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Deleterious effects of wastewater on the health status of fish: A field caging study

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ABSTRACT

We carried out a caging field experiment to assess the potential effects of an untreated sewage effluent on the health status of *Prochilodus lineatus*. We analyzed multiple biomarker responses, which included morphological indices, biochemical and hematological parameters as well as oxidative stress markers. In addition, we investigated the energetic demand of that exposure. Our findings showed that fish caged at the effluent showed a differential physiologic profile, suggesting a strong impact on fish health. Particularly, mortality, monocytosis, transaminase increase, antioxidant enzyme activation, lipid oxidative damage in several tissues and hepatic and muscle glycogen depletion were observed. According to multivariate analysis, oxidative stress markers and metabolic parameters were key biomarkers to contribute in separating fish caged at effluent site from those caged at upstream and downstream sites. So, these biomarkers allied to a caging strategy are recommended for future environmental monitoring assessments.

1. Introduction

Sewage effluents represent an important point source of water pollution since their discharges consisting of complex mixture of chemicals. Chemicals like metals, polycyclic aromatic hydrocarbons, pesticides, organotins, volatile organic compounds, chlorobenzenes, phthalates and alkylphenols have been reported in wastewater, as well as certain pharmaceuticals and hormones (Abessa et al., 2005; Gasperi et al., 2008; Bolong et al., 2009; Metcalfe et al., 2010).

Although chemical analyses are able to measure many of these compounds qualitatively and quantitatively, it is not viable to quantify all the pollutants that are potentially present in the sewage water. Furthermore, chemical analyses alone do not reveal the impact of chemical pollution on the aquatic environment because of potential synergistic/antagonistic effects of complex mixtures of chemical pollutants (Kerambrun et al., 2011). In this context, alternative monitoring methods involving biomarkers have been developed in order to provide a reliable assessment of the environmental quality (van der Oost et al., 1996). However, there is no single biomarker that can give a complete diagnosis of the effects of effluent exposure on organisms. Consequently, the use of a battery of complementary biomarkers is recommended to gain an understanding of how an organism responds to the total pollution load in an area (Lavado et al., 2006; Cazenave et al., 2009).

In this way, the use of biological markers in transplanted organisms is an integrative tool that measures the toxic effect of an effluent mixture as a whole. The development of caging field experiments are useful in effluent monitoring as they permit to assess the habitat quality in the outfall discharge area and the impact of contamination on biota. Furthermore, caging strategies are one of the techniques that integrate true ambient conditions over the chemical exposure and allow an interpretation of the exposure effects to complex mixtures (de la Torre et al., 2000). Thus, active biomonitoring using cages offer several advantages: the precise knowledge of the place and the precise duration of exposure, and the selection of a representative species and its particular developmental stage and genetic background (constancy of the test organism) (Oikari, 2006; Wepener, 2008). So, results from different sites are validly comparable.

In the present caging experiment the chosen species was *Prochilodus lineatus*, a neotropical fish representative of the water body of the region, with ecological relevance and economic importance. Besides, previous studies have demonstrated that *P. lineatus* gives a rapid response to an early exposure of various pollutants

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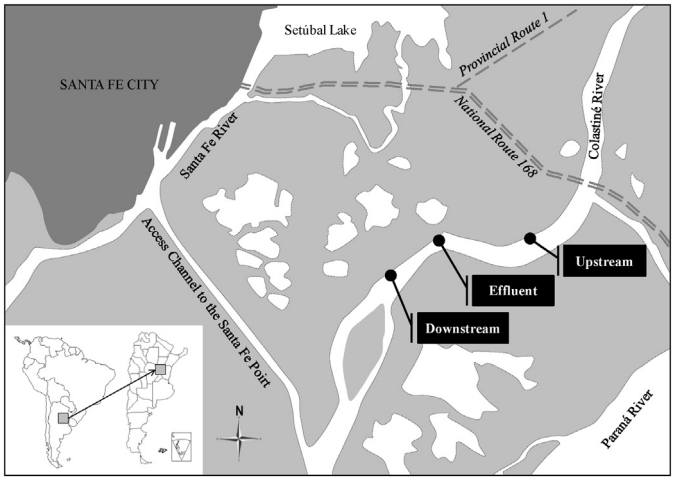

Fig. 1. Map showing study area and location of caging sites on the Colastiné River, Santa Fe, Argentina.

in both laboratory and field studies (Parma et al., 2007; Camargo and Martinez, 2006; Langiano and Martinez, 2008; Simonato et al., 2008; Cazenave et al., 2009; Lombardi et al., 2010; Bacchetta et al., 2011a; Paulino et al., 2012a,b; Troncoso et al., 2012; among others).

