

Interspecific Differences in the Metabolic Rate, Gill Dimension and Hematology of Fish in an Amazonian Floodplain Lake

Wallice P. Duncan

Laboratório de Morfologia Funcional, Universidade Federal do Amazonas. Avenida Rodrigo Otavio, 6200. Coroado I, 69.080-900, Manaus AM, Brasil. E-mail: wduncan@ufam.edu.br

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Abstract

It has been hypothesized that respiratory physiology in fish is closely associated with ecological traits. Therefore, data on gill morphometrics (lamellae frequency, gill surface area and barrier thickness), metabolic rate (oxygen consumption) and blood oxygen capacity (hematology) were analyzed in several fish, including benthic, benthopelagic and pelagic species collected in an Amazon floodplain lake. Similar to other teleostean species, the 2nd and 3rd gill arches have numerous large filaments in both pelagic and benthic species, as these characteristics tend to increase the gill surface area. A large gill area (4 to 7 cm² g⁻¹, mass-specific) is associated with a high (100 to 300 mg O₂ h⁻¹ kg⁻¹) routine oxygen consumption rate and has been observed in active pelagic swimmers, such as *Cichla monoculus* and *Pygocentrus nattereri*. Benthic dwelling fish (e.g., *Pterygoplichthys pardalis* and *Sorubim lima*) have low metabolic rates (20 to 50 mg O₂ h⁻¹ kg⁻¹), small gill dimensions (2 to 3 cm² g⁻¹, mass-specific), low hemoglobin levels (3 to 5 g dL⁻¹), reduced numbers of circulating red blood cells (1 to 2 × 10⁶ mm⁻³) and low hematocrit values (25 to 35%) compared to pelagic species. These results demonstrated that pelagic fish have high routine oxygen consumption rates compatible with their large gill surface area and high blood oxygen capacity, whereas benthic species have low metabolic rates, small gill dimensions and reduced blood oxygen capacity.

Keywords: benthic fish, pelagic fish, gill area, oxygen uptake, blood parameters

1. Introduction

It has been suggested that pelagic fish are generally more active than benthic species (Roberts 1975; Johnston et al. 1988). The high energy demand in pelagic fish is attributable to active swimming, which is in contrast with the sluggish behavior of benthic species (Childress 1995; Killen et al. 2010). Thus, it is expected that active fish have (i) a high gill surface area to

enable more effective oxygen uptake in supplying their high-energy demand (Wegner et al. 2010); (ii) high oxygen consumption rates (as indirect indicator of metabolic rates) to yield aerobic ATP (Val & Almeida-Val 1995); (iii) a high blood oxygen capacity to carry oxygen through the blood (Nikinmaa & Soivio 2005); and (iv) adjusted functional integration between gill area, oxygen consumption rates and hematological profile (Weibel 2000). In other words, the routine oxygen rate is a function of the gill surface area, assuming that all extracted oxygen from the gill epithelium is carried through the blood.

The species-specific anatomy and dimensions of the gills often reflect the performance of the fish under various environmental conditions (Ong et al. 2007; Matey et al. 2011). The measurement of respiratory area is based on the number and length of the filaments, surface area of lamellae and spacing of the lamellae along the filaments (Hughes & Morgan 1973). These authors have extensively described the relationship between the gill dimensions and lifestyle of several fish species. In addition, it has been reported that variations are correlated with high tolerances to hypoxia or notably high energy demand (De Jager & Dekker 1975). It has been argued that gill dimensions appear to be closely matched to the requirement for oxygen (Nilsson & Östlund-Nilsson 2008; Wegner et al. 2010). Thus, it is expected that gill surface area has a linear relationship with oxygen uptake (Rogers et al. 2016). In addition, the diffusion distance between blood and water is an important variable linking branchial morphology to the capacity for oxygen consumption (Hughes 1984, Hughes et al. 1986).

If the gill structures are matched to oxygen uptake in water, it is expected that blood oxygen carrying capacity is also adjusted to metabolic rate. The oxygen transport in the blood may depend directly on (i) the number of circulating red blood cells and hemoglobin concentration and (ii) the functional properties of hemoglobin and its allosteric modulators (Nikinmaa & Soivio 2005; Brunori 2014). It has been reported that active swimming fish have more circulating red cell blood and high hemoglobin levels, while sluggish fish and/or those living in low oxygen environments tend to have the functional properties of hemoglobin modulated by a high affinity for oxygen (Powers 1980; Wells 2009; Brunori 2014). Considering all the arguments outlined above, there is support for the concept of symmorphosis, which is the capacity of structural elements to match but not exceed the functional requirements of an animal (Weibel 2000). This hypothesis was tested initially in the mammalian respiratory system (Weibel et al. 1991) and is expected to have similar effects on fish respiration (Rombough & Moroz 1997), considering that gill area and oxygen consumption rate increase according to fish growth (Pauly 1981, Hughes et al. 1986). At present, it is not clear whether gill area, oxygen consumption rate, and blood oxygen capacities are closely matched in the Amazon teleostean species, since these fish employ different mechanisms to tolerate hypoxia.

Several biochemical, physiological, behavioral and morphological adaptations to survival in low oxygen concentrations have been reported in many Amazon fish species (Val & Almeida-Val 1995; Campos et al. 2016). A survey of tolerance to hypoxia in Amazon fish indicated large degrees of variation among and within some species. Some cichlids possess a specialized metabolism to survive hypoxia or even anoxia (Muusze et al. 1998, Chippari-Gomes et al. 2005, De Boeck et al. 2013). Several species increase opercular movements to increase oxygen uptake when concentrations decrease to 1 mg L⁻¹ (Saint-Paul

& Soares 1987). Characins, such as *Colossoma macropomum* Cuvier (1816), have hemoglobin with a great affinity for oxygen (Saint-Paul 1984), while several species display aquatic surface respiration when the dissolved oxygen reaches 0.5 mg L^{-1} (Saint-Paul & Soares 1987). Considering this background information, the present study examines the relationship between routine oxygen consumption rates, gill area and blood oxygen-carrying capacity in fish from a floodplain lake in the Central Amazon. In addition, we tested the hypothesis of whether pelagic active swimming fish have higher metabolic rates than benthic sluggish fish.

