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SOCIAL BEHAVIOR AND HEMATOLOGICAL PROFILE OF THE DISCUS FISH *Symphysodon* spp.: SUBSIDIES FOR AQUARIUM PRODUCTION

MAIKO WILLAS SOARES RIBEIRO

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Advisor: Ph.D. Adriano Teixeira de Oliveira

Co- Advisor: Ph.D. Thaís Billalba Carvalho

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ABSTRACT

The fishing of Amazonian ornamental fish has been a source of income for traditional Amazonian peoples. Among the Amazonian species of fish, cichlids stand out for their diversity of body color patterns, territorial behavior, and good acclimatization to different breeding systems, which interest ornamental aquaculture. Within this group, the discus fish *Symphysodon* spp. stands out as a species of great interest. The manipulation of abiotic factors can favor the production of native species in an artificial system, making knowledge of the biological aspects of these species essential for the development of suitable management practice protocols. This study aimed to understand how manipulating factors such as conductivity and luminosity can affect the behavior of this species *Symphysodon* spp.. In addition, the hematological profile for the species *S. discus* was outlined. Based on the results observed, the reduction in acute conductivity can negatively influence the complexity of the behavioral repertoire of two discus species. However, high light intensity can favor the complexity of the social behavior of *S. discus*, which can favor hierarchical stability and, consequently, the choice of partners. Thus, the hematological profile of *S. discus* suggests that the species is sensitive to stress, which can be attributed to what occurs during capture. Therefore, this study contributes scientific information on the behavior and health of the species, which can be used for management protocols aimed at animal health and welfare.

Keywords: Behavior, Abiotic factors, Welfare, Fish.

RESUMO

A pesca de peixes ornamentais amazônicos tem sido uma fonte de renda para povos tradicionais da Amazônia. Dentre as espécies de peixes amazônicos, os ciclídeos se destacam pela diversidade de padrões de coloração corporal, comportamento territorial e boa aclimatação a diferentes sistemas de criação, o que interessa à aquicultura ornamental. Dentro deste grupo, os peixes discos *Symphysodon* spp. se destacam como espécies de grande interesse. A manipulação de fatores abióticos pode favorecer a produção de espécies nativas em sistema artificial, tornando o conhecimento dos aspectos biológicos destas espécies essenciais para o desenvolvimento de protocolos de práticas de manejo adequados. Este estudo teve como objetivo entender como a manipulação de fatores como condutividade e luminosidade podem afetar o comportamento de *Symphysodon* spp.. Além disso, foi delineado o perfil hematológico para a espécie *Symphysodon discus*. Com base nos resultados observados, a redução da condutividade aguda pode influenciar negativamente na complexidade do repertório comportamental de duas espécies de discus. No entanto, a alta intensidade luminosa pode favorecer a complexidade do comportamento social de *S. discus,* o que pode favorecer a estabilidade hierárquica e, consequentemente, a escolha de parceiros. Dessa forma, o perfil hematológico de *S. discus* sugere que a espécie é sensível ao estresse, o que pode ser atribuído ao que ocorre durante a captura. Portanto, este estudo contribui com informações científicas sobre o comportamento e a saúde da espécie, que podem ser utilizadas para protocolos de manejo voltados à saúde e bem-estar animal.

Palavras chaves: comportamento, fatores abióticos, bem-estar, peixe.

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LIST OF ACRONYMS

ABINPET: Brazilian Pet Products Industry Association

BW: Body weight

Cl^-: Chloride

DW: Disk width

Hb: Hemoglobin

Ht: Hematocrit

IBAMA: Brazilian Institute of Environment and Renewable Natural Resources

MCH: Mean Corpuscular Hemoglobin

MCHC: Mean Corpuscular Hemoglobin Concentration

MCV: Mean Corpuscular Volume

RBC: Erythrocyte counts

SD: Standard deviation

SECEX: Foreign Trade Secretariat

TC: Total length

SUMÁRIO

ORGANIZATION

This thesis was developed between 2021 and 2025. It is structured in 5 chapters, and the first chapter is a literature review that was converted to a book chapter (in edition) that will be published soon. The second chapter is a narrative review of the species studied. It was submitted to the journal Acta Amazonica (under review) with an impact factor of 0.8. The third chapter deals with the hematological profile of the discus (*Symphysodon discus*). This article has already been published in the Brazilian Journal of Biology with an impact factor of 1.6.

The fourth chapter contains an experiment in a controlled environment on the effect of reducing conductivity on the social behavior of two species of discus. This article is submitted to the journal Applied Animal Behavior Science (under review) with an impact factor 2.2. Finally, we have the fifth chapter, an experiment in an artificial environment on the influence of light on the social behavior and territorial defense of the discus fish *Symphysodon discus*. This article will also be submitted to the Journal of Applied Animal Behavior Science, which has an impact factor of 2.2.

1 INTRODUCTION

In the State of Amazonas, the exploration and sale of ornamental fish has been taking place for more than six decades, mainly along the middle River Negro basin, Amazonas, which has a great diversity of fish and constitutes the largest ornamental fishing area in Brazil (Prang et al., 2013; Ladislau et al., 2021). This activity plays an important economic, environmental, and social role, providing employment and income generation for riverside communities (Prang et al., 2013; Tribuzy-Neto et al., 2020; Ladislau et al., 2021).

However, as it is an extractive and rustic activity for current times, the commercialization of Amazonian ornamental fish has lost competitiveness in the international market (Santos & Fujimoto, 2015; Tribuzy-Neto et al., 2020). Over the past 30 years, the Amazon has lost a substantial portion of its international ornamental fish market to Asian countries such as Singapore, Thailand, and Malaysia, as well as to breeders in the United States and Europe (Prang et al., 2013; Tribuzy-Neto et al., 2020). These countries have breeding technology and can supply the global market with some of the Amazonian species, with higher quality and lower prices (Santos & Fujimoto, 2015; Tribuzy-Neto et al., 2020).

Because of this scenario, research focused on freshwater aquaculture and improving the efficiency of native species production is considered essential. In this sense, cultivating ornamental fish is regarded as one of the most profitable sectors of fish farming. This potential has stimulated and driven the sector, which has expanded rapidly due to the growing global and Brazilian demand (ABINPET, 2022).

According to Tribuzy-Neto et al. (2020), between 2006 and 2015, 375 species were exported, most of which went to European and Asian countries, which are currently the largest consumers. With the new normative instruction No. 10 of April 17, 2020 (IBAMA, 2020), we have a new legal framework for ornamental fish, replacing the old list of 725 permitted species with a negative list of prohibited species. The states of Amazonas and Pará in Brazil stand out as the places of origin of most of the fish sold to the regional, national, and international markets (SECEX, 2017), and it can be said that the aquarium market is one of the most promising for aquaculture, combined with the national pet market (Evans, 2016; ABINPET, 2022). Among the Amazonian fish species, cichlids, which are characterized by a divided lateral line and spines on the dorsal and anal fins, stand out in the world trade of ornamental fish due to their beauty, diversity of body color patterns, and also due to their territorial behavior and good acclimatization to farming systems (Selvatici et al., 2017). These animals have trophic and survival adaptations, inhabiting places with large amounts of decomposing plant material, rapids, areas of dense submerged vegetation, floodplain lakes, whitewater rivers (rich in nutrients and with neutral pH) and blackwater rivers (poor in nutrients and with acidic pH) (Lowe-McConell, 1999).

Representatives of the Cichlidae family also present a social organization based on the hierarchy of dominance and territorial defense, which are defined through aggressive interactions (Baerends & Baerends-Van, 1950). These behavioral repertoires are exhibited in the natural environment and are directly related to reproduction, as they involve mate selection and competition for mating sites, spawning and/or parental care (Baerends & Baerends-Van, 1950; Crampton, 2008; Mattos et al., 2016). However, aggressive behavior can be exacerbated by confinement, overcrowding and handling of individuals under conditions resulting from production systems and neutrophilia (Gauy et al., 2018; Solomon-Lane & Hofmonn, 2019).

Some cichlid species are easily kept under confined conditions, being of great interest for ornamental fishing and presenting great potential for national aquaculture. Therefore, it is essential to know the biological aspects of species of commercial interest to develop suitable management practice protocols that can contribute to maintaining organic balance and, consequently, to animal welfare (Gauy et al., 2018; Solomon-Lane & Hofmonn, 2019; Swain et al., 2020a; Swain et al., 2020b).

Environmental stimuli are crucial in modulating the life cycle. Thus, changes related to the environment can affect animals' physiological and behavioral parameters (Wingfield, 2013; Brandão et al., 2018; Ribeiro et al., 2021). According to Ribeiro & Moreira (2012), abiotic factors can be decisive for reproductive success. Factors such as water quality, light intensity, hydrological cycles and temperature are crucial in maintaining the natural reproductive cycle of fish both in natural environments and in artificial systems (Maitra & Hasan, 2016; Navarro & Navarro, 2017; Brandão et al., 2018; Shahjahan et al., 2020; Ribeiro et al., 2021).

Environmental factors are also essential in aquaculture practices, and it is common to manipulate these conditions to improve the production of species of commercial interest. However, these environmental modifications can influence growth, physiology, survival, behavior and reproduction, which are essential for production in artificial environments (Navarro & Navarro, 2017). Therefore, understanding how it is possible to improve management and reduce the stress caused by confinement, combined with good nutrition and water quality, can be decisive for the reproductive success of animals kept in artificial environments (Navarro & Navarro, 2017).

According to Schulz & Miura (2002) and Almeida (2013), teleosts depend on environmental stimuli to initiate gametogenesis, spawning and offspring development. Thus, ecological manipulation can be used to control the reproduction of animals both for maturation and to suppress spawning (Taranger et al., 2006).

It is important to emphasize that specific changes in environmental factors can interfere with biological rhythm synchronization (López-Olmeda et al., 2006; Bayarri et al., 2009). The light-dark cycle plays a vital role in influencing the daily rhythms of fish's behavioral and physiological functions (Almeida et al., 2018).

Given the above, it is considered that understanding environmental stimuli is essential for maintaining the reproductive cycle, as these factors can act as stimuli for the animal, signaling the appropriate time for reproduction (Nissling et al., 2006). According to Almeida (2013), it is essential to study how different fish species react to environmental stimuli and how the association of these other factors can contribute to understanding the physiology associated with reproduction. However, few studies evaluate the effect of the association of different factors on the reproductive success of fish. Thus, this thesis aims to evaluate the influence of light conductivity and intensity on the social behavior and hematological parameters of discus fish (*Symphysodon* spp.).

2 OBJECTIVES

2.1 General Objective

To evaluate the influence of light conductivity and intensity on the social behavior and hematological parameters of discus fish (*Symphysodon* spp.).

2.2 Specific Objective

- Assess the hematological profile of discus fish *Symphysodon* spp.
- Assess whether the reduction in water electrical conductivity modulates the display of agonistic behavior and territorial defense in discus fish.
- Assess whether increased light intensity modulates the display of agonistic behavior and territorial defense in discus fish.
- Assess whether conductivity and light intensity can influence the display of reproductive parameters (establishment of territory, choice of partner, and courtship) in discus fish.
- Assess whether social behavior (territorial and reproductive) has a daily rhythm in discus fish.
- To analyze whether environmental factors (conductivity and light intensity) modulate the daily rhythm of social behavior in discus fish.

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CHAPTER 1

Social Behavior and welfare of Fish of interest to Fish Farming

Maiko Willas Soares Ribeiro[1,2], Thaís Billalba Carvalho[3], Adriano Teixeira de Oliveira[1,2]

[1]Faculty of Agricultural Sciences, Federal University of Amazonas – UFAM, Manaus, Brazil.

[2]Morphophysiology Laboratory, Federal Institute of Education, Science and Technology of Amazonas – IFAM, Manaus, Brazil.

[3]Laboratory of Feeding Behavior and Fish Nutrition, Center of Agricultural Sciences, Environmental and Biological, Campus Cruz das Almas, Federal University of Recôncavo Bahia – UFRB.

1 LITERATURE REVIEW

1.1 Social behavior and welfare of fish of interest to fish farming

Environmental factors that modulate behavior, such as light, temperature, rainfall, and physical and chemical changes in the water, can influence the behavior of fish, especially those that depend on the hydrological cycle for reproduction (Anjos & Anjos, 2006; Crampton, 2008; Bueno et al., 2008; Ramos et al., 2015). As the competition for resources and sexual partners increases, aggressive behavior can be exacerbated (Kua et al., 2020; Santos et al., 2020; Swain et al., 2020; Krishnakumar et al., 2020).

But how can behavior be quantified? Behavioral science is not limited to descriptive procedures for animal behavior (Yamamoto & Volpato, 2006; Brandão et al., 2021). We need a quantitative method: units of measurement for behavior. With a quantitative methodology, we can design experimental studies, test hypotheses, and conduct comparative and associative analyses on aspects of behavior. In this sense, the behavior ethogram can be this tool (Yamamoto & Volpato, 2006; Brandão et al., 2021).

The ethogram can be understood as a list of behavioral units of a given species (Brandão et al., 2021). Each unit corresponds to a verb that indicates an action, such as capture (Brandão et al., 2021). It can also be described as a behavioral state, such as sleep or rest Brandão et al., 2021). Verbal compliments such as chase, flee, and attack can also be included (Brandão et al., 2021). After this structuring, each unit receives a detailed description (Yamamoto & Volpato, 2006; Brandão et al., 2021).

Every description must be replicable, and the ethogram development phase is crucial. If behavioral units are described ambiguously, this can result in errors that compromise the quality of the information (Pinho-Neto et al., 2014; Brandão et al., 2018; Gauy et al., 2018; Brandão et al., 2021). An accurate and comprehensive ethogram is a reference for scientists studying the species for which the ethogram was developed (Yamamoto & Volpato, 2006; Brandão et al., 2018; Gauy et al., 2018; Brandão et al., 2021).

Behavioral measures can be measured using latency, frequency, duration and intensity and are described as follows (Yamamoto & Volpato, 2006; Pinho-Neto et al., 2014; Brandão et al., 2021):

- **Latency:** corresponds to the time it took to display a specific behavior. Analyzing animal behavior latency is essential to understanding the duration between a stimulus and an organism's behavioral response. This measure provides valuable insights into the speed or slowness of certain behaviors.
- **Frequency** refers to the number of times a particular event occurred in a given unit of time. For example, the number of times an animal attacks another is quantified in attacks per minute.
- **Duration** refers to the time the animal displays a specific behavioral unit.
- **Intensity:** this is related to the strength of the behavior displayed. We can understand it in the following way: we can measure the intensity of the aggressiveness of several individuals. For example, an individual who presents with a higher frequency

of attacks will be considered the most aggressive and spend the most energy in the clashes.

However, we currently have several species cultivated for production and ornamental purposes. Therefore, we need a representative sample of these populations for behavioral studies. To standardize, we have some methodologies for behavioral quantification. Brandão et al. (2021) suggest some examples below.

- **Ad libitum sampling**: the observer records everything he can and observes, as well as what he finds relevant. However, the observer must be careful not to be distracted by more active individuals in the group.
- **Focal animal sampling**: the observer randomly chooses a single individual from the group to observe, recording all the behaviors displayed by that individual. This method may be complicated in a natural environment because the observed animal may escape the observer's field of vision.
- **Scanning sampling**: Several regular time intervals are determined within a period. At the end of each interval, the behavioral activities of all individuals in sight are instantly recorded.
- **Behavioral sampling**: the observer seeks to record a specific behavior, providing all the details about the individuals involved.

For social animals, the dominance hierarchy will dictate those who have priority access to resources and, consequently, reproductive success. Understanding which hierarchical position within the group is fundamental for hierarchical stability, and at this point, environmental and biological factors influence hormonal synthesis, which can increase or decrease activities and behavioral patterns. Due to this, dominant animals can reach sexual maturity (Huntingford & Tuner, 1987; Pinho-Neto et al., 2014; Brandão et al., 2018; Gauy et al., 2018; Brandão et al., 2021).

Understanding this dynamic can be fundamental for the successful farming of farm animals confined to artificial environments with limited resources. One requirement for profitability in fish farming is the homogeneity of size and weight, so the breeder must understand that animals with a dominant hierarchy in their social organization will always have animals of different sizes and weights since the dominant animals will have priority access.

Another factor that the breeder must take into account if the desired species can be kept in large groups is that the farming environment can be very stressful and can influence animal welfare (Pinho-Neto et al., 2014; Brandão et al., 2018; Gauy et al., 2018; Brandão et al., 2021). Therefore, it is essential to understand what welfare is. In its definition, welfare is the state of the individual facing or trying to face the difficulties in their environment, including their health and feelings. It must be understood that welfare is a characteristic of the individual, not the environment. In this way, the environment can provide this state to the animal or not.

Another factor about animal welfare is that it is not an absolute state but can vary over time and can be understood on a scale from very bad to very good (Pinho-Neto et al., 2014; Gauy et al., 2018). To understand welfare problems, it is essential to understand animal behavior. Through daily observations, we can associate behavioral displays with this characteristic. For example, we can observe the following factors: accelerated or no ventilation (in the case of fish, the opercular beat), heart rate, and physiological analyses such as adrenal gland function, brain chemistry and immune activity (Pinho-Neto et al., 2014; Gauy et al., 2018).

Can associate well-being with stress, need, freedom, feeling, boredom, suffering and health (Brandão et al., 2021). These factors can be measured by observation, so studying behavior is essential to meet the need for quality of life and success in raising fish in captivity.

Behavioral, physiological, immunological, and disease incidence assessments can guide the breeder in understanding whether a healthy and quality environment is provided to the raised animals and that an unbalanced farming environment can favor disturbances, recurring stress and low immune function (Brandão et al., 2021).

Records such as these of the levels of certain steroids in the blood or feces are known to be associated with stress (since they are secreted in situations involving physical injuries or the need to deal with environmental challenges) (Brandão et al., 2018; Brandão et al., 2021). Stressful environmental stimuli can cause the animal to lose hemostasis, leading to a pathological condition of endocrine imbalance (Pinho-Neto et al., 2014; Brandão et al., 2018; Gauy et al., 2018).

In a natural environment, the animal's equilibrium point can vary, providing a certain elasticity adapting to conditions that may be searching for or fleeing from predators (Makori et al., 2017; Gauy et al., 2018; Kua et al., 2020; Santos et al., 2020; Swain et al., 2020). In a captive environment, this change can subject it to constant stress, leading to the loss of the adaptive capacity found in nature and a process of biochemical, molecular, systemic, psychological and behavioral damage that can gradually undermine the organism's resistance.

Environmental enrichment's main objective is reducing stress in captive animals (Brandão et al., 2021). Stereotyped behaviors may indicate inadequate conditions. Observations of repetitive, lethargic or species-inappropriate behaviors may also be indicative. Therefore, observing the animals will help determine whether the strategy works.

