Biol.* **207**, 3381–3390. doi:10.1242/jeb.01144
- Brauner, C. J., Shartau, R. B., Damsgaard, C., Esbaugh, A. J., Wilson, R. W. and Grosell, M. (2019). Acid-base physiology and CO<sub>2</sub> homeostasis: regulation and compensation in response to elevated environmental CO<sub>2</sub>. In *Fish Physiology*, Vol. 37 (ed. M. Grosell, P. L. Munday, A. P. Farrell and C. J. Brauner), pp. 69–132. Elsevier.
- Braz-Mota, S., Fé, L. M. L., De Lunardo, F. A. C., Sadauskas-Henrique, H., Almeida-Val, V. M. F. and Val, A. L. (2017). Exposure to waterborne copper and high temperature induces the formation of reactive oxygen species and causes mortality in the Amazonian fish *Hoplosternum littorale*. *Hydrobiologia* **789**, 157–166. doi:10.1007/s10750-016-2847-y
- Brodie, J., Post, E. and Laurence, W. (2012). Climate change and tropical biodiversity: a new focus. *Trends Ecol. Evol.* **27**, 145–150. doi:10.1016/j.tree.2011.09.008
- Caldas, L. R. (1990). Um pigmento nas águas negras. *Ciência Hoje* **11**, 55–57.
- Campbell, P. G. C., Twiss, M. R. and Wilkinson, K. J. (1997). Accumulation of natural organic matter on the surfaces of living cells: implications for the interaction of toxic solutes with aquatic biota. *Can. J. Fish. Aquat. Sci.* **54**, 2543–2554. doi:10.1139/f97-161
- Campos, D. F., Jesus, T. F., Kochhann, D., Heinrichs-Caldas, W., Coelho, M. M. and Almeida-Val, V. M. F. (2017). Metabolic rate and thermal tolerance in two congeneric Amazon fishes: *Paracheirodon axelrodi* Schultz, 1956 and *Paracheirodon simulans* Géry, 1963 (Characidae). *Hydrobiologia* **789**, 133–142. doi:10.1007/s10750-016-2649-2
- Campos, D., Val, A. L. and Almeida-Val, V. M. F. (2018). The influence of lifestyle and swimming behavior on metabolic rate and thermal tolerance of twelve Amazon forest stream fish species. *J. Therm. Biol.* **72**, 148–154. doi:10.1016/j.jtherbio.2018.02.002
- Campos, D., Braz-Mota, S., Val, A. L. and Almeida-Val, V. M. F. (2019). Predicting thermal sensitivity of three Amazon fishes exposed to climate change scenarios. *Ecol. Indicators* **101**, 533–540. doi:10.1016/j.ecolind.2019.01.051
- Castello, L. and Macedo, M. N. (2016). Large-scale degradation of Amazonian freshwater ecosystems. *Glob. Change Biol.* **22**, 990–1007. doi:10.1111/gcb.13173
- Catania, K. (2014). The shocking predatory strike of the electric eel. *Science* **346**, 1231–1234. doi:10.1126/science.1260807
- Chaussé, T. C., Santos Brandão, C., Silva, L. P., Spanghero, P. E. S. F. and Silva, D. M. (2016). Evaluation of nutrients and major ions in streams – implications of different timescale procedures. *Environ. Monit. Assess.* **188**, 1–16. doi:10.1007/s10661-015-5034-0
- Chippari-Gomes, A. R., Gomes, L. C., Lopes, N. P., Val, A. L. and Almeida-Val, V. M. F. (2005). Metabolic adjustments in two Amazonian cichlids exposed to hypoxia and anoxia. *Comp. Biochem. Physiol.* **141B**, 347–355. doi:10.1016/j.cbpc.2005.04.006
- Clifford, A. M., Goss, G. G. and Wood, C. M. (2021). Functional evidence for a novel K<sup>+</sup>-dependent Na<sup>+</sup> uptake mechanism during low pH exposure in adult zebrafish (*Danio rerio*): new tricks for old dogma. *Acta Physiologica* **234**, e13777.
- Costa, J. C. and Val, A. L. (2020). Extreme climate scenario and parasitism affect the Amazonian fish *Colossoma macropomum*. *Sci. Total Environ.* **726**, 138628. doi:10.1016/j.scitotenv.2020.138628
- Cox-Fernandes, C. (1997). Lateral migration of fishes in Amazon floodplains. *Ecol. Freshw. Fishes* **6**, 36–44. doi:10.1111/j.1600-0633.1997.tb00140.x
- Crémazy, A., Wood, C. M., Smith, D. S., Ferreira, M. S., Johannsson, O. E., Giacomini, M. and Val, A. L. (2016). Investigating copper toxicity in the tropical fish cardinal tetra (*Paracheirodon axelrodi*) in natural Amazonian waters: measurements, modeling, and reality. *Aquat. Toxicol.* **180**, 353–363. doi:10.1016/j.aquatox.2016.10.011
- Crémazy, A., Wood, C. M., Scott Smith, D. and Val, A. L. (2019). The effects of natural suspended solids on copper toxicity to the cardinal tetra in Amazonian river waters. *Environ. Toxicol. Chem.* **38**, 2708–2718. doi:10.1002/etc.4586
- Cruz, A., Pedretti, A. and Fernandes, M. (2008). Stereological estimation of the surface area and oxygen diffusing capacity of the respiratory stomach of the air-breathing armored catfish *Pterygoplichthys anisitsi* (Teleostei: Loricariidae). *J. Morphol.* **270**, 610–614.
- Dagosta, F. C. P. and De Pinna, M. (2019). The fishes of the Amazon: distribution and biogeographical patterns, with a comprehensive list of species. *Bull. Am. Mus. Nat. Hist.* **2019**, 1–163. doi:10.1206/0003-0090.431.1.1
- Dai, A. (2012). Increasing drought under global warming in observations and models. *Nat. Clim. Change* **3**, 52–58. doi:10.1038/nclimate1633
- Dal Pont, G., Domingos, F. X. V., Fernandes-de-Castilho, M. and Val, A. L. (2017). Potential of the biotic ligand model (BLM) to predict copper toxicity in the white-water of the Solimões-Amazon River. *Bull. Environ. Contam. Toxicol.* **98**, 27–32. doi:10.1007/s00128-016-1986-1
- De Boeck, G., Wood, C. M., Iftikar, F. I., Matey, V., Scott, G. R., Sloman, K. A., Paula-Silva, M. N., Almeida-Val, V. M. F. and Val, A. L. (2013). Interactions between hypoxia tolerance and food deprivation in Amazonian oscar, *Astronotus ocellatus*. *J. Exp. Biol.* **216**, 4590–4600.
