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The scale epithelium as a novel, non-invasive tool for environmental assessment in fish: Testing exposure to linear alkylbenzene sulfonate

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ABSTRACT

Increasing pollution levels have turned our attention to assessing lethal and sublethal effects of toxic agents using the most informative techniques possible. We must seek non-invasive or non-lethal sampling methods that represent an attractive alternative to traditional techniques of environmental assessment in fish. Detergents are amongst the most common contaminants of water bodies, and LAS (Linear Alkylbenzene Sulfonate) is one of the most used anionic surfactant on the market. Our study analyzed morphological alterations (histological and histochemical) of the scale epithelium of *Prochilodus lineatus* under exposure to two concentrations of LAS, 3.6 mg/L and 0.36 mg/L, for a period of 30 days and evaluated at 14, 21 and 30 days. In order to establish morphological analysis of the scale epithelium as a new non-lethal environmental assessment tool that is reliable and comparable to classic methods, the relative sensibility of this technique was compared to a commonly used method of environmental assessment in fish, the estimation of the effects of pollutants upon branchial morphology. Two experiments were carried out, testing animals in tanks, and in individual aquariums. Results of analyses on gill tissue show that exposure to 3.6 mg/L of surfactant caused severe damage, including hyperplasia, hypertrophy and fusion at 14 days, with aneurisms at 21 and 30 days; while exposure to 0.36 mg/L had lighter effects on the organ, mainly lower incidence of fusion and hyperplasia. Aditionally, scale morphology was altered severely in response to 3.6 mg/L of LAS, consistently showing increased mucous and club cell production. Epithelial thickness was the most variable parameter measured. Scale epithelium sensibility has the potential to be a reliable environmental marker for fish species since it has the advantage of being less invasive when compared to traditional methods. However, more studies are required to increase the robustness of the technique before it can be generally applied.

1. Introduction

There is a growing sense of urgency regarding global pollution which drives the need to develop the most informative diagnostic techniques to assess lethal and sub-lethal effects of water contaminants (Anderson et al., 1994) and search for non-invasive or non-lethal sampling techniques that represent an attractive alternative to traditional monitoring methods in fish (Heltsley et al., 2005). In many developing countries water quality is assessed primarily through physical and chemical criteria, with little information concerning effects of several types of contaminants (Camargo and Martinez, 2007).

Morphological analysis is a common, efficient tool for identifying the effects of contaminants on target-organs through the evaluation of lesions and adaptive changes (Schwaiger et al., 1997) and fish scales have long been used for such purpose in bioaccumulation studies (e.g.: Abdullah et al. (1976), Burguer et al. (2013), Dua and Gupta (2005)). Several authors also explore the potential of epithelial tissues as pollution indicators in fish, measuring mucous production and epithelium thickness (Dowling and Mothersill, 2001; Kilemade and Mothersill, 2000; Pretti et al., 2008; Rajan and Banerjee, 1991). Whereas other studies/authors show that alternative structures in the epithelial tissue can be evaluated. For example, club cells, which are unicellular epidermal glands characteristic of several orders of fish responsible for the production of pheromones in response to toxic and stress agents (Brown, 2003; Chivers et al., 2007; Zaccone et al., 2001). Their abundance has been associated with the occurrence of mechanical

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damage to the skin (Chivers et al., 2007). Yet, no attempts have been made to use fish scales as a non-invasive method to obtain samples of epithelial tissue for morphological analysis.

Gills are considered the target-organs of several types of contaminants and changes in its structure are largely used for contamination assessment (Mallatt, 1985), which makes them a reliable standard to validate the sensibility of the scale epithelium as an environmental assessment technique.

Detergents represent a great portion of the most common aquatic contaminants and linear alkylbenzene sulfonates (LAS) are one of the major anionic surfactants used in detergents on the market (Hera, 2009). The toxic effects of LAS in different concentrations and its persistence in several types of water and effluents have been extensively studied (Konnecker et al., 2011; Mungray and Kumar, 2009). Data on aquatic toxicity of LAS is rich and has been compiled by the International Association for Soaps, Detergents and Maintenance Products and the European Chemical Industry Council. Toxic levels for many species have been recorded in European studies −0.25 up to 4.1 mg/L in chronic exposure, with Predicted no effect concentration (PNEC) of 0.27 mg/L (Hera, 2009). However, neotropical regions have significantly fewer studies on the matter, which makes it an important aquatic toxicant that needs to be evaluated for neotropical environments.

Only a few neotropical fish species can be used as models in environmental assessment, one of them is *Prochilodus lineatus*, a specie commonly used in toxicity studies (e.g.: Cerqueira and Fernandes (2002), Palermo et al. (2015), Paulino et al. (2012), Pereira et al. (2012, 2014) and Simonato et al. (2008)). It is abundant in South American basins (Reis et al., 2003) and is present in rivers that continuously receive surfactant contaminants from domestic and industrial dejects (Meschiatti and Arcifa, 2009). Therefore, it represents a solid neotropical bioindicator for environmental monitoring.

