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# Plasma energetic substrates and hepatic enzymes in the four-eyed fish *Anableps anableps* (Teleostei: Cyprinodontiformes) during the dry and rainy seasons in the Amazonian Island of Maracá, extreme north of Brazil

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*Anableps anableps* is a viviparous teleost typical from Amazon Delta estuaries. It is representative of this biome in Maracá, which offers a potential for biomonitoring. The aim of this study is to apply different biomarkers to males and females of this species and verify possible seasonal influences on their physiology. To collect fish, three expeditions were carried out from the rainy season of April 2018 to the rainy season of February 2019. Biometric parameters and gonadosomatic (GSI), hepatosomatic (HSI), and viscerosomatic (VSI) indexes were calculated, and blood samples were taken to measure triglycerides, total proteins, glucose, and activity of the enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). The GSI of males is higher in the rainy season and of females in the dry season. This is probably related to the embryogenesis process. Males show an increase in biomass during the dry season, a metabolic homogeneity, and females show an increase in plasma glucose, triglycerides, and ALT activity. The tested biomarkers are potential for biomonitoring, preliminarily suggesting that there is a seasonal asynchronism between males and females of *A. anableps* as for the allocation of energy resources at different times of their life cycle.

Keywords: Amapá, Delta, Fish transaminases, Tralhoto, Viviparity.

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*Anableps anableps* é um teleósteo vivíparo típico de estuários do Delta do Rio Amazonas, sendo representativo deste bioma na ilha de Maracá, com potencial para o biomonitoramento. O objetivo deste estudo foi aplicar diferentes biomarcadores em machos e fêmeas desta espécie e verificar a possível influência sazonal em aspectos de sua fisiologia. Para a coleta dos peixes foram realizadas três expedições, de abril/2018 (estação chuvosa), setembro/2018 (estação seca) até fevereiro/2019 (estação chuvosa). Foram obtidos parâmetros biométricos, índices gonadossomático (IGS), hepatossomático (IHS), viscerossomático (IVS) e amostras de sangue para dosagem de triglicerídeos, proteínas totais, glicose e a atividade das enzimas aspartato aminotransferase (AST), alanina aminotransferase (ALT) e fosfatase alcalina (ALP). O IGS dos machos foi maior na estação chuvosa e das fêmeas na estação seca, isso provavelmente ocorreu devido ao processo de embriogênese. Os machos aumentaram a biomassa na estação seca e apresentaram homogeneidade metabólica, já as fêmeas apresentaram hiperglicemia, hipertrigliceridemia e maior atividade da ALT. Os biomarcadores testados mostraram-se promissores para o biomonitoramento, sugerindo de forma preliminar que há um assincronismo sazonal entre machos e fêmeas de *A. anableps* na alocação de recursos energéticos em diferentes momentos do seu ciclo de vida.

Palavras-chave: Amapá, Delta, Peixes transaminases, Tralhoto, Viviparidade.

## INTRODUCTION

Currently, several types of anthropogenic threats are affecting aquatic ecosystems globally, including chemical pollutants, climate change, fishing, introduction of exotic species, human population growth, and pathogens (Häder *et al*., 2020). In this scenario, recent ecological disasters have occurred in the neotropical region and are especially marked in Brazil, among which the mining accident in Mariana (Foesch *et al*., 2020), fires in all biomes (da Silva Junior *et al*., 2020), and deforestation (for example in the Amazon River Basin region; Exbrayat *et al*., 2017), and river silting (Santos *et al*., 2020).

Some of these impacts occur in conservation and integral protection units, as an example, we mention the marine coastal island of Maracá. This island is susceptible to all the environmental threats mentioned above, since the Amazon Delta Estuary is vulnerable to multiple environmental and anthropogenic pressures (Mansur *et al*., 2016).

Therefore, the biomonitoring of aquatic ecosystems is an important tool to allow an ecophysiological assessment of biodiversity and of landscape in face of unpredictable or unspecified environmental threats. It requires the verification and interpretation of biological and physiological responses of target organisms (Costa, Teixeira, 2014). One of the main challenges in implementing biomonitoring programs is the selection of a species or a taxonomic group to be considered as a bioindicator. This is a decisive factor in its applicability (Ruaro *et al*., 2016).