Effects of wastewater have been evaluated on individual analysis of serum chemistry (Bernet et al., 2001), hematology (Maceda-Veiga et al., 2010, 2013), oxidative stress (Almroth et al., 2008), some reproductive parameters (Akande et al., 2010; Galus et al., 2013), histopathology and infectious agents (Escher et al., 1999; Bernet et al., 2000; Fontaínhas-Fernandes et al., 2008). However, to obtain a holistic and integrative overview of how untreated sewage effluent exposure affects fish health we carried out a field caging experiment to analyze multiple biomarker responses, which included morphological indices, biochemical and hematological parameters as well as oxidative stress markers. Additionally, we investigated the energetic cost of that exposure.

2. Materials and methods

2.1. Study area and exposure sites

Colastine River, the main tributary of the Middle Parana River, is 35 km long, has a mean depth of 11 m, and its discharge at high water is about 2800 $m^3\ s^{-1}$ (Iriondo, 1975). This river supplies drinking water to Santa Fe city (more than 525,000 inhabitants). On the other hand, untreated domestic wastewater is discharged directly into the same watercourse.

In order to assess the effects of this wastewater effluent, fish were caged in the Colastiné River at the following three sites: at a reference site, located 2 km upstream from the sewage effluent (site upstream) (a natural area without having any known industrial and domestic sewage); at immediately (0.2 km; site effluent) and 2 km downstream (site downstream) from the sewage effluent site (Fig. 1). The experiment was carried out during the wet season in May 2011 (average monthly water level of 4.51 m).

2.2. Test organisms and experimental caging

Juveniles *P. lineatus* (three-month-old; $n = 60$) were obtained from a local hatchery at one week before the caging experiment, and held in two 500 L-tanks. Then, fish were transported from the laboratory to the exposure sites (in boat for <1 h) in large plastic bags (100 L) of oxygenated water.

Once at the field sites, fish were selected randomly and they were placed into the cages. We used polyethylene cages (0.60 m × 0.30 m × 0.36 m, 65-dm^3), perforated with many holes to allow water circulation through the cage. Cages were completely immersed (depth ≤ 1.5 m) near the sediment and they were firmly anchored at the sites to prevent their displacement. At each site, two cages (separated by approximately 3 m) were paced for 96 h (10 individuals per cage).

During the field exposure, water quality was evaluated three times (at 0, 48 and 96 h) at each site exposure. We recorded in situ water temperature, dissolved oxygen, conductivity, pH and transparency. Additionally, 2 L of water samples were taken for each station and transported to the laboratory at 4 °C in clean plastic bottles. The following parameters were measured according to standard procedures (APHA and AWWA, 1998): chemical oxygen demand, nitrates, nitrites, ammonia, total phosphorus, calcium, magnesium, and hardness. Besides, water samples for bacteriological analyses were kept in sterilized recipients and then total and fecal coliforms were determined by the More Probable Number (MPN) method.

Following the exposure period, fish were retrieved and they were rapidly transported (in river water with aeration) back to the laboratory for sample processing.

2.3. Biomarkers

Prior to blood sampling and dissection, fish were anaesthetized in benzocaine as described by Parma de Croux (1990). Body weight (g) and total length (cm) were recorded for each individual. Blood was collected immediately from the caudal vessel, and plasma separated via centrifugation (at 1409 × *g* for 10 min). The brain, liver, kidney, gill, intestine and muscle were dissected and quickly frozen in liquid nitrogen and subsequently stored at −80 °C until biochemical determinations. Before freezing, the wet weight of the liver was determined.

2.3.1. Condition indexes

Condition factor (CF) was calculated according to Goede and Barton (1990): $CF = BW/L^3 \times 100$, with BW = body weight (g), L = total length (cm). The liver somatic index (LSI) was calculated as: $LSI = LW/BW \times 100$, with LW = liver weight (g).

2.3.2. Hematology

Red blood cells (RBC) counts were performed with a Neubauer chamber. Hematocrit (Ht) values were determined by the micromethod using capillary tubes and centrifuged at 1409 × *g* for 10 min. Hemoglobin concentration (Hb) was measured by the cyanomethaemoglobin method at 546 nm (Houston, 1990). Mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) were calculated from primary indexes (Cazenave et al., 2005).

A drop of freshly collected blood was smeared on clean slides to estimate the total white blood cells (WBC) counts and for determination of leukocyte frequency according to Tavares-Dias and de Moraes (2007).

2.3.3. Transaminases and alkaline phosphatase

Liver and kidney enzyme extracts were prepared according to Bacchetta et al. (2011b). Aspartate aminotransferase (AST) (L-Aspartate-2-oxaloglutarate aminotransferase) and alanine aminotransferase (ALT) (L-Alanine-2-oxaloglutarate aminotransferase) activities were measured spectrophotometrically at 505 nm following the protocol described by Reitman and Frankel (1957). Alkaline phosphatase (ALP) (Orthophosphoric monoester phosphohydrolase) activity was determined colorimetrically using a commercial kit. Each sample was measured by triplicate and the