- Dejours, P. (1975). *Principles of Comparative Respiratory Physiology*. Amsterdam: North-Holland Publishing Company.
- de la Vega, E., Chalk, T. B., Wilson, P. A., Bysani, R. P. and Foster, G. L. (2020). Atmospheric CO<sub>2</sub> during the mid Pliocene warm period and the M2 glaciation. *Sci. Rep.* **10**, 11002. doi:10.1038/s41598-020-67154-8
- Drake, T. W., Van Oost, K., Barthel, M., Bauters, M., Hoyt, A. M., Podgorski, D. C., Six, J., Boeckx, P., Trumbore, S. E., Ntaboba, L. C. et al. (2019). Mobilization of aged and biolabile soil carbon by tropical deforestation. *Nature Geoscience* **12**, 541–546. doi:10.1038/s41561-019-0384-9
- Duarte, R. M., Menezes, A. C. L., Rodrigues, L. S., Almeida-Val, V. M. F., Val, A. L. (2009). Copper sensitivity of wild ornamental fish of the Amazon. *Ecotoxicol. Environ. Saf.* **72**, 693–698. doi:10.1016/j.ecoenv.2008.10.003
- Duarte, R. M., Ferreira, M. S., Wood, C. M. and Val, A. L. (2013). Effect of low pH exposure on Na<sup>+</sup> regulation in two cichlid fish species of the Amazon. *Comp. Biochem. Physiol. A* **166**, 441–448. doi:10.1016/j.cbpa.2013.07.022
- Duarte, R. M., Scott Smith, D. S., Val, A. L. and Wood, C. M. (2016). Dissolved organic carbon from the upper Rio Negro protects zebrafish (*Danio rerio*) against ionoregulatory disturbances caused by low pH exposure. *Sci. Rep.* **6**, 20377–20386. doi:10.1038/srep20377
- Duarte, R. M., Wood, C. M., Val, A. L. and Scott Smith, D. (2018). Physiological protective action of dissolved organic carbon on ion regulation and nitrogenous waste excretion of zebrafish (*Danio rerio*) exposed to low pH in ion-poor water. *J. Comp. Physiol. B* **188**, 793–807. doi:10.1007/s00360-018-1169-y
- Duran, N., Justo, G. Z., Ferreira, C. V., Melo, P. S., Cordi, L. and Martins, D. (2007). Violacein: properties and biological activities. *Biotechnol. Appl. Biochem.* **48**, 127–133. doi:10.1042/BA20070115

- Dutra, G., Freitas, T., Prudente, B., Salvador, G., Leão, M., Peixoto, L., Mendonça, M., Neto-Ferreira, A., Silva, F., Montag, L. et al. (2020). Rapid assessment of the ichthyofauna of the southern Guiana Shield tributaries of the Amazonian River in Pará, Brazil. *Acta Amazonica* **50**, 24-36. doi:10.1590/1809-4392201901402
- Dymowska, A., Hwang, P. P. and Goss, G. G. (2012). Structure and function of ionocytes in the freshwater fish gill. *Respir. Physiol. Neurobiol.* **184**, 282-292. doi:10.1016/j.resp.2012.08.025
- Farrell, A. P. and Richards, J. G. (2009). Defining hypoxia: an integrative synthesis of the responses of fish to hypoxia. In *Fish Physiology*, Vol. 27 (ed. J. G. Richards, A. P. Farrell and C. J. Brauner), pp. 487-503. Elsevier.
- Fé-Gonçalves, L. M., Paula-Silva, M. N., Val, A. L. and Almeida-Val, V. M. F. (2018). Differential survivorship of congeneric ornamental fishes under forecasted climate changes are related to anaerobic potential. *Genet. Mol. Biol.* **41**, 107-118. doi:10.1590/1678-4685-gmb-2017-0016
- Figueiredo, R. O., Markewitz, D., Davidson, E. A., Schuler, A. E., Watrin, O. S. and Silva, P. S. (2010). Land-use effects on the chemical attributes of low-order streams in the eastern Amazon. *J. Geophys. Res. Biogeosci.* **115**, G04004. doi:10.1029/2009JG001200
- Frommel, A. Y., Kwan, G. T., Prime, K. J., Tresguerres, M., Lauridsen, H., Val, A. L., Gonçalves, L. U. and Brauner, C. J. (2021). Changes in gill and air-breathing organ characteristics during the transition from water- to air-breathing in juvenile *Arapaima gigas*. *J. Exp. Zool. A* **335**, 801-813. doi:10.1002/jez.2456
- Furch, K. (1984). Water chemistry of the Amazon basin: the distribution of chemical elements among freshwaters. In *The Amazon. Limnology and Landscape Ecology of a Mighty Tropical River and its Basin* (ed. H. Sioli), pp. 167-199. Dordrecht: Dr W Junk Publishers.
- Furch, K. and Junk, W. J. (1997). Physicochemical conditions in the floodplains. In *The Central Amazon Floodplain. Ecology of a Pulsing System*, Vol. 126 (ed. W. J. Junk), pp. 69-108. Heidelberg: Springer Verlag.
- Gaffney, J. S., Marley, N. A. and Clark, S. B. (1996). Humic and fulvic acids and organic colloidal materials in the environment. In *Humic/Fulvic Acids and Organic Colloidal Materials in the Environment* (ed. J. S. Gaffney, N. A. Marley and S. B. Clark), pp. 2-16. Washington, DC: American Chemical Society.
