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Behavioral analysis of fish in response to chondroitin and its applicability in the protection of ichthyofauna in the hydroelectric sector

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ABSTRACT

Alarm signals are released into the environment by the epidermal cells of aquatic communities to indicate danger situations. Chondroitin sulfate is indicated as one of the active compounds of alarm substances which can induce behavioral changes of avoidance in specimens that have chemical stimuli receptors. The potentiality of chondroitin for the development of a behavioral barrier to deter ichthyofauna was previously evaluated in laboratory tests (1, 2 and 5 µg L^{-1}) and subsequently in the draft tube (6 µg L^{-1}) of bulb-type turbines at a Hydroelectric Power Plant, on the Madeira River, in Rondônia, Brazil. In tests under controlled conditions with the Matrinxã and Tambaqui species, fast swimming and grouping were the most frequent alarm reactions in both species, with a greater number of episodes being observed at higher concentrations. In the draft tube, the injection of the concentrated chondroitin solution caused a decrease (55%) in the movement of confined fish, which indicates the deterrence of the ichthyofauna. The tests showed the potentiality of the sustainable use of chondroitin sulfate in the protection of ichthyofauna in hydroelectric projects.

KEYWORDS: Alarm substances. Confined ichthyofauna. Behavioral barrier.

1 **INTRODUCTION**

The transfer of information between animals can occur through visual, sound, tactile, electromagnetic, chemical means and also through temperature waves. The aquatic environment is favorable for chemical communication, as chemical signals can easily dissolve in water. In addition, aquatic animals, especially fish, have a high sense of smell for detecting chemical stimuli (Da Silva, 2010).

Alarm signals are released into the environment by the epidermal cells of aquatic communities to indicate danger situations, changing the behavior of exposed individuals. These substances are present in teleosts of the superorder Ostariophysi, which includes about 55 families of fish (Pfeiffer, 1963). However, similar reactions were found in Perciformes (Percidae), inferring a wide distribution (Smith and Lawrence, 1991; Smith, 1979a and 1992b). The detection of these chemical signals can result in the search for shelter, immobility, schooling, fast swimming, in addition to other alarm reactions (Mathuru et al., 2012).

Studies dedicated to identifying the chemical composition of the substances responsible for producing the alarm signals indicated chondroitin sulfate and hyaluronic acid in the mucus of *Salmo gairdner* (Brown et al. 2000, Van de Winkel et al., 1986). Experiments performed by Mathuru et al. (2012) using biochemical fractionation indicated that the alarm substances include chondroitin oligosaccharides, with a minimum size of a tetrasaccharide (∼1,000 Daltons). Other studies suggest the presence of chondroitin sulfate as one of the components of alarm substances (Farnsley et al., 2016; Brown et al., 2000; Hintz et al., 2017).

Chondroitin is a sulfated glycosaminoglycan composed of an unbranched polysaccharide chain with a repeating disaccharide structure (N-acetylgalactosamine and glucuronic acid), being bound to proteins as part of a proteoglycan (Zhou et al., 2010). Chondroitin is distributed in various human and animal tissues, being an important component of skin, cartilage, tendons and other tissues, and is also related to the regulation of blood clotting and inflammation, and to the regeneration of the central nervous system (Guidan et al., 2014 , Krylov et al., 2011; Guidan et al., 2014).

The study of this substance's repulsive potential may result in important future applications that aspire to protect the ichthyofauna in the hydroelectric sector. During scheduled or untimely maintenance of the generating units of hydroelectric plants, drainage

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often occurs, requiring the insertion of gates or stop log panels upstream and downstream of the unit to isolate the hydraulic circuit. However, before the closure is completed, fish may enter the draft pipe, probably attracted by the reduction in flow and/or by the turbulence of water coming from adjacent generating units in operation (Cemig, 2015). Rescue operations must then be quickly triggered, but they are always manual in places of difficult access and confined space, which results in a costly process (Perry et al., 2014).