Therefore, it is essential to understand that the physical environment influences animal well-being and health. An environment that respects size and accommodation, including its microclimate, must be provided. One of the main errors in raising fish in an artificial environment is overpopulation, leading to increased water nitrogen compounds. Reducing space may lead to more frequent clashes over resources, a breakdown in hierarchical stability, stress, and a drop in immunity.

Environmental enrichment can provide animal well-being. According to Carlstead et al. (1994), it is an animal management principle that seeks to improve the quality of care for captive animals by identifying and using the environmental stimuli necessary for their optimal psychological and physiological well-being. Covering specific techniques to keep captive animals constantly stimulated through stimuli from an enriched environment.

In this way, environmental enrichment will allow captive animals to behave as closely as possible to what they would in nature. It is also a way of optimizing the use of the environment, providing the animal with the possibility of more significant interaction with it (Makori et al., 2017; Gauy et al., 2018; Santos et al., 2020; Swain et al., 2020).

Another factor to be taken into account is the social environment. Social species need a social environment and company. Fish with gregarious behavior need large groups to feel safe and fed. Others, such as cichlids, need enriched environments that encourage the choice of territory mating sites or dens for protection (Teresa & Gonçalves-de-Freitas, 2009; Mattos et al., 2016; Brandão et al., 2018; Gauy et al., 2018; Brandão et al., 2021). Therefore, environmental enrichment can promote well-being.

Combined with a good physical space, a good management routine can favor productive success (Brandão et al., 2021). A good management routine allows animals to be conditioned to the farming environment. Although many fish stocks farmed for cutting and ornamental purposes today were born in captivity, their ancestors came from nature (Brandão et al., 2021). Conditioning animals to feeding routines and reducing space is essential to provide well-being and quality of life for these animals.

Animal conditioning must follow schedules and days to get used to handling biometrics or water changes in tanks or aquariums, which can be stressful events and trigger diseases. Therefore, everyone who handles animals must understand that routine handling will condition the animals to the farming environment, improving the quality of life of these individuals.

Therefore, the study of behavior and well-being must be understood and taught critically. We still lack behavioral studies aimed at well-being and how we can understand how well-being significantly influences the lives of animals, especially in a farming environment.

2.1 Factors that modulate behavior in fish

Hydrological cycles are essential for the reproductive maintenance of tropical species; these animals require environmental stimuli as a signal for spawning (Navarro & Navarro, 2017). During periods of frequent rain, hydrological systems change considerably, and water levels, conductivity, pH, turbidity, salinity and light intensity can be understood as stimuli for reproduction (Ribeiro & Moreira, 2012).

Aggressive behavior highlights competition for resources (e.g., territory and reproductive partner), where dominant animals maintain hierarchical control and priority access to resources (Huntingford & Tuner, 1987). Thus, territorial defense and aggressive interaction are fundamental in the reproductive context (Alcazar et al., 2016).

Light intensity can be considered an environmental signal to modulate social behavior and, consequently, fish reproduction (Ribeiro & Moreira, 2012; Carvalho et al., 2012; Carvalho et al., 2013). However, this regulation can be variable for different species. Light intensity can strongly affect the sexual maturation of individuals and the synthesis and secretion of sex hormones in fish. Thus, the timing, period and duration of light intensity maintain a seasonality in the reproductive activity of fish (Norberg et al., 2004; Zhu et al., 2014; Elisio et al., 2014a; Elisio et al., 2015b; Navarro & Navarro, 2017; Shahjahan et al., 2020). Korf (2006) observed that the pineal organ acts as a translator of changes in luminosity, assisting the functions of the retina and other sensory systems and regulating the circadian cycle as well as the hormone melatonin, which acts as a regulator in ovarian development (Falcon et al., 2003).

Reproduction in tropical fish depends on the synchronization of several factors, such as water quality, since seasonality and the rainy season are fundamental for these animals (Kirschbaum & Schugardt, 2002; Anjos & Anjos, 2006; Crampton, 2008; Ribeiro & Moreira, 2012; Orfão, 2013; Ramos et al., 2015). Furthermore, factors such as temperature, pH, dissolved oxygen, turbidity and conductivity undergo considerable changes during these periods (Borghezan et al., 2020). They can directly affect the display of social behavior in fish (Makori et al., 2017; Gauy et al., 2018; Kua et al., 2020; Santos et al., 2020; Swain et al., 2020; Krishnakumar et al., 2020). In the Amazon environment, with rising water levels, conductivity is strongly affected by a reduction; this factor can be understood as the signaling agent for spawning and is favorable to offspring (Kirschbaum & Schugardt, 2002; Crampton, 2008). This response has been observed for several tropical species (Kirschbaum & Schugardt, 2002; Anjos & Anjos, 2006; Crampton, 2008; Bueno et al., 2008; Ramos et al., 2015), indicating that reduced conductivity can stimulate reproduction in fish.

Amazonian rivers, especially blackwater rivers, have low ion concentrations, maintaining low electrical conductivity (Borghezan et al., 2020). Anjos (2006) conducted tests with artificial rainfall for cardinal tetra (*Paracheirodon axelrodi*), where he noted that reduced electrical conductivity and pH, associated with the level of the breeding tanks, stimulated spawning for the species. Crampton (2008) also observed the stimulation of spawning in the discus fish *Symphysodon aequifasciatus*, where the spawning of this species is synchronized with the reduction of electrical conductivity and the increase in water level in a natural environment.

Thus, it is expected that the reduction in electrical conductivity of the water can be used as a stimulus to modulate the reproductive behavior of *Symphysodon* spp. Kept in an artificial system. These results, in turn, can be used in association with the variation of another environmental variable (luminosity) to enhance the display of social behavior in discus fish.

2.2. *Symphysodon* spp. acarás ornamentals

Symphysodon spp. (Figure 1) is a genus of Neotropical cichlids characterized by a disc-shaped body; 12 to 20 cm in standard length and 25 cm in height in adulthood; smallmouth, feeding on small fish, live worms and microcrustacean nauplii; split spawning; biparental care; and inhabiting streams, lakes and riverbanks (Rossoni et al., 2014; Jesus et al., 2022) with water temperatures ranging from 26 to 30 ºC (Riehl & Baensch, 1991). According to Celik et al. (2008), *Symphysodon* needs to maintain stable water parameters since factors such as pH, conductivity and hardness are essential in the commercial breeding of discus fish.

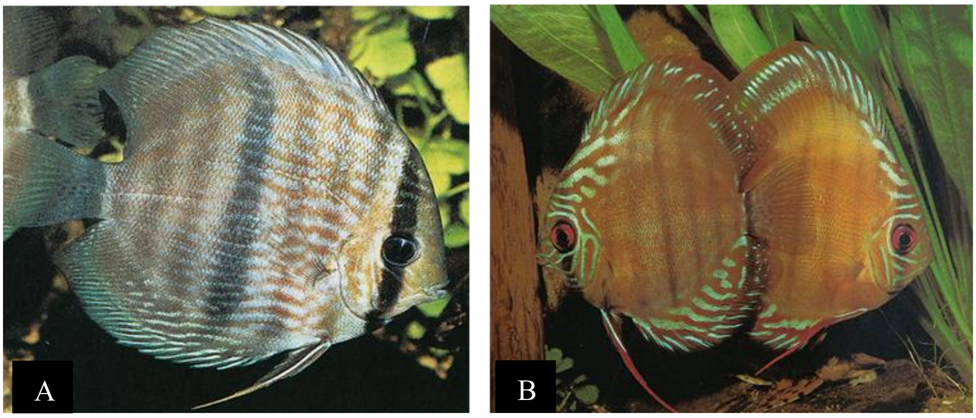

Figure 1. Discus species. (A) *Symphysodon discus* and (B) *Symphysodon aequifasciatus*. (Source: www.fishbase.org).

According to Chellappa et al. (2005), the species *S. aequifasciatus* and *S. discus* present a social organization based on a hierarchy of dominance and territoriality, involving competition for partners and spawning areas, in addition to elaborate courtship and mating behaviors. The social hierarchy is established through aggressive interactions, and these conflicts usually begin with low-intensity, energy-efficient behavioral units that are performed at a greater distance between the animals, such as displays, and costly behavioral units performed with more excellent proximity between the animals, such as threats (Maan et al., 2001). Attacks and chases, on the other hand, are usually carried out in the final phases of conflicts and contribute to their resolution, which occurs after reaching high levels of escalation and when one of the opponents gives up the conflict (Teresa & Gonçalves-de-Freitas, 2009; Mattos et al., 2016). Female discus fish choose larger mates who defend better-quality territories (Mattos et al., 2016).

During courtship and mating, females are observed to approach dominant males with territories that offer more resources (Mattos et al., 2016). Mattos et al. (2016) also describe the reproductive behavior of *S. aequifasciatus* in the following behavioral units: female permanence in the male's territory, cleaning the substrate before spawning, spawning, egg aeration, division of parental care, cleaning the substrate after hatching, and changes in body coloration of males and females. As the larvae develop, they swim erratically and may move away from the territory/substrate. At this stage, the couple displays darker coloration, which may be related to negative phototaxis behavior, that is, discus larvae are attracted to darker objects, and the coloration of adults may serve as guidance for the direction of the substrate. Parental care is performed by both parents and is characterized by the offspring's defense and the larvae's feeding with mucus produced by the couple's body (Chong et al., 2005).

This genus is widely used as an ornamental fish due to its body coloration pattern and behavioral characteristics (Tribuzy-Neto et al., 2021; Jesus et al., 2022) and, therefore, presents excellent interest for production and economic viability for ornamental aquaculture. Its price in the aquarium market can reach a high value in the foreign market, reaching a unit price of 50 to 170 dollars depending on its variant, being commercially classified by the consumer market as royal, non-royal or common (Anjos et al., 2009; Rossoni et al., 2014; Tribuzy-Neto et al., 2021; Jesus et al., 2022).

Its importance has been observed by the numbers recorded between 2006 and 2015, with exports corresponding to 0.16% of all ornamental fish exports (Tribuzy-Neto et al., 2021). Due to its importance and beauty, some studies have been directed to the genus *Symphysodon*, for example, in studies focused on biology (Cramptom, 2008), cultivation (CeliK et al., 2008; Livengood et al., 2009; Zuanon et al., 2011; ElGhany et al., 2014; Tibile et al., 2016) nutrition (Chong, et al., 2000; Chong et al., 2005; Chong, et al. 2008; Zuanon et al., 2011), embryonic development (Swain et al., 2020; Swain et al., 2020), intestinal microbiota (Zheng, et al., 2021), physiological responses to stressors (Wen et al., 2017), environmental contamination (Lemgruber et al., 2013; Huang et al., 2022), reproductive behavior (Chellappa et al. 2005; Mattos, et al., 2016), hematological profile (Paixão, et al., 2017, Ribeiro, et al. 2024), diversification patterns (Farias & Hrbek, 2008), polymorphism (Silvano, 2009), genetic diversity and phylogeny (Koh et al., 1999; Hercos, et al., 2017), parasitism (Zuanon et al., 2011), taxonomic studies (Ready et al., 2006; Bleher et al., 2007; Amado et al., 2011) and ethnobiological knowledge (Rossoni et al., 2014; Begossi et al., 2006; Mendonça & Camargo, 2006; Silvano, 2004; Ladislau et al., 2021).

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CHAPTER 2

Overview of the Discus Fish (*Symphysodon* spp.): Narrative Review Combined with Potential for Ornamental Aquaculture.

Maiko Willas Soares Ribeiro[1], Thaís Billalba Carvalho[2]; Thayson Pinto Lima[3]; Adriano Teixeira de Oliveira[3]

[1]Faculty of Agricultural Sciences, Federal University of Amazonas – UFAM, Manaus, Brazil.

[2]Laboratory of Feeding Behavior and Fish Nutrition, Center of Agricultural Sciences, Environmental and Biological, Campus Cruz das Almas, Federal University of Recôncavo Bahia – UFRB.

[3]Morphophysiology Laboratory, Federal Institute of Education, Science and Technology of Amazonas – IFAM, Manaus, Brazil.

ABSTRACT

The discus fish (*Symphysodon* spp.) is a species of Amazonian cichlid with great appeal in the ornamental fish market owing to its distinctive shape, coloration, and behavior. This study aims to conduct a narrative review on the discus fish. It is found in acidic waters and waters close to neutral in their natural environment, with low levels of nitrogenous compounds and considered warm waters. Thus, it is often regarded as a fish that demands high water quality. However, due to its genetic variability, it exhibits a wide range of adaptability, making it an excellent candidate for breeding systems. Despite being a well-known and beloved species in the aquarium hobby, large-scale breeding is still considered difficult and surrounded by myths regarding the species, discouraging professional breeders' interest.

Keywords: Discus fish, ornamental fish, cichlid, aquarism.

INTRODUCTION

In the state of Amazonas, the exploitation and sale of ornamental fish have been taking place for over six decades, mainly concentrated along the middle River Negro basin, which boasts a great diversity of fish and constitutes the largest ornamental fishery area in Brazil (Prang et al. 2013; Ladislau et al. 2021). This activity plays an important economic, environmental, and social role, providing employment and income generation for riverside communities (Prang et al. 2013; Tribuzy-Neto et al. 2020; Ladislau et al. 2021). According to Tribuzy-Neto et al. (2020), between 2006 and 2015, 375 species of ornamental fish were exported, with a significant portion going to European countries (Germany, United Kingdom, Portugal, Spain), Asian countries (China, Singapore, Indonesia), and the United States, which are currently the largest consumers. However, being an extractive and rustic activity by today's standards, the commercialization of Amazonian ornamental fish has been losing competitiveness in the international market (Santos and Fujimoto 2015; Tribuzy-Neto et al. 2020).

With the new Normative Instruction No. 10 of April 17, 2020, established by the Brazilian government (IBAMA 2020), there was a new legal framework for ornamental fish, replacing the old list of 725 permitted species with a negative list of prohibited species, facilitating the trade of ornamental fish and streamlining the process of selling and shipping fish for decorative purposes. The states of Amazonas and Pará stand out as the primary sources of fish traded in the regional, national, and international markets (SECEX 2017). Therefore, it is possible to affirm that the aquarium market is one of the most promising sectors for aquaculture, combined with the national pet market (ABINPET 2022).

Among the species of Amazonian fish, cichlids, characterized by a divided lateral line and spines in the dorsal and anal fins, stand out in the global ornamental trade due to their beauty, diverse body coloration patterns, territorial behavior, and good adaptation to cultivation systems (Selvatici et al. 2017). These animals have trophic and survival adaptations, inhabiting areas with abundant decomposing plant material, rapids, dense submerged vegetation, floodplain lakes, whitewater rivers (nutrient-rich with neutral pH), and blackwater rivers (nutrient-poor with acidic pH) (Lowe-McConell 1999).

Some species of cichlids are easily maintained under confinement conditions, making them of great interest for ornamental fishing and presenting significant potential for national aquaculture (Selvatici et al. 2017). Therefore, it is essential to understand the biological aspects of commercially important species to develop protocols of good management practices that can contribute to maintaining organic balance and, consequently, animal welfare (Gauy et al. 2018; Solomon-Lane and Hofmann 2019).

Among these commercially traded cichlid species is the discus fish *Symphysodon* spp. stands out. It is widely used as an ornamental fish due to its body coloration pattern and behavioral characteristics (Tribuzy-Neto et al. 2021; Jesus et al. 2022). Hence, it presents excellent interest for production and economic viability in ornamental aquaculture. Given the above, this study aims to conduct a narrative review on the discus fish *Symphysodon* spp.

Diversity, behavior, and reproduction

Symphysodon spp. is a genus of Neotropical cichlid characterized by a disc-shaped body, 12 to 20 cm in standard length and 25 cm in height in adulthood; small mouth, no sexual dimorphism, feeding on small fish, live worms, and microcrustacean nauplii; batch spawning; biparental care; and inhabiting streams, lakes, and riverbanks (Rossoni et al. 2014; Jesus et al. 2022) with water temperature ranging between 26 to 30°C (Riehl and Baensch 1991). It is now understood that the Symphysodon genus comprises three distinct species: *S. aequifasciatus*, *S. discus*, and *S. tarzoo*, along with other subspecies (Bleher et al. 2007; Amado et al. 2011).

In their natural environment, discus fish form large shoals near fallen trees and branches during the dry season and small shoals within flooded forests during the wet season (Crampton 2008). According to Celik et al. (2008), *Symphysodon* species require the maintenance of stable water parameters, as factors such as pH, conductivity, and hardness are essential in the commercial breeding of discus fish.

Crampton (2008) suggests that discus fish are iteroparous, reaching sexual maturity within a year, with spawning starting at the beginning of the flood season, influenced by changes in water conductivity. This reproductive strategy indicates a concern for ensuring that offspring mature by the next dry season. In this sense, spawning is influenced by biotic parameters (such as high predation on eggs, larvae, and young adults) and local and regional environmental dynamics (such as sudden increases in river water levels, locally known as "repiques"). Thus, it can be affirmed that females spawn only one batch of eggs at a time, with the most developed ones being released, while the rest are held back in case of high predation on eggs, larvae, or fry (Rossoni et al. 2014).

According to Chellappa et al. (2005), *S. discus* species exhibit social organization based on dominance hierarchy and territoriality, involving competitions for mates and spawning areas and elaborate courtship and mating behaviors (Figure 1). The social hierarchy is established through aggressive interactions, with conflicts typically starting with low-intensity, energetically economical behavioral units performed at a greater distance between the animals, such as displays, and escalating to more costly behavioral units completed at closer proximity, such as threats (Maan et al. 2001). Attacks and chases usually occur in the final stages of conflicts and contribute to their resolution after reaching high escalation levels and when one of the opponents gives up the conflict (Teresa and Gonçalves-de-Freitas 2003; Mattos et al. 2016).

Female discus fish choose more significant partners and defend territories of better quality (Rossoni et al. 2014; Mattos et al. 2016). During courtship and mating, females approach dominant males with territories that offer more resources (Mattos et al. 2016). Mattos et al. (2016) also describe the reproductive behavior of *S. aequifasciatus* in the following behavioral units: female staying in the male's territory, substrate cleaning before spawning, spawning, egg aeration, parental care division, substrate cleaning after hatching, and changes in male and female body coloration. As the larvae develop, they swim erratically and may move away from the territory/substrate. In general, discus larvae exhibit characteristics similar to those of other cichlid species and are altricial, meaning that when the larvae start swimming, they already have all functional structures (Onal et al. 2009; Mattos et al. 2015; Satoh et al. 2016).

