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Laser-induced fluorescence in fish scales to evaluate the environmental integrity of ecosystems

Cristiane Ávila Santana, Dinorah Machado Vaz Lima, Luis Humberto da Cunha Andrade, Yzel Rondon Súarez, Sandro Marcio Lima *

Programa de Pós-Graduação em Recursos Naturais, Universidade Estadual de Mato Grosso do Sul, C.P. 351, Dourados, MS, Brazil

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ABSTRACT

The aim of this study was to evaluate the fluorescence of *Astyanax lacustris* fish scales when excited in the UV-A and blue regions for use as a bioindicator of aquatic ecosystems. This species was firstly defined as *Astyanax altiparanae*, popularly known as "lambari-do-rabo-amarelo". Currently, abiotic/biotic environmental integrity is generally assessed using limnological and physicochemical parameters related to biological indicators in streams or rivers, which are not sufficient to evaluate the real environmental conditions: in some cases, these parameters can be strongly dependent on local weather conditions. In this study, after the fish scales were excited with UV-A (360 nm) and blue (405 nm) photons, a strong and broad visible fluorescence band (from blue to red) could be observed that was mainly related to collagen and hydroxyapatite, independent of whether the excitation was applied to the inside or outside of the scale. Selected emission wavelengths were used as variables and the fluorescence intensities were interpreted using multivariate discriminant statistical analysis to compare streams with known different levels of integrity. The fluorescence data showed strong correlation with the electrical conductivity of the water, indicating that the scales of *A. lacustris* could be employed as bioindicators of environmental integrity on water chemistry monitoring programs.

1. Introduction

The streams in a basin are characterized by low water flow, shallow depth and short length. Their integrity can be checked by the diversity of animal and vegetable species, as well as by soil quality and by the presence of riparian vegetation. Due their particular qualities, the streams are most sensible to degradation of biotic and abiotic factors [1]. In streams, the impacts of the anthropogenic actions lead to the loss of integrity, which is often assessed by using limnological and physicochemical characteristics. However, in some cases, these parameters can be variable, as they are dependent on weather conditions, and not sufficient to evaluate the integrity of water chemistry.

The use of biological indicators offers an attractive alternative, and fish or structures of their bodies have been used for this purpose as integrated measure of water quality over longer time periods. Studies have shown that fish scales can be used as bioindicators of water quality, due to the ability of scales to incorporate pollutants in their matrices [2–4]. The analysis of scales can therefore provide information concerning pollution events in the environment inhabited by the fish.

Fish scales are composed of organic compounds, water, and mineral salts, and have two distinct faces: the inside face, containing a higher concentration of collagen, both smooth and fibrillary; and the outside face, with a bony and rough appearance, composed of a mineral phase consisting primarily of calcium-deficient hydroxyapatite (HAp) [5–8]. Previous studies of fish scales have described their structural arrangement, shape, size, and architecture, as well as the formation of organic and inorganic components and the possible applications of fish scales as bioindicators [9–11]. Environmental studies involving fish scales are gaining importance, with scales used to understand fish diet, phylogeny, growth, species differentiation, and sexual dimorphism, as well as for DNA extraction and evaluation of the pollution status of water bodies [12–14]. However, most of these studies have been based only on morphometric measurements.

Astyanax lacustris popularly known as "lambari-do-rabo-amarelo", of the Characidae family, is commonly found in the Upper Paraná Basin in Brazil. It is one the most abundant fish species and exhibits a wide distribution, being present in different types and sizes of aquatic systems (streams, lakes, and rivers) [15]. It was firstly known as *Astyanax altiparanae* and after a review by de Lucena and Soares [16], it was named as *Astyanax lacustris*. These fish are small in size, with length up to ~15–20 cm, and their diet reveals that they are a generalist and opportunist species [17]. They are highly adaptable to environmental changes and show elevated plasticity in response to resource availability [18,19]. In recent work, the chemical composition of scales from *A. lacustris* was monitored by infrared spectroscopy in

* Corresponding author.
E-mail address: smlima@pesquisador.cnpq.br (S.M. Lima).

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order to distinguish environments and populations, and the results indicated a high correlation between the chemical signature and the habitat [20,21].

Fluorescence is an important spectroscopic tool that can be employed to characterize many different compounds. This technique is highly sensitive and has been extensively exploited for a variety of purposes [22–27], including the characterization of water quality in rivers [28]. It can be used complementary with the conventional physicochemical parameters because the fluorescence gives real information on the dynamic interaction among the organic matter and the water [29]. Although the fluorescence methodology is not new, it has turned more used in the last years mainly because new cheapest instruments are being commercialized.

In this work fluorescence spectroscopy was used to assess the environmental characteristics of streams, with fish scales as bioindicators. The *A. lacustris* fish species was chosen due the reasons listed above and its previous identification as a potential bioindicator [30]. A systematic description of the methodology used is provided, including investigation of the best excitation wavelengths and scale side (internal or external) for fluorescence measurements, as well as the best part of the body for extraction of the scales submitted to fluorescence analysis.

2. Materials and methods

The *A. lacustris* fish were sampled in three streams in the Ivinhema River Basin, Upper Paraná River, Brazil. The names and locations of these streams near Dourados city are provided in Fig. 1. The streams were chosen because they exhibited differences in terms of their water quality integrity, mainly related to their proximity to the city. A multi-probe field instrument (Model 556, YSI) was used to measure the electrical conductivity, dissolved oxygen, pH and turbidity of the water in these streams. Differences in riparian vegetation were also observed, when urban streams (#3) have smaller riparian coverage. Domestic and industrial sewage as another pollution source in this stream and are moderately present in stream #2.