- Galvez, F., Donini, A., Smith, S., O'Donnell, M. and Wood, C. M. (2008). A matter of potential concern: Natural organic matter alters the electrical properties of fish gills. *Environ. Sci. Technol.* **42**, 9385-9390. doi:10.1021/es8005332
- Glover, C. N. and Wood, C. M. (2005). The disruption of *Daphnia magna* sodium metabolism by humic substances: mechanism of action and effect of humic substances sources. *Physiol. Biochem. Zool.* **78**, 1005-1016. doi:10.1086/432858
- Glover, C. N., Pane, E. F. and Wood, C. M. (2005). Humic substances influence sodium metabolism in the freshwater crustacean *Daphnia magna*. *Physiol. Biochem. Zool.* **78**, 405-416. doi:10.1086/430036
- Gonsior, M., Valle, J., Schmitt-Kopplin, P., Hertkorn, N., Bastviken, D., Luek, J., Harir, M., Bastos, W. and Enrich-Prast, A. (2016). Chemodiversity of dissolved organic matter in the Amazon Basin. *Biogeosciences* **13**, 4279-4290. doi:10.5194/bg-13-4279-2016
- Gonzalez, R. J. and Prest, M. R. (1999). Ionoregulatory specializations for exceptional tolerance of ion-poor, acidic waters in the neon tetra (*Paracheirodon innesi*). *Physiol. Biochem. Zool.* **72**, 156-163. doi:10.1086/316651
- Gonzalez, R. J. and Wilson, R. W. (2001). Patterns of ion regulation in acidophilic fish native to the ion-poor, acidic Rio Negro. *J. Fish Biol.* **58**, 1680-1690. doi:10.1111/j.1095-8649.2001.tb02322.x
- Gonzalez, R., Wood, C., Wilson, R., Patrick, M., Bergman, H., Narahara, A. and Val, A. (1998). Effects of water pH and calcium concentration on ion balance in fish of the Rio Negro, Amazon. *Physiol. Zool.* **71**, 15-22. doi:10.1086/515893
- Gonzalez, R., Wilson, R. and Wood, C. (2006). Ionoregulation in tropical fishes from ion-poor, acidic blackwaters. In *The Physiology of Tropical Fishes*, Vol. 21 (ed. A. L. Val, V. M. F. Almeida-Val and D. J. Randall), pp. 397-442. San Diego: Elsevier/Academic Press.
- Gonzalez, R. J., Brauner, C. J., Wang, Y., Richards, J., Patrick, M., Xi, W., Matey, V. and Val, A. L. (2010). Impact of ontogenetic changes in branchial morphology on gill function in *Arapaima gigas*. *Physiol. Biochem. Zool.* **83**, 322-332. doi:10.1086/648568
- Gonzalez, R., Jones, S. and Nguyen, T. (2017). Ionoregulatory characteristics of non-Rio Negro characiforms and cichlids. *Physiol. Biochem. Zool.* **90**, 407-414. doi:10.1086/690306
- Gonzalez, R. J., Cradeur, A., Guinnip, M., Mitchell, A. and Redura, V. (2018). South American characids share very similar ionoregulatory characteristics. *Comp. Biochem. Physiol.* **226A**, 17-21. doi:10.1016/j.cbpa.2018.06.025
- Gonzalez, R. J., Wilson, R. W., Wood, C. M., Patrick, M. L. and Val, A. L. (2002). Diverse strategies for ion regulation in fish collected from the ion-poor, acidic Rio Negro. *Physiol. Biochem. Zool.* **75**, 37-47. doi:10.1086/339216
- Gonzalez, R. J., Hsu, R., Mahaffey, L., Rebagliatti, D. and Shami, R. (2020). Examination of ionoregulatory characteristics of South American cichlids. *Comp. Biochem. Physiol.* **253A**, 110854.
- Gonzalez, R. J., Patrick, M. L., Duarte, R. M., Casciato, A., Thackeray, J., Day, N. and Val, A. L. (2021). Exposure to pH 3.5 water has no effect on the gills of the Amazonian tambaqui (*Colossoma macropomum*). *J. Comp. Physiol. B* **191**, 493-502. doi:10.1007/s00360-021-01349-x
- Graham, J. B. (1997). *Air-breathing Fishes. Evolution, Diversity and Adaptation*. San Diego: Academic Press.
- Groff, A., Silva, J., Nunes, E., Ianistcki, M., Oliveira, A., Oliveira, C., Val, A. L. and Henriques, J. (2010). UVA/UVB induced lesion repair and genotoxicity in the Amazonian fishes *Colossoma macropomum* and *Arapaima gigas*. *Aquat. Toxicol.* **99**, 93-99. doi:10.1016/j.aquatox.2010.04.003
- Gross, L. (2018). Confronting climate change in the age of denial. *PLoS Biol.* **16**, e3000033. doi:10.1371/journal.pbio.3000033
- Harter, T. S., Shartau, R. B., Baker, D. W., Jackson, D. C., Val, A. L. and Brauner, C. J. (2014). Preferential intracellular pH regulation represents a general pattern of pH homeostasis during acid-base disturbances in the armoured catfish, *Pterygoplichthys pardalis*. *J. Comp. Physiol. B* **184**, 709-718. doi:10.1007/s00360-014-0838-8
- Heisler, N. (1982). Intracellular and extracellular acid-base regulation in the tropical fresh-water teleost fish *Synbranchus marmoratus* in response to the transition from water breathing to air breathing. *J. Exp. Biol.* **99**, 9-28. doi:10.1242/jeb.99.1.9
- Heisler, N. (1986). Acid-base regulation in fishes. In *Acid-Base Regulation in Animals* (ed. N. Heisler), pp. 309-356. Amsterdam: Elsevier.
- Herrera-R, G., Oberdorff, T., Anderson, E., Brosse, S., Carvajal-Vallejos, F., Frederico, R., Hidalgo, M., Jézéquel, C., Maldonado, M., Maldonado-Ocampo, J. et al. (2020). The combined effects of climate change and river fragmentation on the distribution of Andean Amazon fishes. *Glob. Change Biol.* **26**, 5509-5523. doi:10.1111/gcb.15285
- Hess, L. L., Melack, J. M., Affonso, A. G., Barbosa, C., Gastil-Buhl, M. and Novo, E. M. L. M. (2015). Wetlands of the lowland Amazon basin: extent, vegetative cover, and dual-season inundated area as mapped with JERS-1 Synthetic Aperture Radar. *Wetlands* **35**, 745-756. doi:10.1007/s13157-015-0666-y
- Hilty, J. A. (2012). *Climate and Conservation: Landscape and Seascape Science, Planning, and Action*. Island Press.
- Holland, A., Wood, C. M., Smith, D. S., Correia, T. G. and Val, A. L. (2017). Nickel toxicity to cardinal tetra (*Paracheirodon axelrodi*) differs seasonally and among the black, white and clear river waters of the Amazon basin. *Water Res.* **126**, 21-29. doi:10.1016/j.watres.2017.06.044
- Iha, P., Schiesari, L., Yanagawa, F. I., Jankowski, K. and Navas, C. A. (2018). Deforestation and stream warming affect body size of Amazonian fishes. *PLoS One* **13**, e0196560. doi:10.1371/journal.pone.0196560
- IPCC (2014). Climate Change 2014: Synthesis Report. In *Contribution of Working Groups I, II and III to the fifth assessment report of the Intergovernmental Panel on Climate Change* (ed. Core Writing Team, R. K. Pachauri and L. A. Meyer). Geneva: Intergovernmental Panel on Climate Change.
