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A preliminary report on the application of *Macrobrachium amazonicum* Heller, 1862 (Decapoda: Palaemonidae) as a biomarker

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Abstract

A method is described for the rapid evaluation of water quality through monitoring the respiratory response of *Macrobrachium amazonicum* a dominant benthic macroinvertebrate of Northeast Brazil constituting a source of nutrients for fish and humans. By maintaining the organism in a respiratory chamber supplied with test water (gravity-feed) at a rate of 10 ml min^{-1}, it is possible to identify when the organism is in stress. Respiration rates were determined before (control) and after test concentrations were introduced. The herbicide paraquat was used because of its popularity with sugar cane cultivators and reported low toxicity. There was a discrete increase in respiration associated with acute toxicity at all concentrations tested above 0.035 μg l^{-1}. At 0.01 μg l^{-1} no increase in respiration was observed and prawn survival continued until the experiment was terminated (25 days).

Introduction

Water quality monitoring has passed through a series of developmental stages. Initially, up to the seventies it was viewed fundamentally as a chemical technology. Presently, approaches such as bioassay and field surveys have been refined to reproducible semi-quantitative sciences. These methodologies, however, require a great investment of money and time.

A more recent approach, which is considerably faster, involves the use of early warning detectors, or biomarkers (Chapman, 1996; Schlend, 1996), for ecological risk assessments. Preliminary research presented herein, indicates that the authors may have identified such a biomarker in the respiratory response of *Macrobrachium amazonicum* to the herbicide paraquat.

Paraquat and *Macrobrachium*

Paraquat, a dipyrimidene herbicide, is commonly used in the culture of sugar cane. Its wide acceptance throughout the world stems from its reported acute toxicity to weeds, rapid degradation and almost negligible impact on animals (Hallawell, 1986). Preliminary unpublished toxicity data generated in our laboratory with aquatic macroinvertebrates indicate, however, that paraquat may not be as benign to tropical aquatic invertebrates as reported for temperate species.

The prawn genus *Macrobrachium* constitutes a major component of the benthic biota in Northeast Brazil. Of the 26 American species comprising this genus (Guest, 1979), five were identified in the Gramame Reservoir in Paraíba and *M. amazonicum* was the dominant species (Aragão, 1995). Originating in the Amazon Region, it was introduced (Bragagnoli & Grotta, 1995) to Northeast Brazil with the objective of serving as forage for carnivorous fish. It has since achieved a wide distribution and has been found to consist of as much as 60% of carnivorous fishes's diet (Medeiros et al., 1995). As such *M. amazonicum* occupies a significant intermediate position in the food web (Pinto, 1977).

It is also ecologically dominant, ubiquitous in its distribution, sensitive to herbicides and is a truly freshwater species (Scaico & Bragagnoli, 1989). Thus,

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we decided to investigate its potential as a standard laboratory organism for toxicity testing (bioassay) in Northern and Northeastern Brazil's inland waters.

Methodology

Testing was implemented in two stages: 1. Preliminary 96 h LC50 acute toxicity with four replicates to determine the range appropriate for chronic toxicity. Specimens were collected from the outfall of the Gramame Reservoir Dam in Alhandra, Paraíba, transferred to a 250 l Brasilit® tank and allowed to acclimate for 4 weeks. They were fed daily (10% body weight) on a meal consisting of 40% fish and 20% each of soy, wheat and corn with vitamins and minerals. Upon acclimation, 96 juveniles (0.5 g $\pm$ 0.1 g) were distributed among six test concentrations and a control. Each replicate containing 1 l of test solution housed four prawn. The acute toxicity test duration was the standard 96 h. To achieve a ranging dose approximation, concentrations of paraquat were tested at decimal intervals and were renovated midway through the test. Dissolved oxygen was determined by the Alsterberg-Azide modification of the Winkler method. All procedures conformed to that described for fish in Standard Methods of Waste Water Analysis (Eaton et al., 1995). 2. Definitive 21-day chronic toxicity test with serial replicate readings for each concentration and control. Chronic toxicity testing was performed in the flow-through gravity-feed apparatus described by Aragão et al. (1998). Respiration was reported as the difference between dissolved oxygen concentrations in bottles flanking a respiratory chamber containing *Macrobrachium amazonicum*. Flow was maintained at 10 ml min^{-1} in each of two (250 ml) respiratory chambers. Testing began at low concentrations and progressed to higher concentrations. The same organisms were used throughout the test period or until death occurred. Exposure to test concentrations was maintained for 6 h and testing was conducted each h until at least four similar values were measured. Before exposure to the next highest concentration, the specimens were maintained at least 24 h in the control water. Testing was resumed when pre-exposure control respiration levels were achieved for four consecutive hourly readings. To establish whether the enhanced respiration rates were stress related or merely compensatory adaptations, continued exposure was maintained for controls and those concentrations immediately above and below that concentration eliciting a heightened respiratory response (0.01, 0.035, 0.5 and 0.1 μg l^{-1}). Testing continued until death or termination of the experiment.

