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Physiological Responses of Juvenile White Mullet, *Mugil curema*, Exposed to Benzene

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The potential effects of water-soluble aromatic hydrocarbons contained in refined and crude oil on aquatic organims have increased in recent years. Thus, the incidence of accidental oil spills from vessels, drilling operation, terminals, and especially refinery and petrochemical effluents have been threatened marine life with the hydrocarbons pollution of bays and estuaries (Baker 1971a; Moulder and Varley 1971; Brocksen and Bailey 1973; Anderson et al. 1974; Clark and Macheod 1977b). Baker (1971a) has suggested the importance to study the biological effects of oil industrial effluents, because the area near an effluent is subjected to continuous but low concentrations of oil and other compounds for a long time.

Most of the studies have focused on the impact of aromatic hydrocarbons in temperate marine and freshwater species (Nelson-Smith 1970; Anderson 1974; and Neff et al. 1976). Since petroleum production, refinement and transportation is increasing in tropical areas, it is important to conduct comparative hydrocarbon toxicity studies using tropical/semitropical fish species (Correa and Venables 1985).

This investigation was designed to determine some physiological responses for the white mullet (Mugil curema) to benzene stress. Thus, the oxygen (O_2)consumption and ammonia nitrogen (N) excretion rate were determined to assess the short-term impact of this compound on this species. The hypothesis is based on the generalization that an organism stressed by an environmental contamination factor, will mobilize a compensatory homeostatic mechanism manifested in some physiological change.

MATERIAL AND METHODS

Juvenile specimens of Mugil curema were collected by seining from sandy beaches in Carenero near Cumaná in eastern Venezuela (Figure 1). Upon arrival they were identified and selectively sorted for uniform size (0.5-1.0 g). They were subsequently maintained in well aerated 100 L tank and acclimated at 20 ± 2°C in filtered sea water at 35 ppt S for 10 d. During the period of acclimation the fish were fed commercial fish food ad libitum.

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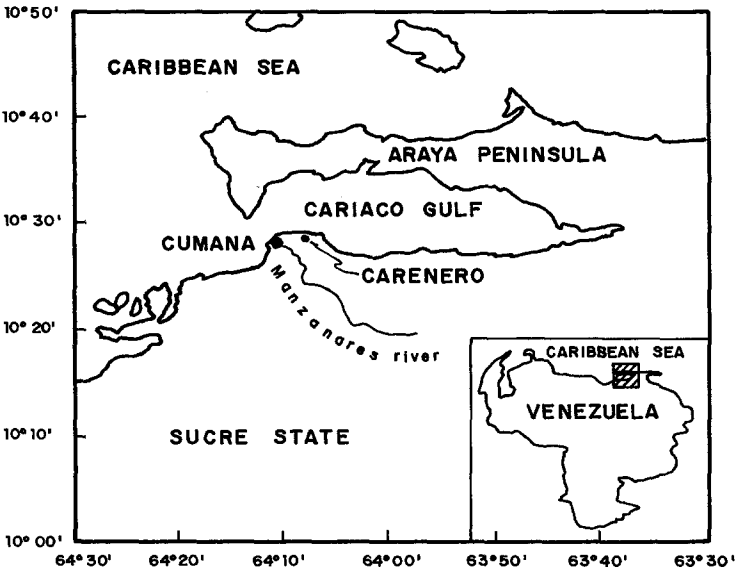

Figure 1. Location of Cumaná in eastern Venezuela

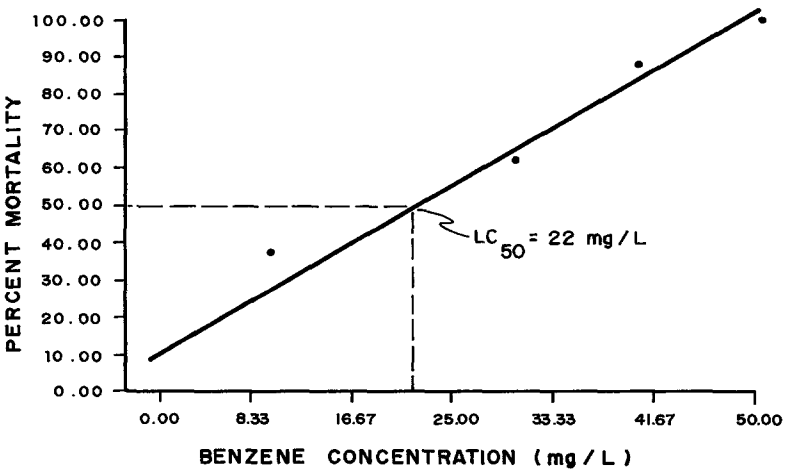

Figure 2. 48-h LC_{50} acute toxicity data for Mugil curema exposed to benzene (mg/L)

Pilot 48-h LC50 bioassay to determine the lethal concentration of benzene was conducted in a static system using glass bottles (2.5 L capacity). The water was aerated before benzene was added to eliminate the need for aeration of the exposure tank water. This minimized the benzene volatilization rate. Benzene concentrations were derived on a volumen basis from the stock solution (1000 ppm). The emulsified sample was then poured into the test aquaria. Each aquarium was stirred with a glass rod for 10 sec to distribute the benzene in the water column. The above procedure was repeated every 24 h during the LC50 test. The cessation of breathing and body movements were used as criteria for death.

Oxygen consumption rates of individual fish were monitored for 24 h during exposure to 0.00, 1.00, 5.00 and 10.00 mg/L of benzene. A flow through system described by Correa et al. (1983) was used. The respiration rate in the chamber is derived from integrating the differences in dissolved oxygen concentrations between "downstream" and "upstream" bottles with a regulated flow rate of 10 ± 0.5 mL/min. The oxygen concentration were determined using an oxygen meter model 9070 Extech (Boston, USA). Seven replicates per benzene concentration were employed.