During this parental care phase, the pair displays darker coloration, possibly related to negative phototaxis behavior. This means that discus larvae are attracted to darker objects, and the coloration of adults can serve as guidance toward the substrate. Parental care is carried out by both parents and is characterized by defending the offspring and feeding the larvae with mucus produced by the couple's bodies (Chong et al. 2005; Khong et al. 2009; Wen et al. 2020; Wei et al. 2021).

Buckley et al. (2009) suggest that parental care occurs equally for males and females during this phase. As the offspring consume parental mucus and bites, the exchange between parents increases the frequency of parental care shifts, with no significant difference between males and females. The mucus provided at this stage presents interesting peculiarities; studies in this regard have observed that when parents are caring for the offspring, the mucus exhibits different protein concentrations and even differences between sexes, providing a source of immunity, nutrition, and hormones essential for the survival of the offspring in the acidic and ion-poor Amazon environment.

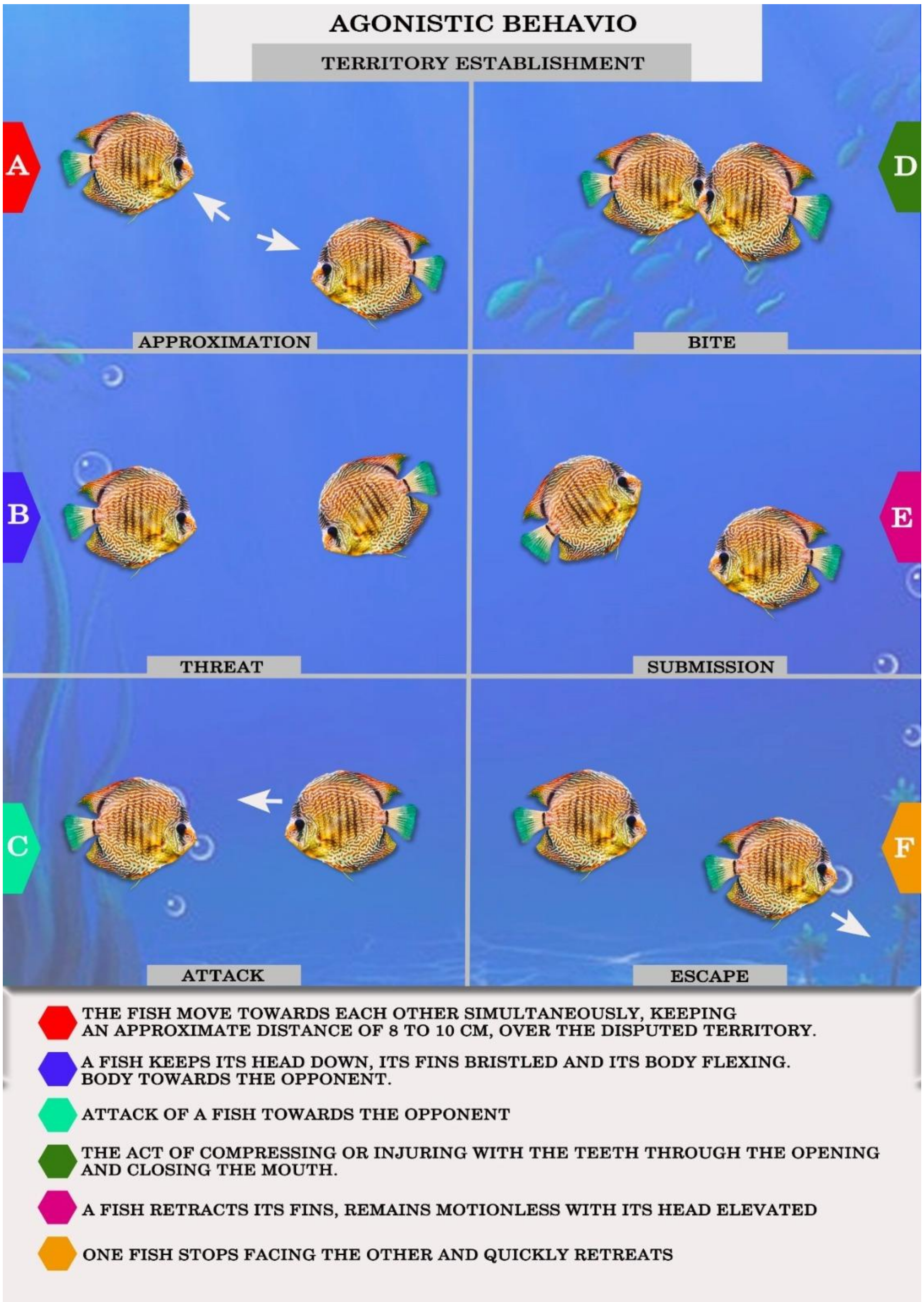

Figure 1. Social hierarchy is established through aggressive interactions.

This peculiarity suggests that the discus fish exhibits similarities in parental care to that of mammals and birds, demonstrating a high level of complexity in its unique behavioral repertoire (Buckley et al. 2010). Understanding such peculiarities can guide professional breeders, as it is understood that offspring require adequate nutrition during this critical phase. Based on this requirement, artificial mucus with these properties can be provided, increasing the efficiency of artificial breeding. While natural mucus is essential for the survival and growth of the offspring, it can also be a source of contaminants and parasites from the parents (Maunder et al. 2013; Sylvain and Derome 2017), making artificial mucus an alternative in captive production.

Taxonomy

Since its description, the genus *Symphysodon* has had a confusing taxonomic classification. Until 2006, it was understood that there were two species recognized in the scientific literature: *Symphysodon discus* Heckel, 1840, and *Symphysodon aequifasciatus* Pellegrin, 1904 (Kullander 1986, 1996), and four subspecies in popular literature: *S. discus willischwartzi* Burgess, 1981 (pineapple phenotype), *S. discus* tarzoo Lyons, 1959 (green phenotype), *S. aequifasciatus haraldi* Schultz, 1960 (blue phenotype), and *S. aequifasciatus axelrodi* Schultz, 1960 (brown phenotype), with the nominal subspecies *S. discus* Heckel, 1840 (Heckel phenotype), and *S. aequifasciatus aequifasciatus* Pellegrin, 1904 (green phenotype), being restricted to just one central phenotype (Amado et al. 2011).

Revisions in 2007 suggest the existence of three species in the genus *Symphysodon*: *Symphysodon discus* Heckel, 1840, *Symphysodon aequifasciatus* Pellegrin, 1904, and *Symphysodon haraldi* Schultz, 1960 (Bleher et al. 2007), with new revisions in 2011 understanding Symphysodon to comprise five significant evolutionary units: *S. discus* (Heckel and pineapple phenotypes), *S. aequifasciatus* (brown phenotype), *S. tarzoo* (green phenotype), *Symphysodon* sp. 1 (blue phenotype), and *Symphysodon* sp. 2 (Xingu group) (Amado et al. 2011).

Morphological and molecular analyses suggest a phylogenetic relationship of *Symphysodon* with *Uaru*, *Heros*, *Mesonauta*, and *Pterophyllum* (Farias et al. 2000; Amado et al. 2008; Mesquita et al. 2008; Schneider et al. 2015). Analyses of discus fish diversification indicate that the species is undergoing diversification, with taxonomic classification remaining controversial (Farias and Hrbek 2008; Silva et al. 2008; Gross et al. 2010b; Li et al. 2015a; Li et al. 2015b; Yu et al. 2015) (Table 1). Such diversity helps us understand the requirements regarding habitat water parameters such as pH and conductivity.

Another genetic peculiarity of this genus is that males and females express genes from their testes and ovaries differently. This difference can be very efficient in captive breeding, especially considering the species does not exhibit sexual dimorphism (Lin et al. 2017; Fu et al. 2020). Genetic tools can also be highly effective in selecting breeding stock.

Gross et al. (2010) suggest that *S. discus* is the oldest species, which could have hybridized with an ancestral species of discus that may now be extinct. This helps us understand that crossings between species occur naturally in the natural environment. Understanding this supports the assertion that the discus fish is not a fragile species; on the contrary, due to its genetic diversity, it exhibits plasticity in responding to changes in the physicochemical properties of water, favoring its adaptation to artificial cultivation environments (Gross et al. 2010).

Table 1. Common names and taxonomic classification of discus fish modified from (Livengood et al. 2010).

Common Name	Scientific Name	Region	Author
Common Disc	*Symphysodon* spp.	Amazon basin	Farias and Hrbek 2008
Heckel	*Symphysodon discus*	Rio Negro basin and central-northern Amazon basin	Shultz 1960; Burgess 1981; Kullander 1996; Bleher et al. 2007; Amado et al. 2011
Abacaxi	*Symphysodon discus willischwartzi* (phenotypes Heckel e abacaxi)	Central and southern Amazon basin	Shultz 1960; Burgess 1981, Amado et al. 2011
Blue and Brown	*Symphysodon aequifasciatus*	Central and eastern Amazon basin	Pellegrin 1904; Shultz 1960; Burgess 1981; Kullander 1996, 2003; Bleher et al. 2007, Amado et al. 2011
Blue (Green phenotype)	*Symphysodon aequifasciatus aequifasciatus*	Central Amazon basin	Pellegrin 1904; Shultz 1960; Burgess 1981; Amado et al. 2011

Brown (Blue phenotype)	*Symphysodon aequifasciatus axelrodi*	Eastern Amazon basin	Shultz 1960; Burgess 1981; Amado et al. 2011
Blue and Brown	*Symphysodon haraldi*	Central and eastern Amazon basin	Bleher et al. 2007
Green	*Symphysodon aequifasciatus haraldi*	Western Amazon basin	Shultz 1960; Burgess 1981; Bleher et al. 2007
Tarzoo (Green phenotype)	*Symphysodon tarzoo*	Western Amazon basin	Ready et al. 2006; Bleher et al. 2007; Amado et al., 2011
Xingu	*Symphysodon* sp. 1 (Blue phenotype) e *Symphysodon* sp. 2 (Xingu group)	Eastern Amazon (Xingu River)	Farias and Hrbek 2008; Amado et al. 2011

Physiology

Similar to other Amazonian cichlids, the discus fish exhibits significant adaptability because the Amazonian environment undergoes constant changes due to its different seasonal periods. In this sense, the discus fish adapts to moderate hypoxia conditions, where the heart undergoes suppression of oxidative metabolism, followed by activation of anaerobic glycolysis, as observed by Chippari-Gomes et al. (2005) (Figure 2). They also suggest that such adaptation is related to its habitat preference, as it favors well-oxygenated areas.

Studies focused on exposure to low pH have demonstrated that *S. discus* is more tolerant to acidic waters, being more efficient in maintaining ionic balance under acidic and ion-poor conditions (Duarte et al. 2013). This observation aligns with what breeders have empirically noted, which is that *S. discus* requires acidic water in its management.

Another peculiarity of discus physiology is its resistance to temperature-related oxidative stress. Jin et al. (2021) observed a specific thermal resistance of the species. Their experiments involving temperature reduction in two discus species (*S. haraldi* found in the central region of the Amazon basin - Manacapuru River, and *S. aequifasciatus* found in the western region of the Amazon basin - Tefé River) determined that *S. haraldi* exhibits more excellent resistance to temperature reduction than *S. aequifasciatus*. Depending on the geographical area and speciation, different discus species may show distinct responses and tolerance to thermal stress (Wen et al. 2018; Jin et al. 2021). In this regard, it is understood that a widely spread notion among discus breeders that discus fish must be kept at 28°C is inaccurate. Discus fish have a wide range of thermal tolerance, capable of modifying metabolic pathways for physiological regulation in response to cold stress or temperature changes (Wen et al. 2018). However, being a tropical cichlid, they prefer warm waters.

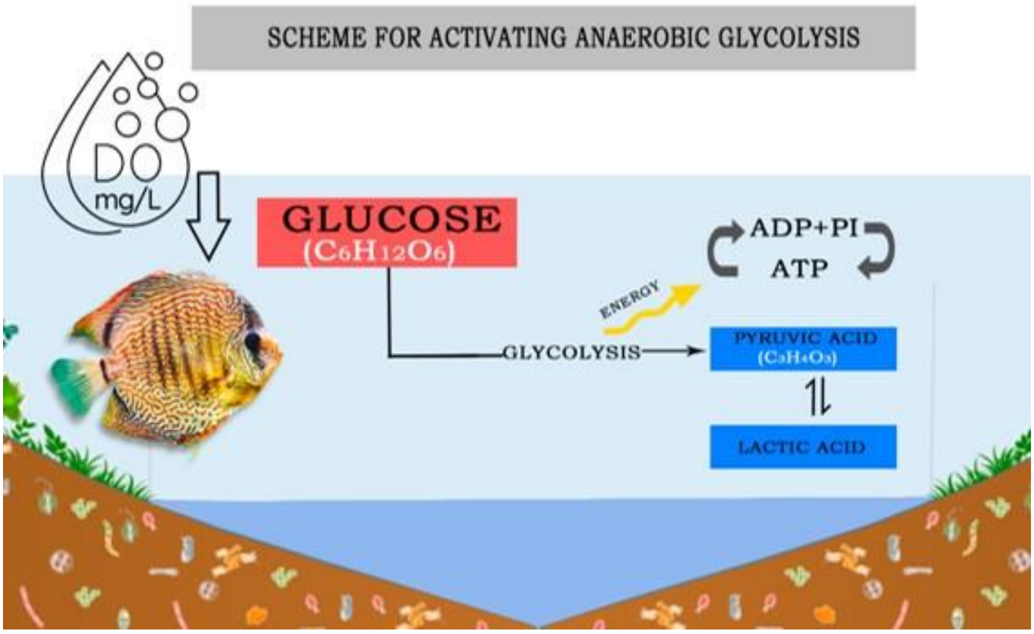

Figure 3. Metabolic adaptability of discus fish under low oxygen conditions.

The discus fish is also well-known for its striking coloration. There are various colors in both wild specimens and hybrids. Recent studies have observed that discus coloration is linked to metabolism. Yang et al. (2021) found that discus fish have specific chromophores in the skin associated with metabolic pathways. This is particularly interesting when related to behavioral displays observed in discus fish. A notable color change occurs when the fish is stressed or shows signs of illness. Such results aid in understanding the composition of body coloration and even discus behavior (Ng et al. 2023).

Studies on blood parameters demonstrate that discus fish naturally have a low number of monocytes and neutrophils, indicating that discus fish have lower resistance to pathogens than other cichlids (Paixão et al. 2017). This suggests that discus breeders face challenges, as these animals are prone to illness in captivity. However, it is not necessarily that the species is more fragile than others, but rather, it is more related to improper handling and prolonged stress during capture and management (Rossoni et al. 2014; Paixão et al. 2017).

Diseases

Among the ailments affecting discus fish, the most common are those caused by gill parasites, such as (*Sciadicleithrum* spp.) monogeneans, protozoa (*Hexamita intestinalis*) hole-in-the-head disease, (*Ichthyophthirius multifiliis*), and bacteria, mainly of the genus Aeromonas, as well as other intestinal parasites (Paull and Matthews 2001; Yanong et al. 2004; Moravec and Laoprasert 2008; Guz and Szczepaniak 2009; Rahmati-Holasoo et al. 2010; Onal et al. 2011; Aquaro et al. 2012; Mohammadi et al. 2012; El-Ghany et al. 2014; Košuthova et al. 2015; Roh et al. 2019; Amesberger-Freitag et al. 2019; Satora et al. 2022).

Parasites, combined with improper management of feeding and water quality, favor the onset of diseases and productivity loss due to the discus fish's lower resistance to pathogens, leading to the loss of entire batches. Another issue for breeders is combating these pathogens, for which there is still no specific literature on treatment (Yanong et al. 2004; El-Ghany et al. 2014).

Trials conducted by El-Ghany and colleagues (2014) with two chemotherapeutic agents and physical management indicate the effectiveness of metronidazole against a mixed infection of flagellated protozoa. According to the study, when tested with 5 ppm of metronidazole for 12 hours, for 3 days, with 50% water changes before medication administration each day, the animals recovered from the infection within 2 weeks after treatment, with complete disappearance of clinical signs. For Group 2, treated with ciprofloxacin (5 ppm for 5 days and 50% water changes before medication administration), it was observed that they did not fully recover from the infection, and two weeks after treatment, the mortality rate was 50% of the fish.

The treatment results indicated that the physical-chemical management with metronidazole and partial water changes effectively overcame parasitic and bacterial infections, with no morbidity and mortality after two weeks (El-Ghany et al. 2014). It is worth noting that it is not only the use of chemotherapeutic agents that will combat such diseases; the water is naturally inhabited by bacteria, fungi, and omnipresent parasites, and any stress factors can cause diseases in fish (El-Ghany et al. 2014; Paixão et al. 2017).

Studies on managing discus stocks raised in captivity demonstrate that sanitary control of stocks is fundamental. Aquaro et al. (2012) observed infestation of Dactylogyridae due to improper management, as young animals were kept with adults, which ended up infesting them, as the adults were asymptomatic about the parasite, leading to significant losses. Therefore, physical management and chemical treatment should be applied if the disease becomes established (El-Ghany et al. 2014).

Nutrition

In their natural environment, discus fish feed on small crustaceans, insect larvae, periphyton, and algae (Crampton 2008; Rossoni et al. 2014; Jesus et al. 2022) (Figure 3). Therefore, in a captive environment, a balanced and varied diet can promote good production and health of these animals.

From their larval stage, discus fish require a diet rich in protein. Studies on the feeding of offspring indicate that parents, during the parental care phase, alter the protein composition of the mucus, indicating the need for diets with high protein concentrations (Khong et al. 2009; Buckley et al. 2010; Wen et al. 2020; Zhang et al. 2021). Studies involving the inclusion of probiotics in the diet of young individuals demonstrate enhanced fish immunity (Sanaya 2022), indicating the need for diets rich in proteins and amino acids, considering that discus fish are susceptible to low immunity under stress.

In this regard, protein-rich diets can pose challenges for breeders as they increase production costs in captivity. However, plant-based diets consisting of soybean and wheat flour can be efficient for the nutrition of the animals (Chong et al. 2002, 2003). Along with an efficient protein diet, the addition of vitamins C and E, natural carotenoids (such as astaxanthin), and minerals can be adequate for growth, improved immunity, and coloration of discus fish (Liu et al. 2014, 2018; Song et al. 2016; Haque et al. 2020; Liu et al. 2021). Studies on protein requirements determine a crude protein diet ranging from 44.9% to 50.1% (beef heart). Therefore, a diet rich in animal protein should be included in the feeding of these animals, with both dry and moist feeds being effective for captive breeding (Chong et al. 2000; Sales and Janssens 2003; Wen et al. 2018a, 2018b; Santos et al. 2022).