A sentinel species, that is, the one chosen for aquatic biomonitoring, must meet some premises: it must indicate environmental disturbances and be a good ecological and biodiversity indicator (Costa, Teixeira, 2014); at the same time, it should meet some additional postulates, such as relative abundance, ease of capture, sampling and maintenance in laboratory, representativeness of the studied site, long life cycles, and relevance in the food chain (Zhou *et al*., 2008). In addition, this species must also meet some ecotoxicological requirements and have a relative physiological sensitivity to environmental stressors, allowing the basal and primary detection of effects and impacts on the food chain.

In this context, *Anableps anableps* (Linnaeus, 1758) Cyprinodontiformes: Anablepidae, commonly known by its indigenous name ("tralhoto"), or four-eyed fish, is a viviparous teleost species. Therefore, it is a livebearer species, in which superfetation is relatively absent or unusual and sexual dimorphism is evident, as the male has a tubular gonopod (Turner, 1938; Knight *et al*., 1985). The genus *Anableps* has the largest representatives in this order. They are pelagic animals distributed in the equatorial portion of the neotropical region (Ikeda *et al*., 2005), which includes the Amazon River Delta, Brazil.

This species resides in estuaries and performs intertidal migration as a surface swimming species (Krumme *et al*., 2014); however, it does not migrate across long distances, being restricted to environments of low salinity (Watanabe *et al*., 2014). Since it is a brackish water fish and inhabits mangrove areas, this species has morphological and jaw adaptations that allow it to jump out of the water and capture prey on land, especially in mudbanks (Michel *et al*., 2015). For this reason, they are notoriously common on the island of Maracá. In water columns or in substrates, it feeds on detritus, red algae, insects, fish, and Grapsidae crabs (Brenner, Krume, 2007). Such a food plasticity reveals a generalist strategy (Figueiredo *et al*., 2019).

Cavalcante and coauthors (2012) report studies that show the ecological importance, the reproductive strategy, and the abundance of *A. anableps* as its main characteristics. Therefore, it can be considered as a potential bioindicator species, especially because of its low occurrence in anthropically impacted environments. In addition to an appropriate choice of the bioindicator species for biomonitoring, the selection of biomarkers that express the physiological condition and the health of an organism is essential for an ecophysiological prognosis of an ecosystem (Hook *et al*., 2014), as they are defined as biological, biochemical, physiological responses, among others, presented by organisms in face of an environmental stressor. Hence, it can be used as a tool to support ecological assessment programs (Milinkovitch *et al*., 2019).

In light of the above, from the perspective of conservation physiology, it is possible to predict changes and impacts on wild animal populations through physiological metrics and approaches in order to contribute to conservation outcomes and management programs (Madliger *et al*., 2016). Among such metrics, fish metabolism and energetic substrates stand out. A study comparing individuals of yellow perch *Perca flavescens* (Mitchill, 1814) captured in different seasons (spring and autumn) in six lakes in Québec-Canada found seasonal changes in condition factor, plasma fatty acids, and several liver and metabolic biomarkers (Levesque *et al*., 2002).

Another field studies compared females of *Hoplias malabaricus* (Bloch, 1794) (Gomes *et al*., 2015) and *Astyanax fasciatus* (currently *Psalidon fasciatus* (Cuvier, 1819)) (Tolussi *et al*., 2018) in a reservoir (reference area) with another reservoir impacted by domestic sewage, industrial effluents, deforestation, and soil occupation in four different seasons in state of São Paulo (Brazil), showed the applicability of plasma biomarkers, as well as body index markers, such as gonadosomatic (GSI), hepatosomatic (HSI), evidencing negative impacts on the physiology of these species due to the interaction between chemical contaminants and seasonal variations.