- Isaacks, R. E., Kim, H. D., Bartlett, G. R. and Harkness, D. R. (1977). Inositol pentaphosphate in erythrocytes of a freshwater fish, pirarucu (*Arapaima gigas*). *Life Sci.* **20**, 987-990. doi:10.1016/0024-3205(77)90285-5
- Jacox, M., Alexander, M., Bograd, S. and Scott, J. (2020). Thermal displacement by marine heatwaves. *Nature* **584**, 82-86. doi:10.1038/s41586-020-2534-z
- Jaffé, R., Nunes, S., Santos, J. F., Gastauer, M., Giannini, T. C., Nascimento, W., Jr, Sales, M., Souza, C. M., Jr, Souza-Filho, P. W. and Fletcher, R. J., Jr (2021). Forecasting deforestation on the Brazilian Amazon to prioritize conservation efforts. *Environ. Res. Lett.* **16**, 084034. doi:10.1088/1748-9326/ac146a
- Jézéquel, C., Tedesco, P. A., Darwall, W., Dias, M. S., Frederico, R. G., Hidalgo, M., Huguely, B., Maldonado-Ocampo, J., Martens, K., Ortega, H. et al. (2020). Freshwater fish diversity hotspots for conservation priorities in the Amazon Basin. *Conserv. Biol.* **34**, 956-965. doi:10.1111/cobi.13466
- Jung, E. H., Brix, K. V., Richards, J. G., Val, A. L. and Brauner, C. J. (2020). Reduced hypoxia tolerance and survival at elevated temperatures may limit the ability of Amazonian fishes to survive in a warming world. *Sci. Total Environ.* **748**, 141349. doi:10.1016/j.scitotenv.2020.141349
- Junk, W. J., Bayley, P. B. and Sparks, R. E. (1989). The flood pulse concept in river-floodplain systems. In *Proceedings of the International Large River Symposium*, Vol. 106 (ed. D. P. Dodge), pp. 110-127. Canada: Can. Spec. Publ. Fish. Aquat. Sci.
- Kjelland, M. E., Woodley, C. M., Swannack, T. M. and Smith, D. L. (2015). A review of the potential effects of suspended sediment on fishes: potential dredging-related physiological, behavioral, and transgenerational implications. *Environ. Syst. Decis.* **35**, 334-350. doi:10.1007/s10669-015-9557-2
- Korzelecka-Orkisz, A., Szalast, Z., Pawlos, D., Smaruj, I., Tański, A., Szulc, J. and Formicki, K. (2012). Early ontogenesis of the angelfish, *Pterophyllum scalare* Schultzze, 1823 (Cichlidae). *Neotrop. Ichthyol.* **10**, 567-576. doi:10.1590/S1679-62252012005000017
- Kramer, D. L., Lindsey, C. C., Moodie, G. E. E. and Stevens, E. D. (1978). The fishes and the aquatic environment of the central Amazon basin, with particular reference to respiratory patterns. *Can. J. Zool.* **56**, 717-729. doi:10.1139/z78-101
- Kullberg, A., Bishop, K. H., Hergeby, A., Jansson, M. and Petersen, R. C. (1993). The ecological significance of dissolved organic carbon in acidified waters. *Ambio* **22**, 331-337.
- Lapointe, D., Cooperman, M. S., Chapman, L. J., Clark, T. D., Val, A. L., Ferreira, M. S., Balirwa, J. S., Mbabazi, D., Mwanja, M., Chhom, L. et al. (2018). Predicted impacts of climate warming on aerobic performance and upper thermal

- tolerance of six tropical freshwater fishes spanning three continents. *Conserv. Biol.* **6**, coy056.
- Larsen, B. K. and Jensen, F. B. (1997). Influence of the ionic composition on acid-base regulation in rainbow trout (*Oncorhynchus mykiss*) exposed to environmental hypercapnia. *Fish Physiol. Biochem.* **16**, 157-170. doi:10.1007/BF0004672
- Latrubesse, E. M., Stevaux, J. C. and Sinha, R. (2005). Tropical rivers. *Geomorphology* **70**, 187-206. doi:10.1016/j.geomorph.2005.02.005
- Leenheer, J. A. (1980). Origin and nature of humic substances in the waters of the Amazon River basin. *Acta Amazonica* **10**, 513-526. doi:10.1590/1809-43921980103513
- Lefreve, S., Wang, T. and McKenzie, D. J. (2021). The role of mechanistic physiology in investigating impacts of global warming on fishes. *J. Exp. Biol.* **224**, jeb238840. doi:10.1242/jeb.238840
- Leitão, R. P., Zuanon, J., Mouillot, D., Leal, C. G., Hughes, R. M., Kaufmann, P. R., Villéger, S., Pompeu, P. S., Kasper, D., Paula, F. R. et al. (2018). Disentangling the pathways of land use impacts on the functional structure of fish assemblages in Amazon streams. *Ecography* **41**, 219-232. doi:10.1111/ecog.02845
- Lenoir, J., Gégout, J., Marquet, P., Ruffray, P. d. and Brisse, H. (2008). A significant upward shift in plant species optimum elevation during the 20th century. *Science* **320**, 1768-1771. doi:10.1126/science.1156831
- Lloyd, E. and Shepherd, T. G. (2020). Environmental catastrophes, climate change, and attribution. *Ann. N. Y. Acad. Sci.* **1469**, 105-124. doi:10.1111/nyas.14308
- Lopes, I. G., Araújo-Daikiri, T. B., Kojima, J. T., Val, A. L. and Portella, M. C. (2018). Predicted 2100 climate scenarios affects growth and skeletal development of tambaqui (*Colossoma macropomum*) larvae. *Ecol. Evol.* **2018**, 1-10. doi:10.1002/ece3.4429
- Lovejoy, T. and Nobre, C. (2018). Amazon tipping point. *Sci. Adv.* **4**, eaat2340. doi:10.1126/sciadv.aat2340
- Lundberg, J., Pérez, M., Dahdul, W. and Aguilera, O. (2010). The Amazonian neogene fish fauna. In *Amazonia: Landscape and Species Evolution. A Look into the Past* (ed. C. Hoorn and F. P. Wesselingh), pp. 281-310. Oxford: Wiley-Blackwell.
- Marcon, J. L., Chagas, E. C., Kavassaki, J. M. and Val, A. L. (1999). Intraerythrocytic phosphates in 25 fish species of the Amazon: GTP as a key factor in the regulation of Hb-O<sub>2</sub> affinity. In *Biology of Tropical Fish* (ed. A. L. Val and V. M. F. Almeida-Val), pp. 229-240. Manaus: INPA.
