IMPLEMENTATION AND INSTRUMENTALIZATION OF THE AQUARIUM FOR EXPERIMENTS RELATED TO ICHTHYOFAUNA

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Miguel Vieira de Melo Neto[1], Ézio Sargentini Júnior[2], Marcos Alexandre Bolson[3], Tania Machado da Silva[4] and Luiz Fabrício Zara[5]

ABSTRACT
The analysis of the behaviors of aquatic organisms in the face of environmental stressors is a way to assess environmental quality and reduce negative impacts. In this study, the use of aquariums to observe the behavior of fish exposed to behavioral barriers was proposed. For this, water circulation, oxygenation, injection and liquid sample dispersion systems were installed in a set of 3 aquariums, in addition to a remote monitoring system with image capture. Software was developed to identify the position of the fish in the aquariums and control the introduction of the sample. The results showed that this method is effective for analyzing fish behavior and can be adapted to study other stressful stimuli.

Keywords: Controlled environment. Air bubbles. Ichthyofauna. Behavioral barriers.

[1] Master in Environment and Rural Development
University of Brasilia
Brasilia, Federal District, Brazil
E-mail: miguelv17@gmail.com
[2] Doctor in Chemistry
São Paulo State University Júlio de Mesquita Filho
Manaus, Amazonas, Brazil
E-mail: eziosargentini@gmail.com
[3] Master of Science
University of São Paulo
Manaus, Amazonas, Brazil
E-mail: mabolson@gmail.com
[4] Doctor in Environmental Sciences
University of Brasilia
Brasilia, Federal District, Brazil
E-mail: taniams.venturo@gmail.com
[5] Doctor in Analytical Chemistry
São Paulo State University Júlio de Mesquita Filho
Brasilia, Federal District, Brazil
E-mail: fabriciozara@gmail.com

INTRODUCTION

The growing need for clean and renewable electricity generation to supply industry, agriculture, commerce, and residence results in the construction of hydroelectric power plants (FRIGOTTO *et al*, 2023). These plants require scheduled and untimely maintenance in order to maximize energy generation and minimize costs. However, during these stops, the reduced operational flow can facilitate the entry of fish into the suction pipes, the water intake galleries and the sewage wells (PERRY *et al*., 2014). As a result, it is necessary to develop behavioral barrier systems suitable for different species, in order to mitigate or even prevent the entry of these fish into undue areas (DA SILVA *et al*., 2022). Behavioral systems have demonstrated efficacy, but each species reacts differently, requiring further investigation (ZIELINSKI *et al*., 2014; DENNIS *et al*., 2019). This need is accentuated because they are tropical species, which live in conditions different from those found in temperate environments (MURCHY *et al*., 2022; DE MENDONÇA MAROJA *et al*., 2023).

The use of controlled environments in carrying out experiments is common in science, as it is used as a pilot in order to adjust the procedures in order to simulate the environmental conditions as much as possible. In addition to reducing the operational costs inherent to the implementation of the bubble system and the respective experiment in the field (Da Silva, 2010; Zielinski *et al*., 2014; Zielinski & Sorensen, 2015). Although they are not fully effective, there is the importance of developing behavioral systems with as much effectiveness as possible, for this it is necessary to develop aquariums to be used as controlled environments in experiments (Zielinski & Sorensen, 2015). In this study, we proposed the development of aquariums instrumentalized to observe the behavior of fish exposed to behavioral barriers.