Energetic substrates, such as proteins, lipids and carbohydrates from plasma and tissues, and the body indexes mentioned above, have also been used as biomarkers in bioassays with fish exposed to metals, such as aluminum, and have been shown to be adequate when evidencing physiological impacts on reproduction, metabolism, and the endocrine system (Correia *et al*., 2010; Vieira *et al*., 2013). Also, for several decades many fish hepatic enzymes, such as transaminases and alkaline phosphatase, have been used to monitor ecosystems and water quality (Gill *et al*., 1990). Currently, studies on the same subject continue to be carried out and they have pointed out that these enzymes, considered as biomarkers, are powerful tools in environmental biomonitoring of aquatic ecosystems (Ebrahimzadeh *et al*., 2021).

The present study aims to investigate in different seasons the metabolism of energy substrates and activity of liver enzymes in the plasma of males and females of *A. anableps* in the estuarine ecosystem of island of Maracá. The reasons for this study are that the island of Maracá encompasses an aquatic ecosystem susceptible to anthropic influences and abiotic seasonal variations, the potential of the *A. anableps* for the context presented here, and the fact that there is no information available on characteristics of the basic physiology of this species, except for some data on zootechnical parameters, feeding and eye physiology.

## MATERIAL AND METHODS

**Study area and field expeditions.** The state of Amapá is in the extreme north of the Brazilian territory, inserted inside the Amazon region. According to the National Waters Agency, the city of Amapá has a portion of its territory in the Araguari River basin and in coastal hydrographic basin. The latter includes the Maracá Island (ICMBio, 2017). The west side of the island is separated from the continent by the Carapori or Varadouro canal, and the east side gives direct access to the Atlantic Ocean (ICMBio, 2017).

The Maracá Island (study area; Fig. 1) is a federal conservation and integral protection unit called Ecological Station of Maracá-Jipióca (01°49'51" and 02°12'54"N 50°11'02" and 50°34'22"W) under the management of the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio). It is a coastal marine biome that covers the islands of Maracá and Jipióca (submerged). There is a small stream (Igarapé do Inferno) that divides the Maracá Island into north and south (ICMBio, 2017).

Three field expeditions were conducted to the ES of Maracá-Jipióca in an annual seasonal cycle: April 2018 (rainy season), September 2018 (dry season), and February 2019 (rainy season). It is important to note that there are only two distinct seasons in the ES: dry (August to November) and rainy (December to July) (ICMBio, 2017). The fish were collected in the island using artisanal fishing technology, that is, fixed gillnets and cast nets (cone-shaped nets, locally known as "tarrafa") (Mesquita, Isaac-Nahum, 2015), with dimensions of 30 m/40 mm and 4 m/30 mm, respectively (ICMBio, 2019). The collection was performed during the passing from the high tide to the low tide around the mangrove stream areas for about an hour so that the passage of fish through the tidal canal was obstructed by the fishing nets (Ikeda *et al*., 2015).