- Marengo, J., Souza, C., Thonicke, K., Burton, C., Halladay, K., Betts, R., Alves, L. and Soares, W. (2018). Changes in climate and land use over the Amazon region: current and future variability and trends. *Front. Earth Sci.* **6**, 228. doi:10.3389/feart.2018.00228
- Markewitz, D., Davidson, E. A., Figueiredo, R. D. O., Victoria, R. L. and Krushe, A. V. (2001). Control of cation concentration in stream waters by surface soil processes in an Amazonian watershed. *Nature* **410**, 802-805. doi:10.1038/35071052
- Matey, V., Iftikar, F. I., De Boeck, G., Scott, G. R., Sloman, K. A., Almeida-Val, V. M. F., Val, A. L. and Wood, C. M. (2011). Gill morphology and acute hypoxia: responses of mitochondria-rich, pavement, and mucous cells in the Amazonian oscar (*Astronotus ocellatus*) and the rainbow trout (*Oncorhynchus mykiss*), two species with very different approaches to the osmo-respiratory compromise. *Can. J. Zool.* **89**, 307-324. doi:10.1139/z11-002
- Matsuo, A. Y. O., Playle, R. C., Val, A. L. and Wood, C. M. (2004). Physiological action of dissolved organic matter in rainbow trout in the presence and absence of copper: sodium uptake kinetics and unidirectional flux rates in hard and softwater. *Aquat. Toxicol.* **70**, 63-81. doi:10.1016/j.aquatox.2004.07.005
- Matsuo, A. Y. O. and Val, A. L. (2007). Acclimation to humic substances prevents whole body sodium loss and stimulates branchial calcium uptake capacity in cardinal tetras *Paracheirodon axelrodi* (Schultz) subjected to extremely low pH. *J. Fish Biol.* **70**, 989-1000. doi:10.1111/j.1095-8649.2007.01358.x
- McDonald, D. G., Hobe, H. and Wood, C. M. (1980). The influence of calcium on the physiological responses of the rainbow trout, *Salmo gairdneri*, to low environmental pH. *J. Exp. Biol.* **88**, 109-131. doi:10.1242/jeb.88.1.109
- McGeer, J. C., Szebedinszky, C., McDonald, G. and Wood, C. M. (2002). The role of dissolved organic carbon in moderating the bioavailability and toxicity of Cu to rainbow trout during chronic waterborne exposure. *Comp. Biochem. Physiol. C* **133**, 147-160.
- McWilliams, P. G. (1982). The effects of calcium on sodium fluxes in the brown trout, *Salmo trutta*, in neutral and acid water. *J. Exp. Biol.* **96**, 439-442. doi:10.1242/jeb.96.1.439
- Menezes, J., Confalonieri, U., Madureira, A., Duval, I., Santos, R. and Margonari, C. (2018). Mapping human vulnerability to climate change in the Brazilian Amazon: the construction of a municipal vulnerability index. *PLoS One* **13**, e0190808.
- Milligan, C. L. and Wood, C. M. (1982). Disturbances in haematology, fluid volume distribution and circulatory function associated with low environmental pH in the rainbow trout, *Salmo gairdneri*. *J. Exp. Biol.* **99**, 397-415. doi:10.1242/jeb.99.1.397
- Morris, C., Val, A. L., Brauner, C. J. and Wood, C. M. (2021). The physiology of fish in acidic waters rich in dissolved organic carbon, with specific reference to the Amazon basin: Ionoregulation, acid-base regulation, ammonia excretion, and metal toxicity. *J. Exp. Zool. A* **335**, 843-863. doi:10.1002/jez.2468
- Moura, N. A. and Val, A. L. (2019). Migração lateral de peixes e a vulnerabilidade da baía do Chacororé, pantanal de Barão de Melgaço, Mato Grosso, Brasil. *J. Neotrop. Biol.* **16**, 1-8. doi:10.5216/rbn.v16i1.46394
- Muusse, B., Marcon, J. L., Van den Thillart, G. and Almeida-Val, V. M. F. (1998). Hypoxia tolerance of Amazon fish. Respiratory and energy metabolism of the cichlid *Astronotus ocellatus*. *Comp. Biochem. Physiol.* **120A**, 151-156. doi:10.1016/S1095-6433(98)10023-5
- Nelson, J. A. (2015). Pickled fish anyone? In *Extremophile fishes: Ecology, Evolution and Physiology of Teleosts in Extreme Environments* (ed. R. Riesch, M. Tobler and M. Plath), pp. 193-215. Heidelberg: Springer Cham.
- Niyogi, S. and Wood, C. M. (2004). The Biotic ligand model, a flexible tool for developing site-specific water quality guidelines for metals. *Environ. Sci. Technol.* **38**, 6177-6192. doi:10.1021/es0496524
- Nobre, C., Sampaio, G., Borma, L., Castilla-Rubio, J., Silva, J. and Cardoso, M. (2016). Land-use and climate change risks in the Amazon and the need of a novel sustainable development paradigm. *Proc. Natl Acad. Sci. USA* **113**, 10759-10768. doi:10.1073/pnas.1605516113
- Oliveira, A. M. and Val, A. L. (2017). Effects of climate scenarios on the growth and physiology of the Amazonian fish tambaqui (*Colossoma macropomum*) (Characiformes: Serrasalminidae). *Hydrobiologia* **789**, 167-178. doi:10.1007/s10750-016-2926-0
- Pabón-Caicedo, J., Arias, P., Carril, A., Espinoza, J., Borrel, L., Goubanova, K., Lavado-Casimiro, W., Masiokas, M., Solman, S. and Villalba, R. (2020). Observed and projected hydroclimate changes in the Andes. *Front. Earth Sci.* **8**, 61. doi:10.3389/feart.2020.00061
- Pelster, B. and Wood, C. M. (2018). Ionoregulatory and oxidative stress issues associated with the evolution of air-breathing. *Acta Histochemica* **120**, 667-679. doi:10.1016/j.acthis.2018.08.012
- Pelster, B., Giacomin, M., Wood, C. M. and Val, A. L. (2016). Improved ROS defense in the swimbladder of a facultative air-breathing erythrinid fish, jeju, compared to a non-air-breathing close relative, traira. *J. Comp. Physiol. B* **186**, 615-624. doi:10.1007/s00360-016-0981-5
- Pelster, B., Wood, C. M., Jung, E. and Val, A. L. (2018). Air-breathing behavior, oxygen concentrations, and ROS defense in the swimbladders of two erythrinid fish, the facultative airbreathing jeju, and the non-air-breathing traira during normoxia, hypoxia and hyperoxia. *J. Comp. Physiol. B* **188**, 437-449. doi:10.1007/s00360-017-1142-1