2. Materials and Methods

2.1 Study Area and Fish

Lake Janauacá is located in the municipality of Careiro Castanho ($03^{\circ}23'S$; $63^{\circ}12'W$) approximately 40 km from Manaus city (Fig. 1). This lake is typically muddy-type water covering approximately 18 km^2 of flooded area. The lake is connected with the Solimões/Amazon River through a small canal 12 km long. In this study, fish collections were performed at this small canal. The limnological variables of the lake are strongly influenced by the main canal of the Solimões River. This river is also classified as white-type water (muddy) with high total values of dissolved particles ($\sim 90 \text{ mg L}^{-1}$) and electrical conductivity ($60\text{-}75 \text{ }\mu\text{S cm}^{-1}$) and circumneutral pH (6-7). All variables were measured with a handheld multiparameter water quality meter (Consort C535 multiparameter, Consort bvba, Turnhout, Belgium).

During the field study in October 2013, the water level in the river decreased. Fish were caught with four different sizes of gillnets (24-60 mm mesh). The nets were maintained at different water depths to sample both benthic and pelagic (and benthopelagic) fish during each hour of collection. The healthy fish without signs of injury or diseases were immediately placed in tanks (1,000 liters) supplied with water from a location near to where the fish were collected. The physical and chemical characteristics of the water used in the experiments were as follows: pH (6.6 ± 0.4), conductivity ($91.6\pm 4.1 \text{ }\mu\text{S cm}^{-1}$), total dissolved solids ($48.8\pm 3.7 \text{ mg L}^{-1}$), dissolved oxygen ($4.5\pm 0.7 \text{ mg O}_2 \text{ L}^{-1}$), temperature ($27.5\pm 0.8 \text{ }^{\circ}\text{C}$), Na^+ ($135\pm 11.8 \text{ }\mu\text{M}$), K^+ ($16.6\pm 2.1 \text{ }\mu\text{M}$), and Ca^{2+} ($176.2\pm 21.9 \text{ }\mu\text{M}$). Conductivity, dissolved solids, dissolved oxygen and temperature were measured using a Consort C535 multiparameter analyzer (CONSORT bvba, Turnhout, Belgium), while Na^+ , K^+ and Ca^{2+} levels were analyzed by flame photometry (Digimed DM-62, Campo Grande, Brazil).

To examine the relationship between routine oxygen consumption rates, blood oxygen capacity and gill dimensions, the following fish were studied: *Pinirampus pinirampu* Spix & Agassiz, 1829 ($n = 8$), *Pterygoplichthys pardalis* Castelnau, 1855 ($N = 8$), *Hemisorubim platyrhynchos* Valenciennes, 1840 ($n = 8$), *Sorubim lima* Bloch & Schneider, 1801 ($n = 10$), *Ageneiosus ucayalensis* Castelnau, 1855 ($n = 9$), *Pygocentrus nattereri* Kner, 1858 ($N = 7$), *Mylossoma duriventre* Cuvier, 1818 ($N = 8$), *Psectrogaster amazonica* Eigenmann & Eigenmann, 1889 ($n = 8$), *Astronotus ocellatus* Agassiz, 1831 ($n = 9$) and *Cichla monoculus* Agassiz, 1831 ($n = 7$). One individual of each species was included as a voucher specimen in the fish collection at the Laboratory of Functional Morphology, Federal University of

Amazonas (Manaus, Brazil). All procedures followed the Brazilian animal welfare standards (Ethical Committee of Animal Experimentation, CEUA/UFAM protocol N° 014/2014).

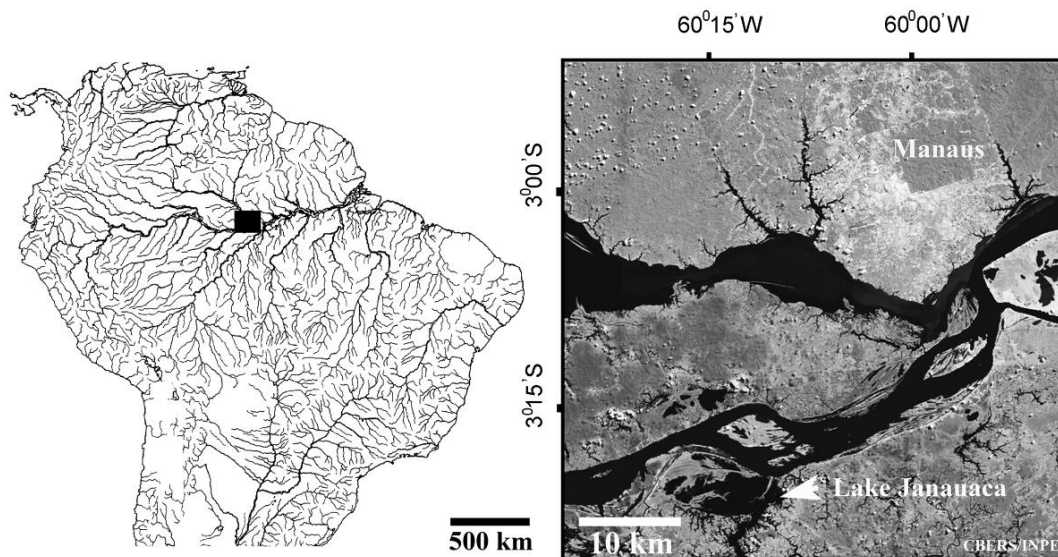


Figure 1. Map of the study site indicating Lake Janauacá (a floodplain lake in the Central Amazon) near Manaus city (~40 km)

2.2 Routine Oxygen Consumption Rate and Opercular Movement Measurements

Fish were kept in their native water 24 hours prior to initiating the experiments. The routine oxygen consumption rate (as indirectly measured by the routine metabolic rate) and opercular beat frequency were measured. To estimate the metabolic rate, a closed respirometric system was built. A Clark electrode was coupled to a rectangular chamber (10 liters), and oxygen content was monitored with an oxygen probe sensor (SZ10T, Consort C535 multiparameter, Consort bvba, Turnhout, Belgium). Individual fish were placed into the chamber and allowed to acclimate for 30 min. The water oxygen content was adjusted to approximately 5 mg L⁻¹ by air pumps. The air supply was later turned off, and the system was closed to monitor the routine oxygen consumption rate through fish respiration for 20 min at an environmental temperature (~27 °C). To avoid sources of variation in the oxygen consumption measurements due to progressive hypoxia and hypercapnia, the O₂ concentration in the chamber was not below ~2 mg L⁻¹. The oxygen uptake rate was calculated as $MO_2 = (\Delta \text{mg O}_2 \text{ h}^{-1}) \times V_{\text{resp}} M$, where $\Delta \text{mg O}_2 \text{ h}^{-1}$ is the change in ambient oxygen content over time, V_{resp} is the respirometer volume and M is the fish mass. The opercular movement was determined by direct count of operculum beating for 1 min over 10-min intervals. The measurements were performed 3 times and expressed as the number of opercular beats min⁻¹.