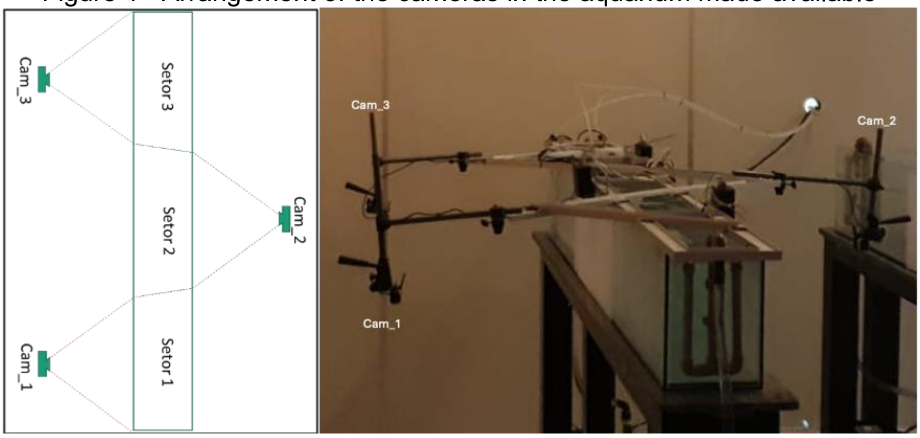
MATERIALS AND METHODS

AQUARIUMS INSTRUMENTALIZED FOR ANALYSIS OF FISH BEHAVIORS

The evaluation of the effect of the bubble on the behavior of the ichthyofauna was used by 03 aquariums with a capacity of 100 L and dimensions 20x30x200 cm (W x H x D), equipped with water circulation and recirculation and oxygenation systems. Each aquarium has an approximate useful capacity of 100 liters and a water column of 26 cm. The recirculation system present in the aquariums has a 24V Aw500s pump, capable of pumping 10 liters per minute, and an FY-B7 flow sensor based on the Hall Effect (Hall *et al.*, 1879). These devices were connected to the aquarium using 3/4-inch crystal polyethylene tubing. Flow control is handled by a 350 W power supply that offers regulated voltages of 5, 12, 17 and 24 V, with flow rates of 3.5; 6,6; 8,8; and 10.6 L/min, respectively. It is essential to highlight that the integration of the liquid sample insertion system with the circulation system is crucial. This system consists of: peristaltic flow pump from Ismatec UK Ltd., model MC-MS/CA8: equipment that has the ability to accurately control the rate of liquids and has the functionality of being operated remotely (Formato et al. 2019); interaction and management device: the gadget_TTGO T-Display, equipped with a 32 MHz chip_ESPRESSIF 240 MHz, is capable of being configured to receive guidance from a computer and transform them into electrical signals to activate the peristaltic pump.

To monitor the behavior of the fish, an image capture system was developed with 3 Full HD cameras, with a resolution of 1080p/30fps, arranged in a mobile aluminum profile in a "V" shape, with the central camera opposite the cameras at the ends, avoiding image overlap (Figure 1). Specific software detects and records the position of the specimens throughout the aquarium, as well as controls the introduction of bubbles and permanent lights. The software uses C# computational language (C Sharp) and the features of the open source computer vision library OpenCvSharp. The aquariums are located at the National Institute for Research in the Amazon - INPA.

Figure 1 - Arrangement of the cameras in the aquarium made available



Source: Prepared by the authors.

EXPERIMENTAL PROTOCOL

The species Tambaqui (*Colossoma macropomum*); Matrinxã (*Brycon*); Amazonian angelfish (*Cichlasoma amazonarum*); Dianema Tiger (*Dianema urostriatum*); Mandi (*Pimelodus blochii*) were commercially acquired and kept in an acclimatization tank (5,000 L) for 60 days at an average temperature of 26 ± 1 °C, being fed with commercial feed and exposed to the natural photoperiod of 12/12 hours. To perform the assays, 30 individuals were randomly selected and transferred from the acclimatization tank to the instrumentalized aquariums 18 hours in advance, being kept fasting. Previously, the aquariums received 50% (v/v) of the water from the acclimatization tank, minimizing significant changes in the physical-chemical parameters of the water, and, consequently, changes in behavior and/or diseases in the fish. In the first stage of the tests, the bubble system was turned off for 15 minutes, and images of the fish's behavior were acquired. In the sequence (second stage), the bubble systems were turned on and images of the fish's behavior were acquired for 15 minutes. In the last step, the bubble systems were turned off and images of the fish's behavior were acquired for 15 minutes.

FULL BUBBLE CURTAIN SYSTEM AT THE BOTTOM IN THE AQUARIUM (20 CM)

The generation of bubble barrier by the air compressor (Vulcan Trent VC 25), working with a pressure of 2.5 bar) linked to a micro-perforated hose for gas diffusion with a bubble size of 50 to 500 µm.

To generate permanent light, in the upper part of the aquarium, at the same point where the bubble curtain system was installed, a structure with dimensions of 20 x 30 cm was adapted with a 3 mm slit to generate a light slit on the bubble dispersion axis. Inside this box there was a Taschibra light-emitting diode lamp with a power of 9 W and bivolt, which was used in yellow (warm, 3000 K).

In addition to the use of bubble curtains and permanent light, the aquariums were prepared to integrate a liquid sample insertion system. This system consists of a peristaltic flow pump from Ismatec UK Ltd., model MC-MS/CA8, equipment capable of precisely controlling the rate of liquids and which can be operated remotely (FORMATO et al. 2019). The interaction and management device used is the T-Display gadget_TTGO, equipped with a 240 MHz ESPRESSIF 32 chip, configured to receive commands from a computer and transform them into electrical signals to activate the peristaltic pump.

It is important to note that the instrumentalized aquariums are located in a laboratory at the National Institute for Research in the Amazon (INPA). This laboratory has light and temperature control, in order not to interfere with the behavior of the fish used in the experiments.