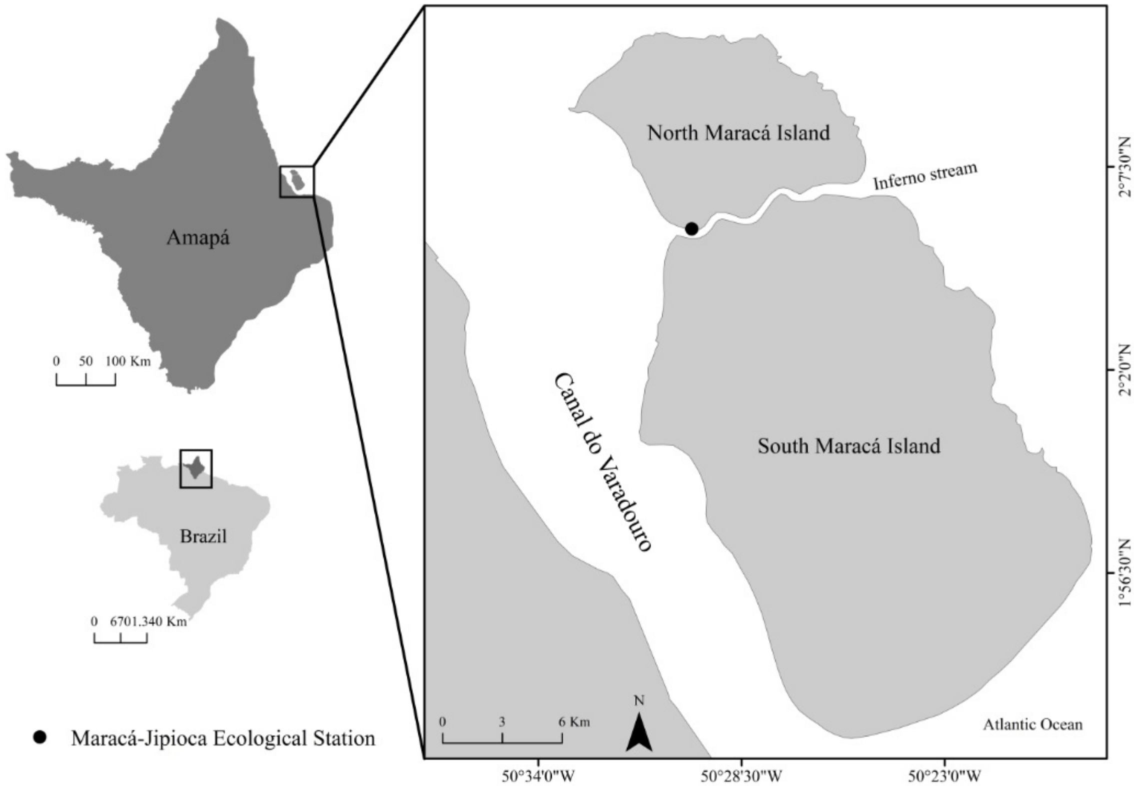


**FIGURE 1** | Ecological Station of Maracá-Jipióca. Black dot shows the location of the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) research base.

**Sampling procedures.** After the fish have been caught (voucher number: LABOT-UNIFAP #003), they were anesthetized with benzocaine 100 mg $L^{-1}$ still at the collection site. A blood sample was obtained by venipuncture of the dorsal caudal vessel considering the lateral line of the fish as an anatomical reference using 3ml disposable syringes and needles (0.80 x 25 mm) – Becton Dickinson (NJ, EUA). The syringes contained 5% EDTA-Na ($C_{10}H_{14}N_2Na_2O_8.2H_2O$, Sigma) as anticoagulant. Then, the samples were centrifuged for 5 minutes at 655.1 g to separate the plasma, and then immediately stored in a freezer and kept frozen at -20 °C until further analysis in the laboratory facilities of the Universidade Federal do Amapá (UNIFAP).

Biometric parameters, such as total length and body weight, were recorded. The liver, gonads (embryos and testicles) and all digestive tract organs in the fish abdominal cavity were removed and weighed to determine the body indexes (%): hepatosomatic –HSI; gonadosomatic – GSI (embryos for females) according to Vazzoler (1996), and celomic fat for viscerosomatic (VSI) following Silva *et al*. (2015). Condition factor (CF) was calculated as Fulton (1904): $K = 100\ W/L^3$ (W (g) = body weight, L (cm) = total length).

**Plasma biochemistry analysis.** The plasma energetic substrates glucose, total proteins, and triglycerides and the activity of the hepatic enzymes alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were measured using commercial kits for humans (Life Biotechnologies, MG, Brazil). Total protein content followed the Biuret reaction (Gornall *et al*., 1949); glucose (Trinder, 1969) and triglycerides (Bucolo, David, 1973) followed a colorimetric enzyme test. The protocols of activity of AST and ALT kits followed the method described by Bergmeyer (1974), and ALP followed the method of McComb *et al*. (1979). The analyses were validated according to the manufacturer's instructions in 1.5 mL glass cuvettes on a digital spectrophotometer (UV-Visible light, 5100 Tecnal SP, Brazil).

The lowest detection level and intra- and inter-assay coefficients of variation (% CV) were 45.4 ± 14.36 mg $dL^{-1}$, 8.05% (n = 3) and 11.77 % (n = 5) for glucose; 37.01 ± 0.05 mg $dL^{-1}$, 3.46 % (n = 3) and 16.03 (n = 4) for triglycerides; 1.30 ± 0.17 g $dL^{-1}$, 8.63 % (n = 4) and 17.50 % (n = 6) for total proteins; 45.97 ± 13.64 U $L^{-1}$, 14.00 % (n = 4), and 16.74 % (n = 4) for AST; 8.72 ± 0.82 U $L^{-1}$, 6.96% (n = 2), 17.80% (n = 4) for ALT; and 26.58 ± 1.29 U $L^{-1}$, 15.51 % (n = 4), 20.23 ± (n = 4) for ALP.