2.3 Blood Sampling and Hematological Analyses

After the respirometric study, the fish were kept in their native water for 2 hours to recover. Fish were euthanized with an overdose of benzocaine (0.8 g L⁻¹), and blood was sampled from the caudal vessel using a heparinized syringe to avoid hemocoagulation. Samples were kept on ice until analysis in hematological studies. The following variables of blood structure

were obtained: hematocrit, concentration of hemoglobin and circulating red blood cell count. Hematocrit was measured by collecting blood in microheterocrit tubes and then centrifuging for 10 min at 10,000 rpm, immediately followed by hematocrit measurements. The hemoglobin concentration was measured by the cyanmethemoglobin method: 10 μL of blood was placed into 2 mL of Drabkin's solution and gently mixed by inversion, and the absorbance was measured at 546 nm using a microplate Multiskan™ Go Spectrophotometer (Thermo Scientific, Waltham, MA, USA). The hemoglobin concentration is reported as g dL^{-1} . The number of circulating red blood cells (erythrocytes $\times 10^6 \mu\text{L}^{-1}$) was determined by transferring 10 μL of blood (well-mixed) into 2 mL of formalin-citrate buffer solution. The red blood cells were counted using a Neubauer chamber under a light microscope at 400x. The following erythrocyte indices were calculated: mean corpuscular hemoglobin (hemoglobin $\times 10/\text{red blood cell count}$), mean corpuscular hemoglobin concentration (hemoglobin $\times 100/\text{hematocrit}$) and mean corpuscular volume (hematocrit $\times 10/\text{red blood cell count}$).

2.4 Light and Scanning Electron Microscopy

Gill samples were dehydrated through a graded ethanol series and embedded in methacrylate (Historesin, Leica, Heidelberg, Germany). The tissue was sectioned at 3 μm sagittally using a SLEE Medical GmbH microtome (model CUT 5062, Mainz, Germany) and stained with toluidine blue (0.13%). Digital images were acquired using a Carl Zeiss camera (AxioCam ERc5s Göttingen, Germany) mounted on a Carl Zeiss light microscope (Primo Star) and analyzed using NIH ImageJ 1.45s computer software (Wayne Rasband, National Institute of Mental Health, Bethesda, USA) to determine the lamellar frequency and water-blood barrier (magnified at 1,000x). The thickness of the water-blood barrier was estimated using a test line of a semicircular grid (Merz's grid), as described by Hughes *et al.* (1986). For each animal, the barrier thickness was calculated as 2/3 of the harmonic mean length. The anatomical diffusion factor of the water-blood barrier was estimated as the quotient of the mass-specific respiratory surface area (S_{gill}/M_b) and 2/3 of the harmonic mean of diffusion barrier thickness. To estimate the bilateral lamellar area, gill samples were processed for scanning electron microscopy procedures. Intact secondary lamellae of each animal were dehydrated using a graded ethanol series followed by immersion in 1,1,1,3,3,3-hexamethyl-disilazane (Sigma Co., Steinheim, Germany), and air-dried. The filaments were coated with gold (99%) and examined under an FEI Quanta 250 scanning electron microscope at 25 kV. The digital images acquired (magnified at 500x) of the lamellae were analyzed to estimate the lamellar area.

2.5 Gill Morphometrics

The four gill arches were immediately removed and fixed by immersion in 2.5% phosphate-buffered glutaraldehyde solution, pH 7.4. The gill surface area of each individual arch was estimated using the method established by Hughes (1984) and calculated by the

equation $S_{\text{gill}} = \text{LTF} \cdot F_{\text{lam}} \cdot S_{\text{lam}}$, where S_{gill} is the total surface area, LTF is the total length

of the gill filaments, F_{lam} is lamellar frequency (for both sides of each filament) and S_{lam} is the mean bilateral surface area of the average secondary lamella. All filaments on the four gill arches of each hemibranch were counted. The length of every 10th filament was estimated, including the first and last filaments. Total filament length was calculated as the product of the mean filament length and total number of counted filaments, multiplied by two to account for both sides of the animal.

2.6 Statistical Analysis

All data are expressed as the means \pm standard errors. Principal component analysis (PCA) was applied to the correlation matrix (Pearson) formed by the log (x+1)-transformed data of gill morphometrics, routine oxygen consumption rate and opercular movement. Similar procedures were performed with hematological data. The principal component axes retained for interpretation were those with eigenvalues larger than 1.0. Linear regression was used to verify the relationship between gill morphometrics, routine oxygen consumption rate, opercular movement and hematological data. The uniformity of the data was tested using the Kolmogorov-Smirnov test. Comparisons of physiological variables among fish species were made with the Kruskal-Wallis nonparametric ANOVA test. The comparisons with routine metabolic rate and gill surface area were made with mass-specific adjusted values. Alternatively, predicted values of routine metabolic rate (RMR) measurements of all ten species were generated by the equation $RMR = 1.55M^{0.64}$ for a neotropical characin, *Colossoma macropomum*, at 30°C (Saint-Paul 1983). The PCA ordination diagrams and statistics were performed using PAST software (<http://folk.uio.no/ohammer/past>), while the linear regressions and the graphics were performed by using SigmaPlot™ version 11 (Systat Software, Inc., San Jose, California, USA) at the 5% level of significance.

3. Results

3.1 Routine Oxygen Consumption (RMR) and Opercular Breathing Frequency

With the exception of *Astronotus ocellatus*, pelagic fish such as *Pygocentrus nattereri*, *Mylossoma duriventre*, *Psectrogaster amazonica* and *Cichla monoculus* have high values of mass-specific metabolic rates compared to benthic/benthopelagic species, such as *Pterygoplichthys pardalis*, *Hemisorubim platyrhynchos*, *Sorubim lima* and *Ageneiosus ucayalensis* (Fig. 2A). As a pelagic predator, *C. monoculus* has a resting metabolic rate 5-fold greater than that of *Pinirampus pirinampu*, the most active benthic fish already studied over the years. However, using values predicted by equation $RMR = 1.55 M^{0.64}$ (Saint-Paul 1983), this difference was only 1.5-fold. In addition, the predicted RMR values show notably similar results among species (Table 1).

The oxygen consumption measurement was not associated with opercular movement. For example, *S. lima* had an opercular frequency of 166 beats/min and an RMR of $<20 \text{ mg O}_2 \text{ h}^{-1} \text{ kg}^{-1}$, while *A. ucayalensis* had an opercular frequency of 130 beats/min and an RMR $> 40 \text{ mg O}_2 \text{ h}^{-1} \text{ kg}^{-1}$ (Fig. 2B).