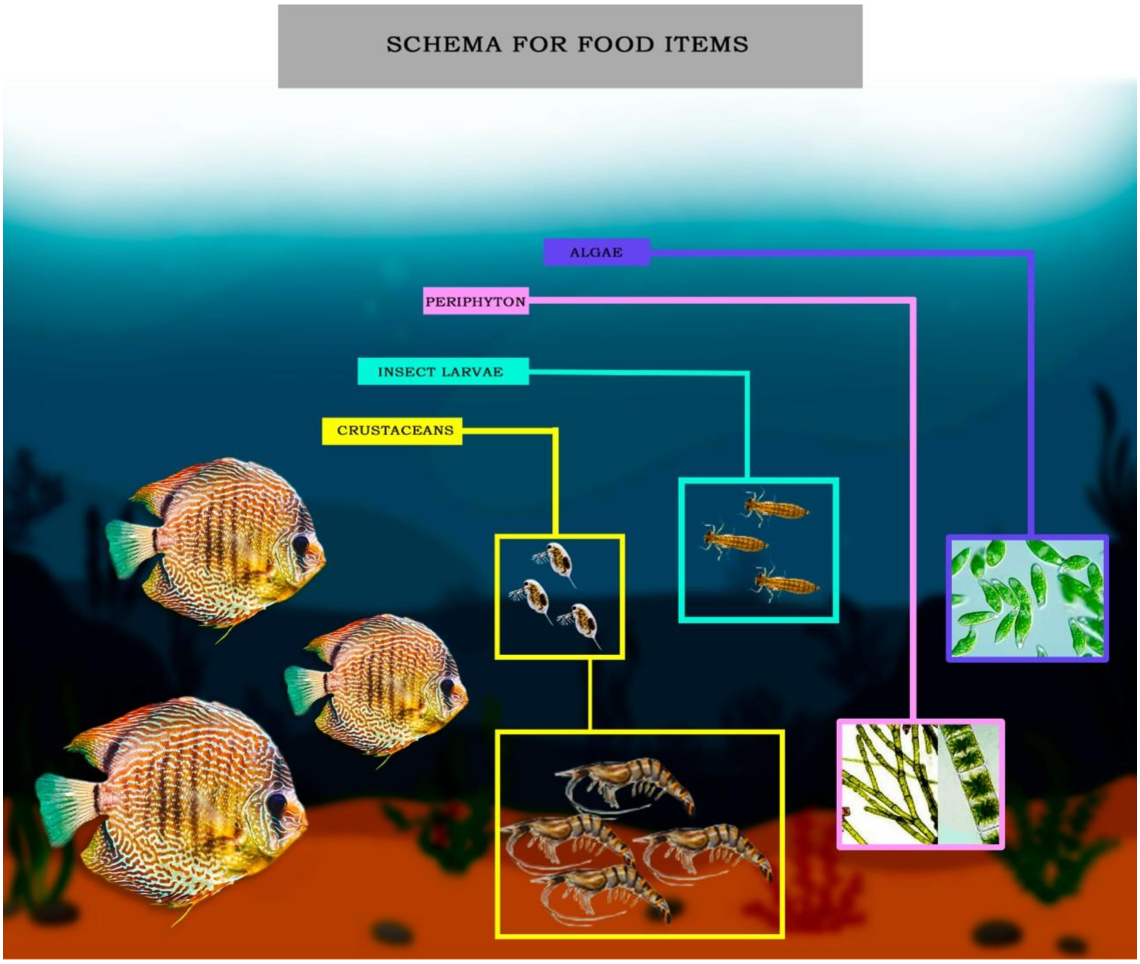

Figure 4. Main foods of discus fish in their natural habitat.

Water Quality

Discus fish also require excellent water quality (Celik et al. 2008). In this regard, breeders worldwide invest in sophisticated filtration systems. Like other fish species, discus fish need good water and stable parameters (Celik et al. 2008). Therefore, the aquarist or breeder must understand how aquatic systems function. In Amazonian aquatic systems, soft water with low salt content is observed, high oxygenation, no nitrogenous compounds, and low-temperature variation (Lowe-McConell 1999; Crampton 2008; Celik et al. 2008). Thus, discus fish require similarity in parameters in an artificial environment.

Due to its presence in different Amazonian environments, discus fish exhibit a certain plasticity regarding parameters, with a pH tolerance range between 4.5 to 7 (Duarte et al. 2013), showing good adaptability in slightly acidic water (pH 6.8) in artificial environments (Celik et al. 2008). However, in static systems with low water renewal, pH levels close to neutral or even slightly alkaline can lead to the accumulation of nitrogenous compounds, potentially causing stress and even mortality, as discus fish have a low tolerance to high concentrations of ammonia and nitrite in the water (Celik et al. 2008).

In this sense, breeding systems for discus fish should be designed to provide a stable environment with minimal water quality variation and the least possible amount of nitrogenous compounds (Din et al. 2002; Livengood et al. 2010; Celik et al. 2008; Kristiany and Prabowo 2022), which can be static recirculation systems, provided there are good water exchange regimes and proper management.

Market and Production

Discus fish are sought after by all aquarium enthusiasts due to their coloration, shape, peaceful behavior, and high value. However, their captive production is challenging (Din et al. 2002; Livengood et al. 2010; Kristiany and Prabowo 2022), as they are captured in different environments where the water is acidic, black, and clear with a pH of 5 or below, as well as poor in nutrients, and also in white waters with pH close to neutral. Thus, it can be very challenging for breeders to provide a favorable environment for reproduction (Riehl and Baensch 1991; Crampton 2008; Livengood et al. 2010; Farias and Hrbek 2008; Gross et al. 2009; Rossoni et al. 2014).

The larviculture of the species in captivity becomes another challenge in production because these animals exhibit parental care, with the larvae being dependent on the initial feeding provided by the parents (Rossoni et al. 2014; Mattos et al. 2016). Additionally, there are no significant differences between males and females, making choosing and selecting breeding stock difficult (Livengood et al. 2010). Despite these difficulties, Asian countries such as China, Singapore, Indonesia, and Thailand have excelled in producing this species (Din et al. 2002).

In the quest for large-scale production, breeders have used different breeding systems, from closed systems to recirculation systems (in aquariums), considering the species' requirement for high water quality. Recirculation systems have proven to be efficient in breeding (Din et al. 2002; Livengood et al. 2010; Kristiany and Prabowo 2022). Different strategies to encourage spawning are employed by breeders, such as significant water changes, temperature increase, slight pH reduction, and use of deionized and reverse osmosis water (Livengood et al. 2010; Celik et al. 2008). However, there is still no definitive protocol for discus fish reproduction, so each producer uses their reproductive management, causing confusion and the creation of myths around discus fish production in captivity.

Regarding economic value, discus fish are among the top 10 species for the ornamental fish trade (Livengood et al. 2009). Their price in the aquarium market can reach a high value in the foreign market, ranging from $50 to $170 per unit depending on the variant, commercially classified by the consumer market as royal, semi-royal, or typical (Figure 4) (Livengood et al. 2010; Anjos et al. 2009; Rossoni et al. 2014; Tribuzy-Neto et al. 2021; Jesus et al. 2022).

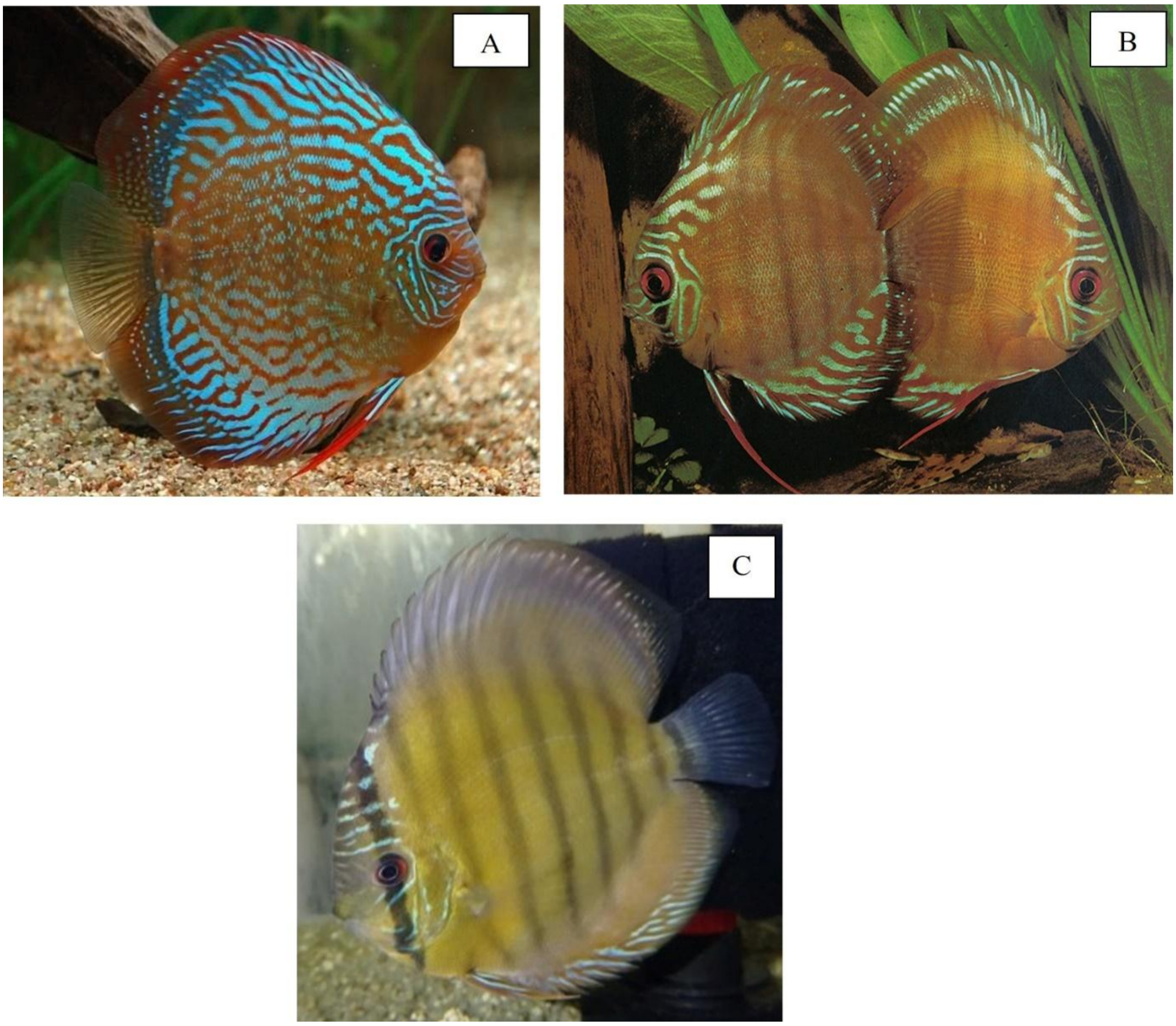

Figure 5. Classification commonly used by traders. (A) Royal; (B) Semi-royal; (C) Common.

Its importance has been observed in the numbers recorded between 2006 and 2015, with exports accounting for 0.16% of all ornamental fish exports from Brazil (Tribuzy-Neto et al., 2021). Unlike other producing countries such as China, Thailand, Singapore, the USA, and Germany, Brazil only exports wild specimens because it does not have significant captive production, failing to meet domestic demand.

Perspectives

When we observe national production, it is evident that we need to improve. We have not yet fully mastered the breeding and larviculture of the species, which means it does not attract the interest of professional breeders. This is due to a lack of scientific information. Although we have various studies on discus fish, these works remain confined to academia with low visibility.

In recent years, the market for discus fish has grown within Brazil. The interest in good quality wild and hybrid specimens has attracted the attention of national breeders and hobbyists. With the growth of social media, groups, and forums dedicated to discus breeding have encouraged interest and stimulated the consumer market. Currently, Brazil has a Brazilian Discus Fish Confederation (CBRAD), which, in partnership with the Federal University of Rio Grande do Norte - UFRN, organizes exhibition contests for national breeders and hobbyists, facilitating outreach activities between academia and the production market. This has strengthened the hobby and fostered interest in the activity.

However, we still lack information and educational materials for breeders and hobbyists. In this regard, universities and research institutes can provide scientifically based materials with language tailored to this audience. Through pamphlets, technical notes, and easily accessible booklets, the need for reliable information about this species, which arouses interest among hobbyists and breeders worldwide, can be met.

Conclusion

Among the Amazonian species with ornamental interest, the discus fish is an excellent candidate for large-scale production. However, its larviculture remains a bottleneck for most commercial breeders. With research focused on reproduction, nutrition, and health, developing an appropriate and replicable management protocol is possible. Due to the prices practiced in the ornamental fish market, investment in aquaculture technologies and systems is favorable for the commercial production of this species, considered the king of aquariums.

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CHAPTER 3

Hematology in ornamental discus fish *Symphysodon discus* from Amazonian, Brazil

Maiko Willas Soares Ribeiro[a], Ariany Rabello da Silva Liebl[b]; Adriano Teixeira de Oliveira[a,b]

[a]Graduate Program in Animal Science and Fisheries Resources (PPGCARP), Faculty of Agricultural Science (FCA), Federal University of Amazonas (UFAM), University Campus, Manaus, Amazonas, Brazil.

[b]Animal Morphophysiology Laboratory, Analytical Center, Federal Institute of Education, Science and Technology of Amazonas (IFAM), Manaus Centro Campus (CMC), Manaus, Amazonas, Brazil

*Correspondence: adriano.oliveira@ifam.edu.br Avenue 7 de setembro, 1975, Centro, 69020-120.

Abstract

Symphysodon discus is known in the world of aquariums for its market value, beauty, and behavior. However, more substantial information about its physiology and biology must be available, which can hinder its development and maintenance in breeding systems. The study evaluated the blood biochemistry and erythrogram of 20 specimens of *S. discus* captured in the municipality of Barcelos, Amazonas, with an average weight of 89.80 ± 7.13 g and an average length of 13.48 ± 0.55 cm. The erythrogram evaluated variables such as hematocrit (Ht), hemoglobin (Hb), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Blood biochemistry was analyzed, including cholesterol levels, total proteins, triglycerides, glucose, urea, and chlorides. Intra-specific variations were observed between the surveyed individuals about the Hb, MCV, and MCH values. The values of triglycerides, cholesterol, and chlorides were elevated compared to other cichlids. This study may be useful to serve as a parameter to indicate the normal health conditions of this Amazonian cichlid. It can be applied in studies for ornamental fish farming and actions for managing and conserving the species.

Keywords: physiology; blood; wild specimens; cichlid.

Introduction

The main drivers of the ornamental fish trade are the aquarium consumers, who determine the species to be produced in aquacultures or caught in the wild (Din et al., 2002; Ribeiro et al., 2021). In general, characteristics such as striking colors, the rarity of the species, and behavioral peculiarities make the species attractive to aquarists (Jesus et al., 2020; Lemos et al., 2015; Santos et al., 2012).

The Amazonian species that comprise the genus Symphysodon (*S. aequefaciatus, S. discus*, and *S. tarzoo*) attract aquarists worldwide due to their body coloration pattern and behavioral characteristics (Rossoni et al., 2014; Yang et al., 2021). These cichlids have disk-shaped bodies, and small mouths, inhabit igarapés, lakes, and riverbanks with water temperatures ranging between 26 and 30 ºC, and have good resistance to thermal variability (Rossoni et al., 2014).

The discus fish *Symphysodon discus* is economically significant in the national and international ornamental fish market (Tribuzy-Neto et al., 2021). However, scientific information on its physiology, health, and adaptation in aquaculture is scarce. The high mortality when out of the natural environment, due to stress and low immunity, problematizes the production of *S. discus* (Tavares-Dias et al., 2004).

Among the tools used to understand animal physiology and health, the analysis of the hematological profile, which includes erythrogram and blood biochemistry, stands out. Hematology allows practical and helpful identification of the stress level, enabling the correction in management and ensuring animal welfare (Magro et al., 2015; Oliveira et al., 2021; Santos et al., 2024; Ranzani-Paiva et al., 2013). In addition to stress, factors such as life stage, type of environment, population genetics, and diet can influence the responses of hematological analyses (Tavares-Dias et al., 2004). The current study verified the hematology of wild specimens of discus fish S. discus captured in the natural environment in the municipality of Barcelos, Amazonas, Brazil.

Material and Methods

Specimens of discus fish of *S. discus* (N= 20) were captured with "rapiché" (hand net) in flooded areas of the Daracuá community and Mariuá Archipelago, located in the municipality of Barcelos, Amazonas, Brazil (Figure 1). The experimental procedures adopted during these animals' capture and blood analysis were registered and approved by the Brazilian Institute of the Environment and Renewable Natural Resources-IBAMA under Process No. 15116-1. The research was developed and approved by the Ethics Commission on the Use of Animals (ECUA) of the Federal University of Amazonas under Process No. 2015/010.02.0905. All experiments were conducted according to local and ARRIVE guidelines (Percie du et al., 2020).

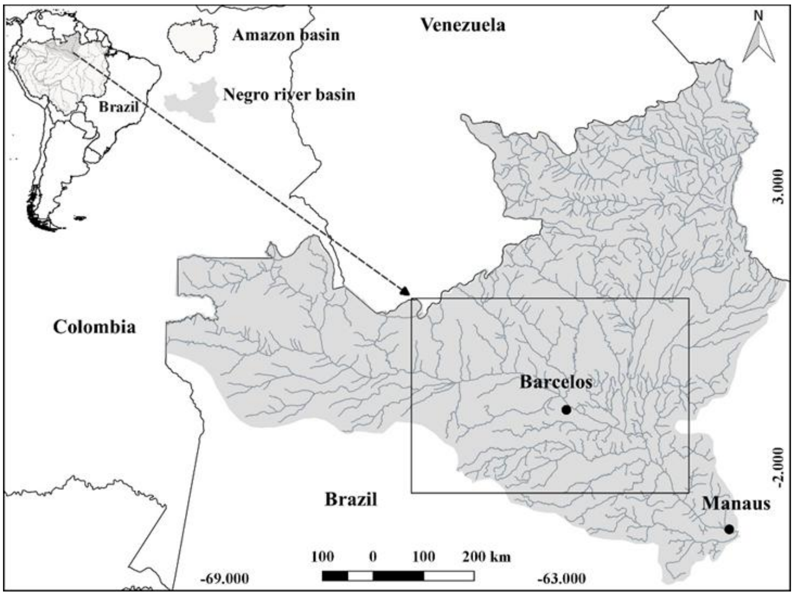

Figure 6. Location of the study area Barcelos, Amazonas, Brazil.

