

ASSESSING CHEMICAL STRESS IN NEOTROPICAL FISH:

A PHYSIOLOGICAL APPROACH

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EXTENDED ABSTRACT ONLY - DO NOT CITE

The impact of pollutants on aquatic biota can be studied by toxicity tests, which are used to detect and evaluate the potential toxicological effects of chemicals on aquatic organisms. Nevertheless, little research has been done on the impact of contaminants on tropical ecosystems. In order to extrapolate meaningful, relevant and ecologically significant results from aquatic toxicity tests, not only appropriate tests but also appropriate organisms should be used. Whenever possible, species should be studied which are indigenous to or representative of the ecosystem that may receive the impact. However, not many native fish species have been employed in toxicity tests in Brazil. Thus, there is a real lack of results concerning the sensitivity of Brazilian fish species to chemicals that are potential pollutants in tropical freshwater ecosystems, as well as the effects of these toxic agents on fish health. It was thus considered of interest to investigate the sublethal effects of pesticides (the herbicide Trifluralin and the insecticide Azodrin) and metals (lead and aluminium) on *Prochilodus lineatus* and *Astyanax bimaculatus*, two freshwater fish species found in a variety of polluted waterways in the south of Brazil. Fish health was assessed by some physiological parameters such as: hematocrit, sodium and glucose plasma concentrations.

Material and Methods

Juveniles of *Prochilodus lineatus* and adults of *Astyanax bimaculatus* were obtained from the University Hatchery Station. After acclimation, short-term static toxicity tests (24h for Trifluralin and 96h for Azodrin and metals) were carried out. Animals were exposed to concentrations corresponding to 25 and 75% of the LC₅₀ (median lethal concentration). Experiments with metals were performed in an 80 L glass aquarium and with pesticides in a 140 L tank. Water was continuously aerated, temperature was kept at 21±1°C and pH 7.5. Only the experiments with aluminium were conducted in acidified water (pH 5.0). Blood samples were taken from caudal vein after 6, 12, 24, 48, 72 and 96 hours of exposure to pollutant and after 96 hours of exposure only to the dilution water (control group). Experiments with Trifluralin lasted only 24 hours and blood samples were taken after 6, 12 and 24h. After blood centrifugation, hematocrit was determined using a microhematocrit scale. Plasma sodium concentrations were measured by flame photometry and glucose concentrations by spectrophotometric enzymatic assay. Differences among groups exposed to the same pollutant concentration, for different time periods, and the control group were tested for significance by one way ANOVA and multiple range test (Student-Newman-Keuls). Means were considered different where $P < 0.05$.

Results

As it is shown in Table 1 *P. lineatus* exposed to the highest concentrations of Azodrin, lead nitrate and aluminium sulfate showed significant reduction on plasma sodium concentrations and a return to sodium control levels after 72 hours. Fish exposed to both metals concentrations, as well as to 0.19 ppm of Trifluralin, showed significant increase on blood glucose that lasted longer in animals exposed to aluminium. Only animals exposed to the highest aluminium concentration showed a significant variation on hematocrit: an increase after 6 and 48 hours of exposure.

The results obtained for *Astyanax bimaculatus* are presented in Table 2. An initial decrease on blood sodium concentration, followed by a significant increase, was observed in fish exposed to both lead concentrations. Aluminium

exposure during 24 and 48 hours led to a decrease on this parameter. Animals exposed to both Azodrin concentrations and to the lowest Trifluralin and lead concentrations showed a significant increase on blood glucose. On the other hand, *A. bimaculatus* exposed to the highest concentrations of the herbicide and aluminium showed blood glucose reduction.

Table 1 - Significant increase (↑) or decrease (↓) on hematocrit, blood sodium and glucose concentrations of *P. lineatus* after the exposure to sublethal concentrations of Azodrin, Trifluralin, lead nitrate or aluminium sulfate, in relation to the control group.

| Pollutant | Hematocrit | Sodium | Glucose |
|---|-------------|-------------------|------------------|
| Azodrin | | | |
| 7.0 ppm | - | - | - |
| 21.0 ppm | - | ↓ 6h ↑ 12 and 48h | - |
| Trifluralin | | | |
| 0.06 ppm | - | - | - |
| 0.19 ppm | - | - | ↑ 6, 12 and 24 h |
| Pb (NO₃)₂ | | | |
| 38.0 ppm | - | - | ↑ 6, 12 and 24 h |
| 114.0 ppm | - | ↓ 48h | ↑ 6 and 24 h |
| Al₂(SO₄)₃ | | | |
| 0.1 ppm | - | - | ↑ 6, 48 and 72 h |
| 1.0 ppm | ↑ 6 and 48h | ↓ 6 and 24h | ↑ from 6 to 96 h |

Discussion and Conclusion

The observed decrease in Na⁺ plasma concentration in both fish species exposed to the insecticide and metals might be indicating a decrease in sodium influx rate in consequence of the inhibition of Na,K,ATPase in gills. The posterior return of sodium concentrations to control values suggests a recovery of osmoionic homeostasis, even with continued pollutant exposure, where cortisol might be playing a key role. The observed hyperglycemia in both species exposed to pesticides and lead reinforces this cortisol role. Blood glucose reduction in *A. bimaculatus* exposed to Trifluralin and aluminium might reflect glucose urinary loss, probably in response to renal reabsorption disruption. The hematocrit increase showed by both species exposed to aluminium might be

rather related to the low pH than to the metal itself. In conclusion, the present study points out that sublethal concentrations of Azodrin, lead and aluminium interfere on sodium regulation and glucose metabolism of *P. lineatus* and *A. bimaculatus* and these parameters might be useful tools in monitoring pesticides and metal effects.

Table 2 - Significant increase (↑) or decrease (↓) on hematocrit, blood sodium and glucose concentrations of *A. bimaculatus* after exposure to sublethal concentrations of Azodrin, Trifluralin, lead nitrate or aluminium sulfate, in relation to the control group.

| Pollutant | Hematocrit | Sodium | Glucose |
|---|--------------|--------------------|------------------|
| Azodrin | | | |
| 253.0 ppm | - | - | ↑ 6h |
| 758.0 ppm | - | ↑ 72h | ↑ 12, 24 and 48h |
| Trifluralin | | | |
| 0.30 ppm | - | - | ↑ 12 and 24h |
| 0.91 ppm | - | - | ↓ 6, 12 and 24h |
| Pb (NO₃)₂ | | | |
| 33.0 ppm | - | ↓ 24h; ↑ 48h | ↑ 12 and 24h |
| 100.0 ppm | - | ↓ 6h; ↑ 72 and 96h | - |
| Al₂(SO₄)₃ | | | |
| 0.1 ppm | ↑ 6h; ↓ 72h | ↓ 24h and 48h | - |
| 1.0 ppm | ↑ 48 and 96h | ↓ 24h | ↓ 24 and 48h |

- indicates no significant variation

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