

## **FUNCTIONAL PARAMETERS IN CAGED FISHES**

### **EXPERIENCING CONTAMINANT STRESS**

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### **EXTENDED ABSTRACT ONLY – DO NOT CITE**

#### **Introduction**

Tropical ecosystems are currently threatened by environmental degradation, however little research has been done on the impact of contaminants on tropical aquatic biota. Knowledge of the effects of contaminants on biota is critical for pollution monitoring, impact assessment and environmental management. The measurement of biochemical, physiological and morphological responses to chemical contaminants in fish may serve to improve the assessment of biologically significant exposures to toxic anthropogenic chemicals, and to enhance the ability to assess the risk of effects on the health and survival of contaminant-exposed populations.

Caging of fishes has been used to assess biological exposure to pollutants in the field. This technique offers advantages over simple field collection of organisms since it gives more realistic results in studies concerning functional effects of contaminants on fish. The objective of the present study was to compare biochemical, physiological and histological parameters of the freshwater neotropical fish species *Prochilodus lineatus* caged in two lakes with impaired water quality with the same parameters from fishes caged in a reference stream.

## Material and Methods

Juveniles of *P. lineatus*, supplied by the University Hatchery Station, were held in cubic 125 L cages (6 fishes per cage) for 7 days. Cages were placed on sediment floor at two lakes: Igapó I and Igapó IV, that receive point source discharge of mixed contaminants, and a reference site: Apertados stream. All sites are located in the city of Londrina, Paraná State, Southern Brazil. Three periods of caging were considered: one in March 2001 (“summer caging”), one in May/June 2001 (“autumn caging”) and one in July /August 2001 (“winter caging”). At the end of the caging period cages were lifted up and fishes were immediately sampled. Blood was taken from the caudal vein into a heparinized syringe. Subsequently gills, liver and kidney were taken from each fish for histological and biochemical analysis. A small amount of blood was used for hemoglobin determination by the cyanmethemoglobin method. After blood centrifugation, plasma glucose and total lipids concentrations were measured by spectrophotometric method using commercial available KIT. Fish livers were homogenized and centrifuged (14,000g for 20 min at 4°C). Supernatants were used for glutathione S-transferase (GST) and catalase (Cat) measurements according to the following spectrophotometric procedures: GST was measured at 340 nm using 1-chloro-2,4-dinitrobenzene as substrate; Cat at 240 nm by the decay of hydrogen peroxide levels. Gills, kidney and liver samples were fixed in Bouin’s, dehydrated in ethanol and embedded in paraplast. Tissue was sectioned (5 µm) and stained with hematoxylin-eosin. Histopathological alterations were classified according to two criteria: 1) semi quantitatively by ranking the severity of tissue lesions (Schwaiger et al., 1997). On the basis of these data mean assessment value (MAV) of lesions was calculated for gills, kidney and liver for each caging site; 2) based on the scope for repair of the lesions (Poleksć & Mitović-Tutundžić, 1994). With this method it was possible to compare the degree of tissue change (DTC) in fishes from different caging sites and to correlate the intensity of pollution with the intensity of changes found. For each parameter differences between caging site were tested with a one-way ANOVA followed by the Student-Newman-Keuls (SNK) multiple range test. Means were considered different where  $P < 0.05$ .

## Results

During the summer, fishes caged in lake Igapó I presented a significant reduction in hemoglobin content and catalase activity when compared to those caged in the reference site. GST activity was significantly higher in fishes caged in both lakes than in those placed in the reference site. During the autumn,

plasma lipid concentrations of fishes caged in the lakes were significantly lower compared with the values found in reference site. In the winter, animals from Igapó IV presented a significantly higher plasma lipid concentration and animals from Igapó I showed a significantly higher catalase activity in relation to the ones from the reference site (Table 1).

In *P.lineatus* a variety of histopathological changes were found in the organs examined. The severity and frequency of organ lesions were found to be much more pronounced in fishes caged in the lakes than in those caged in the reference site, as evidenced by statistical evaluation of MAV and DCT (Fig.1).

### **Conclusions**

Data obtained on functional parameters measured in fishes placed in cages showed clear differences between cages located in the lakes and in the reference site. The clearest indications of environmental stress were observed with GST induction as a measure of organic compounds exposure in both lakes and hemoglobin reduction as a response to high levels of lead in Igapó I sediment and water. The histopathological results revealed strong differences in the quantity and severity of lesions between fishes caged in the lakes and in the reference stream making more reliable functional responses in fishes exposed to environmental stressors. Overall, the present study demonstrates functional measurements in caged fish as a promise approach for evaluating effects of pollutants in neotropical fishes.