COMPUTER PROGRAM FOR ACQUISITION, CONTROL AND TREATMENT OF AQUARIUM IMAGES

The elaboration of a computer program in the C# computer language (C Sharp), which was compiled in Microsoft Visual Studio, using the tools available in the open source computer vision library OpenCvSharp. The aforementioned program has the functionality of detecting and recording the position of the fish in the aquarium, as well as controlling the bubbles.

In order to locate the fish, the software analyzes the images captured by the cameras, putting them together to create a single view of the aquarium. This process goes through six steps: (1) grayscale transformation and noise reduction using the Gaussian Blur filter. This step smooths the image and prevents false detections; (2) using a copy of the original image, a background image is created with the Gaussian Blur filter adjusted differently; (3) the difference between the two images is calculated to highlight the objects of interest; (4) the resulting image is then binarized with the Threshold filter, converting it to black and white; (5) the Dilate filter is applied to the binarized image to improve the definition and correct internal flaws in the objects; (6) In the last stage, the fish are counted and recorded in Cartesian coordinates.

The information of the detection process is stored in a document in .csv format at a frequency of ten frames per second (10 Hz), in which each line records the date, time and location of each fish identified in each frame analyzed. Simultaneously, a file in .mp4 format is generated to record all frames in a streaming video with a rate of 10 Hz, allowing the choice of whether or not to include markers for the fish.

EXPERIMENTAL PROTOCOL FOR TESTING WITH BUBBLE CURTAIN

The species Tambaqui (*Colossoma macropomum*), Matrinxã (*Brycon spp.*), Amazonian angelfish (*Cichlasoma amazonarum*), Dianema Tigre (*Dianema urostriatum*) and Mandi (*Pimelodus blochii*) were commercially acquired and kept in a 5,000 L acclimatization tank for 60 days, at an average temperature of 26 ± 1 °C. During this period, they were fed commercial feed and exposed to the natural 12/12-hour photoperiod.

To perform the trials, 30 individuals were randomly selected and transferred from the acclimatization tank to the instrumentalized aquariums 18 hours in advance, being kept fasting. Previously, the aquariums received 50% (v/v) of the water from the acclimatization tank, minimizing significant changes in the physical-chemical parameters of the water and, consequently, changes in behavior and/or diseases in the fish.

In the first stage of the trials, the bubble system was kept off for 15 minutes, and images of the fish's behavior were acquired. Then (second step), the bubble curtain system was turned on, and the images of the fish behavior were captured for 15 minutes. In the final step, the bubble curtain system was turned off again, and the images of the fish's behavior for another 15 minutes.

RESULTS

To verify the functioning of the bubble curtain system, an initial test was carried out before the behavioral tests. To this end, the compressor, operating at a pressure of 2.5 bar, was connected to the microperforated hose for gas diffusion, in order to evaluate the formation of the bubble curtain. The results were satisfactory, with the bubble curtain being formed as expected and the test recording confirming the efficacy of the system (Figure 2).

Figure 2 – Image of the curtain formation test in the bubble curtain system.

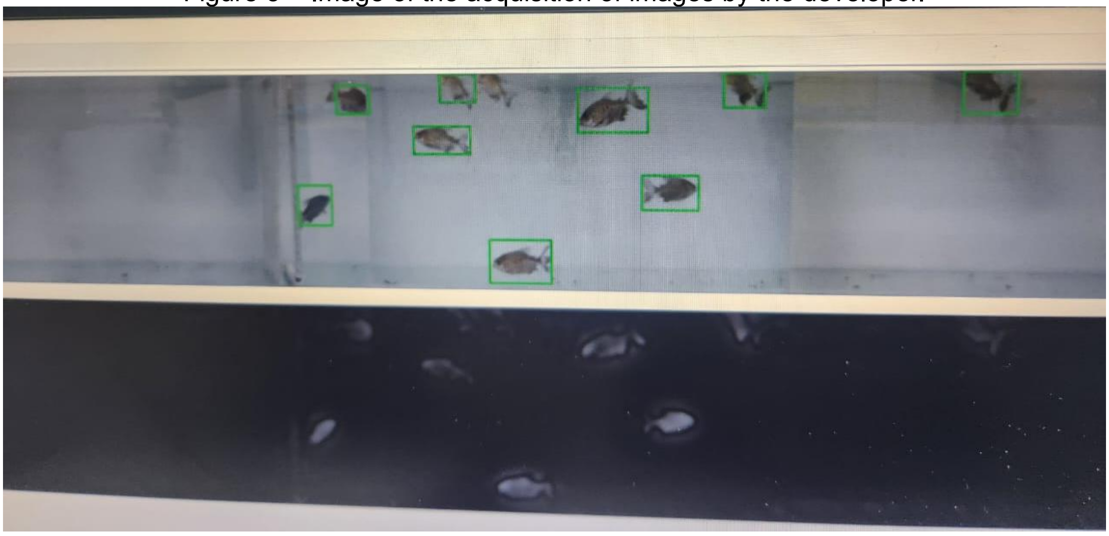
Source: Prepared by the authors.