- Pelster, B., Wood, C. M., Braz-Mota, S. and Val, A. L. (2020a). Gills and air-breathing organ in O<sub>2</sub> uptake, CO<sub>2</sub> excretion, N-waste excretion, and ionoregulation in small and large pirarucu (*Arapaima gigas*). *J. Comp. Physiol. B* **190**, 569-583. doi:10.1007/s00360-020-01286-1
- Pelster, B., Wood, C. M., Campos, D. F. and Val, A. L. (2020b). Cellular oxygen consumption, ROS production and ROS defense in two different size-classes of an Amazonian obligate air-breathing fish (*Arapaima gigas*). *PLoS One* **15**, e0236507. doi:10.1371/journal.pone.0236507
- Perry, S. F., Gilmour, K. M., Duarte, R. M., Wood, C. M., Almeida-Val, V. M. F. and Val, A. L. (2021). The effects of dissolved organic carbon on the reflex ventilatory responses of the neotropical teleost (*Colossoma macropomum*) to hypoxia or hypercapnia. *Chemosphere* **277**, 130314. doi:10.1016/j.chemosphere.2021.130314
- Petrere, M., Jr, Barthem, R., Córdoba, E. and Gómez, B. (2004). Review of the large catfish fisheries in the upper Amazon and the stock depletion of piraíba (*Brachyplatystoma filamentosum* Lichtenstein). *Rev. Fish Biol. Fish.* **14**, 403-414. doi:10.1007/s11160-004-8362-7
- Pörtner, H. O., Scholes, R. J., Agard, J., Archer, E., Arneith, A., Bai, X., Barnes, D., Burrows, M., Chan, L., Cheung, W. L. et al. (2021). IPBES-IPCC co-sponsored workshop report on biodiversity and climate change. *IPBES and IPCC*, 28 p. doi:10.5281/zenodo.4782538
- Potts, W. T. W. (1984). Transepithelial potentials in fish gills. In *Fish Physiology*, Vol. 10B (ed. W. S. Hoar and D. J. Randall), pp. 105-128. San Diego: Academic Press.
- Preest, M. R., Gonzalez, R. J. and Wilson, R. W. (2005). A pharmacological examination of Na<sup>+</sup> and Cl<sup>-</sup> transport in two species of freshwater fish. *Physiol. Biochem. Zool.* **78**, 259-272. doi:10.1086/427058
- Randall, D. J., Baumgarten, D. and Malysz, M. (1972). The relationship between gas and ion transfer across the gills of fishes. *Comp. Biochem. Physiol. A* **41**, 629-637. doi:10.1016/0300-9629(72)90017-5
- Randall, D. J., Burggren, W. W., Farrell, A. P. and Haswell, M. S. (1981). *The Evolution of Air-Breathing Vertebrates*. Cambridge: Cambridge University Press.
- Rantin, F. T. and Kalinin, A. L. (1996). Cardiorespiratory function and aquatic surface respiration in *Colossoma macropomum* exposed to graded and acute hypoxia. In *Physiology and Biochemistry of the Fishes of the Amazon* (ed. A. L. Val, V. M. F. Almeida-Val and D. J. Randall), pp. 169-180. Manaus: INPA.
- Rasera, M. D. F. L., Krusche, A. V., Richey, J. E., Ballester, M. V. and Victoria, R. L. (2013). Spatial and temporal variability of pCO<sub>2</sub> and CO<sub>2</sub> efflux in seven Amazonian Rivers. *Biogeochemistry* **116**, 617-620.

- Richey, J. E., Melack, J. M., Aufdenkampe, A. K., Ballester, V. M. and Hess, L. L. (2002). Outgassing from Amazonian rivers and wetland as a large tropical source of atmospheric CO<sub>2</sub>. *Nature* **416**, 617–620. doi:10.1038/416617a
- Robertson, L. M., Val, A. L., Almeida-Val, V. M. F. and Wood, C. M. (2015). Ionoregulatory aspects of the osmoregulatory compromise during acute environmental hypoxia in 12 tropical and temperate teleosts. *Physiol. Biochem. Zool.* **88**, 357–370. doi:10.1086/681265
- Rocha, J. C., Sargentini, E., Jr, Toscano, I. A. S., Rosa, A. H. and Burba, P. (1999). Multi-method study on aquatic humic substances from the “Rio Negro” - Amazonas state/Brazil: emphasis on molecular-size classification of their metal contents. *J. Braz. Chem. Soc.* **10**, 169–175. doi:10.1590/S0103-50531999000300002
- Röpke, C. P., Amadio, S. A., Zuanon, J. A. S., Ferreira, E. J., Deus, C. P., Pires, T. H. and Winemiller, K. O. (2017). Simultaneous abrupt shifts in hydrology and fish assemblage structure in a floodplain lake in the Central Amazon. *Sci. Rep.* **7**, 40170. doi:10.1038/srep40170
- Sá-Leitão, C. S., Souza, E. M. S., Santos, C. H. A., Val, P., Val, A. L. and Almeida-Val, V. M. F. (2021). River reorganization affects populations of dwarf cichlid species (*Apistogramma* genus) in the lower Negro River, Brazil. *Front. Ecol. Evol.* **9**, 760287. doi:10.3389/fevo.2021.760287
- Saint-Paul, U. (1984). Physiological adaptation to hypoxia of a neotropical characid fish *Colossoma macropomum*, Serrasalminae. *Environ. Biol. Fishes* **11**, 53–62. doi:10.1007/BF00001845
- Sanchez, A. P., Giusti, H., Bassi, M. and Glass, M. L. (2005). Acid-base regulation in the South American lungfish *Lepidosiren paradoxa*: effects of prolonged hypercarbia on blood gases and pulmonary ventilation. *Physiol. Biochem. Zool.* **78**, 908–915. doi:10.1086/432859
- Scanes, E., Scanes, P. R. and Ross, P. M. (2020). Climate change rapidly warms and acidifies Australian estuaries. *Nat. Commun.* **11**, 1803. doi:10.1038/s41467-020-15550-z
- Schindler, D. W. (1988). Effects of acid rain on freshwater ecosystems. *Science* **239**, 149–157. doi:10.1126/science.239.4836.149
- Schöngart, J. and Junk, W. J. (2007). Forecasting the flood-pulse in Central Amazonia by ENSO-indices. *J. Hydrol.* **335**, 124–132. doi:10.1016/j.jhydrol.2006.11.005
- Schöngart, J. and Junk, W. J. (2020). Clima e hidrologia nas várzeas da Amazônia Central. In *Várzeas Amazônicas: Desafios para um Manejo Sustentável* (ed. W. J. Junk, M. T. F. Piedade, F. Wittmann and J. Schöngart), pp. 44–65. Manaus: INPA.