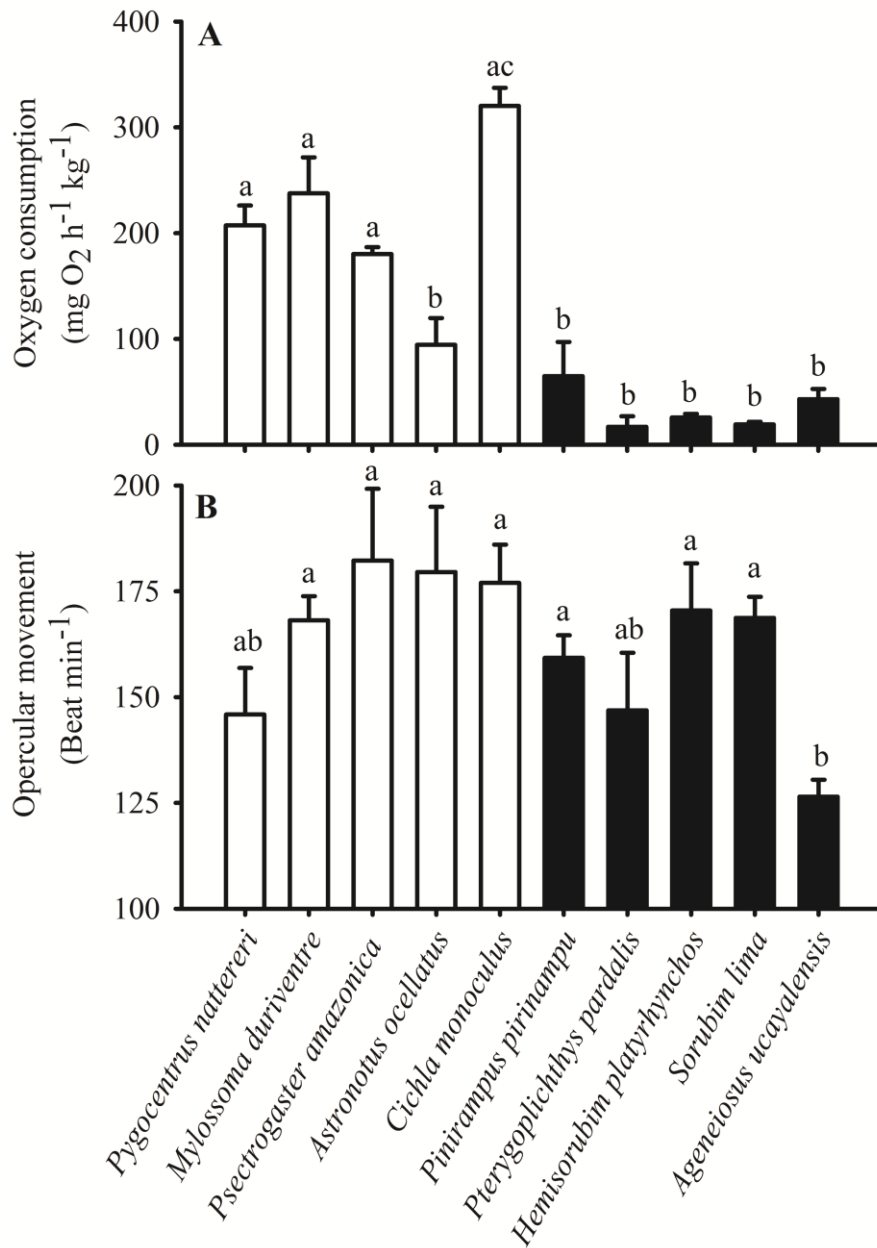


Figure 2. Interspecific variations in oxygen consumption rate (A) and opercular movement (B) in several fish species analyzed from Lake Janauacá. The routine metabolic rate (as oxygen consumption, mg O₂ h⁻¹ kg⁻¹) was measured in a closed respirometer at ~27°C. The open bars represent the pelagic species, and the filled bars represent benthic/bentho-pelagic species. Different lowercase letters indicate significant differences among the species ($p < 0.05$) by Kruskal-Wallis nonparametric ANOVA multiple comparisons test

Table 1. Routine oxygen consumption rate (RMR per fish) in different fish species from a floodplain lake (Lake Janauacá). (‡) Predicted RMR per fish was calculated according to the equation $RMR = 1.55 M^{0.64}$. The relationship between RMR and body mass has previously been reported by Saint-Paul (1983) for neotropical characin, *Colossoma macropomum*, at 30°C

Species	Mass (g)	RMR (mg O ₂ h ⁻¹ per fish)	Predicted RMR (mg O ₂ h ⁻¹)‡
<i>Pygocentrus nattereri</i>	167.5 ± 10.4	34.7 ± 9.2	41.1
<i>Mylossoma duriventre</i>	149.5 ± 9.5	34.9 ± 11.3	38.2
<i>Psectrogaster amazonica</i>	196.2 ± 11.7	36.0 ± 12.1	45.5
<i>Astronotus ocellatus</i>	67.3 ± 4.1	6.3 ± 2.4	22.9
<i>Cichla monoculus</i>	571.4 ± 48.6	182.8 ± 35.6	90.1
<i>Pirirampus pirinampu</i>	312.9 ± 13.8	18.3 ± 3.8	61.3
<i>Pterygoplichthys pardalis</i>	151.7 ± 12.2	2.0 ± 1.2	38.6
<i>Hemisorubim platyrhynchos</i>	198.8 ± 10.0	5.0 ± 1.3	45.8
<i>Sorubim lima</i>	237.0 ± 17.7	4.4 ± 1.3	51.3
<i>Ageneiosus ucayalensis</i>	241.3 ± 19.4	10.8 ± 2.9	51.9

3.2 Gill Dimensions

The average values for gill measurements are shown in Table 1. With the exception of *M. duriventre* and *A. ocellatus*, pelagic species (e.g., *P. nattereri*, *P. amazonica* and *C. monoculus*) have a lamellae density (40-66 lamellae mm⁻¹) greater than benthic fish (18-24 lamellae mm⁻¹). On the other hand, benthic and pelagic fish have similar lamellar areas. Therefore, a larger lamellar area is not enough to increase both the total respiratory gill surface (Table 2) and mass-specific gill area (Fig. 3A). For example, *H. platyrhynchos* has a lamellar area 1.6

times greater than that of *P. amazonica*. However, this characin presents more lamellae per millimeter of filament than does catfish. Thus, the gill surface area estimated in catfish is twice as large, although the fish have the same size (Table 2). The water-blood barrier to gas exchange is highly variable and is not associated with either pelagic or benthic environments. Some species, such as *C. monoculus* and *P. pirinampu*, have a diffusion barrier significantly thicker than the other species. On the other hand, the anatomical diffusion factor (a ratio between mass-specific gill area and diffusion barrier) was higher (Fig. 3B) in pelagic fish ($0.9\text{-}3.3\text{ cm}^2\text{ g}^{-1}\text{ }\mu\text{m}^{-1}$) than in benthic fish ($0.6\text{-}1.1\text{ cm}^2\text{ g}^{-1}\text{ }\mu\text{m}^{-1}$). Analysis of the secondary lamellae from each arch, regardless of lifestyle, indicates that the 2nd and 3rd gill arches possess larger surface areas than the other arches. In general, the gill arches of benthic fish have smaller surface areas than pelagic fish (Table 3).