**Statistical analysis.** The results are expressed as mean ± standard deviation in a 95% confidence level (p <0.05). Shapiro-Wilk normality test and a Brown-Forsythe homoscedasticity test were applied to treat the data. One-way analysis of variance (ANOVA) was performed to compare three or more groups (different seasons and same sex), two-way analysis of variance (ANOVA) compared three or more groups (seasons and sex), and Student t test to compare between two groups. The nonparametric data were submitted respectively a test of Kruskal-Wallis followed by Dunn's test and Mann-Whitney test. The relationship between biometric and plasma parameters was analyzed by Pearson´s correlation test (p <0.05).

## RESULTS

We collected thirty-three specimens of *A. anableps* during the three expeditions: the rainy season in April 2018 (only females n = 9), the dry season in September 2018 (males n = 5 and females n = 7), and the rainy season in February 2019 (males n = 5 and females n = 7). All results presented are considered preliminary because this is a study with a small sample size.

The biometric analysis (Tab. 1) showed that the females in the rainy season (April 2018) have a longer total length compared to males in both seasons (P = 0.029) and females in the dry season (P = 0.029). Regarding rainy season females (2018), a higher body weight was recorded compared to males (P = 0.002) in the rainy season (2019) and females of the dry (2018) and rainy season (2019) (P = 0.008). Females in the dry season have a lower CF compared to females in the rainy seasons (P = 0.001). However, regarding body indexes, females in the dry season compared to females in the other seasons showed a high GSI (P = 0.012) and a higher VSI (P = 0.011) compared to females in the rainy season (2018). There was no statistical difference for HSI (P = 0.32) between females. The number of embryos between seasons was not significantly different (P = 0.28).

Comparing males, the body weight of dry season animals was greater than that in the rainy season (P = 0.002). There were no statistical differences for total length (P = 0.899), HSI (P = 0.392), VSI (P > 0.999), and CF (P = 0.732). However, GSI was higher in males in rainy season (P = 0.011).

**TABLE 1** | Biometric analysis and body index of males and females of *Anableps anableps* during seasonal cycle. Total length and body weight were compared inter-sex and gonadosomatic index (GSI), hepatosomatic index (HSI), viscerosomatic index (VSI), and Fulton´s condition factor (K) was compared between animals of the same sex. Results are expressed as mean and standard deviation. Different letters indicate statistical differences (Kruskal-Wallis with a Dunn's *post hoc* test, P < 0.05; Mann-Whitney test P < 0.05 for GSI, HSI, VSI, and K males).

| | Rainy season (April 2018) | | Dry season (September 2018) | | Rainy season (February 2019) | |
|---|---|---|---|---|---|---|
| | Female | Male | Female | Male | Female | Male |
| Total length (cm) | 36.20 ± 2.1 [a] | – | 29.14 ± 2.9 [b] | 26.00 ± 4.5 [b] | 31.07 ± 3.6 [ab] | 26.60 ± 1.3 [b] |
| Weight (g) | 383.06 ± 90.7 [a] | – | 198.00 ± 52.5 [bc] | 235.00 ± 155.0 [ac] | 196.42 ± 54.2 [bc] | 118.32 ± 37.7 [b] |
| GSI (%)<br>Number of embryos | 2.87 ± 0.7 [b]<br>9.00 ± 2.5 | – | 14.66 ± 2.0 [a]<br>20.66 ± 5.7 | 0.41 ± 0.1 [a]<br>– | 4.05 ± 0.8 [b]<br>9.60 ± 5.0 | 2.97 ± 0.7 [b]<br>– |
| HSI (%) | 2.02 ± 0.2 | – | 1.42 ± 0.8 | 1.68 ± 0.7 | 1.45 ± 0.3 | 1.16 ± 0.3 |
| VSI (%) | 3.99 ± 1.0 [a] | – | 10.63 ± 3.8 [b] | 5.93 ± 2.5 | 7.08 ± 1.7 [ab] | 6.21 ± 2.1 |
| K (100 x g $cm^{-3}$) | 1.21 ± 0.2 [a] | – | 0.75 ± 0.1 [b] | 1.00 ± 0.5 | 1.05 ± 0.0 [a] | 0.62 ± 0.1 |