There are techniques that can protect the ichthyofauna by preventing their entry into these facilities, such as physical barriers consisting of screens or grids (Perry et al., 2014; Cemig, 2015) and behavioral barriers using sounds, bubble curtains, lights and electric current (Zielinski et al., 2014; Dias et al., 2020; Figueiredo et al., 2021). However, behavioral barriers in many situations can be influenced by the species and its body size and/or by environmental variables such as turbidity (Wisenden & Smith, 1997; Perry et al., 2014).

Recently, Da Silva et al. (2022) studied an operative maneuver to raise the hydraulic flow in a bulb-type turbine (3.6 to 11.0 m/s), which is highly efficient in minimizing the confinement of the ichthyofauna in the draft tubes during the shutdown of generating units. By reducing the confinement of the ichthyofauna by around 87%, this maintenance procedure, combined with real-time monitoring in the draft tube, has a positive impact on the conservation of the fauna and on the economic costs related to the shutdown of the generating unit, in addition to reducing the workload of the teams responsible for rescuing ichthyofauna in confined spaces.

The development of the hydroelectric sector in the Amazon region requires practices to protect the ichthyofauna. In this sense, this study investigates the potential of chondroitin sulfate for the development of a behavioral barrier aimed at deterring ichthyofauna during operational maneuvers in the hydroelectric sector.

2 MATERIALS AND METHODS

2.1 Studies in controlled environment

2.1.1 Remote system for behavioral analysis of fish

Glass aquariums measuring 20 x 30 x 200 cm (W x H x L) were individually equipped with oxygenation, recirculation, injection and liquid sample injection systems (Figure 1). An aluminum profile structure was developed in order to be attached to each aquarium during the tests, maintaining the adjustments for capturing video images. In this arrangement, 3 (three) USB cameras, Logitech brand and model C920e Full HD, were arranged alternately in order to avoid overlapping of images and/or footage outside the field of view of the cameras. The movement pattern of individuals was analyzed considering three sectors, which were defined according to the camera images, sector 1 being closest to the dispersion of the alarm substance, sector 2 in the central portion and sector 3 farthest from the introduction of the substance. All of the aquarium's operational equipment and the acquisition of images from the cameras were

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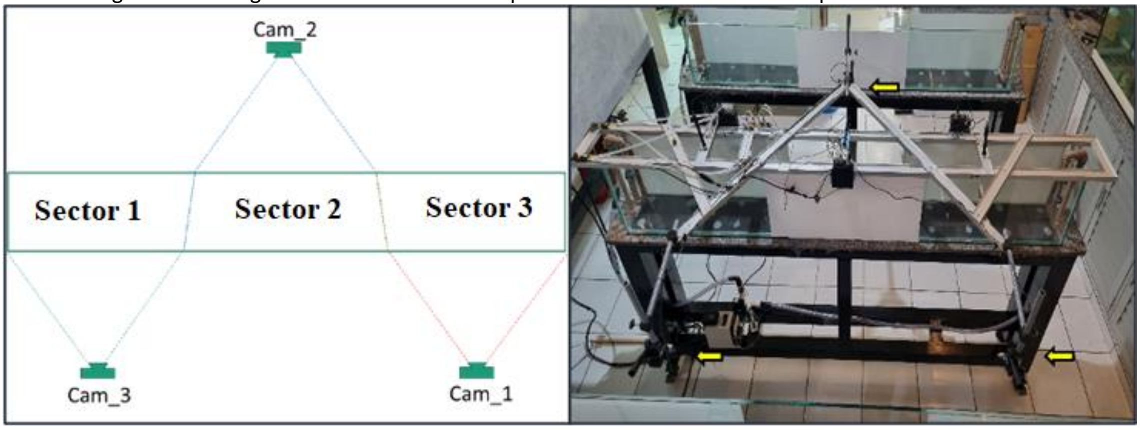
managed by software developed with the computational language C# (C Sharp) and the open source library of computational vision "OpenCvSharp".

Figure 1 – Arrangement of the cameras disposed over the instrumented aquarium. Cam: Camera.



Source: Own authorship.

2.1.2 Experiment protocol

Species selection was guided by ease of handling and adaptability to laboratory conditions, good sensitivity of the species to external stimuli and abundance in the Amazon region. Matrinxã (*Brycon cephalus*) and Tambaqui (*Colossoma macropomum*) specimens were commercially purchased and kept in aerated acclimatization tanks (5,000 L). The fish were fed daily with commercial fish food, up to 24 hours before the tests, and exposed to a natural photoperiod of 12/12 hours (light and dark) and an average temperature of 26 ± 1 °C for 60 days.