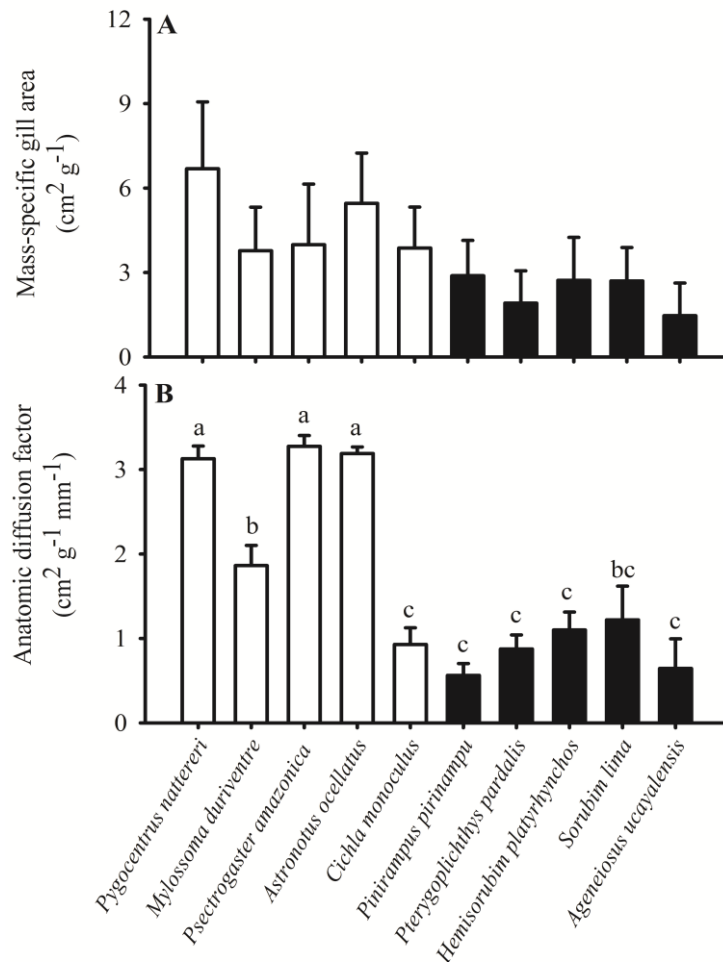


Figure 3. Mass-specific gill area (A) and anatomical diffusion factor (ADF) (B) among fish species collected from Lake Janauacá. The open bars represent the pelagic species, and the filled bars represent benthic/bentho-pelagic species. Different lowercase letters indicate significant differences among the species ($p < 0.05$) by Kruskal-Wallis nonparametric ANOVA multiple comparisons test

Table 2. Gill dimensions in different fish species from a floodplain lake (Lake Janauacá). Different lowercase letters indicate significant differences among the species ($p < 0.05$) by Kruskal-Wallis nonparametric ANOVA multiple comparisons test. Lamellae area and total gill area were not tested due to size-dependent effects

Species		Lamellae frequency (number mm^{-1})	Lamellae area ($\times 10^{-3}$ mm^2)	Barrier thickness $\frac{2}{3}$ harmonic mean (μm)	Total gill area [†] (cm^2)*
	<i>Pygocentrus nattereri</i>	66.7 ± 3.1^a	91.6 ± 2.3	2.1 ± 0.3^a	1094.4 ± 42.8
Pelagic species	<i>Mylossoma duriventre</i>	31.4 ± 1.8^b	107.1 ± 8.4	1.4 ± 0.2^b	360.7 ± 22.6
	<i>Psectrogaster amazonica</i>	40.6 ± 2.2^c	77.6 ± 6.5	1.3 ± 0.2^b	876.4 ± 54.1
	<i>Astronotus ocellatus</i>	24.0 ± 1.3^b	100.1 ± 7.2	1.2 ± 0.2^b	266.3 ± 21.7
	<i>Cichla monoculus</i>	44.6 ± 4.4^c	116.8 ± 9.6	2.7 ± 0.3^c	1326.6 ± 89.2
Benthic/	<i>Pinirampus pirinampu</i>	24.9 ± 2.5^b	121.3 ± 10.8	2.5 ± 0.3^c	456.8 ± 25.0
Benthopelagic species	<i>Pterygoplichthys pardalis</i>	23.3 ± 2.1^b	128.0 ± 10.3	1.7 ± 0.2^{ab}	214.2 ± 17.6
	<i>Hemisorubim platyrhynchos</i>	24.3 ± 1.9^b	127.6 ± 11.0	1.8 ± 0.2^{ab}	405.0 ± 35.9
	<i>Sorubim lima</i>	24.5 ± 2.0^b	145.1 ± 12.5	1.6 ± 0.3^b	571.7 ± 35.1
	<i>Ageneiosus ucayalensis</i>	18.4 ± 1.9^b	138.9 ± 9.7	2.2 ± 0.2^a	322.3 ± 20.8

*Both sides of the gill apparatus

Table 3. Variations in respiratory area ($\text{cm}^2 \text{g}^{-1}$) estimated in each gill arch for fish species collected from Lake Janauacá. Different lowercase letters indicate significant differences among the species ($p < 0.05$) by Kruskal-Wallis nonparametric ANOVA multiple comparisons test