The collected fish were stocked in "curries" (pens) (wooden tanks) until they were transported in canoes with "caçapas" (plastic containers with about 20 L^{-1} of water) to the city of Barcelos, Amazonas. After that, the animals were anesthetized with eugenol (0.2 $g.L^{-1}$) for blood collection by caudal puncture, using disposable syringes previously moistened with the anticoagulants heparin 2500 IU. After blood withdrawal, standard length (cm) and weight (g) were determined using tape and portable scales, respectively.

The collected blood was divided into two aliquots: one for determining red blood parameters and the other for obtaining plasma and performing assays on biochemical constituents. Erythrocyte counts (RBC) were conducted in a Neubauer chamber after dilution in formalin-citrate solution; hematocrit (Ht) was determined using the microhematocrit method;

and the hemoglobin (Hb) concentration was found using the cyanmethemoglobin method. Through these data, the following red cell indexes were calculated: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) (Anselmo et al., 2021; Bussons et al., 2021; Oliveira et al., 2017; Aride et al., 2020).

Plasma was obtained after centrifugation and frozen in liquid nitrogen (-86 ºC) until the biochemical analyses. Glucose, triglycerides, total cholesterol, total protein, urea, and chloride (Cl^-) concentrations were determined using enzyme-colorimetric methods, with quantification using commercial kits (Labtest®, Brazil) (Oliveira et al., 2016; Castro et al., 2016). The data were tabulated, and descriptive statistics were used, using mean, standard deviation (SD), and maximum and minimum values.

Results

In the biometric variables of the fish analyzed, a little intra-specific alteration was observed in total length (TC) and body weight (BW), with TC varying between 12.40 and 14.00 cm and BW varying between 77.00 and 104.00g (Table 1).

The erythrogram of the acará disco (Table 2) showed wide variation in the values of Hb (Hemoglobin; minimum of 2.51 and a maximum of 21.11 g dL^{-1}), MCV (Mean corpuscular volume; minimum of 101.82 and a maximum of 718.31 fL), and HCM (Mean Corpuscular Hemoglobin; minimum of 9.98 and maximum of 91.64 pg). The levels of cholesterol, total proteins, triglyceride, glucose urea and chloride are demonstrated in Table 3.

Table 2. Biometric variables of specimens of *Symphysodon discus* captured in Barcelos, Amazonas, Brazil.

Variables	Mean	Standard Deviation	Minimum	Maximum
Standard length (cm)	13.48	0.55	12.40	14.00
Weight (g)	89.80	7.13	77.00	104.00

Table 3. Erythrogram of specimens of *Symphysodon discus* captured in Barcelos, Amazonas, Brazil.

Variables	Mean	Standard Deviation	Minimum	Maximum
Ht (%)	34.11	10.23	20.00	51.00
Hb (g dL^{-1})	7.08	4.27	2.51	21.11

RBC (millions µL^{-1})	1.80	0.76	0.56	2.99
VCM (fL)	245.37	164.9	101.82	718.31
HCM (pg)	44.78	23.73	9.98	91.64
CHCM (g dL^{-1})	21.45	11.59	9.80	49.10

Table 4. Plasma metabolites of specimens of *Symphysodon discus* captured in Barcelos, Amazonas, Brazil.

Variables	Mean	Standard Deviation	Minimum	Maximum
Cholesterol (mg dL^{-1})	298.97	81.32	95.71	442.57
Total Proteins (g dL^{-1})	2.08	1.14	0.63	4.51
Triglyceride (mg dL^{-1})	119.28	44.82	33.33	213.07
Glucose (mg dL^{-1})	53.98	34.80	11.74	140.41
Urea (mg dL^{-1})	31.85	7.20	6.96	43.01
Chloride (mEq L^{-1})	141.68	18.12	81.21	169.07

Discussion

According to Rossoni et al. (2014), the adult *S. discus* has a body measuring between 12.00 and 20.00 cm. Thus, the data in Table 1 (total length: 13.48 ± 0.55 cm) show that the *S. discus* captured for the current study is in the adult phase. The fishes analyzed presented high hematocrit (Ht: 34.11 ± 10.23 %) and mean corpuscular volume (MCV: 245.37 ± 164.9 fL) about the cichlids *S. aequifasciatus* (blue Discus; MCV: 121.80 ± 45.67 fL and Ht: 17.11 %) and *Geophagus brasiliensis* (papa terra acará; MCV: 121.80 ± 45.67 fL and Ht: 5.68 ± 0.87 %) reared outside the natural environment and studied by Paixão et al. (2017) and Romão et al. (2006), respectively. However, when compared to the cichlids *Cichla monoculus* (yellow tucunaré), *Cichla temensis* (tucunaré açu), and *Cichla vazzoleri*, in the natural environment, verified by Castro et al. (2020) regarding Ht (between 40.37 ± 1.17 % and 40.40 ± 1.06 %) and VCM (between 222.94 ± 25.94 fL and 246.11 ± 20.46 fL), the data of the present study are shown to be lower. Lower hematological values show the animal has lentic behavior (Tavares-Dias et al., 2004). At the same time, high values can be attributed to the constant movement of the animal for foraging or escape from predators. The comparison between the cichlids shows the possible influence of the natural environment and the aquaculture production environment on the hematological parameters of *S. discus*.

The mean corpuscular hemoglobin (44.78 pg) and the corpuscular mean hemoglobin concentration (21 45 g.dL^{-1}) of *S. discus* (Table 2) showed lower values than those of tucunarés *C. monoculus*, *C. temensis*, and *C. vazzoleri* (Castro et al., 2020). Although both are cichlids in the natural environment, this difference can be attributed to factors that influence hematological parameters such as temperature, dissolved oxygen, seasonal cycle, stress, poor nutrition, and the sex of individuals (Castro et al., 2020).

In the plasma biochemistry analysis, *S. discus* showed high values for triglycerides, cholesterol, and chlorides about other Amazonian cichlids, such as *Cichla* spp. (Castro et al., 2020). Those above may be related to the type of feeding of *S.discus* in the natural environment, which is based on protein- and lipid-rich foods such as small fish, live worms, nauplii of microcrustaceans and periphyton, and the availability and nutritional composition of these foods in the localities where the fish were collected (Rossoni et al., 2014).

The high glucose and urea levels (53.98 mg dL^{-1} and 31.85 mg dL^{-1}, respectively; Table 3) suggest that the animals were stressed, which can be attributed to the traditional processes of capture, stocking in pens and pens, and transport to the distribution center in Manaus city (Erdal et al., 1991; Ranzani-Paiva et al., 1999). The stocking process can be long and without control over the ideal water quality levels (up to 48 hours without aeration in caçapas), which provides hypoxia and contributes to high values of plasma metabolites (Rossoni et al., 2014; Ramos et al., 2015; Ladislau et al., 2021).

Conclusion

In conclusion, the hematological responses obtained in this study with S. discus suggest that the species is sensitive to stress caused by environmental disturbances. Standardized hematological studies with S. discus in different environments are needed to confirm this. This understanding is fundamental to providing adequate acclimatization of animals under production, and maintaining animal welfare and health, since S. discus naturally presents low immunity, and stressful conditions can worsen this condition and lead to mortality. The current study profiles the hematological variables of S. discus under the described conditions and serves as a parameter for further physiological studies with the species.

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CHAPTER 4

Reducing conductivity increases the aggressiveness of the discus fish *Symphysodon* spp.

Maiko Willas Soares Ribeiro[1], Ana Caroliny Cerdeira Lopes[2], Thaís Billalba Carvalho[3], Adriano Teixeira de Oliveira[1,4]

[1]Graduate Program in Animal Science and Fisheries Resources (PPGCARP), Faculty of Agricultural Science (FCA), Federal University of Amazonas (UFAM), University Campus, Manaus, Amazonas, Brazil

[2]Postgraduate Program in Zoology - PPG-ZOO - PPG-ZOO, Federal University of Amazonas (UFAM), University Campus, Manaus, Amazonas, Brazil

[3]Laboratory of Feeding Behavior and Fish Nutrition, Center of Agricultural Sciences, Environmental and Biological, Campus Cruz das Almas, Federal University of Recôncavo Bahia (UFRB)

[4]Animal Morphophysiology Laboratory, Academic Department of Teacher Training (DAEF), Federal Institute of Education, Science and Technology of Amazonas (IFAM), Manaus Centro Campus (CMC), Manaus, Amazonas, Brazil

Abstract

Reduction in conductivity can signal the spawning period and favor offspring, as observed in studies on tropical fish. This phenomenon is particularly relevant for fish native to the Amazon, which depend on environmental stimuli, such as changes in conductivity, for reproduction. Reproduction in tropical fish is synchronized with seasonality and the rainy season, which alter water quality and several physical-chemical parameters. Changes in these conditions can affect the social and reproductive behavior of fish, such as temperature and dissolved oxygen. Despite its importance, there are few detailed behavioral studies on wild fish, such as the discus (*Symphysodon* spp.), a Neotropical cichlid valued in the aquarium hobby. The objective of the study was to evaluate the effect of reduced conductivity on the aggressive behavior and territorial defense of the discus. The agonistic profile was evaluated in pairs of discus fish subjected to a gradual reduction in water conductivity (30 – 14 µS cm-1) and a control condition (30 µS.cm-1). For this, the individuals were measured, weighed and then isolated for 72 hours. After this period, the animals were paired for 24 hours for 7 days in a neutral aquarium for both individuals, for observation and recording of aggressive behavior. The research revealed that the reduction in conductivity increased the frequency of aggressive interactions in both species studied, *S. discus* and *S. aequifasciatus*. This increase in agonistic behavior may be related to the influence of conductivity on social hierarchy and territoriality, which are crucial for reproduction. The results showed that the reduction in conductivity influences social behavior and competition for territory and reproductive partners.

Keywords: Aquarium, behavior, conductivity, reproduction.

Introduction

In the Amazon environment, with rising water levels, a reduction in conductivity affects fish reproduction (Kirschbaum & Schugardt, 2002). This factor can be understood as the signaling agent for spawning, favoring offspring (Kirschbaum & Schugardt, 2002; Crampton, 2008). This response has been observed for several tropical species (Kirschbaum & Schugardt, 2002; Anjos & Anjos, 2006; Crampton, 2008; Bueno et al., 2008; Ramos et al., 2015), indicating that the reduction in conductivity can stimulate reproduction in fish.

For fish species native to the Amazon, understanding how hydrological cycles are essential for the reproductive maintenance of tropical species requires environmental stimuli as a signal for spawning (Navarro & Navarro, 2017). During rainy seasons, hydrological systems change, with water levels, conductivity, pH, turbidity, salinity and light intensity being stimuli for fish reproduction (Oliveira et al., 2011, 2017, 2017a, Ribeiro & Moreira, 2012).

Reproduction in tropical fish depends on the synchronization of several factors, such as water quality, since seasonality and the rainy season are fundamental (Kirschbaum & Schugardt, 2002; Anjos & Anjos, 2006; Crampton, 2008; Ribeiro & Moreira, 2012; Ramos et al., 2015). In addition, factors such as temperature, pH, dissolved oxygen, turbidity, and conductivity undergo changes during these periods (Borghezan et al., 2021; Douglas et al., 2024) and can affect the display of social behavior in fish (Makori et al., 2017, Gauy et al., 2018, Kua et al., 2020, Santos et al., 2020, Swain et al., 2020, Krishnakumar et al., 2020).

Behavioral studies with wild fish specimens with a reproductive focus are scarce, leading to a lack of interest in wild varieties (Rossoni et al., 2014). *Symphysodon* spp. is a genus of Neotropical cichlid with a disc-shaped body, 12 to 20 cm in standard length and 25 cm in height in adulthood, a small mouth, feeding on small fish, live worms and microcrustacean nauplii, split spawning, biparental care and inhabiting streams, lakes and riverbanks (Rossoni et al., 2014, Jesus, et al., 2022). This genus is used as an ornamental fish due to its body color pattern and behavioral characteristics (Ladislau et al., 2020; Tribuzy-Neto et al., 2021; Jesus et al., 2022; Ribeiro et al., 2024), presenting interest in production and economic viability for ornamental aquaculture (Ribeiro et al., 2024). Its value in the aquarium market varies from 50 to 170 dollars, depending on the color variant, in which it is commercially classified as royal, non-royal or common (Anjos et al., 2009; Rossoni et al., 2014; Tribuzy-Neto et al., 2021; Jesus et al., 2022).

According to Chellappa et al. (2005), *Symphysodon discus* presents a social organization based on a hierarchy of dominance and territoriality, involving competition for partners and spawning areas and elaborate courtship and mating behaviors. Aggressive behavior evidences competition for resources (e.g., territory and reproductive partner), where dominant animals maintain hierarchical control and priority access to resources (Huntingford & Tuner, 1987). Thus, territorial defense and aggressive interaction are fundamental in the reproductive context (Alcazar et al., 2016). Thus, the present study aimed to understand how changes in conductivity can influence the social behavior of the discus fish *Symphysodon* spp., which is of great interest in the ornamental fish market.

Material and methods

The animals were collected from bodies of water near Manaus, Amazonas (3° 17' 59" S 60° 37' 14" W). All specimens were acclimated at the Experimental Laboratory of Physiology and Behavior of Aquatic Animals - LEFCAQ (Department of Physiological Sciences/UFAM) in aquariums measuring 78 X 37 X 45 cm (132 liters) (1 animal $5L^{-1}$) for at least 15 days prior to the experiments. During this period, the average temperature was controlled at 27.88 ± 0.73 °C, and the photoperiod was 12 hours of light (07:00 h to 19:00 h) with light intensity (174.77 ± 0.62 lux). Water quality was maintained in adequate conditions through the use of biological filters, partial water changes every two days, and monitoring of water physicochemical parameters (dissolved oxygen 5 ± 0 mg L^{-1}, pH 6.01 ± 0.67 and ammonia 0.001 ± 0.001 mg L^{-1}). The animals were fed commercial food for ornamental fish and offered until satiation in the morning and late afternoon.

The agonistic profile was evaluated in pairs of discus fish subjected to a gradual reduction in water conductivity (30 – 14 µS cm^{-1}) and a control condition (30 µS cm^{-1}). For this purpose, the individuals were measured, weighed, and isolated for 72 hours. After this period, the animals were paired for 24 hours for 7 days in a neutral aquarium for both individuals to observe and record aggressive behavior. The pairs were formed by individuals of approximate weight (27 ± 14.81 g) and size (8 ± 1.34 cm) (independent t-test, t= 8.05; p= 2.53), as this parameter interferes with the frequency of aggressive interaction in fish (Beeching, 1992). The individuals were identified by different color patterns and by cuts in the caudal fin. The animals were isolated in neutral aquariums of 49 x 29 x 40 cm (59 liters) lined with opaque blue plastic on three sides to avoid visual contact with animals from neighboring aquariums in the laboratory. The gradual reduction in conductivity was achieved by means of a deionizing filter, with partial changes of 30% of the volume of the aquariums, maintained with constant aeration. The animals were fed with ornamental fish food in the proportion of 1% of the biomass offered once, on alternate days, considering that excess feeding can interfere with the gradual reduction of conductivity.

The frequency of agonistic behavior was recorded in a film corresponding to the pairing time, with 10 minutes of each recorded hour being quantified; the recording began immediately after the animals were included in the neutral aquarium. Frequency is the number of times the animals exhibit a specific behavior, classified in the behavioral ethogram (Table 1). Thus, an ethogram for the agonistic behavior of the discus was elaborated, based on the work of Câmara (2004) and Mattos et al. (2016; 2017) carried out for *S. discus* and *S. aequifasciatus*, respectively, and Yamamoto et al. (1999) for *Pterophyllum scalare* (Table 1). In addition, the latency for confrontations and the quantification of aggressive interaction for each individual and for the pair were evaluated. Latency is the time the animals take to exhibit the first characteristic behavior classified in the behavioral ethogram. The animals were classified as winners and losers, according to Falter (1983), from the moment one of the animals stops attacking (loser) and starts to flee from the other individual (winner).

Table 5: Ethogram of behavioral categories for discus fish *Symphysodon* spp. Behavioral Category Behavioral Unit Description Agonistic behavior (establishment of territory)

Behavioral Category	Behavioral Unit	Description
Agonistic behavior	Approximation	The fish move towards each other simultaneously,

(territory establishment)		maintaining a distance of approximately 8 to 10 cm, over the disputed territory.
	Threat	A fish keeps its head down, its fins expand, and its body flexes toward its opponent.
	Attack	A fish lunges at its opponent.
	Submission	A fish retracts its fins and remains motionless with its head raised.
	Escape	One fish stops looking at the other and retreats quickly and hastily.

Results

For the latency, no statistical difference was observed in the test conditions [Mann-Whitney w= 3, p= 0.1 (*S. aequifaciatus*), w= 9, p= 0.657 (*S. discus*); Mann-Whitney, $p < 0.05$]. For *S. discus*, ten behavioral units were observed, two of which were described for the first time for the species (Table 2). For *S. aequifaciatus*, nine behavioral units were observed, two of which were described for the first time for the species (Table 3).

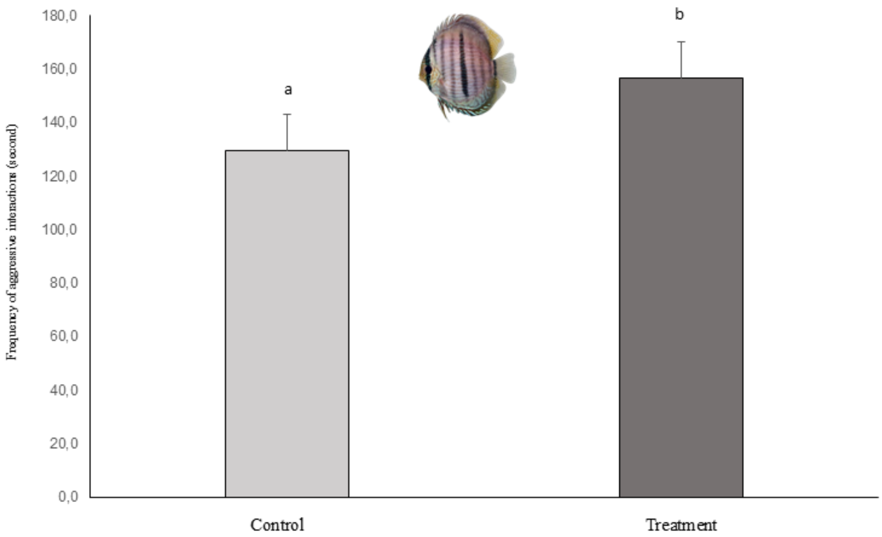

Figure 7: Frequency of total aggressive interactions for discus species (*S. discus*) (Mann-Whitney, $p < 0.05$) (Different letters indicate statistical difference).