On the day before the experiments, 10 fish were randomly selected and placed in each aquarium, being kept in fasting. The tests took place in two stages. In the first one, filming was performed for 20 min with interruption of oxygenation and adjustment of the water flow to 1.7 L min^{-1}. In the second stage, chondroitin sulfate solution was injected (2 min) and another 20 min interval was monitored. Chondroitin sulfate sodium salt synthesized from shark cartilage was purchased commercially (Sigma C4384, St. Louis, MO). Assays took place in triplicates at chondroitin concentrations of 1; 2 and 5 µg L^{-1}, considering the dilution of the substance in the total volume of the aquarium (100 L), following the protocol by Mathuru et al. (2012).

2.1.3 Behavioral patterns

The stimuli caused by exposure to chondroitin sulfate in the Matrinxã and Tambaqui species were analyzed from the manual calculation per minute of episodes of grouping, fast swimming and immobility in the first and second stages of the tests. These alarm behaviors are frequent in studies that prove the existence of alarm substances in ichthyofauna (Brown & Godin, 1997; Brown et al., 2000; Ide et al., 2003; Goulart, 2010). Clustering consists of forming groups with 3 or more individuals; fast swimming occurs when fish energetically move their fins; and immobility is the suspension of movements in their entirety for periods longer than 30 seconds (Lawrence & Smith, 1989).

2.2 Studies in the draft tube of HPP Jirau

2.2.1 Study area

The Jirau Hydroelectric Power Plant (HPP Jirau) is located in the Amazon region, on the Madeira River (Figure 2). HPP Jirau has an installed capacity of 3,750 MW, making it the fourth largest generator of electricity in Brazil, representing about 3.7% of all hydroelectric power in the country. The plant has 50 bulb-type generating units, distributed in two powerhouses (CF1 and CF2), with a nominal discharge of 550 m^3/s and physical guarantee of 2,211.6 MW (Jirau Energia, 2022).

Figure 2 - Structure of the study areas at HPP Jirau, on the Madeira River, Rondônia.

Source: Jirau Energia (2022).

2.2.2 Chondroitin injection and dispersion system in the draft tube

The bulb-type generating units at HPP Jirau have an aeration system built into the lower part of the draft tube, allowing the introduction and dispersion of the chondroitin solution. This system consists of 8 hexagonal injector nozzles (3 cm), with 4 nozzles distributed on each side with a spacing of 6.0 m. In the supply line of this system, a by-pass was installed with a 3/4" valve and a 10 bar pressure gauge, allowing the introduction of liquid and later transport by the compressed air drag flow (7 bar). The solutions of reference (80 L of distilled water) and concentrated chondroitin solution of 0.34 g L^{-1} (80 L), were previously pressurized at 10 bar for injection into the system (Figure 3).