Table 1. Average (n=4) respiration rates (mg O_2 g^{-1} h^{-1}) with confidence intervals (CI) and weighing for seven specimens of *M. amazonicum* when exposed to indicated concentrations of paraquat

	Weight (g)		Respiration (CI) (mg O_2 g^{-1} h^{-1})	
Concentration	1	2	1	2
Control	1.20	1.40	0.37 (0.04)	0.27 (0.04)
0.01 μg l^{-1}	1.20	1.40	0.39 (0.04)	0.32 (0.04)
Control	1.20	1.40	0.35 (0.03)	0.32 (0.04)
0.035 μg l^{-1}	1.20	0.37	0.48 (0.08)	0.63 (0.07)
Control	1.20	1.40	0.35 (0.05)	0.33 (0.05)
0.05 μg l^{-1}	1.20	1.40	0.40 (0.04)	0.47 (0.06)
Control	1.20	1.40	0.37 (0.03)	0.56 (0.27)
0.1 μg l^{-1}	1.20	1.40	0.65 (0.10)	1.20 (0.08)[a]
Control	1.00	0.57	0.32 (0.05)	0.30 (0.04)
0.5 μg l^{-1}	1.00	0.57	0.60 (0.12)	0.49 (0.01)
Control	1.00	0.57	0.35 (0.03)	0.32 (0.04)
1.0 μg l^{-1}	2.60	1.60	0.65 (0.10)	0.56 (0.10)
Control	2.60	1.60	0.35 (0.09)	0.30 (0.08)
10.00 mg l^{-1}	2.60	1.60	0.65 (0.09)[b]	0.56 (0.07)[b]
Average (n=14) respiration of all controls with CI			0.35 (0.47)	
Average (n=7) respiration of treatments between 0.1 μg l^{-1} and 10.0 mg l^{-1}			0.59 (0.10)	

[a] Moulted (not included in calculations).
[b] Died after 5 h of exposure.

Feeding was scheduled at the end of the day after all readings were made. Respiration under this regime was designated as standard metabolism. Sampling intervals were based on that period required to achieve a minimum of 95% replacement of water in the respiratory chamber (Sprague, 1969). At no point did the oxygen saturation level of the inflow water drop below 60%. Test water (pH 6.9, alkalinity <15 mg l^{-1} and hardness <20 mg$CaCO_3$ l^{-1}) was supplied from Gramame Reservoir (Alhandra-PB). In all seven juveniles were used ranging in weight from 0.37 g to 2.6 g.

Results and discussion

A review of the data indicates that at a concentration between 0.035 and 0.05 μg l^{-1}respiration increased 29% over control levels (Table 1). At those tested con-

centrations between 0.1 μg l^{-1} and 10 mg l^{-1} respiration rates leveled off at a consistent 169% of control levels. It is interesting that this observed increased respiration rate was not proportional to treatment levels. In all instances when exposure was maintained above threshold concentrations, mortality occurred in less than a week. At 0.01 μg l^{-1}, however, respiration was equal to control levels and no mortality was observed in the 3 week test period. These results suggest that the increase in respiration is a consequence of the cost of mobilization of some homeostatic mechanism that permits survival over a limited interval. The respiratory response is biologically significant because the organism can not sustain itself at this heightened level indefinitely. While the data base does not support statistically significant conclusions, it does justify the need for a more controlled replicated study.

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