When comparing the conditions for *S. discus*, we can observe that among the total number of aggressive interactions (Figure 1), the treatment condition had a higher average than the control condition, indicating that the reduction in conductivity increases aggressive behavior for the species. However, this relationship is not observed for behavioral units; it is observed that the control group had a higher average in almost all behavioral units (Table 2).

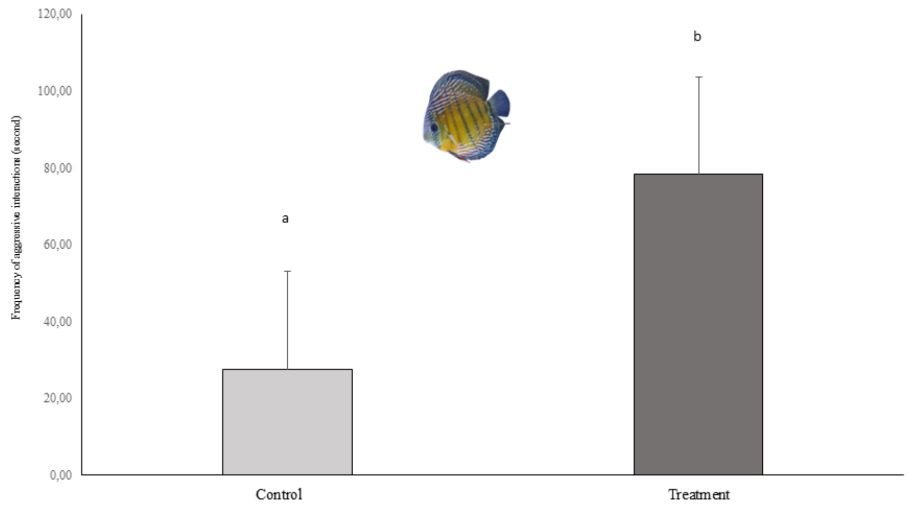

Figure 8: Frequency of total aggressive interactions for discus fish species (*S. aequifaciatus*) (Mann-Whitney, p< 0.05) (Different letters indicate statistical difference).

For *S. aequifaciatus*, the same relationship was observed for the total number of interactions, where the treatment condition had a higher average number of interactions than the control condition. This indicates that the reduction in conductivity increases the frequency of agonistic behavior (Figure 2). The same pattern was observed for all behavioral units, with the treatment group having a higher average number of interactions (Table 3).

The same effect was also observed over the days for *S. discus* and *S. aequefaciatus*. Both species start with low interaction intensity, which increases over the days, reaches a peak, and then decreases.

For *S. discus* under the control condition, there were no statistical differences over the days, with the frequency of interactions remaining low (Figure 3). For the treatment condition, a statistical difference was observed on the third day, with the maximum peak of interactions, followed by a decrease reaching zero on the seventh day (Figure 3).

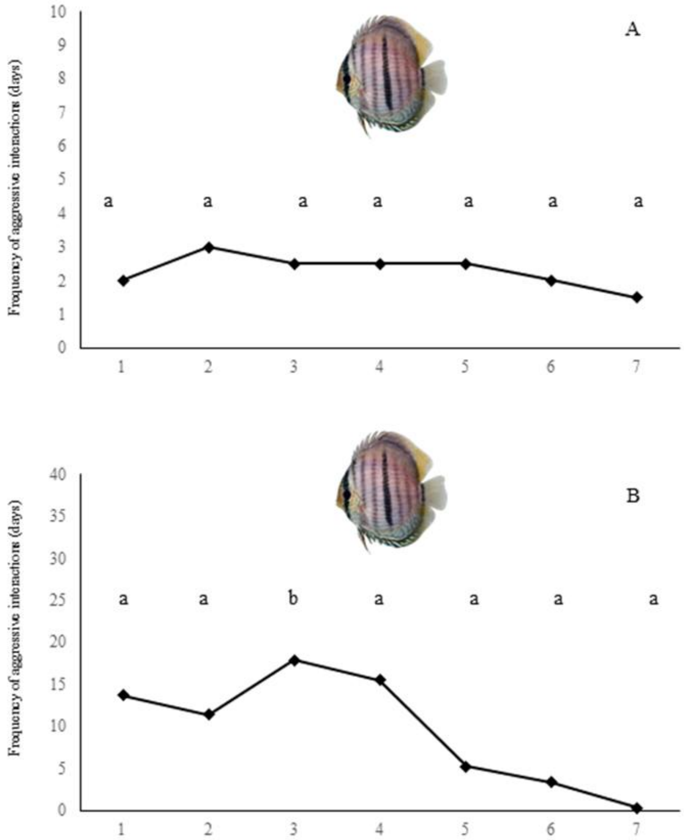

Figure 9: Frequency of total aggressive interactions for discus species (*S. discus*) (A control, Friedman's ANOVA $p > 0.05$), (B treatment, Friedman's ANOVA $p < 0.05$ (Different letters indicate statistical difference).

For *S. aequefaciatus* in the control condition, there was no statistical difference during the days, maintaining the low frequency of interactions (Figure 4). For the treatment condition, a statistical difference was observed from the fifth day onward, with the maximum peak of interactions (Figure 4).

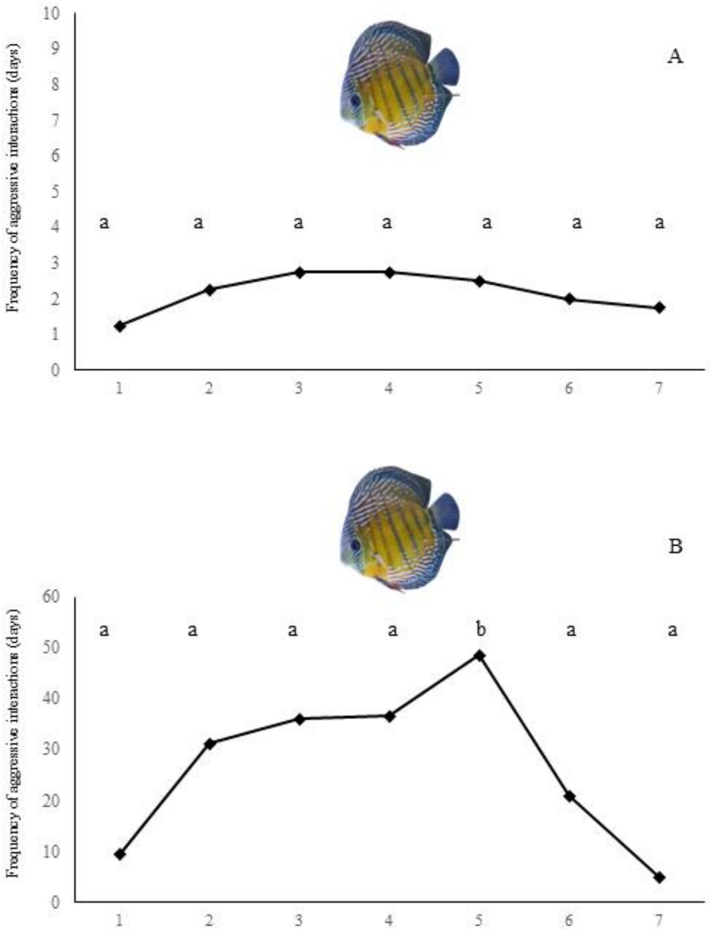

Figure 10: Frequency of total aggressive interactions for discus fish species (*S. aequefaciatus*) (A control, Friedman's ANOVA $p > 0.05$), (B treatment, Friedman's ANOVA $p < 0.05$ (Different letters indicate statistical difference).

A statistical difference was observed for the animals considered winners for S. discus, for the behavioral units of tail beat and total interactions (Table 4). For the animals considered losers, a statistical difference was observed for the following behavioral units: display, tail beat, and total interactions (Table 6).

For *S. aequefaciatus*, the winners showed statistical differences in the behavioral units of attack, pursuit, tremor, and total interactions (Table 5). For the losers, differences were observed between the units of escape, color change, and total interactions (Table 7).

Table 6. Mean (+ standard error) of the frequency of aggressive interactions for the discus pair in the treatment and control conditions for *Symphysodon discus*.

	Behavioral Units									
	Frontal Attack	**Display**	**Color change**	**Attack**	**Persecution**	**Escape**	**Threat**	**Tail flapping**	**Tremors**	**Total**
Control	0,25 ± 0,35	40,12 ± 9,62	36,31 ± 7,99	44 ± 4,24	21,5 ± 3,18	19,37 ± 1,23b	1,12 ± 0,53	12,25 ± 13,08b	NE	135,81 ± 7,08b
Treatment	NE	56,93 ± 13,18	39,93 ± 13,52	28,12 ± 14,97	6,56 ± 3,86	2,25 ± 1,10a	3,75 ± 2,20	0,12 ± 0,17a	NE	156,5 ± 30,07a

(Mann-Whitney, $p < 0.05$) (Different letters indicate statistical difference).

Table 7. Mean (+ standard error) of the frequency of aggressive interactions for the discus fish pair in the treatment and control conditions for *Symphysodon aequefaciatus*.

	Behavioral Units									
	Frontal Attack	**Display**	**Color change**	**Attack**	**Persecution**	**Escape**	**Threat**	**Tail flapping**	**Tremors**	**Total**
Control	NE	15,86 ± 3,09	4,81 ± 1,30	3,93 ± 1,63b	0,75 ±	1,75 ± 0,79	0,25 ±	0,62 ± 0,37	0,87 ±	27,68 ± 6,23b

					0,35b		0,23		0,67	
Treatment	NE	20,25 ± 3,60	7,56 ± 2,36	19,37 ± 6,54a	7,81 ± 2,33a	7 ± 5,04	0,56 ± 0,52	0,87 ± 0,62	NE	78,37 ± 18,30a

(Mann-Whitney, $p < 0.05$) (Different letters indicate statistical difference).

Table 8. Mean (+ standard error) frequency of aggressive interactions for winner in treatment and control condition for *Symphysodon discus*.

	Behavioral Units									
	Frontal Attack	**Display**	**Color change**	**Attack**	**Persecution**	**Escape**	**Threat**	**Tail flapping**	**Tremors**	**Total**
Control	0,12 ± 0,17	22,65 ± 3,26	18,53 ± 2,75	12,56 ± 3,77	1,62 ± 2,10	1,75 ± 0,79	1,03 ± 0,64	3,43 ± 1,41b	0,03 ± 0,04	71,12 ± 8,85b
Treatment	NE	38,40 ± 13,15	13,59 ± 5,75	11,34 ± 6,55	1,59 ± 1,13	3,75 ± 2,76	2,18 ± 1,09	0,09 ± 0,13a	NE	99,68 ± 18,07a

(Mann-Whitney, $p < 0.05$) (Different letters indicate statistical difference).

Table 9. Mean (+ standard error) frequency of aggressive interactions for winner in treatment and control condition for *Symphysodon aequefaciatus*.

	Behavioral Units									
	Frontal Attack	**Display**	**Color change**	**Attack**	**Persecution**	**Escape**	**Threat**	**Tail flapping**	**Tremors**	**Total**
Control	NE	9,06 ± 1,37	0,75 ± 0,25	2,15 ± 1,63b	0,06 ± 0,06b	NE	0,06 ± 0,06	0,06 ± 0,06	0,50 ± 0,50b	14,12 ± 1,53b
Treatment	NE	9,37 ± 3,14	7,12 ± 4,05	36,18 ± 11,89a	9,68 ± 3,55a	NE	1,93 ± 1,20	NE	NEa	60,62 ± 15,85a

(Mann-Whitney, $p < 0.05$) (Different letters indicate statistical difference).

Table 10. Mean (+ standard error) frequency of aggressive interactions for the loser in treatment and control condition for *Symphysodon discus*.

	Behavioral Units									
	Frontal Attack	**Display**	**Color change**	**Attack**	**Persecution**	**Escape**	**Threat**	**Tail flapping**	**Tremors**	**Total**
Control	NE	12,81± 4,94b	10,43 ± 2,61	5,68± 1,68	NE	2,56±0,88	4,18± 1,52	2,06 ± 0,46b	0,12 ± 0,12	47,62 ± 5,91b

Treatment	NE	22,12 ± 2,83a	21,93 ± 2,49	17,56 ± 7,07	2,62± 2,01	4,12 ± 2,12	3,43± 2,16	NEa	NEa	80,81± 10,38a

(Mann-Whitney, $p < 0.05$) (Different letters indicate statistical difference).

Table 11. Mean (+ standard error) frequency of aggressive interactions for the loser in treatment and control condition for *Symphysodon aequefaciatus*.

	Behavioral Units									
	Frontal Attack	**Display**	**Color change**	**Attack**	**Persecution**	**Escape**	**Threat**	**Tail flapping**	**Tremors**	**Total**
Control	NE	4,12 ± 0,41	1,75 ± 0,25b	NE	NE	0,87 ± 0,51b	NE	NE	NE	7 ± 0,40b
Treatment	NE	13,93 ± 2,93	6,68 ± 2,26a	NE	NE	8,5±2,53a	NE	NE	NE	31,37 ± 7,54a

(Mann-Whitney, $p < 0.05$) (Different letters indicate statistical difference).

Discussion

The discus fish demonstrated behavioral similarity with other cichlid species such as *Astronotus ocellatus*, *P. scallare, Geophagus proximus, Laetacara fulvipinnis* (Beeching, 1997; Gonçalves-de-Freitas & Mariguela 2006; Teresa & Gonçalves-de-Freitas 2003; Carvalho et al., 2012; Sarmento et al., 2017; Teresa & Gonçalves-de-Freitas, 2011).

It was noted that the species present a complex behavioral repertoire, exhibiting 9 different behavioral units, corroborating the studies of Chellappa et al. (2005). The species *S. aequifasciatus* and *S. discus* present a social organization based on the hierarchy of dominance and territoriality, and social interactions begin with energetically less expensive behavioral units (Maan et al., 2001).

The reduction in conductivity increased the frequency of behavioral repertoire about aggressive behavior in both species. These results demonstrate that changes in the environment can influence social behavior, a fact observed for other cichlid species such as *Apistograma agazzizi*, *P. scal*ar, *Laetacara fulvipinnis*, *and Astronotus ocellatus* (Kokhan et al., 2019; Ribeiro et al., 2022; Sarmento et al., 2017; Beeching, 1997).

However, when comparing the number of behavioral units, the control condition has more units than the treatment condition for both species (Table 2, Table 3), indicating that the animals in the control condition had more complex behavior. According to Chellappa et al. (2005), *Symphysodon discus* presents a social organization based on the hierarchy of dominance and territoriality, involving competition for partners and spawning areas, in addition to elaborate courtship and mating behaviors.

In which aggressive behavior highlights competition for resources (e.g. territory and reproductive partner), where dominant animals maintain hierarchical control and priority access to resources (Huntingford & Tuner, 1987). Thus, territorial defense and aggressive interaction play a fundamental role in the reproductive context (Alcazar et al., 2016).

When comparing the effect of conductivity reduction between the two species, it can be understood that the effect is much more felt in the species *S. aequefaciatus* than in *S. discus*. This situation can be attributed to the habitat preferences of each species. S. discus originates from the Rio Negro basin and the north-central Amazon basin, where black waters are predominantly poor in ions and have low conductivity (Shultz, 1960; Burgess, 1981; Kullander, 1996; Bleher et al., 2007; Amado et al., 2011). *S. aequefaciatus*, present in the central and eastern Amazon basin, inhabits slightly acidic waters, with pH values ranging from 6.0 to

6.8, with higher conductivity (Pellegrin 1904; Shultz 1960; Burgess 1981; Kullander 1996). 2003; Bleher et al. 2007, Amado et al., 2011).

Similar to other Amazonian cichlids, the discus acara is highly adaptable to environmental changes. However, the loss of ions with reduced conductivity can cause stressful conditions for the animals. Duarte and collaborators (2013) suggest that S. discus has a greater tolerance to the loss of ions from the water, efficiently maintaining ionic balance in acidic water conditions that are poor in ions. This effect is evident when we observe the frequency of interactions between the two species.

However, for the physiology of behavior, the stability of the social hierarchy is established through aggressive interactions, with conflicts normally starting with low-intensity, energy-saving behavioral units that are performed at greater distances between the animals, such as displays and costly behavioral units performed at greater proximity between the animals, such as threats (Maan et al., 2001).

Attacks and pursuits are usually carried out in the final stages of conflicts and contribute to their resolution, which occurs after reaching high levels of escalation and when one of the opponents gives up the conflict (Teresa & Gonçalves-de-Freitas, 2003, Mattos et al., 2016). The acute reduction in conductivity increased the aggressiveness of the discus fish, however it reduced the complexity of the behavioral repertoire, indicating that a rapid reduction in conductivity can negatively influence the hierarchical stability of the species, and consequently in reproduction.

Changes in the environment can significantly affect the hierarchical stability of the group, being understood as a disturbance to stability. This was observed for *Apistograma agazzizi,* an Amazonian cichlid, with changes in temperature as well as oxygen in the water, there was a breakdown in hierarchical stability, leading to constant changes in dominance positions in the group (Kokhan et. al., 2019). Aggressiveness in cichlids is observed at different stages of the individual's life as well as in both sexes (Pinho-Neto et al., 2014, Brandão et al., 2018; Gauy et al., 2018; Brandão et al., 2021). Through fights, the dominant ones have priority access to resources that can be food, sexual partners, and mating sites (Huntingford & Tuner, 1987).

Studies in natural environments conducted by Crempton (2008) observed that for the discus fish, the reduction in conductivity favors reproduction. The discus fish synchronized spawning during periods of frequent rain, a factor that promotes a reduction in conductivity according to the increase in the water level of the rivers. This observation was also reported for the leaf fish (Ramos et al., 2015), where low conductivity favored the spawning of the species. Leaffish pairs subjected to low conductivity spawned more often than pairs with high conductivity (Ramos et al., 2015).

However, changes in conductivity may favor spawning. In the social reproductive context of cichlids, for which spawning occurs, a complex behavioral repertoire must occur, which is the choice of partner as well as hierarchical dominance (Teresa & Gonçalves-de-Freitas, 2003). A reduction in conductivity for the discus caused a reduction in the behavioral repertoire.

This condition can be attributed to increased aggression, which triggers social stress in animals. This condition stimulates the hypothalamic axis, with secretion and increased cortisol, which can impact the growth, feeding, reproduction and immunity of animals (Wendelaar-Bonga, 1997; Johnsson et al., 2006, Winberg et al., 2016; Brandão et al., 2021). Cortisol levels can be higher in dominant animals as well as in subordinates. During fights, the cortisol levels of both animals can increase in different types of social context (Corrêa et al., 2003; Øverli et al., 1999; Brandão et al., 2021).