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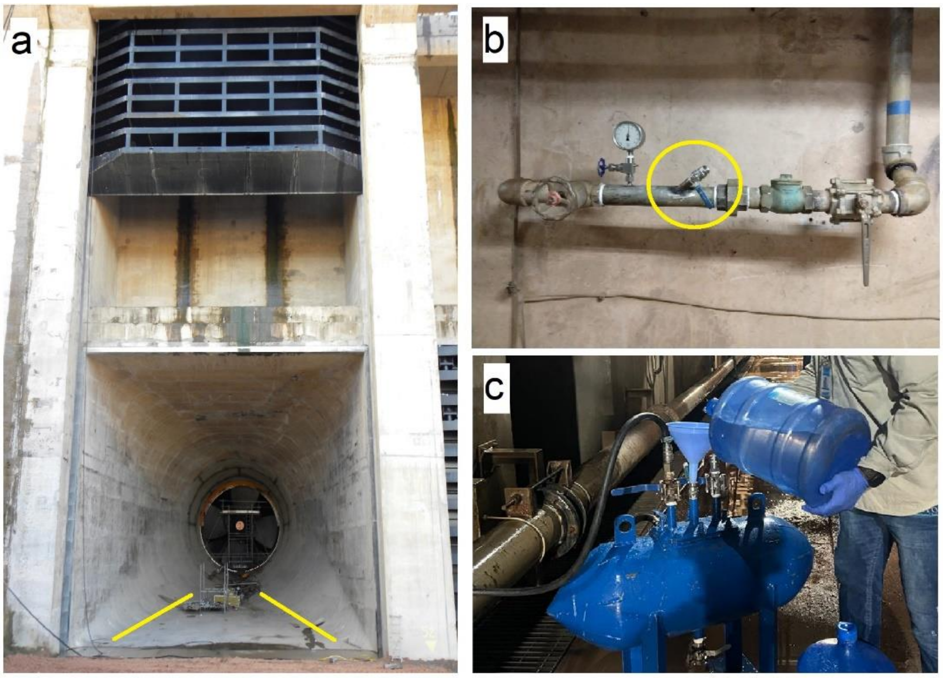
Figure 3 – Adapted system for injecting liquid samples into the draft tube of a generating unit at HPP Jirau. **a** – Draft tube with indication of the location of the aeration system piping; **b** – Aeration system with manual actuation valves and manometer, with by-pass indication and; **c** – Technical arrangement of pressurization for injection of substances.



Source: Own authorship.

2.2.3 Fish monitoring system in the draft tube

For real-time evaluations of the movement of the ichthyofauna in the draft tube of the generating units, a hydroacoustic system of low and high frequency sweeps and a measurement tool were used. The miniaturized and robust system developed by the company Venturo Consultoria Ambiental is composed of two transducers fixed on a support, about 9 m above the lower part of the unit's sill, so that the transducer is in the center of the draft tube. The transducer cabling was connected to the display, in a safe area, on the upper slab of the face downstream of the powerhouse. The hydroacoustic system was adjusted to frequencies from 350 to 400 kHz, with the "side Imaging" module having a horizontal beamwidth of 0.9°, a vertical beamwidth of 39° and a vertical inclination angle of 26° (Da Silva et al., 2022 and Santana et al., 2022). Acquisition of underwater images occurred with injection of water (1st Stage) and concentrated chondroitin solution (2nd Stage), for 25 minutes, with the draft tube completely open. The images were later analyzed using software for viewing and counting the movement of ichthyofauna.

3 RESULTS

In a controlled environment, 18 behavioral tests were carried out with the Matrinxã (*Brycon cephalus*) and Tambaqui (*Colossoma macropomum*) species, totaling 12 hours of footage for manual analysis per minute of episodes of grouping, fast swimming and immobility in the first and second stages.

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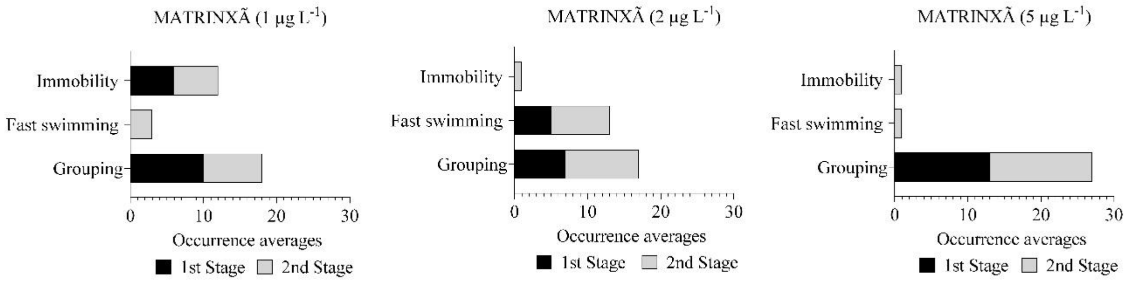
The multivariate statistics of the behavioral evaluation of the Matrinxã species under the effect of chondroitin in an environment with concentrations of 1; 2 and 5 µg L^{-1} showed no difference for the grouping, fast swimming and immobility stimuli, these behaviors being recurrent before and after the injection of the substance. However, other alarm reactions, such as lethargy and stereotyped movements, were more prevalent in the 2nd stage of the experiments (Figure 4).