- Scott, G. R., Wood, C. M., Sloman, K. A., Iftikar, F. I., De Boeck, G., Almeida-Val, V. M. F. and Val, A. L. (2008). Respiratory responses to progressive hypoxia in the Amazonian oscar, *Astronotus ocellatus*. *Respir. Physiol. Neurobiol.* **162**, 109–116. doi:10.1016/j.resp.2008.05.001
- Scott, G. R., Matey, V., Mendoza, J., Gilmour, K. M., Perry, S. F., Almeida-Val, V. M. F. and Val, A. L. (2017). Air breathing and aquatic gas exchange during hypoxia in armored catfish. *J. Comp. Physiol. B* **187**, 117–133. doi:10.1007/s00360-016-1024-y
- Sentinella, A., Warton, D., Sherwin, W., Offord, C. and Moles, A. (2020). Tropical plants do not have narrower temperature tolerances, but are more at risk from warming because they are close to their upper thermal limits. *Glob. Chang. Biogeography* **29**, 1387–1398. doi:10.1111/gcb.13117
- Seyler, P. T. and Boaventura, G. R. (2003). Distribution and partition of trace metals in the Amazon basin. *Hydrol. Process.* **17**, 1345–1361. doi:10.1002/hyp.1288
- Shartau, R. B., Baker, D. W., Crossley, D. A. and Brauner, C. J. (2016). Preferential intracellular pH regulation: hypothesis and perspectives. *J. Exp. Biol.* **219**, 2235–2244. doi:10.1242/jeb.126631
- Silva, E. A. and Stewart, D. J. (2017). Reproduction, feeding and migration patterns of *Prochilodus nigricans* (Characiformes: Prochilodontidae) in northeastern Ecuador. *Neotrop. Ichthyol.* **15**, e160171.
- Silva, G. S. D., Bisinoti, M. C., Fadini, P. S., Magarelli, G., Jardim, W. F. and Fostier, A. H. (2009). Major aspects of the mercury cycle in the Negro River basin, Amazon. *J. Braz. Chem. Soc.* **20**, 1127–1134. doi:10.1590/S0103-50532009000600019
- Sioli, H. (1984). *The Amazon. Limnology and Landscape Ecology of a Might Tropical River and its Basin*. Dordrecht: Dr W. Junk Publishers.
- Sloman, K. A., Wood, C. M., Scott, G. R., Wood, S., Kajimura, M., Johannsson, O. E., Almeida-Val, V. M. F. and Val, A. L. (2006). Tribute to R. G. Boutilier: The effect of size on the physiological and behavioural responses of oscar, *Astronotus ocellatus*, to hypoxia. *J. Exp. Biol.* **209**, 1197–1205. doi:10.1242/jeb.02090
- Sloman, K. A., Sloman, R., De Boeck, G., Scott, G. R., Iftikar, F. I., Wood, C. M., Almeida-Val, V. M. F. and Val, A. L. (2009). The role of size in synchronous air breathing of *Hoplosternum littorale*. *Physiol. Biochem. Zool.* **82**, 625–634. doi:10.1086/605936
- Somero, G. N. (2010). The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine ‘winners’ and ‘losers’. *J. Exp. Biol.* **213**, 912–920. doi:10.1242/jeb.037473
- Stropp, J., Umbelino, B., Correia, R. A., Campos-Silva, J. V., Ladle, R. J. and Malhado, A. C. M. (2020). The ghosts of forests past and future: deforestation and botanical sampling in the Brazilian Amazon. *Ecography* **43**, 979–989. doi:10.1111/ecog.05026
- Tencatt, L., Muriel-Cunha, J., Zuanon, J., Ferreira, M. and Britto, M. (2020). A journey through the Amazon Middle Earth reveals *Aspidoras azaghal* (Siluriformes: Callichthyidae), a new species of armored catfish from the Rio Xingu basin, Brazil. *J. Fish Biol.* **97**, 1–15. doi:10.1111/jfb.14467
- Thurman, E. (1985). *Geochemistry of Natural Waters*. Dordrecht, Boston: Martinus Nijhoff/Dr W Junk Publishers.
- Tippling, E. (1998). Humic ion-binding model VI: an improved description of the interactions of protons and metal ions with humic substances. *Aquat. Geochem.* **4**, 3–48. doi:10.1023/A:1009627214459
- Val, A. L. (2000). Organic phosphates in the red blood cells of fish. *Comp. Biochem. Physiol.* **125A**, 417–435. doi:10.1016/S1095-6433(00)00184-7
- Val, A. L. and Almeida-Val, V. M. F. (1995). Fishes of the Amazon and their environments. Physiological and Biochemical Features. Zoophysiology Series (ed. S. D. Bradshaw, W. Burgren, H. C. Heller, S. Ishii, H. Langer, G. Neuweiller and D. J. Randall), Vol. 32, 224p. Heidelberg: Springer Verlag. doi:10.1007/978-3-642-79229-8
- Val, A. L. and Oliveira, A. M. (2021). *Colossoma macropomum* – a tropical fish model for biology and aquaculture. *J. Exp. Zool. A* **2021**, 1–10.
- Val, A. L., Almeida-Val, V. M. F. and Affonso, E. G. (1990). Adaptive features of Amazon fishes: hemoglobins, hematology, intraerythrocytic phosphates and whole blood Bohr effect of *Pterygoplichthys multiradiatus* (Siluriformes). *Comp. Biochem. Physiol.* **97**, 435–440.
- Val, A. L., Affonso, E. G., Souza, R. H. S., Almeida-Val, V. M. F. and Moura, M. A. F. (1992). Inositol pentaphosphate in erythrocytes of an Amazonian fish, the pirarucu (*Arapaima gigas*). *Can. J. Zool.* **70**, 852–855. doi:10.1139/z92-121
- Val, A. L., Silva, M. N. P. and Almeida-Val, V. M. F. (1998). Hypoxia adaptation in fish of the Amazon: a never-ending task. *South African J. Zool.* **33**, 107–114. doi:10.1080/02541858.1998.11448459
- Val, A., Almeida-Val, V. and Randall, D. (2006). Tropical environment. In *The Physiology of Tropical Fishes*, Vol. 21 (ed. A. L. Val, V. M. F. Almeida-Val and D. J. Randall), pp. 1–45. San Diego: Elsevier/Academic Press.