**TABLE 2** | Concentration of plasma energetic substrates and plasma activity of liver enzymes of males and females of *Anableps anableps* during expeditions in the dry (September 2018) and rainy seasons (February 2019). Results are expressed as mean and standard deviation. Different letters indicate statistical differences for intra and inter-sex comparisons in different seasons. Hepatic enzymes alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST).

| | Dry season (September 2018) | | Rainy season (February 2019) | |
|---|---|---|---|---|
| | Female | male | Female | male |
| Total proteins (g $dL^{-1}$) | 2.51 ± 0.5 | 1.99 ± 0.1 | 2.24 ± 0.4 | 2.35 ± 0.4 |
| Glucose (mg $dL^{-1}$) | 228.71 ± 39.0 [a] | 82.61 ± 33.6 [b] | 95.58 ± 37.2 [b] | 77.30 ± 29.5 [b] |
| Triglycerides (mg $dL^{-1}$) | 277.26 ± 60.4 [a] | 125.91 ± 36.4 [bc] | 196.49 ± 34.5 [b] | 103.48 ± 30.0 [c] |
| AST (U $L^{-1}$) | 136.19 ± 30.0 | 93.56 ± 28.1 | 106.79 ± 23.4 | – |
| ALT (U $L^{-1}$) | 27.35 ± 13.9 [a] | 18.33 ± 5.7 [ab] | 12.88 ± 5.7 [b] | 15.42 ± 4.5 [ab] |
| ALP (U $L^{-1}$) | 33.30 ± 5.8 | 38.5 ± 6.3 | 51.79 ± 22.2 | 23.8 ± 6.5 |

The analysis of plasma energetic substrates (Tab. 2) showed no statistical differences for total proteins between all groups (P = 0.156). However, females in the dry season have a higher concentration of glucose (P < 0.005) and triglycerides (P < 0.001) compared to animals of both sexes in the dry and rainy seasons. Females in the rainy season have a higher triglycerides concentration compared to males in the same season (P < 0.050).

The activity of plasma liver enzymes (Tab. 2) showed that only females in the dry season have a higher ALT than females in the rainy season (P < 0.05). However, for this enzyme, there were no differences in the other comparisons between males and females (P = 0.274). There were also no differences for AST (P = 0.256) and ALP (P = 0.268). Defrosting and logistical difficulties in preserving the samples in the island of Maracá (rainy season of 2018, females) and hemolysis in AST samples for males of the rainy season of 2019 impeded these analyses.

The Pearson's correlation analysis showed a significative positive correlation only between total length and body weight of males and females in the rainy season in 2019 (Tab. 3).

**TABLE 3 |** Pearson correlation test for biometric parameters, body indexes, and plasma of males and females of *Anableps anableps* in a seasonal cycle. P < 0.05 indicates a significative correlation. Alanine aminotransferase (ALT), Gonadosomatic (GSI); viscerossomático (IVS); Viscerosomatic (VSI).