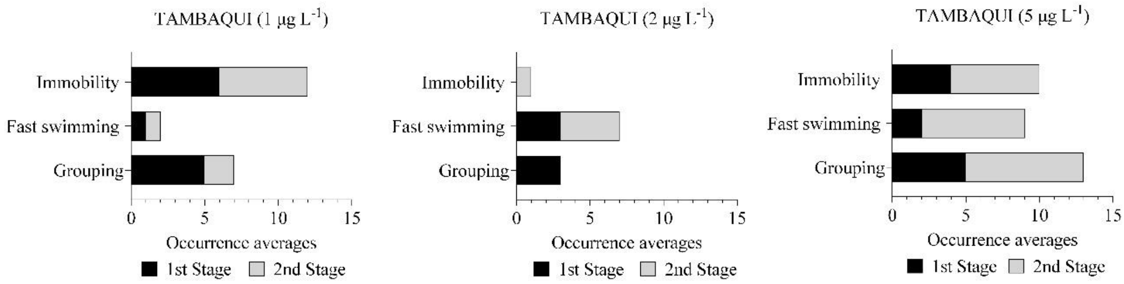
Under the effect of chondroitin, the Tambaqui species showed behaviors similar to those observed with the Matrinxã species, making it possible to identify the expansion of alarm reactions in the environment with 5 µg L^{-1} of chondroitin, with emphasis on the fast swimming effect (Figure 5).

Figure 4 – Mean value of occurrences of clustering, fast swimming and immobility alarm reactions for the Matrinxã species, before (1st Stage) and after exposure to chondroitin sulfate (2nd Stage).



Source: Own authorship.

Figure 5 – Mean value of occurrences of clustering, fast swimming and immobility alarm reactions for the Tambaqui species, before (1st Stage) and after exposure to chondroitin sulfate (2nd Stage).



Source: Own authorship.

For the tests in the draft tube of the bulb-type generating unit at HPP Jirau, the volume for chondroitin dispersion of approximately 4,500 m^3 was considered. Therefore, a concentrated solution was injected (0.34 g L^{-1}) in order to reach an environment with a concentration of about 6 µg L^{-1} of chondroitin sulfate along the draft tube. It is important to highlight that the adjacent generating units also remained inoperative.

Movement patterns of neotropical migratory fish throughout the day are still not fully understood. Some species of the Siluriformes order, which includes catfish, pimelodid catfish, leopard catfish and even suckermouth armored catfish, have nocturnal habits. The order of Characiformes, made up of approximately 270 genera and over 1,700 species such as piranha,

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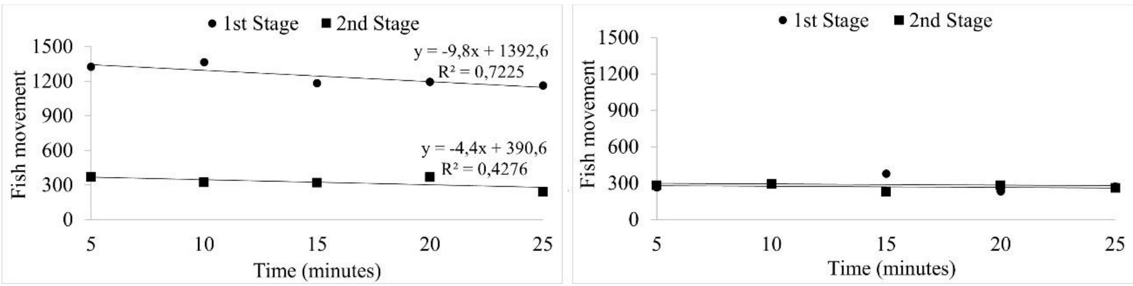
lambari fish, streaked prochilod and dorado, present a different pattern (Pompeu & Martinez, 2006).

Injections of concentrated chondroitin solutions occurred in two different scenarios regarding the presence of ichthyofauna inside the draft tube (Figure 6), the first with a greater presence of specimens (morning period) and the second with a smaller presence of specimens (afternoon period), with the second scenario about 75% smaller when compared to the first scenario.