- Val, A. L., Gomes, K. R. M. and Almeida-Val, V. M. F. (2015). Rapid regulation of blood parameters under acute hypoxia in the Amazonian fish *Prochilodus nigricans*. *Comp. Biochem. Physiol.* **184A**, 125–131. doi:10.1016/j.cbpa.2015.02.020
- Val, A. L., Paula-Silva, M. N., Almeida-Val, V. M. F. and Wood, C. M. (2016). *In vitro* effects of increased temperature and decreased pH on blood oxygen affinity of 10 fish species of the Amazon. *J. Fish Biol.* **89**, 264–279. doi:10.1111/jfb.13009
- Weber, R., Fago, A., Val, A. L., Bang, A., Van Hauwaert, M. L., De Wilde, S., Zal, F. and Moens, L. (2000). Isohemoglobin differentiation in the bi-modal-breathing Amazon catfish *Hoplosternum littorale*. *J. Biol. Chem.* **275**, 17297–17305. doi:10.1074/jbc.M001209200
- Wilson, R. W., Wood, C. M., Gonzalez, R. J., Patrick, M. L., Bergman, H. L., Narahara, A. and Val, A. L. (1999). Ion and acid-base balance in three species of Amazonian fish during gradual acidification of extremely soft water. *Physiol. Biochem. Zool.* **72**, 277–285. doi:10.1086/316672
- Winemiller, K. O., López-Fernández, H., Taphorn, D. C., Nico, L. G. and Duque, A. B. (2008). Fish assemblages of the Casiquiare River, a corridor and Amazon basins. *J. Biogeogr.* **35**, 1551–1563. doi:10.1111/j.1365-2699.2008.01917.x
- Wood, C. M. (1989). The physiological problems of fish in acid water. In *Acid Toxicity and Aquatic Animals* (ed. E. W. Taylor, D. J. A. Brown and J. A. Brown), pp. 125–152. Cambridge: Cambridge University Press.
- Wood, C. M. and Eom, J. (2021). The osmoregulatory compromise in the fish gill. *Comp. Biochem. Physiol. A* **254**, 110895. doi:10.1016/j.cbpa.2021.110895
- Wood, C. M., Al-Reasi, H. A. and Smith, S. (2011). The two faces of DOC. *Aquat. Toxicol.* **105S**, 3–8. doi:10.1016/j.aquatox.2011.03.007
- Wood, C. M., Matsuo, A. Y. O., Gonzalez, R. J., Wilson, R. W., Patrick, M. L. and Val, A. L. (2002). Mechanisms of ion transport in *Potamotrygon*, a stenohaline freshwater elasmobranch native to the ion-poor blackwaters of the Rio Negro. *J. Exp. Biol.* **205**, 3039–3054. doi:10.1242/jeb.205.19.3039
- Wood, C. M., Matsuo, A. Y., Wilson, R. W., Gonzalez, R. J., Patrick, M. L., Playle, R. C. and Val, A. L. (2003). Protection by natural blackwater against disturbances in ion fluxes caused by low pH exposure in freshwater stingrays endemic to the Rio Negro. *Physiol. Biochem. Zool.* **76**, 12–27. doi:10.1086/367946
- Wood, C. M., Kajimura, K., Sloman, K. A., Scott, G. R., Almeida-Val, V. M. F. and Val, A. L. (2007). Rapid regulation of Na<sup>+</sup> and ammonia fluxes in response to acute environmental hypoxia in the Amazonian oscar, *Astronotus ocellatus*. *Am. J. Physiol.* **292**, R2048–R2058.
- Wood, C. M., Iftikar, F., Scott, G. R., de Boeck, G., Sloman, K., Matey, V., Valdez Domingos, F. X., Duarte, R. M., Almeida-Val, V. M. F. and Val, A. L. (2009). Regulation of gill transcellular permeability and renal function during acute hypoxia in the Amazonian oscar (*Astronotus ocellatus*): new angles to the osmoregulatory compromise. *J. Exp. Biol.* **212**, 1949–1964. doi:10.1242/jeb.028464
- Wood, C. M., Wilson, R. W., Gonzalez, R. J., Patrick, M. L., Bergman, H. L., Narahara, A. and Val, A. L. (1998). Responses of an Amazonian teleost, the tambaqui (*Colossoma macropomum*) to low pH in extremely soft water. *Physiol. Zool.* **71**, 658–670. doi:10.1086/515977
- Wood, C. M., Robertson, L. M., Johannsson, O. E. and Val, A. L. (2014). Mechanisms of Na<sup>+</sup> uptake, ammonia excretion, and their potential linkage in native Rio Negro tetras (*Paracheirodon axelrodi*, *Hemigrammus rhodostomus*, and *Moenkhausia diktyota*). *J. Comp. Physiol. B* **184**, 877–890. doi:10.1007/s00360-014-0847-7

- Wood, C. M., Pelster, B., Giacomini, M., Sadauskas-Henrique, H., Almeida-Val, V. M. F. and Val, A. L.** (2016). The transition from water-breathing to air-breathing is associated with a shift in ion uptake from gills to gut: a study of two closely related erythrinid teleosts, *Hoplerythrinus unitaeniatus* and *Hoplias malabaricus*. *J. Comp. Physiol. B* **186**, 431-445. doi:10.1007/s00360-016-0965-5
- Wood, C. M., Gonzalez, R. J., Ferreira, M. S., Braz-Mota, S. and Val, A. L.** (2018). The physiology of the tambaqui (*Colossoma macropomum*) at pH 8.0. *J. Comp. Physiol. B* **188**, 393-408. doi:10.1007/s00360-017-1137-y
- Wood, C. M., Pelster, B., Braz-Mota, S. and Val, A.** (2020). Gills versus kidney for ionoregulation in the obligate air-breathing *Arapaima gigas*, a fish with a kidney in its air-breathing organ. *J. Exp. Biol.* **223**, jeb232694. doi:10.1242/jeb.232694
- Xenopoulos, M., Lodge, D., Alcamo, J., Marker, M., Schulze, K. and Van Vuren, D.** (2005). Scenarios of freshwater fish extinctions from climate change and water withdrawal. *Glob. Change Biol.* **11**, 1557-1564. doi:10.1111/j.1365-2486.2005.001008.x
- Zimmer, A. M., Goss, G. G. and Glover, C. N.** (2021). Chemical niches and ionoregulatory traits: applying ionoregulatory physiology to the conservation management of freshwater fishes. *Conserv. Physiol.* **9**, 1-17. doi:10.1093/conphys/coab066