### **References**

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	Hb mg.dL <sup>-1</sup>	Glucose mg.dL <sup>-1</sup>	Lipids mg.dL <sup>-1</sup>	CAT nM.min <sup>-1</sup> .mgP <sup>-1</sup>	GST nM.min <sup>-1</sup> .mgP <sup>-1</sup>
<b>Summer</b>					
Reference	6.5 ± 1.4 (12)	26.6 ± 6.9 (11)	985.5 ± 291.1 (12)	49.3 ± 8.6 (12)	281.9 ± 64.4 (11)
Igapó I	2.1 ± 1.5* (6)	27.3 ± 11.0 (5)	787.8 ± 84.7 (6)	28.8 ± 13.3 * (6)	446.3 ± 32.8 * (6)
Igapó IV	6.9 ± 1.4 (6)	36.2 ± 8.9 (6)	715.1 ± 57.6 (6)	56.6 ± 8.2 (5)	510.1 ± 42.1 * (5)
<b>Autumn</b>					
Reference	6.3 ± 0.6 (6)	32.9 ± 9.1 (6)	1125.2 ± 55.6 (6)	48.0 ± 11.2 (6)	214.0 ± 55.8 (6)
Igapó I	5.6 ± 1.1 (6)	36.3 ± 5.7 (6)	816.8 ± 69.9 * (6)	57.3 ± 6.5 (5)	230.4 ± 183.4 (5)
Igapó IV	6.4 ± 1.5 (11)	41.9 ± 12.9 (12)	872.2 ± 167.0 * (11)	53.3 ± 8.3 (12)	189.5 ± 96.6 (12)
<b>Winter</b>					
Reference	6.9 ± 1.7 (6)	27.8 ± 12.2 (6)	752.5 ± 260.8 (6)	58.7 ± 9.8 (6)	196.2 ± 49.1 (6)
Igapó I	5.1 ± 1.1 (6)	41.0 ± 11.5 (6)	678.3 ± 192.0 (7)	75.2 ± 14.2 * (6)	203.6 ± 38.9 (6)
Igapó IV	6.7 ± 1.0 (6)	42.4 ± 3.6 (6)	1005.6 ± 86.3 * (6)	49.2 ± 9.0 (6)	131.2 ± 30.6 (6)

All data are expressed as mean ± SD (n). \*different from reference site (P<0.05) in the same season.

Table 1 – Blood hemoglobin (Hb), plasma glucose and lipids concentrations and catalase (CAT) and glutathione S-transferase (GST) activity in liver of *P. lineatus* caged for one week during summer, autumn and winter in two lakes (Igapó I and Igapó IV) and in a reference stream.

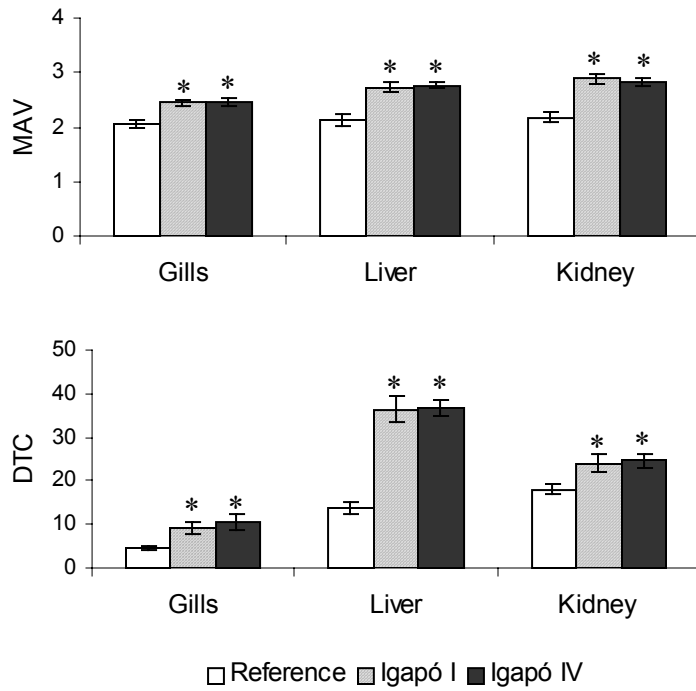


Fig.1 – Mean assessment values (MAV) and degree of tissue change (DTC) of gills, liver and kidney in *P. lineatus* caged in two lakes (Igapó I and Igapó IV) and in a reference stream. \* different from reference site (P<0.05).