Our research aimed to investigate the use of morphological analysis of the scale epithelium as a reliable tool for environmental assessment in fish by testing the effects of a common aquatic contaminant on the number of specialized cells (*club cells* and *mucous cells*) and thickness of the epithelium of a well-known neotropical specie – *P. lineatus*. Additionally, a comparison was made of this new technique to effects on gill morphology, a well established technique in toxicity studies.

2. Material and methods

2.1. Experiments and sampling

Two experiments were carried out, one in 310 L tanks containing 24 animals, and one in 25 L individual aquariums. Tank experiments were designed to sample fish for morphological analyses of gills and scales and aquarium experiments were designed to sample fish for scales and food consumption. Tank experiments only would originally suffice for morphological analyses, but the aquarium experiments were designed as another set of tests to obtain feeding data that will not be discussed in this paper. Because the experimental design in aquariums allowed for individual responses to be properly accompanied, we present scale results of those tests in this paper, although we do not present the feeding results.

For the tank experiments, 72 juveniles with 7–10 cm body length and approximately 10 g were obtained on a pisciculture (Piscicultura Polettini – Mogi-Mirim, SP, Brazil), divided into three groups of 24 individuals (control, first concentration of LAS and second concentration) and acclimatized for two weeks prior to the beginning of experiments in 310 L polyethylene tanks equipped with UV filters, mechanical filters, heaters, water pumps and sandy bottoms. The sand was acquired at a local store and consisted of clean, white sand, which was replaced by new, clean sand before the start of every repetition. The same was done with mechanical filters.

Fish densities per tank were not expected to affect fish growth, since the densities in our study are not high if compared with previous studies which indicate that no effects on growth are observed in densities up to 0.5 fish/L for much bigger juveniles (24.9 g).

One experimental group (lower concentration) was exposed to a 0.36 mg/L dilution of LAS (Sigma-Aldrich©-code 289957), a second group, to a dilution of 3.6 mg/L and the third was a control group (control), exposed to water from a well available on *campus* (UNESP Rio Claro – Rio Claro, SP, Brazil). Water quality for all experimental groups can be verified in a physical and chemical characterization available on the original research (Alves, 2015) and details of the methods for collection and measuring physical and chemical parameters are given on a subsequent topic. LAS concentrations were chosen based on Mungray and Kumar (2009), that showed that surfactant concentrations vary from 0.36 to 0.49 mg/L in rivers that receive effluents of sewage treatment plants, and 3.6–4.9 mg/L in effluents directly from sewage treatment plants. Detergent dilutions were made with the same water used in the control groups.

Three sampling periods were executed on days 14, 21 and 30, in order to detect structural changes provoked by low concentrations during prolonged exposure, similarly to experimental designs from other authors (Hera, 2009). The experiment was conducted twice, animals were fed neotropical fish food (Poytara Disco©) which fulfils quality levels of 35% Crude Protein set for animal food and underwent 48 h fasting before every sampling. In every sampling period, eight scales per individual were removed from eight individuals per group using tweezers. They were always removed from the posterior half of the body, near the caudal fin and under the lateral line, due to the fact that juveniles do not shed many scales and even in adult specimens loss is higher above the lateral line (McCart, 1967). Additionally, a selection was made for six of the same animals already sampled for scales in the tank experiments in each group and in every sampling group, to be sampled for gills. Two gill arches were removed from the operculum opening of each of the aforementioned fish. Extraction of gills was performed under anesthesia using a benzocaine solution (0.1 g of benzocaine per milliliter of ethyl alcohol in every 100 mL of deionized water), following approval by the Ethics Committee (Comissão de Ética no Uso de Animais – CEUA – UNESP, *campus* of Rio Claro – Rio Claro, SP, Brazil, Process no. 031/2012).

For aquarium experiments, 12 juveniles were distributed amongst 12 25 L glass aquaria (1 individual/aquarium) and acclimatized for two weeks prior to the beginning of every experiment. Aquariums were equipped with UV filters, mechanical filters, heaters and water pumps. Animals were assigned to 3 groups, each comprising of 4 aquariums. LAS concentrations and sampling periods were the same as in tank experiments. Individuals were fed neotropical fish food of different granulation (Tetra Veggie algae wafer©), and underwent 24 h fasting before sampling took place. Fasting periods were different in an effort to minimize confinement stress, as the target specie swims in shoals. Food with a different granulation was used because the experimental design for aquarium tests was originally outlined to assess food intake which can only be done with a special kind of granulation.

The experiment in aquariums was repeated four times to match sample sizes between both types of experiment. Sampling procedures were similar to the previously mentioned, except that animals were not sacrificed for gill collection during the experiments and the area where scales were removed was treated with iodine to avoid infections and help the regeneration process.