Fifteen fish were sampled in streams #1 and #2, and 30 fish were sampled in stream #3. Immediately after collection, the fish were fixed in 10% formaldehyde for transport to the laboratory, where they were transferred to bottles containing 70% alcohol. After removal, the scales were washed in distilled water and placed between two microscope slides in order to keep them flat. Scales from the same fish were placed on a single glass slide, maintaining conformity in the side of the scale (inside or outside) that was uppermost. All slides were labeled according to the streams from which the fish were collected.

A greater number of fish were sampled in stream #3 to enable a preliminary evaluation of the dependence of scale fluorescence on the size of the fish (and consequently the size of the scale), as well as the relationships among the fluorescence signals obtained for scales removed from different parts of the fish. For this purpose, five scales were removed from the humeral, pelvic, and caudal parts of the body (Fig. 2), totaling 15 scales for each fish, and 450 scales from stream #3. The scales were organized into three groups, according to the size of the fish: 69 ± 5 mm (group I); 79 ± 3 mm (group II); and 89 ± 6 mm (group III). Subsequently, only 15 fishes from stream #3 were used in the next study to compare the fluorescence signals obtained in scales from different streams. For this study, the size of the fish (45 animals, totaling 225 scales) sampled in the streams #1, #2 and #3 was standardized between 77 and 84 mm in order to investigate fishes with approximately the same age.

Excitation spectroscopy was firstly performed by exciting a scale from 250 to 450 nm with a Xenon lamp (Model 66901, Oriel Instruments) coupled to a monochromator (Model 76001, Oriel Instruments) and fluorescence signal was observed with a Horiba JY CCD detector coupled to a Horiba JY iHR320 monochromator. This procedure enabled selection of appropriate wavelengths for excitation of all the scales in the fluorescence measurements. Two excitation wavelengths were employed: 405 nm, using a diode laser, and 360 nm, using an argon laser (Innova 308C, Coherent). A bifurcated optical fiber was used to conduct the excitation light to the scale and to transport the captured fluorescence signal to a portable spectrometer (Model HR4000, Ocean Optics). For each scale, spectra were obtained with excitation of both the outside and inside faces.

Deconvolution of the emission spectra enabled fitting with appropriate Gaussian functions. The relative fluorescence intensities for the Gaussians in each spectrum were used to perform multivariate discriminant statistical analysis. This enabled the identification in each group of Gaussian functions able to discriminate the streams, together with linear combinations of the variables that best explained the differences among the groups [31]. The statistical analyses were employed to characterize the scales in terms of the properties of the streams.

3. Results and discussion

In order to identify the best excitation wavelengths to be used in the fluorescence spectroscopy, a fish scale was excited in different

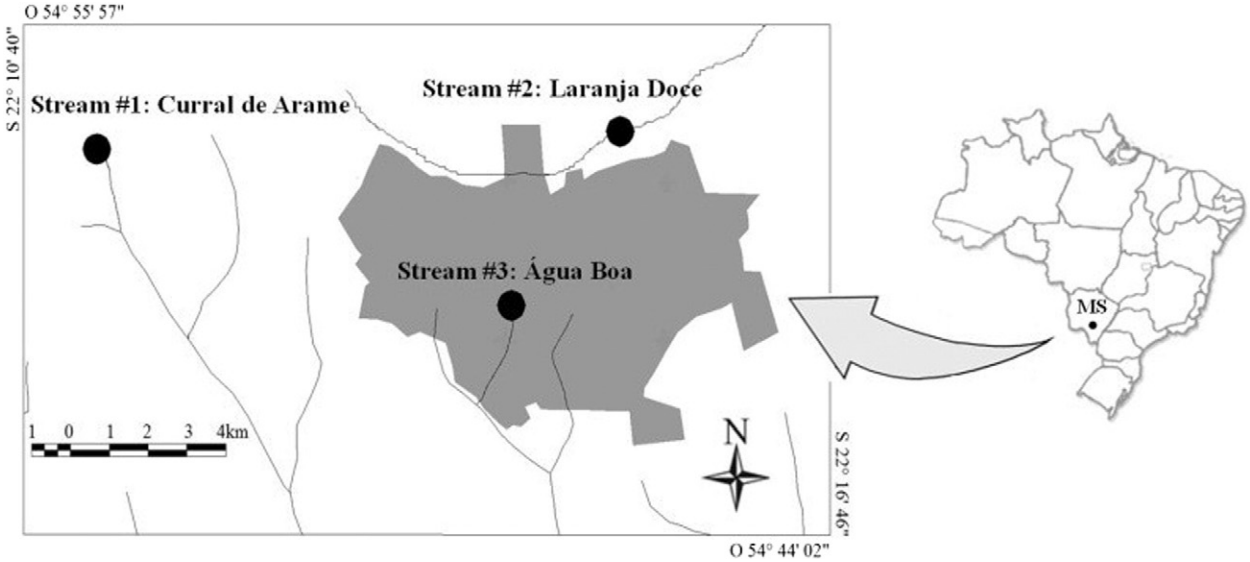

Fig. 1. Locations of the streams around Dourados city (shaded in gray), Mato Grosso do Sul State, Brazil.