Species		Surface areas, $\text{cm}^2 \text{g}^{-1}$ (gill arches)			
		I	II	III	IV
Pelagic fish	<i>Pygocentrus nattereri</i>	1.74 ± 0.05 ^a	1.93 ± 0.06 ^a	1.68 ± 0.04 ^a	1.14 ± 0.07 ^a
	<i>Mylossoma duriventre</i>	0.64 ± 0.08 ^b	0.71 ± 0.10 ^b	0.69 ± 0.07 ^b	0.57 ± 0.05 ^b
	<i>Psectrogaster rutiloides</i>	1.24 ± 0.04 ^a	1.45 ± 0.05 ^a	1.40 ± 0.02 ^a	0.38 ± 0.09 ^b
	<i>Astronotus ocellatus</i>	1.01 ± 0.08 ^a	1.03 ± 0.05 ^a	1.07 ± 0.07 ^a	0.86 ± 0.06 ^a
	<i>Cichla monoculus</i>	0.57 ± 0.09 ^b	0.62 ± 0.07 ^b	0.61 ± 0.05 ^b	0.57 ± 0.04 ^b
Benthic/benthopelagic fish	<i>Pirirampus pirinampu</i>	0.38 ± 0.06 ^b	0.46 ± 0.05 ^b	0.45 ± 0.05 ^b	0.37 ± 0.05 ^b
	<i>Liposarcus pardalis</i>	0.41 ± 0.04 ^b	0.40 ± 0.05 ^b	0.38 ± 0.06 ^b	0.41 ± 0.04 ^b
	<i>Hemisorubim platyrhynchos</i>	0.58 ± 0.04 ^b	0.62 ± 0.07 ^b	0.62 ± 0.06 ^b	0.60 ± 0.04 ^b
	<i>Sorubim lima</i>	0.47 ± 0.05 ^b	0.53 ± 0.06 ^b	0.57 ± 0.04 ^b	0.47 ± 0.05 ^b
	<i>Ageneiosus ucayalensis</i>	0.36 ± 0.05 ^b	0.37 ± 0.09 ^b	0.34 ± 0.04 ^b	0.27 ± 0.06 ^b

3.3 Blood Variables

The hematology was analyzed in the same species studied for RMR and gill morphometry. Similar to gill dimensions, some pelagic species (e.g., *P. nattereri*, *M. duriventre*, *P. amazonica* and *C. monoculus*) have more red blood cells (Fig. 4A), hemoglobin concentration (Fig. 4B) and hematocrit (Fig. 4C) than do benthic fish. The hematological indices (MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; and MCHC, mean corpuscular hemoglobin concentration) are shown in Table 4. The highest and lowest values of MCV were observed in *A. ocellatus* and *C. monoculus*, respectively. Since MCV is an erythrocyte parameter, it depends on the hematocrit/RBC (red blood cells) ratio. Both

species had similar values for hematocrit; however, *C. monoculus* presented more circulating red blood cells than *A. ocellatus*. The values of hematological indices were similar for all studied species.

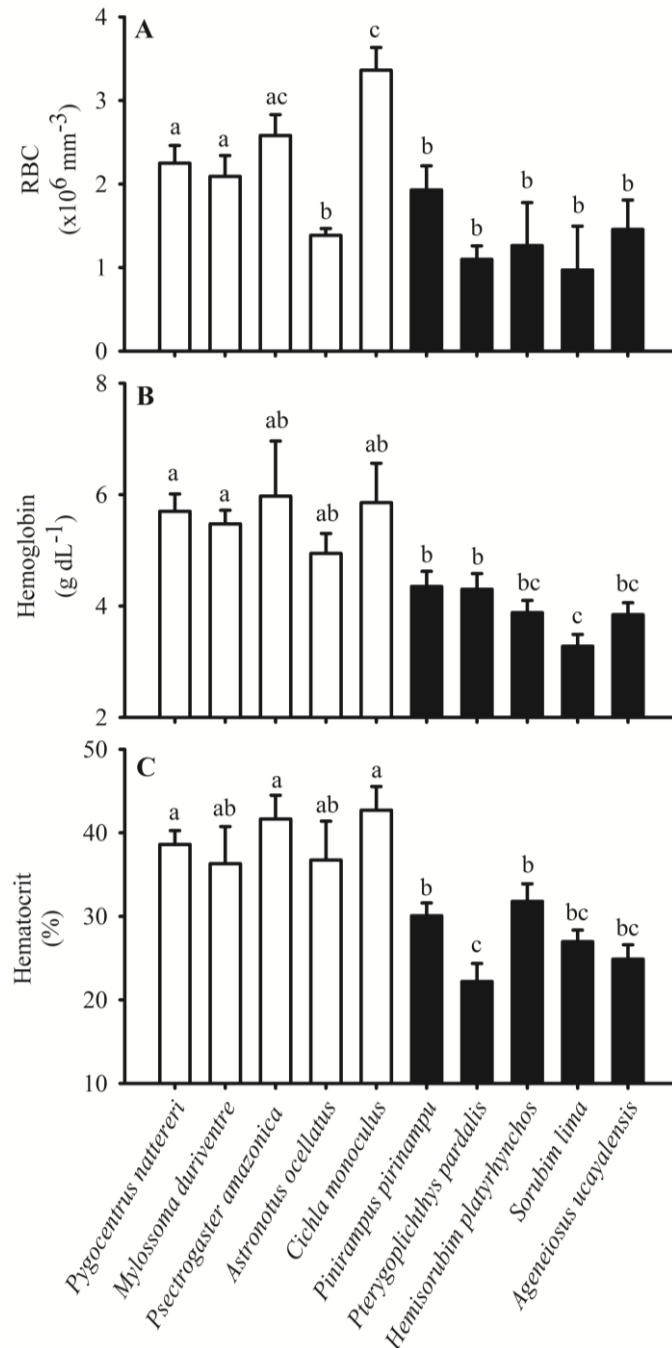


Figure 4. Blood oxygen capacity inferred as red blood cell count, RBC (A), hemoglobin (B) and hematocrit (C) in several fish species from floodplain lakes (Lake Janauacá). The open bars represent the pelagic species, and the filled bars represent the benthic/benthopelagic species. Different lowercase letters indicate significant differences among the species ($p < 0.05$) by Kruskal-Wallis nonparametric ANOVA multiple comparisons test

3.4 Relationship Between Blood Variables, RMR and Gill Dimensions

Clearly, the two first axes of the principal component analysis (PCA) based on the data matrix of gill morphometrics/oxygen consumption rates (MO_2) and blood variables were significantly correlated [PC1-gill morphology and $MO_2 = -1.49 \times 10^{-6} + (0.539 \times PC1\text{-hematological variables})$, $p < 0.001$, Fig. 5A]. According to our expectation, the mass-specific RMR (MO_2) was also related to the first axis of PCA based on the matrix of hematological variables [log mass-specific $MO_2 = 1.834 + (0.199 \times PC1\text{-hematological variables})$, $p < 0.001$, Fig. 5B]. This relationship was also positive when analyzing the first axis of the PCA data matrix of hematological variables and total gill surface area [gill surface area = $2.676 + (0.106 \times PC1\text{-haematological variables})$, $p < 0.001$, Fig. 5C].