After the dispute is resolved, hormone levels may decrease in winners and increase in losers. In a condition of environmental disturbance and hierarchical instability, as observed in the experiment, cortisol levels in dominant animals may increase significantly, and in a group context, all unfavorable animals may present elevated levels (Fox et al., 1997; Øverli et al., 1999; Sloman et al., 2001; Barreto et al., 2015).

Aggressive social interactions are part of the natural life of social individuals, as in the case of cichlids. However, intense and prolonged fights have negative impacts on both the individual and the group, and can cause physiological and reproductive damage to the animals (Brandão et al., 2021).

Therefore, understanding how environmental changes affect animal social behavior can promote management practices and protocols that favor animal welfare and improved production.

Conclusions

Changes in conductivity are an influencing factor in the social behavior of discus fish, acting in the dispute and territorial choice of the species. These two factors determine the stability of the social hierarchy and reproductive success. The condition of acute change may, as proposed by the study, indicate that the species is susceptible to environmental disturbances, which may influence the social and physiological context of the animals. Comprehension through behavioral observation may contribute to improving management practices, water quality and the environment, favoring well-being, and may also guide productive protocols for the species and artificial propagation.

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CHAPTER 5

Increasing light intensity increases aggressiveness in the Amazonian discus fish (*Symphysodon discus*)

Maiko Willas Soares Ribeiro[1], Thaís Billalba Carvalho[2], Philip Dalbert Castro[3], Adriano Teixeira de Oliveira[1,4]

[1]Graduate Program in Animal Science and Fisheries Resources (PPGCARP), Faculty of Agricultural Science (FCA), Federal University of Amazonas (UFAM), University Campus, Manaus, Amazonas, Brazil

[2]Laboratory of Feeding Behavior and Fish Nutrition, Center of Agricultural Sciences, Environmental and Biological, Campus Cruz das Almas, Federal University of Recôncavo Bahia (UFRB)

[3]Federal University of Amazonas (UFAM), University Campus, Manaus, Amazonas, Brazil

[4]Animal Morphophysiology Laboratory, Academic Department of Teacher Training (DAEF), Federal Institute of Education, Science and Technology of Amazonas (IFAM), Manaus Centro Campus (CMC), Manaus, Amazonas, Brazil

Abstract

In an aquatic environment, behavior can be modulated by several factors, such as temperature, dissolved oxygen, pH, turbidity, and light intensity. Increased light intensity can influence hormone synthesis and behavior, may affect aggressive behavior and dominance hierarchy, and produce social instability. *Symphysodon* spp. is a genus of Neotropical cichlids with a disc-shaped body, 12 to 20 cm in standard length and 25 cm in height in adulthood. This genus is used as an ornamental fish due to its body color pattern and behavioral characteristics. *Symphysodon* spp. has a social organization based on dominance and territoriality hierarchy, involving competition for mates and spawning areas and elaborate courtship and mating behaviors. The effect of two light intensities, high light (1,512.85 ± 11.07 lux) and low light (144 ± 0.64 lux), on the agonistic behavior of the discus fish was evaluated. We evaluated the latency and frequency of aggressive interactions and territorial defense and observed a reduction in aggressive behavior at low intensity compared to high intensity. We conclude that light intensity can influence aggressive motivation and territory establishment. Thus, low light intensities can reduce mating success, and therefore, high intensity can be favorable for the species, favor hierarchical stability, and, consequently, favor the selection of a reproductive partner for *S. discus*.

Keywords: Light intensity, Aggressive behavior, Territoriality.

Introduction

Environmental stimuli influence the animal life cycle, acting on growth, feeding, reproduction and hormonal metabolism (Wingfield, 2003). In this sense, different behavioral strategies can emerge with these stimuli to adapt to these changes (Wingfield, 2003). In an aquatic environment, behavior can be modulated by several factors, such as temperature, dissolved oxygen, pH, turbidity, and light intensity (Kirschbaum & Schugardt, 2002, Anjos & Anjos, 2006, Crampton, 2008, Ribeiro & Moreira, 2012, Orfão, 2013, Ramos et al., 2015, Borghezan et al., 2020, Makori et al., 2017, Gauy et al., 2018, Kua et al., 2020, Santos et al., 2020, Swain et al., 2020, Krishnakumar et al., 2020).

Light can be considered as the environmental signal to modulate social behavior and, consequently, fish reproduction (Ribeiro & Moreira, 2012; Carvalho et al., 2012; Carvalho et al., 2013a); however, this regulation can be variable for different fish species. Thus, light can be divided into absorption spectrum, quantity, and duration, and can influence feeding, growth, survival, locomotion, metabolism, and reproduction and aggressive behavior (Norberg et al., 2004, Zhu et al., 2014, Elisio et al., 2014a, Elisio et al., 2015b, Navarro & Navarro, 2017, Shahjahan et al., 2020, Korf, 2006, Biswas et al., 2006, (Reynalte-Tataje et al., 2002, Sheng et al., 2006, Pankhurst & Porter, 2003, Almazán-Rueda et al., 2004, Carvalho et al., 2012, Carvalho et al., 2013).

Light intensity can strongly affect the sexual maturation of individuals and the synthesis and secretion of sex hormones in fish. Thus, the timing, period and duration of light intensity maintain a seasonality in the reproductive activity of fish (Norberg et al., 2004; Zhu et al., 2014; Elisio et al., 2014a; Elisio et al., 2015b, Navarro & Navarro, 2017, Shahjahan et al., 2020). Korf (2006) observed that the pineal organ acts as a translator of changes in luminosity, assisting the functions of the retina and other sensory systems, regulating the circadian cycle, as well as the hormone melatonin, melatonin that acts as a regulator in ovarian development (Falcon et al., 2003). In the reproductive social context, aggressiveness plays a fundamental role in choosing partners and the dispute for mating sites. The light intensity per light period can influence the increase and decrease in agonistic behavior (Carvalho et al., 2012). In this way, the increase in light intensity can influence hormonal synthesis and the behavioral physiology of cichlids. It can affect aggressive behavior and the dominance hierarchy and produce social instability (Carvalho et al., 2013).

In this sense, it is understood that the stability of the social hierarchy is established through aggressive interactions, with conflicts typically starting with low-intensity, energetically economical behavioral units that are performed at greater distances between animals, such as displays and costly behavioral units performed at more excellent proximity between animals, such as threats (Maan et al., 2001). Attacks and pursuits are usually carried out in the final stages of conflicts and contribute to their resolution, which occurs after high levels of escalation have been reached and when one of the opponents gives up the conflict (Teresa & Gonçalves-de-Freitas, 2003, Mattos et al., 2016).

Symphysodon spp. is a genus of Neotropical cichlid with a disc-shaped body, 12 to 20 cm in standard length and 25 cm in height in adulthood, a small mouth, feeding on small fish, live worms and microcrustacean nauplii, split spawning, biparental care and inhabiting streams, lakes and riverbanks (Rossoni et al., 2014, Jesus, et al., 2022). This genus is used as an ornamental fish due to its body color pattern and behavioral characteristics (Tribuzy-Neto et al., 2021; Jesus et al., 2022) and is of interest for production and economic viability for ornamental aquaculture (Ribeiro et al., 2024). Its value in the aquarium market varies from 50 to 170 dollars, depending on the color variant, in which it is commercially classified as royal, non-royal or common (Anjos et al., 2009; Rossoni et al., 2014; Tribuzy-Neto et al., 2021, Jesus et al., 2022).

According to Chellappa et al. (2005), *Symphysodon discus* has a social organization based on a hierarchy of dominance and territoriality, involving competition for partners and spawning areas and elaborate courtship and mating behaviors. Aggressive behavior demonstrates competition for resources. (e.g., territory and reproductive partner), where dominant animals maintain hierarchical control and priority resource access (Huntingford & Tuner, 1987). Thus, territorial defense and aggressive interaction play a fundamental role in the reproductive context (Alcazar et al., 2016).

Due to their commercial importance, studies on the social behavior of discus fish are still scarce. Thus, understanding how environmental factors influence their management in an artificial environment is essential to promote improved management practices. Therefore, the present study aims to understand how increased light intensity can influence the aggressive behavior and territorial defense of the discus fish *Symphysodon discus*.

Material and methods

Acquisition and maintenance of animals

The *S. discus* fish come from water bodies near Manaus, Amazonas (3° 17' 59" S 60° 37' 14" W). All specimens were acclimatized at the Experimental Laboratory of Physiology and Behavior of Aquatic Animals - LEFCAQ (Department of Physiological Sciences/UFAM) in aquariums measuring 78 X 37 X 45 cm (132 liters) (1 animal/5L) for at least 15 days prior to the experiments. During this period, the average temperature was controlled at 27.88 ± 0.73 °C and the photoperiod was 12 hours of light (07:00 h to 19:00 h) with light intensity (174.77 ± 0.62 lux). Water quality was maintained in adequate conditions through the use of biological filters, partial water changes every two days and monitoring of water physicochemical parameters (dissolved oxygen 5 ± 0 mg. L^{-1}, pH 6.01 ± 0.67 and ammonia 0.001 ± 0.001 mg L^{-1}). The animals were fed with commercial food (Maramarpet) for ornamental fish, offered until satiation in the morning and late afternoon.

Experimental design

The agonistic profile was evaluated in pairs of discus fish subjected to a high light intensity condition (1.512,85 ± 11,07 lux) and a low light intensity condition (144 ± 0,64 lux). For this, the individuals were measured, weighed and then isolated for 72 hours. After this period, the animals were paired for 24 hours for 7 days in a neutral aquarium for both individuals, for observation and recording of aggressive behavior. Pairs were formed by individuals of approximate weight (40,2 ± 2,4 g) and size (9,0 ± 0,0 cm) (independent t-test, $t < 113,42$; $p < 2.2$), as this parameter interferes with the frequency of aggressive interaction in fish (Beeching, 1992). Individuals were identified by different color patterns and by cuts in the caudal fin.

The animals were isolated in neutral aquariums measuring 49 x 29 x 40 cm (59 liters) lined with opaque blue plastic on three sides to avoid visual contact with animals from neighboring aquariums in the laboratory. The frequency of agonistic behavior was recorded in a film corresponding to the pairing time, being quantified 10 minutes of each recorded hour;

the recording began immediately after the animals were included in the neutral aquarium. An ethogram for the agonistic behavior of discus fish was thus developed, based on the work of Câmara (2004) and Mattos et al. (2016; 2017) carried out for *S. discus* and *S. aequifasciatus*, respectively, and Yamamoto et al. (1999) for *Pterophyllum scalare* (Table 1).

In addition, the latency for confrontations and the quantification of aggressive interaction for each individual and for the pair were evaluated. Latency is understood as the time it takes for the animals to exhibit the first characteristic behavior classified in the behavioral ethogram. According to Falter (1983), the animals were classified as winners and losers from the moment one of the animals stopped attacking (loser) and started to flee from the other individual (winner).

Table 12. Ethogram of behavioral categories for discus fish *Symphysodon discus*.

Behavioral Category Behavioral	**Unit Description.**	**Description**
Agonistic behavior (territory establishment)	Approximation	Agonistic behavior (establishment of territory) Approach The fish approach each other simultaneously, maintaining a distance of approximately 8 to 10 cm, over the disputed territory.
	Threat	A fish keeps its head down, its fins expand, and its body flexes towards the opponent.
	Attack	A fish lunges towards the opponent.
	Submission	A fish retracts its fins and remains motionless with its head raised.
	Escape	A fish stops facing the other and retreats quickly and hastily.

Results

For *S. discus* under the low-intensity condition, 4 behavioral units were observed (attack, display, tremor and total interactions); for the high-intensity condition, 8 behavioral units were observed (frontal confrontation, attack, display, color change, escape, tail beat, tremor and total interactions).

Interactions (Table 2), when comparing the two conditions, a statistical difference was observed for the total number of interactions between the pairs (Figure 1). For the other behavioral units, a statistical difference was also observed (Table 2), indicating that the frequency of agonistic behavior was higher in the high-intensity condition. The latency for clashes was also observed, and there was a statistical difference between the two conditions, with the time to display agonistic behavior being higher for low intensity compared to high intensity (Mann-Whitney w= 0.5 p= 0.0009) (Figure 1).

Table 13. Average (+ standard error) frequency of aggressive interactions for the discus fish *Symphysodon discus* in low-intensity and high-intensity conditions.

	Behavioral units							
	Frontal confrontation	**Attack**	**Display**	**Color change**	**Escape**	**Tail Beat**	**Tremor**	**Total**
Low	Nea	1,28 ± 0,40[a]	42,75 ± 4,33a	Nea	NEa	Nea	0,37 ± 0,12a	44,51±4,18a
High	0,23 ± 0,15b	6,29 ± 0,46b	55,91 ± 4,49b	12,12 ± 1,38b	2,88 ± 0,22b	1,10 ± 0,02b	0,46 ± 0,30b	74,27 ± 0,28b

NE indicates that the animals did not display the behavioral unit. Different letters indicate statistical differences (Mann-Whitney, p< 0.05).

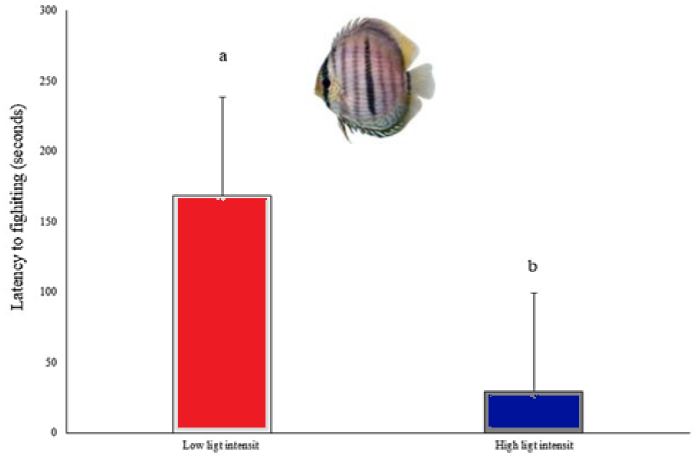

Figure 11: Latency of aggressive interactions for pairs of discus fish *Symphysodon discus*, under low and high light intensity conditions (Mann-Whitney w= 0.5 p= 0.0009). Different letters indicate statistical differences.

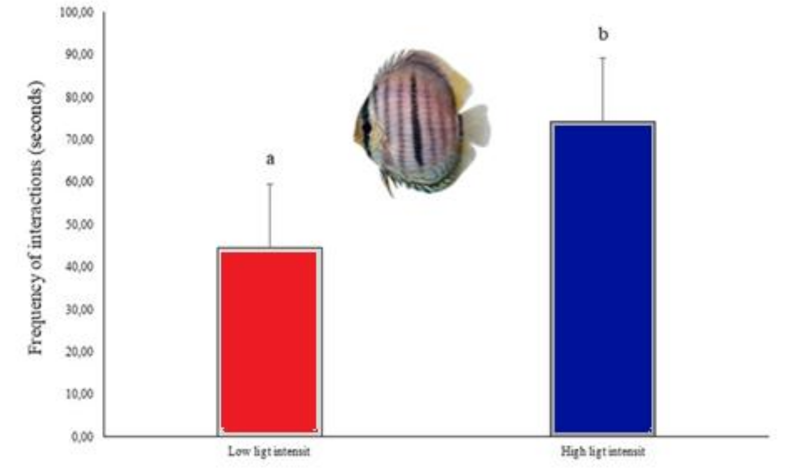

Figure 12: Frequency of total aggressive interactions for pairs of discus fish *Symphysodon discus*, under low and high light intensity conditions (Mann-Whitney w= 64 p= 0.0004). Different letters indicate statistical differences.

When days were compared for low intensity (Friedman ANOVA f= 2.33 p= 0.88) and high intensity (Friedman ANOVA f= 8.78 p= 0.18), no statistical differences were observed.

Comparisons were also made between winning and losing fish under the conditions. For the winner, statistical differences were observed for the following units (frontal confrontation, attack, color change, tail beat) (Table 3). For the loser, statistical differences were observed for the following behavioral units (attack, color change, escape, tail beat, tremor and total interactions) (Table 4). The high-intensity condition exhibited more behavioral units for both the winner and the loser, indicating a larger and more complex behavioral repertoire.

Table 14. Mean (+ standard error) of the frequency of aggressive interactions for the winning discus fish *Symphysodon discus* in low and high-intensity conditions.

	Behavioral units							
	Frontal confrontation	**Attack**	**Display**	**Color change**	**Escape**	**Tail Beat**	**Tremor**	**Total**
Low	Nea	0,17 ± 0,13[a]	3,23 ± 0,58	NEa	NE	0,44 ± 0,35a	NE	3,48 ± 0,66
High	0,05± 0,08b	1,30 ± 0,31b	2,80 ± 0,84	0,46 ± 0,16b	NE	0,30 ± 0,18b	NE	4,36 ± 1,47

NE indicates that the animals did not display the behavioral unit. Different letters indicate statistical differences (Mann-Whitney, $p < 0.05$).

Table 16. Mean (+ standard error) frequency of aggressive interactions for discus loser *Symphysodon discus* in low and high-intensity conditions.

	Behavioral units							
	Frontal confrontation	**Attack**	**Display**	**Color change**	**Escape**	**Tail Beat**	**Tremor**	**Total**
Low	NE	Nea	2,46 ± 0,89	Nea	NEa	NEa	0,13 ± 0,12a	3,48 ± 0,66a
High	NE	0,25 ± 0,20b	2,11± 0,44	1,64 ± 0,26b	1,02 ± 0,58b	0,19 ± 0,07b	NEb	3,68± 0,79b

NE indicates that the animals did not display the behavioral unit. Different letters indicate statistical differences (Mann-Whitney, $p< 0.05$).

Discussion

The behavioral profile of the discus (*S. discus*) showed similarity to other cichlid species such as *Astronotus ocellatus*, *P. scallare*, *Geophagus proximus*, *Laetacara fulvipinnis* (Beeching, 1997; Gonçalves-de-Freitas & Mariguela 2006; Teresa & Gonçalves-de-Freitas 2003; Carvalho et al., 2012; Sarmento et al., 2017; Teresa & Gonçalves-de-Freitas, 2011, Ribeiro et al., 2022). The species presents a social organization based on a dominance hierarchy through less energetically costly clashes, such as initial displays and after more energetic interactions, such as attacks (Maan et al., 2001).