In the environment with a high presence of specimens in the draft tube, the ichthyofauna movement analysis shows a significant decrease of about 55% after the injection of chondroitin (Stage 2), making it possible to infer the deterrence of the ichthyofauna from the draft tube. According to Galhardo & Oliveira (2006), the primary response of animals in situations of stress is flight or immobilization, however, in environments that do not allow escape, it is common to observe behavioral changes such as increased rhythm and swimming pattern, search for shelter and even reduced feeding frequency, and territorial and reproductive behavior. The injection of chondroitin in an environment with a low presence of specimens showed no difference between Stages 1 and 2, and it was not possible to observe the occurrence of alarm reactions.

Figure 6 – Movement of ichthyofauna in two scenarios with higher (left) and lower (right) number of specimens in the draft tube of the generating unit of HPP Jirau, with application of water (1st Stage) and chondroitin sulfate (2nd Stage).



Source: Own authorship.

The underwater images of the movement of the ichthyofauna in the draft tube (Figure 7) in the morning period show the presence of a large number of small specimens and a subsequent decrease in the presence of chondroitin. While the images referring to the test in the afternoon period (Figure 8) show the absence of the occurrence of alarm reactions after the injection of chondroitin. In addition, there is the presence of a small number of individuals and large specimens, which can be predatory fish.

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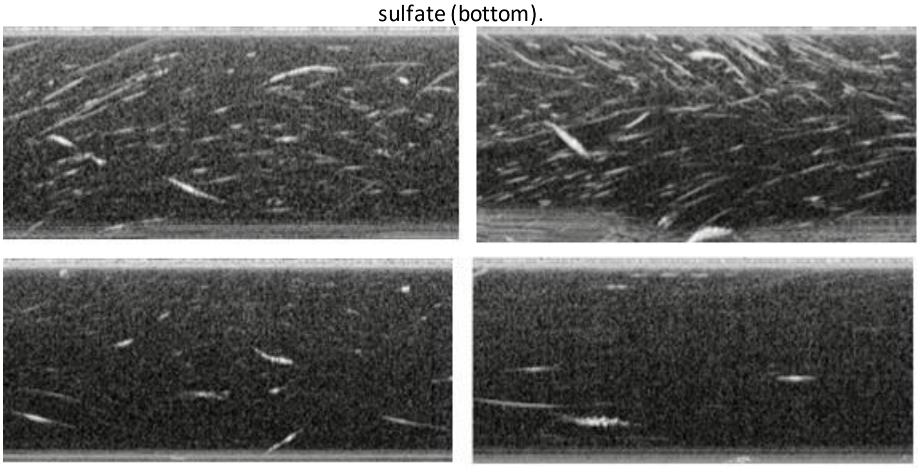
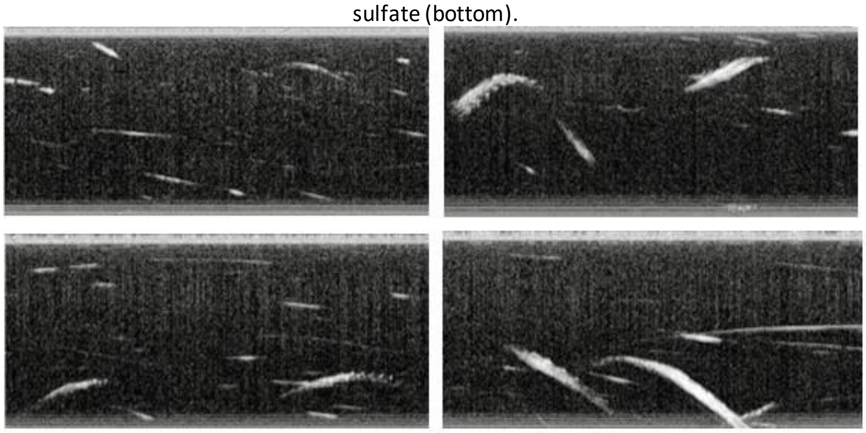
Figure 7 – Underwater images of the movement of ichthyofauna in the scenarios with the highest number of specimens in the draft tube of the generating unit of HPP Jirau, with the application of water (top) and chondroitin sulfate (bottom).