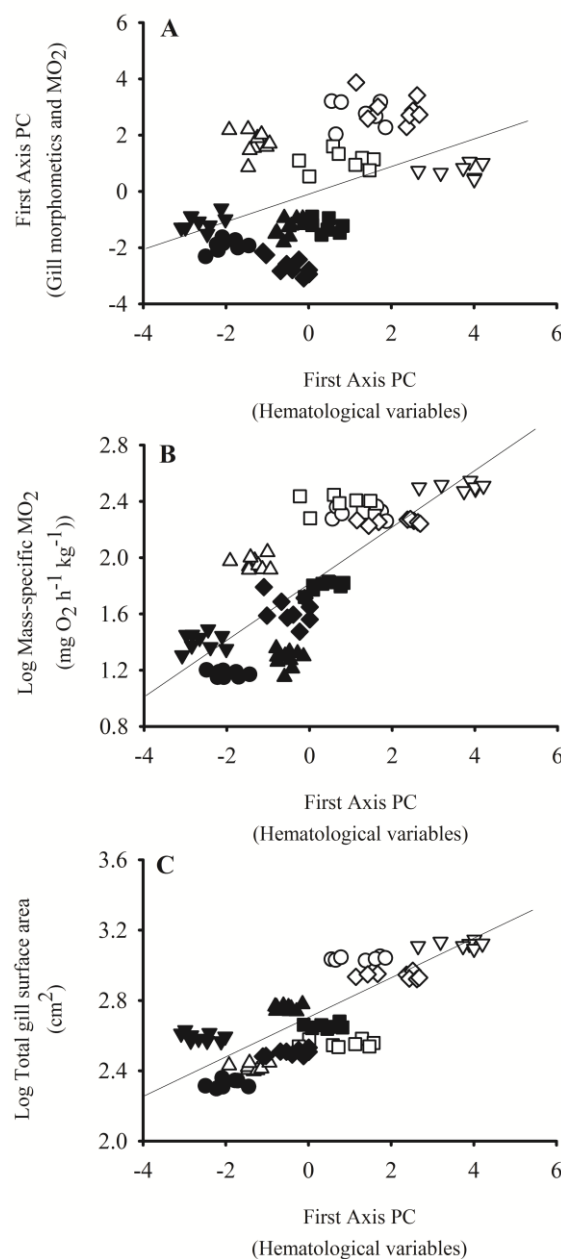


Figure 5. Correlation between the two first axes of PCA based on the data matrix of gill

morphometrics/routine oxygen consumption rates (MO_2) and blood variables (A). Relationship between \log_{10} mass-specific MO_2 and first axis of PCA based on the data matrix of hematological variables (B). Correlation between \log_{10} total gill area and first axis of PCA based on the data matrix of hematological variables (C) analyzed in different fish species from Lake Janauacá: *Pygocentrus nattereri* (○), *Mylossoma duriventre* (□), *Psectrogaster amazonica* (◇), *Astronotus ocellatus* (△) and *Cichla monoculus* (▽), *Pinirampus pirinampu* (■), *Pterygoplichthys pardalis* (●), *Hemisorubim platyrhynchos* (▲), *Sorubim lima* (▼) and *Ageneiosus ucayalensis* (◆). The open symbols represent the pelagic species, and the closed symbols represent the benthic/benthopelagic species

4. Discussion

Several authors have generalized that pelagic fish are more metabolically active than benthic species (De Jager & Dekkers 1975; Powers 1980; Childress 1995), although it has been speculated that the metabolic demand is adjusted to the oxygen-carrying capacity in the blood. Furthermore, these physiological processes may be closely related to gill morphology. This seems to agree with the concept of symmorphosis already well established in the work of Weibel (2000), in which the structural design of the gills is closely adjusted to the oxygen demand by the animal. Considering these points of view and based on the results obtained in this study, this report discusses the following physiological and ecological issues: (i) are the gill dimensions, RMR and the blood oxygen-carrying capacity adjusted to the metabolic demand of the fish as predicted by the symmorphosis hypothesis? and (ii) are pelagic fish metabolically more active than benthic fish (Killen et al. 2010)?

The gill dimensions, such as the total number and length of filaments, density of lamellae on the filament, blood-water barrier thickness and bilateral surface area of lamellae, are adjusted by selective factors that favor the gill area that meets the oxygen demand of the animal (Hughes et al. 1986; Severi et al. 1997; Wegner et al. 2010). Similarly, blood characteristics, such as the number of circulating red blood cells (RBCs), hematocrit, hemoglobin and RBC indices, reflect the oxygen-carrying capacity (Nikinmaa & Soivio 2005) and are also adjusted to the metabolic demands of the fish (Powers 1980). Weibel (2000) argues that "the state of structural design commensurate to functional needs resulting from regulated morphogenesis whereby the formation of structural elements is regulated to satisfy but not exceed the requirements of the functional system". In fact, a clear "functional integration" is one of the three principles of the symmorphosis concept originally described by Weibel et al. (1991). Therefore, this study described a morphofunctional integration of gill structure that is matched to environmental oxygen uptake and gas transport through the blood.

As expected, active pelagic predators (e.g., *C. monoculus* and *P. nattereri*) have high metabolic rates (320 and 207 mg O_2 h^{-1} kg^{-1} , respectively), large mass-specific gill areas (2.3 to 6.7 cm^2 g^{-1} , respectively), densely packed lamellae and a high capacity to carry oxygen in the blood based on hemoglobin concentration, hematocrit and circulating red blood cells. These results are similar to reports for marine teleosts with high energy demands. For example, skipjack tuna (*Katsuwonus pelamis* Linnaeus, 1758) and yellowfin tuna (*Thunnus albacares* Bonnaterre, 1788) have high gill areas (18.5 and 13.3 cm^2 g^{-1} , respectively) to meet

an oxygen uptake of 412 and 286 mg O₂ h⁻¹ kg⁻¹, respectively, during standard metabolic rates (Muir & Hughes 1969, Wegner et al. 2010). The Amazon fish do not have high metabolic demands and gill areas, such as those found in high-speed marine scombrids, but the ratio of the routine metabolic rate and gill area of Amazon pelagic fishes appears consistent with the energy requirement. In the Amazon basin, one of the most active fish is the cichlid *C. monoculus*. This species has a relatively large head and caudal fin to quickly accelerate from rest. Furthermore, this fish is able to sprint and perform fast-start maneuvers during predator-prey interactions (Winemiller et al. 1997). *P. nattereri* is also a highly active predator but is not as fast a swimmer as *C. monoculus*. However, *P. nattereri* has a similar metabolic profile to that described for *C. monoculus*. On the other hand, high metabolic demand is not unique in predatory fish. Many pelagic herbivorous, omnivores or detritivorous fish are also metabolically active. For example, *M. duriventre* and *P. amazonica* have values of mass-specific gill area and routine metabolic rate similar to those observed in carnivorous species (Divya & Ranjeet 2014).