| ***Anableps anableps* (females)** | | | | |
|---|---|---|---|---|
| **Expedition** | **Correlation of variables** | **Pearson (r)** | **P** | **n** |
| Rainy season (April 2018) | Total length and body weight | 0.452 | 0.222 | 9 |
| | GSI and K | 0.857 | 0.143 | 9 |
| | GSI and VSI | -0.645 | 0.355 | 9 |
| Dry season (September 2018) | Triglycerides and glucose | 0.125 | 0.875 | 7 |
| | Triglycerides and ALT | 0.386 | 0.748 | 7 |
| | Triglycerides and VSI | 0.023 | 0.976 | 7 |
| | Triglycerides and GSI | 0.637 | 0.363 | 7 |
| | Glucose and ALT | -0.348 | 0.774 | 7 |
| | GSI and K | 0.560 | 0.440 | 7 |
| | GSI and VSI | 0.777 | 0.223 | 5 |
| Rainy season (April 2019) | Total length and body weight | 0.985 | <0.05 | 7 |
| | GSI and K | 0.704 | 0.206 | 7 |
| | GSI and VSI | 0.295 | 0.705 | 7 |
| | IVS and K | -0.144 | 0.759 | 7 |
| ***Anableps anableps* (males)** | | | | |
| | **Correlation of variables** | **Pearson (r)** | **P** | **n** |
| Dry season (September 2018) | Total length and body weight | 0.367 | 0.633 | 5 |
| | GSI and K | -0.519 | 0.653 | 5 |
| | GSI and VSI | -0.287 | 0.815 | 5 |
| | IVS and K | -0.670 | 0.532 | 5 |
| Rainy season (April 2019) | Total length and body weight | 0.89 | <0.05 | 5 |
| | GSI and K | -0.827 | 0.08 | 5 |
| | GSI and VSI | 0.01 | 0.982 | 5 |
| | IVS and K | -0.166 | 0.790 | 5 |

## DISCUSSION

We designed this field study to test whether the teleost *Anableps anableps* meets the requirements of a species with a potential for ecological biomonitoring in the scope of conservation physiology by assessing basic aspects of the energy metabolism of the plasma in seasonal cycles (rainy and dry seasons) in the island of Maracá. The field approaches present preliminary results, suggesting that males have a stable plasma metabolic physiology between seasons more than females do, which, in turn, showed high body indexes and an increased metabolic profile during the dry season. However, in view of the perspective adopted in this study, a more rigorous and robust biomonitoring approach is needed to confirm the results obtained for this species in the island of Maracá.

The capture and *in situ* handling of *A. anableps* specimens to obtain data on their physiology required specific logistics, in addition to challenges that made the conduction of this study difficult, such as the rusticity of the research support base and the navigation on small boats to collection sites in face of extremely agitated tidal dynamics, including the phenomenon of "pororoca" and the presence of wild animals in the island. In addition, the difficulty of keeping the samples refrigerated in liquid nitrogen cylinders and stored in a freezer at –20 °C using portable generators uninterruptedly was an aggravating factor and resulted in substantial sample losses.

Biometric analyses and body indexes of males of *A. anableps* show that there were no allometric variations, as well in HSI and VSI between the dry and rainy seasons. Together with the very homogeneous plasma results, this preliminarily denotes an apparent physiological condition that is little variable or imperceptible to the biomarker considered. However, GSI, K, and body weight allowed us to infer an interrelation of these parameters with the energy investment and the reproductive cycle of these animals in a seasonal perspective.

It is interesting to note that the males of *A. anableps* in the dry season invested in the increase in body biomass, which suggests that this is not their reproductive peak. This is corroborated by the much lower GSI than that observed in the rainy season. At the same time, the condition factor was inverse to that of GSI in both seasons. Although the correlation between these parameters was not significant according to the Pearson's test, the allocation of energy resources for gonadal development was clearly not prioritized in that season.

According to Nascimento, Assunção (2008), *A. anableps* reproduces throughout the year without a defined period and there is no reproductive synchronism between males and females, that is, the peak of greater gonadal development of both sexes is inverse, which is in agreement with our preliminary findings. While males showed a greater gonadal development in the rainy season, in females this occurred in the dry season. This can be explained since this is a viviparous species and therefore related to the embryogenic process, that is, the gonadal development of females is greater after copulation. Females in this season (September 2018) also showed a higher number of embryos, which is in agreement with Cavalcante and coauthors (2012), who pointed out that September is the reproductive peak of females due to a greater gonadal development and a higher number of embryos. These results, together with the higher concentration of lipids and carbohydrates in the plasma in that season, suggest this period as the possible reproductive peak and the highest energy investment in females.