Based on the preliminary tests, the bubble curtain system and other components of the instrumentalized aquariums are confirmed to be adequate for the execution of the planned experiments. The aquariums, equipped with circulation, water recirculation and oxygenation systems, proved to be efficient in all phases of the tests, making it possible to maintain the stability of the physicochemical properties of the water and the controlled observation of the behavior of the fish. The integration of the bubble system with the remote control center and the ability to insert liquid samples allows the flexibility of the experiments, with the possibility of changing the experimental conditions without significantly interfering in the operation of the other systems.

The *software* developed for image acquisition, control, and processing played a crucial role in capturing and analyzing fish behavior**.** The detection and recording of fish positions throughout the aquariums occurred flawlessly, with the camera system in "V" profile ensuring complete coverage of the environment. Combining the images from the three cameras into a single view of the aquarium allowed for continuous and accurate monitoring of fish movement, avoiding overlaps and maximizing the clarity of observations.

During the experimental phase, the fish were monitored in three stages: with the bubble curtain system turned off, turned on and turned off again. The data collected by the *software*, stored in .csv format and videos in .mp4, allowed a detailed analysis of the behavioral changes induced by the activation of the bubble curtain (Figure 3). The system detected clear changes in swimming patterns and behavior, such as fast swimming, immobility, and lethargy, which were consistently observed across all species tested.

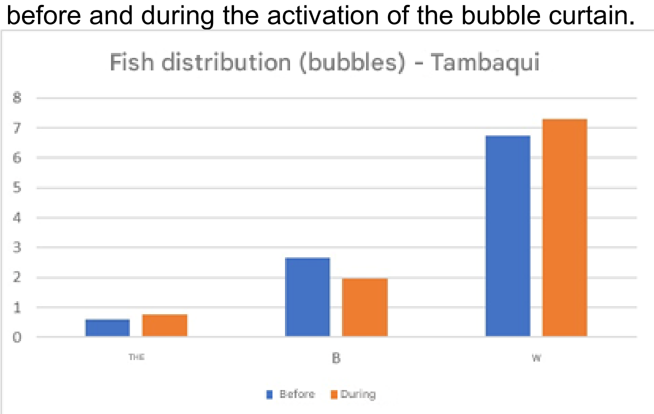
Figure 3 – Image of the acquisition of images by the developer.



Source: Prepared by the authors.

Figure 4 shows the average distribution of the specimens of the five species participating in the experiment, in the three sectors of the instrumentalized aquarium, before and during the activation of the bubble curtain. With the exception of the mandi specimens, the other fish species showed a tendency to move away from the bubble curtain, being mostly positioned in sector 3 of the aquarium. This behavior reflects the efficiency of the bubble curtain as a physical-behavioral barrier for fish. However, the experiment is done in a closed environment. Thus, with the passage of time and without the possibility of escaping to more distant regions, some species tend to "face" the risk and, consequently, the other specimens participating in the experiment follow the "lead fish", as observed in the behavior analysis described above.

Figure 4 – Average distribution of the ichthyofauna specimens used in the study in the three sectors of the instrumentalized aquarium, before and during the activation of the bubble curtain.



Source: Prepared by the authors.

Figueiredo et al. (2023) performed experiments using the liquid sample insertion system in aquariums to introduce hypoxanthine-3-N-oxide, an alarm substance that has been shown to be effective in provoking behavioral responses in fish species of the Osteriophysan group. In the assay with the species Matrinxã and Tambaqui, the concentration of 6.0 µg L^{-1} of hypoxanthine-3-N-oxide was introduced into the aquatic environment, resulting in unusual behaviors such as lethargy, foraging for the bottom and attacks. These results confirm the efficacy of hypoxanthine-3-N-oxide as an inducer of alarm signals in fish, demonstrating the potential of its use in ichthyofauna management practices, especially in areas impacted by hydroelectric activities. The possibility of remote and precise application of the substance using the developed monitoring and control system reinforces the feasibility of future studies and practical applications with this approach.

CONCLUSION

In general, the instrumentalized aquarium, combined with the monitoring software, proved to be highly effective for conducting the planned experiments. The precision in the control of the experimental variables and the software's ability to process and analyze images in real time provided reliable results, demonstrating the feasibility and potentiality of the system for future studies on behavioral and chemical barriers in fish.

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