The metabolic level boundaries hypothesis (MBLH) provides a mechanistic link between the lifestyle of organisms and the scaling of metabolism with body size (Glazier 2014). According to the MBLH, species that live in different habitats will have different ecological needs and may affect the metabolic level and, in turn, potentially affect the scaling exponent (Killen et al. 2010). It has been reported that both the scaling exponent and coefficient vary systematically among species (Ohlberger et al. 2012, Huang et al. 2013). This finding explains, at least in part, the differences between predicted and observed and measured values of RMR among the fish species of Lake Janauacá. Pelagic species have a high energy requirement for swimming activity and, in turn, have the highest metabolic level (scaling coefficient). This elevated maintenance cost enhances the role of physical boundary limits, such as gill surface area (Glazier 2014), resulting in a decrease in the values of the scaling exponent (Killen et al. 2010). These authors reported an inverse relationship between metabolic levels and scaling exponent. Thus, benthic fish had the lowest values for metabolic levels and the highest values of the scaling exponent. In the present study, RMR measurements for benthic species suggest that these fish have low metabolic rates. Therefore, it is possible that the value of the scaling exponent in the benthic species can be higher than those found in pelagic fish. Unfortunately, the limited available data are insufficient to calculate a reliable scaling exponent for each species.

In general, larger gill surface areas and thin water-blood barriers are the main factors that increase oxygen uptake capacity (Crampton et al. 2008, Wegner et al. 2010). The oxygen molecules must cross the thin epithelium, which consists of two cell layers. Pavement cells and flanges of pillar cells constitute the water-blood barrier (Evans et al. 2005, Duncan et al. 2010). As chloride cells (or mitochondria-rich cells) are rarely observed on lamellae (except in *C. monoculus* and *M. duriventre*), most species have a thin water-blood barrier varying from 1.2 to 2.7 μm (2/3 harmonic mean). Thin barriers tend to improve the morphological potential for gas exchange. However, if the water-blood distance is thick, the oxygen uptake can be compensated by increasing the total gill surface area (Hughes et al. 1986). The anatomical diffusion factor (ADF) is a good measure of this capacity normalized to body

mass (Hughes, 1984). The ADF for Amazon pelagic fish was $>3 \text{ cm}^2 \text{ g}^{-1} \mu\text{m}^{-1}$. This value is 3 times greater than those estimated for Atlantic salmon, *Salmo salar* (Perry 1990). Surprisingly, *C. monoculus* and *M. duriventre* have high routine metabolic rates but demonstrated low ADF values. ADF is a quotient of the mass-specific gill surface area and water-blood barrier. Therefore, the presence of numerous chloride cells in the gill epithelia of these fish species results in an increase in the thickness of the gas exchange barrier. However, the large metabolic rate is compensated by the large gill surface area. Thus, these species maintain osmorepiratory control without altering the delicate balance between gas and ion exchange by the gills.

In general, more than 90% of the oxygen transported from the gill epithelium is carried by hemoglobin (Nikinmaa & Soivio 2005). The number of circulating red blood cells, the hemoglobin concentration, the amount of oxygen-binding hemoglobin (modulated by the erythrocyte environment) and the properties of the prevailing oxygen partial pressure all determine the amount of oxygen carried per unit volume of blood (Brunori 2014). Unfortunately, neither the kinetic properties of hemoglobin nor the blood oxygen content were analyzed in this study, but the circulating red cell blood and total hemoglobin content favors species-specific adjustments to carry oxygen, depending on the metabolic demand of each animal.

As previously reported, pelagic predators, such as *C. monoculus* and *P. nattereri*, have higher hematocrit values, hemoglobin contents and circulating red blood cell levels compared with benthic fish. In contrast, fish that live in benthic environments have large cell volumes. It is possible that cell volume may influence hemoglobin-oxygen affinity (Brunori 2014). As the bottom environment in floodplain lakes is usually hypoxic or anoxic (Anjos et al. 2008), it is possible that a higher cell volume may be advantageous for benthic fish. Wells (2009) suggested that fish with an active swimmer lifestyle tend to have a high O_2 -carrying capacity and a relatively low oxygen affinity, and the blood Hb- O_2 affinity is modulated by ATP. On the other hand, fish that live in habitats that have periodically low dissolved oxygen concentrations (hypoxic) may also have high oxygen-carrying capacities, but generally, these species have high blood hemoglobin O_2 affinities, and the affinity is modulated by GTP or ATP (Val & Almeida-Val 1995).

In the Amazon basin, several fish species have developed adaptations to tolerate hypoxia and, occasionally, anoxia. Moreover, some species are more tolerant than others. For example, *A. ocellatus* is highly anoxia-resistant and can survive in severe hypoxia for 20 hours and anoxia for up to 6 hours (Muusze et al. 1998; Almeida-Val et al. 2000). Metabolic arrest and/or activation of anaerobic metabolism have been described as the main strategy for this cichlid to survive in severe hypoxic and anoxic conditions (Almeida-Val et al. 2000; Chippari-Gomes et al. 2005, Scott et al. 2008). Although *A. ocellatus* is closely related to *C. monoculus*, this species has only one-third of the mass-specific RMR estimated for *C. monoculus*. However, *A. ocellatus* has a thin water-blood barrier that can facilitate gas transport through the gill epithelium. The catfish *P. pardalis* also has a low RMR, but this catfish is a facultative air-breathing species (Val & Almeida-Val 1995). It is important to emphasize that in the study of this species, the fish were not given access to atmospheric

oxygen. This loricariid occupies most hypoxia-prone habitats in the Neotropical region, and *P. paradalis* is one of 673 loricariid species that use the stomach for air breathing (Nelson & Dehn 2011).

In summary, pelagic *P. nattereri*, *M. duriventre*, *P. amazonica* and *C. monoculus* have higher metabolic rates, large gill surface areas and high capacities for carrying oxygen through the blood compared to benthic species. The blood characteristics, such as the number of circulating red blood cells, hemoglobin, hematocrit and RBC indices, are correlated with RMR and gill dimensions (surface area, lamellar area and anatomical diffusion factor). This study can help researchers understand the complex relationships between pelagic and benthic fish communities in an Amazon floodplain lake in relation to their physiological ecology. However, more studies are needed to establish further generalizations.

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