As for males, the K showed an inverse relation to GSI. This parameter allows monitoring a series of factors related to the health and ecophysiological status of fish. It is known that it is environmentally influenced by the reproductive cycle, specific characteristics intrinsic to each species, food, and even seasonal variation of abiotic factors and environmental or nonspecific stressors (Famoofo, Abdul, 2020).

The categorized interpretation of the condition factor, according to Le Cren (1951), with the standard value (k = 1.0), the females in the rainy season (2018–2019), and males in the dry season would be in adequate body conditions, while the females in the dry season and males in the rainy season (2019), would be in precarious body conditions.

Matrotrophy is a marked characteristic in *A. anableps*. Specializations in the follicular placenta, which has a portal circulatory system, ensure an adequate supply of nutrients to the embryos, a condition that denotes a high reproductive energy investment (Hagmayer *et al*., 2018). In this scenario, still little explored in the literature on Anablepidae, our results suggest that there is a simultaneous accumulation of visceral fat, a greater gonadal-embryonic development, and a high concentration of energetic substrates in the blood of females in the dry season.

We consider that our physiological results are original, preliminary, and contribute to the development of reference values for *A. anableps*, since basic plasma biochemistry parameters can be used to assess fish health status and are extremely important to compose the biochemical profile of a species (Anderson *et al*., 2010). Therefore, it can be used as baseline data in a future ecophysiological biomonitoring in Amazon Delta.

Usually, the high concentration of glucose in the plasma of teleosts, together with cortisol, is used to identify conditions of physiological stress, which may be due to environmental stressors or capture (Sopinka *et al*., 2016). However, we consider that there may have been a metabolic adjustment in *A. anableps* females, whose increased ALT activity implied gluconeogenesis and plasma hyperglycemia, since transamination is an important biochemical reaction in several metabolic processes, such as the formation of metabolites, substrate amino acids, and gluconeogenesis (Fernandes *et al*., 2008).

Liver enzymes, such as ALT, AST and ALP, have been used in biomonitoring to detect organ damage and impairment to fish health (Akbary *et al*., 2018). Studies have shown that increased ALT activity in teleost plasma is implicated in the catabolism of amino acids and the formation of alanine (Zhang *et al*., 2017). When there is a change in the metabolism of proteins and carbohydrates due to stressful situations, changes in AST and ALT activity occur (Akbary *et al*., 2018).

Although it is not possible to determine the causes for the plasma changes observed here in females in the dry season, whether due to a seasonal physiological adjustment or a non-specific stressor, it is worth mentioning the fact that in a parasitological study, specimens of *A. anableps* were found on the island of Maracá infected by ectoparasites (Esteves-Silva *et al*., 2020) and that parasitism situations as this have been reported as capable of causing a plasma increase in AST and ALT activity (Rastiannasab *et al*., 2016). This cause-effect relationship must be analyzed with caution in view of the considerations presented here.

In conclusion, the GSI of males of *Anableps anableps* is higher in the rainy season and of females in the dry season, and these differences are probably related to embryogenesis. This is in line with the literature, suggesting that the reproductive peak for each sex is in opposite periods. Plasma biomarkers (energetic substrates and liver enzymes) contribute to the composition of reference values for the physiology of this species and indicate that seasonal variation does not affect males. However, females show a greater mobilization of energetic substrates in the dry season, which coincides with the greatest gonadal development and the largest number of embryos. These results, although preliminary, show the applicability of the tested biomarkers and the relevance of *A. anableps* for ecophysiological biomonitoring studies on the island of Maracá and the urgent need for more physiological information on this species.

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## AUTHORS' CONTRIBUTION

**Maria Eduarda Gomes Guedes:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing-original draft, Writing-review and editing.
**Tiago Gabriel Correia:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing-original draft, Writing-review and editing.

## ETHICAL STATEMENT

The fish were euthanized by sectioning the spinal cord at the operculum level and subsequent decapitation according to the animal care protocols approved by the Ethics Committee on Animal Use of CEUA/UNIFAP (protocol no. 021/2018). The specimen collections were authorized by the Sistema de Autorização e Informação em Biodiversidade (SISBIO, license number: 61870–1).

## COMPETING INTERESTS

The authors declare no competing interests.

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