Source: Own authorship.

Figure 8 – Underwater images of the movement of ichthyofauna in the scenarios with the lowest number of specimens in the draft tube of the generating unit of HPP Jirau, with the application of water (top) and chondroitin sulfate (bottom).



Source: Own authorship.

Studies proving the effectiveness of chondroitin in changing behavior in fish are scarce. Farnsley et al. (2016) presents results, similar to the present study, with the species *Fundulus catenatus*. Mathuru et al. (2012) observed, in the species *Danio rerio*, slow swimming, darts and permanence in the bottom. The same authors point out that chondroitin activates the mediodorsal posterior bulb of fish through smell. Ide et al. (2003) identified fast swimming followed by immobility as the main alarm reaction for the species *Brycon cephalus*, a result that corroborates the results observed in this study.

However, the intensity and frequency of alarm reactions in fish exposed to the skin extract is higher when compared to chondroitin, with deceleration reactions, immobility, increased swimming activity and exploration with the application of skin extracts (Lawrence & Smith, 1989; Chivers & Smith, 1994; Brown & Godin, 1999; Da Silva, 2010; Mathuru et al., 2012).

Slow swimming, which consists of movement of approximately 0.5 cm s^{-1}, and stereotyped and short movements of the fish in the horizontal position were observed more frequently in trials with an environment of 5 µg L^{-1} of chondroitin sulfate, for both the Matrinxã

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and Tambaquí species. According to Pfeiffer (1977), these behaviors are characteristic in situations of high stress.

In most laboratory tests there was no specific pattern of repulsion, considering the predilection of individuals for aquarium sectors. The fact that it is a confined space may have tended individuals to go through the same sector repeatedly. Thus, knowledge of ethology, which is the science that analyzes animal behavior patterns, is more relevant for this study (Braithwaite & Huntingford, 2004).

Studies in the draft tube of the bulb-type generating unit at HPP Jirau showed the potential of the application of chondroitin in deterring fish from structures in the hydroelectric sector, favoring the development of new technologies aimed at protecting the ichthyofauna. Dias et al. (2020) analyzed the reports of the Ichthyofauna Conservation Program (PCI) of HPP Jirau over 10 years (March/2009 to April/2019) and found that more than 500 species of fish were identified near the structures of this hydroelectric plant. Not all fish species react to alarm substances, such as fish from cave environments or voracious predators, which are less subject to selective pressure and, therefore, have reduced or lost this ability (Duboc, 2007).

The high olfactory sensitivity of the ichthyofauna for detecting chemical stimuli and the solubilization of alarm substances in water make the aquatic environment favorable to chemical communication (Da Silva, 2010).

The Madeira River has high turbidity, especially in rainy periods, due to the presence of suspended solids, such as inorganic particles (sand, silt, clay) and organic debris, algae and bacteria, plankton, among others (Barbosa et al, 2018). These conditions certainly hinder the homogeneous dispersion of alarm substances, favoring interactions with suspended particulate matter and possible reactions with organic debris.

However, even in this unfavorable aquatic environment, the study in the draft tube showed relevant results in deterring ichthyofauna exposed to chondroitin. Taking into account the sustainability guidelines of the hydroelectric sector, the development of behavioral barriers for fish is of fundamental importance to reduce the possible impacts caused by hydraulic structures.

4 CONCLUSION

In a controlled environment, the alarm reactions of the Matrinxã and Tambaqui species were similar when exposed to chondroitin. However, greater effects were observed on the behavior of the Tambaqui species. The most recurrent behaviors were fast swimming followed by grouping, with a greater number of episodes during exposure to higher concentrations of chondroitin.

Laboratory tests associated with tests in the draft tube of the bulb-type generating unit at HPP Jirau allowed inferring the potential of the sustainable use of chondroitin sulfate to protect ichthyofauna in hydroelectric projects. The strategy of using the by-pass system to introduce alarm substances into the draft tube of generating units, through the built-in compressed air piping, is easily adaptable for implementation in different hydroelectric projects.

5 ACKNOWLEDGMENTS

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