The latency for interactions was greater for low intensity than for high intensity, indicating that the animals took longer to begin exhibiting the behavior (Figure 1). P, for the study, the exhibition of 8 behavioral units was also observed for the highest lux intensity and 4 for the lowest intensity, indicating a significant effect of light intensity on the agonistic behavior of the species (Carvalho et al., 2012, Sarmento et al., 2017, Brandão et al., 2021), the observations made by Chellappa et al. (2005) and Mattos et al. (2016), which indicate that the discus acara presents complex social behavior since it was observed that.

Aggressive behavior plays a fundamental role in controlling and prioritizing access to food as well as territory, and in addition, the animals considered dominant will be those that are able to reproduce. In this way, aggressiveness for cichlids has the function of organizing the social structure of the group (Huntingford & Tuner, 1987, Alcazar et al., 2016).

With the increase in light intensity, an increase in aggressive behavior and the complexity of social interactions were observed in relation to animals subjected to low intensity (Figure 2 or table?). When we think about physiological changes in relation to environmental stimuli, it should be understood that light plays a role in the synthesis and secretion of sex hormones, as well as melatonin and cortisol (Falcon et al., 2003, Korf, 2006).

In this sense, it is essential to understand that light can influence metabolism, sexual maturation and aggression, and can increase aggression and favor the establishment of the dominant hierarchy and choice of sexual partners (Norberg et al., 2004, Zhu et al., 2014, Elisio et al., 2014a, Elisio et al., 2015b, Navarro & Navarro, 2017, Shahjahan et al., 2020). It is noted that in the high-intensity condition, the color change unit was observed, in the social context when this behavior is observed it is an indication of hierarchical stability where the animals begin to have their parents defined as dominant and subordinate, indicating that high intensity favors the establishment of the dominance hierarchy and establishment of territory (Huntingford & Tuner, 1987, Chellappa et al. 2005). Aggression in cichlids is observed at different stages of development. However, aggressive behavior is exacerbated for reproduction. However, intense fights can cause negative impacts on animals, which can lead to hormonal imbalance, increased cortisol levels, reducing growth for both dominant and subordinate animals (Fox et al., 1997; Øverli et al., 1999; Sloman et al., 2001; Correa et al, 2003; Barreto et al., 2015, Brandão et al., 2021).

Studies carried out with the cichlid *Ciclhassoma dimerus* indicate that dominant animals have a gonodaosomatic index with the highest sperm concentration and that subordinate animals do not. This study suggests that animals in a lower condition in the social hierarchy of the group alter their physiology to reduce hormonal synthesis to delay their sexual maturation, as well as their growth for the next change in social position (Scaia et al., 2020). Such compression of social dynamics and what it can cause for social individuals can favor the improvement of well-being for the production of these species in captivity.

For artificial breeding, the production of homogeneous batches where all animals reach a similar size for sale is essential for profitability. The study, in addition to suggesting that increased light intensity can favor aggressiveness, the establishment of social hierarchy and, consequently, reproduction, also observed that low intensity reduces aggressiveness for the discus fish. In the context of production, especially in the growth phase, low light intensity can reduce the dispute for food, reducing clashes and, consequently, energy expenditure, which can be directed towards accelerated growth and homogeneous batches. Therefore, the simple practice of changing light intensity can favor the productive improvement as well as the well-being of the discus fish kept in an artificial environment.

Conclusion

Light intensity can be a factor influencing the social behavior and dominance hierarchy of the discus fish, and can increase and/or decrease aggressiveness for the discus fish. Adoption of management protocols as proposed by this study can favor the reproduction of the species in captivity and promote animal welfare. In this sense, the use of simple measures as proposed by the study can contribute to the production market, as well as to professional and hobbyist breeders of the species.

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CONCLUSION OF THE THESIS

The discus fish is a species of great interest to the ornamental fish market, due to its peculiar characteristics, arousing the interest of hobbyists as well as commercial producers. However, we still lack scientific information to propose the best management protocol for the species, taking into account the role of the necks in its production. The present study suggests that the discus fish is sensitive to changes in the environment, which can lead to a stressful condition leading to a drop in its production. Based on the data, observation of behavior combined with subtle changes such as those proposed in the work can influence the good management and well-being of the species.

ANNEX

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Original Article

Hematology in ornamental discus fish *Symphysodon discus* from Amazonian, Brazil

Hematologia em peixes-disco ornamentais *Symphysodon discus* da Amazônia

M. W. S. Ribeiro[a], A. R. S. Liebl[b] and A. T. Oliveira[a,b]*

[a]Universidade Federal do Amazonas – UFAM, Faculdade de Ciências Agrárias – FCA, Programa de Pós-graduação em Ciência Animal e Recursos Pesqueiros – PPGCARP, Campus Universitário, Manaus, AM, Brasil

[b]Instituto Federal de Educação, Ciência e Tecnologia do Amazonas – IFAM, Laboratório de Morfofisiologia Animal, Campus Manaus Centro – CMC, Manaus, AM, Brasil

Abstract

Symphysodon discus is known in the world of aquariums for its market value, beauty, and behavior. However, more substantial information about its physiology and biology must be available, which can hinder its development and maintenance in breeding systems. The study evaluated the blood biochemistry and erythrogram of 20 specimens of *S. discus* captured in the municipality of Barcelos, Amazonas, with an average weight of 89.80 ± 7.13 g and an average length of 13.48 ± 0.55 cm. The erythrogram evaluated variables such as hematocrit (Ht), hemoglobin (Hb), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Blood biochemistry was analyzed, including cholesterol levels, total proteins, triglycerides, glucose, urea, and chlorides. Intra-specific variations were observed between the surveyed individuals about the Hb, MCV, and MCH values. The values of triglycerides, cholesterol, and chlorides were elevated compared to other cichlids. This study may be useful to serve as a parameter to indicate the normal health conditions of this Amazonian cichlid. It can be applied in studies for ornamental fish farming and actions for managing and conserving the species.

Keywords: physiology, blood, wild specimens, cichlid.

Resumo

Symphysodon discus é conhecido no mundo dos aquários por seu valor de mercado, beleza e comportamento. Informações substanciais sobre a sua fisiologia e biologia devem estar disponíveis, o que pode dificultar seu desenvolvimento e manutenção em sistemas de reprodução. O estudo avaliou a bioquímica sanguínea e o eritrograma de 20 exemplares de *S. discus* capturados no município de Barcelos, Amazonas, com peso médio de 89,80 ± 7,13 g e comprimento médio de 13,48 ± 0,55 cm. O eritrograma avaliou variáveis como hematócrito (Ht), hemoglobina (Hb), contagem de hemácias (hemácias), volume corpuscular médio (VCM), hemoglobina corpuscular média (HCM) e concentração de hemoglobina corpuscular média (CHCM). A bioquímica sanguínea foi analisada, incluindo níveis de colesterol, proteínas totais, triglicerídeos, glicose, uréia e cloretos. Foram observadas variações intraespecíficas entre os indivíduos pesquisados quanto aos valores de Hb, VCM e HCM. Os valores de triglicerídeos, colesterol e cloretos foram elevados em comparação com outros ciclídeos. Este estudo pode ser útil para servir de parâmetro para indicar as condições normais de saúde desse ciclídeo amazônico. Pode ser aplicado em estudos para piscicultura ornamental e ações de manejo e conservação da espécie.

Palavras-chave: fisiologia, sangue, espécimes selvagens, ciclídeos.

1. Introduction

The main drivers of the ornamental fish trade are the aquarium consumers, who determine the species to be produced in aquacultures or caught in the wild (Din et al., 2002; Ribeiro et al., 2023). In general, characteristics such as striking colors, the rarity of the species, and behavioral peculiarities make the species attractive to aquarists (Jesus et al., 2022; Lemos et al., 2015; Santos et al., 2012).

The Amazonian species that comprise the genus Symphysodon (*S. aequefaciatus*, *S. discus*, and *S. tarzoo*) attract aquarists worldwide due to their body coloration pattern and behavioral characteristics (Rossoni et al., 2014; Yang et al., 2021). These cichlids have disk-shaped bodies, and small mouths, inhabit igarapés, lakes, and riverbanks with water temperatures ranging between 26 and 30 °C, and have good resistance to thermal variability (Rossoni et al., 2014).

The discus fish *Symphysodon discus* is economically significant in the national and international ornamental

*e-mail: adriano.oliveira@ifam.edu.br
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fish market (Tribuzy-Neto et al., 2021). However, scientific information on its physiology, health, and adaptation in aquaculture is scarce. The high mortality when out of the natural environment, due to stress and low immunity, problematizes the production of *S. discus* (Tavares-Dias and Moraes, 2004).

Among the tools used to understand animal physiology and health, the analysis of the hematological profile, which includes erythrogram and blood biochemistry, stands out. Hematology allows practical and helpful identification of the stress level, enabling the correction in management and ensuring animal welfare (Magro et al., 2016; Oliveira et al., 2021; Santos et al., 2024; Ranzani-Paiva et al., 2013). In addition to stress, factors such as life stage, type of environment, population genetics, and diet can influence the responses of hematological analyses (Tavares-Dias and Moraes, 2004). The current study verified the hematology of wild specimens of discus fish *S. discus* captured in the natural environment in the municipality of Barcelos, Amazonas, Brazil.

2. Material and Methods

Specimens of discus fish of *S. discus* (N= 20) were captured with "rapiché" (hand net) in flooded areas of the Daracuá community and Mariuá Archipelago, located in the municipality of Barcelos, Amazonas, Brazil (Figure 1). The experimental procedures adopted during these animals' capture and blood analysis were registered and approved by the Brazilian Institute of the Environment and Renewable Natural Resources-IBAMA under Process No. 15116-1. The research was developed and approved by the Ethics Commission on the Use of Animals (ECUA) of the Federal University of Amazonas under Process No. 2015/010.02.0905. All experiments were conducted according to local and ARRIVE guidelines (Percie du Sert et al., 2020).

The collected fish were stocked in "curries" (pens) (wooden tanks) until they were transported in canoes with "caçapas" (plastic containers with about 20 L^{-1} of water) to the city of Barcelos, Amazonas. After that, the animals were anesthetized with eugenol (0.2 $g.L^{-1}$) for blood collection by caudal puncture, using disposable syringes previously moistened with the anticoagulants heparin 2500 IU. After blood withdrawal, standard length (cm) and weight (g) were determined using tape and portable scales, respectively.

The collected blood was divided into two aliquots: one for determining red blood parameters and the other for obtaining plasma and performing assays on biochemical constituents. Erythrocyte counts (RBC) were conducted in a Neubauer chamber after dilution in formalin-citrate solution; hematocrit (Ht) was determined using the microhematocrit method; and the hemoglobin (Hb) concentration was found using the cyanmethemoglobin method. Through these data, the following red cell indexes were calculated: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) (Anselmo et al., 2021; Bussons et al., 2021; Oliveira et al., 2017; Aride et al., 2021).

Plasma was obtained after centrifugation and frozen in liquid nitrogen (-86 °C) until the biochemical analyses. Glucose, triglycerides, total cholesterol, total protein, urea, and chloride (Cl^-) concentrations were determined using enzyme-colorimetric methods, with quantification using commercial kits (Labtest®, Brazil) (Oliveira et al., 2016; Castro et al., 2021). The data were tabulated, and descriptive

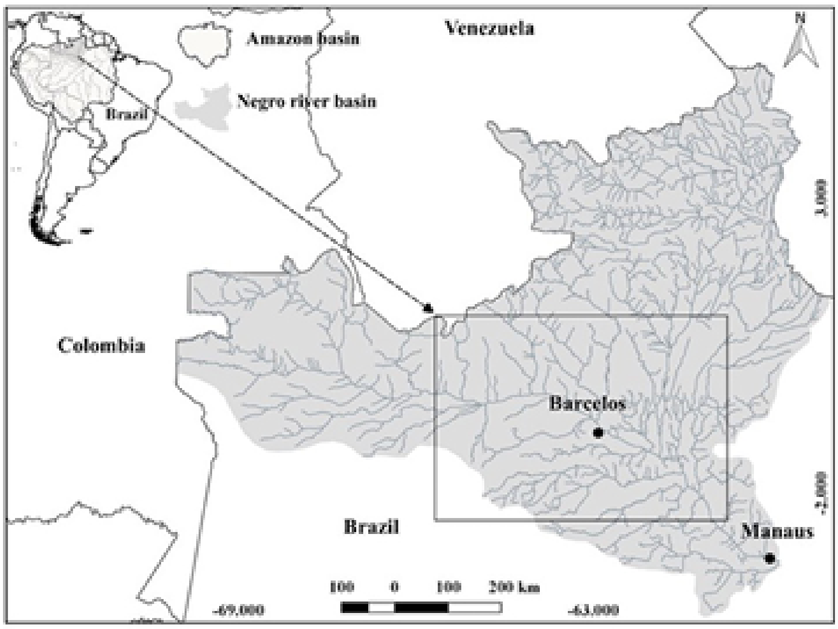

Figure 1. Location of the study area Barcelos, Amazonas, Brazil.

statistics were used, using mean, standard deviation (SD), and maximum and minimum values.

3. Results

In the biometric variables of the fish analyzed, a little intra-specific alteration was observed in total length (TC) and body weight (BW), with TC varying between 12.40 and 14.00 cm and BW varying between 77.00 and 104.00g (Table 1).

The erythrogram of the acará disco (Table 2) showed wide variation in the values of Hb (Hemoglobin; minimum of 2.51 and a maximum of 21.11 $g.dL^{-1}$), MCV (Mean corpuscular volume; minimum of 101.82 and a maximum of 718.31 fL), and HCM (Mean Corpuscular Hemoglobin; minimum of 9.98 and maximum of 91.64 pg). The levels of cholesterol, total proteins, triglyceride, glucose urea and chloride are demonstrated in Table 3.

4. Discussion

According to Rossoni et al. (2014), the adult *S. discus* has a body measuring between 12.00 and 20.00 cm. Thus, the data in Table 1 (total length: 13.48 ± 0.55 cm) show that the *S. discus* captured for the current study is in the adult phase. The fishes analyzed presented high hematocrit (Ht: 34.11 ± 10.23%) and mean corpuscular volume (MCV: 245.37 ± 164.9 fL) about the cichlids *S. aequifasciatus* (blue Discus; MCV: 121.80 ± 45.67 fL and Ht: 17.11%) and *Geophagus brasiliensis* (papa terra acará; MCV: 121.80 ± 45.67 fL and Ht: 5.68 ± 0.87%) reared outside the natural environment and studied by Paixão et al. (2017) and Romão et al. (2006), respectively. However, when compared to the cichlids *Cichla monoculus* (yellow tucunaré), *Cichla temensis* (tucunaré açu), and *Cichla vazzoleri*, in the natural environment, verified by Castro et al. (2021) regarding Ht (between 40.37 ± 1.17% and 40.40 ± 1.06%) and MCV (between 222.94 ± 25.94 fL and 246.11 ± 20.46 fL), the data of the present study are shown to be lower. Lower hematological values show that the animal has lentic behavior (Tavares-Dias and Moraes, 2004). While high values can be attributed to the constant movement of the animal for foraging or escape from predators. The comparison between the cichlids shows the possible influence of the natural environment and the aquaculture production environment on the hematological parameters of *S. discus*.

The mean corpuscular hemoglobin (44.78 pg) and the corpuscular mean hemoglobin concentration (21.45 $g.dL^{-1}$) of *S. discus* (Table 2) showed lower values than those of tucunarés *C. monoculus*, *C. temensis*, and *C. vazzoleri*

Table 1. Biometric variables of specimens of *Symphysodon discus* captured in Barcelos, Amazonas, Brazil.

Variables	Mean	Standard Deviation	Minimum	Maximum
Standard length (cm)	13.48	0.55	12.40	14.00
Weight (g)	89.80	7.13	77.00	104.00

Table 2. Erythrogram of specimens of *Symphysodon discus* captured in Barcelos, Amazonas, Brazil.

Variables	Mean	Standard Deviation	Minimum	Maximum
Ht (%)	34.11	10.23	20.00	51.00
Hb ($g.dL^{-1}$)	7.08	4.27	2.51	21.11
RBC (millions.μL^{-1})	1.80	0.76	0.56	2.99
MCV (fL)	245.37	164.9	101.82	718.31
MCH (pg)	44.78	23.73	9.98	91.64
MCHC ($g.dL^{-1}$)	21.45	11.59	9.80	49.10

Table 3. Plasma metabolites of specimens of *Symphysodon discus* captured in Barcelos, Amazonas, Brazil.

Variables	Mean	Standard Deviation	Minimum	Maximum
Cholesterol ($mg.dL^{-1}$)	298.97	81.32	95.71	442.57
Total Proteins ($g.dL^{-1}$)	2.08	1.14	0.63	4.51
Triglyceride ($mg.dL^{-1}$)	119.28	44.82	33.33	213.07
Glucose ($mg.dL^{-1}$)	53.98	34.80	11.74	140.41
Urea ($mg.dL^{-1}$)	31.85	7.20	6.96	43.01
Chloride ($mEq.L^{-1}$)	141.68	18.12	81.21	169.07

(Castro et al., 2021). Although both are cichlids in the natural environment, this difference can be attributed to factors that influence hematological parameters such as temperature, dissolved oxygen, seasonal cycle, stress, poor nutrition, and the sex of individuals (Castro et al., 2021).

In the plasma biochemistry analysis, *S. discus* showed high values for triglycerides, cholesterol, and chlorides about other Amazonian cichlids, such as *Cichla* spp. (Castro et al., 2021). Those above may be related to the type of feeding of *S.discus* in the natural environment, which is based on protein- and lipid-rich foods such as small fish, live worms, nauplii of microcrustaceans and periphyton, and the availability and nutritional composition of these foods in the localities where the fish were collected (Rossoni et al., 2014).

The high glucose and urea levels (53.98 $mg.dL^{-1}$ and 31.85 $mg.dL^{-1}$, respectively; Table 3) suggest that the animals were stressed, which can be attributed to the traditional processes of capture, stocking in pens and pens, and transport to the distribution center in Manaus city (Erdal et al., 1991; Ranzani-Paiva et al., 1999). The stocking process can be long and without control over the ideal water quality levels (up to 48 hours without aeration in caçapas), which provides hypoxia and contributes to high values of plasma metabolites (Rossoni et al., 2014; Ramos et al., 2015; Ladislau et al., 2021).

5. Conclusion

In conclusion, the hematological responses obtained in this study with *S. discus* suggest that the species is sensitive to stress caused by environmental disturbances. Standardized hematological studies with *S. discus* in different environments are needed to confirm this. This understanding is fundamental to providing adequate acclimatization of animals under production, and maintaining animal welfare and health, since *S. discus* naturally presents low immunity, and stressful conditions can worsen this condition and lead to mortality. The current study profiles the hematological variables of *S. discus* under the described conditions and serves as a parameter for further physiological studies with the species.

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