Histological observations of gills were performed on the paraffin sections (6-8μm) using Periodic acid-Schiff, alcian blue, toluidine blue and Mallory's triple stain (Prasad 1988).

Ammonia excretion rates of individual organism were monitored in a static system; 28 sets of 2.5-L bottle were filled with filtered sea water saturated with O_2 at benzene concentrations (0.00, 1.00, 5.00 and 10.00 mg/L). After 24 h the organisms were removed from the bottles and weighed. Ammonia levels were determined according to the phenolhypochlorite method (Solorzano 1969) using an Spectro Photometer Bausch & Lomb (Model 21) at 640 nm.

The LC50 values and their corresponding 95% confidence intervals were estimated using the graphical method of Litchfield and Wilcoxon (1949). Statistical difference between means of oxygen consumption and ammonia excretion rates at the designated benzene concentrations were evaluated using Dunnett's procedure of analysis of variance (Steel and Torrie 1980).

RESULTS AND DISCUSSION

The 48-h LC50, estimated graphically from deaths in the container, for Mugil curema was 22 mg/L (Figure 2); this value is quite narrow compared with 11 mg/L and 15 mg/L reported by Broncksen and Bailey (1973) and Meyerhoff (1975) for Morone saxatilis and Oncorhynchus tshawytscha, respectively. In the present study, the white mullet, in particular, was found to be less susceptible to

benzene than the other species. Brocksen and Bailey (1973) determined that it is probable that benzene concentration in the range of 0-15 mg/L would exist in the vicinity of an oil spill in fresh and salt water, considering the relative abundance of benzene in most crude oils (20% or more of total aromatics) and its high solubility in water (in excess of 200 mg/L).

Benzene undoubtedly modifies the gas exchange and, therefore, has an influence on the respiration rate. Thus, a high significant differences ($P \leq 0.01$) were found between control and sublethal benzene concentrations in Mugil curema. It was determined that respiration rate increased with raising benzene concentrations. The upper value, at the maximum exposure time, was 1.67 ± 0.04 $mgO_2/h/g$ at the concentration of 10 mg/L (Figure 3). It was also observed in this high sublethal concentration that the opercular movements become more pronounced until breathing was gradually strained. Histological observations to the gills determined an swelling of the gill epithelium and accumulation of mucus secretion on the gill rakers and filaments in organims exposed to 10 mg/L of benzene.

A similar increase in the respiration rate was found by Brocksen and Bailey (1973) using salmon and striped bass exposed to 5 and 10 mg/L of benzene. They found that benzene is absorbed across the gill surface of fish directly into the blood. Being lipid-soluble, the benzene attaches to erythrocytes and to the lipoproteins in the blood. They also determined that bio-oxidation and hydroxylation of benzene to phenol in the liver and other tissues caused the possible inhibition on the enzymatic reaction that requires $NADPH^+$ and O_2. Thus, fish increase their respiration rate to metabolize the benzene. It has been also observed that benzene modified behavior patterns creating an immediate effect upon increased activity. Prolonged exposure generally results in impairment of different functions, followed by narcosis.

Excretion rate in M. curema decreased with increased benzene concentrations. Significant differences were found at $P \leq 0.01$ in organisms exposed to different benzene concentrations. A reduction in the excretion rates of 40% was observed in organisms exposed to 10 mg/L of benzene (Figure 4). The difference in excretion responses, may be due to several variables; thus, the hydroxylation of steroids, oxidation of lipids, and the amount of lipid rich tissue could be some causes for the alteration of the ammonia pathway (Broncksen and Bailey, 1973). However, the lowering of rate of detoxification in this fish could also be due to effects of benzene on the cycle for the catabolism of glucose and on the mechanism of the deamination of the free amino acid when benzene concentrations were increased. The results presented in this study suggested that the deamination pathway of the excretory process has been reduced. Research dealing with the biochemical

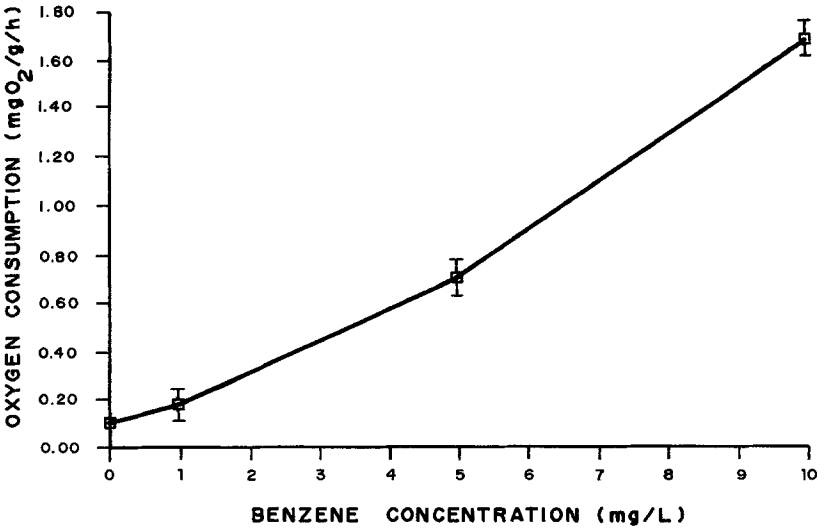

Figure 3. Relationship between wet weight respiration for the white mullet Mugil curema exposed to indicated concentration of benzene during 24h. at 20 ± 2°C

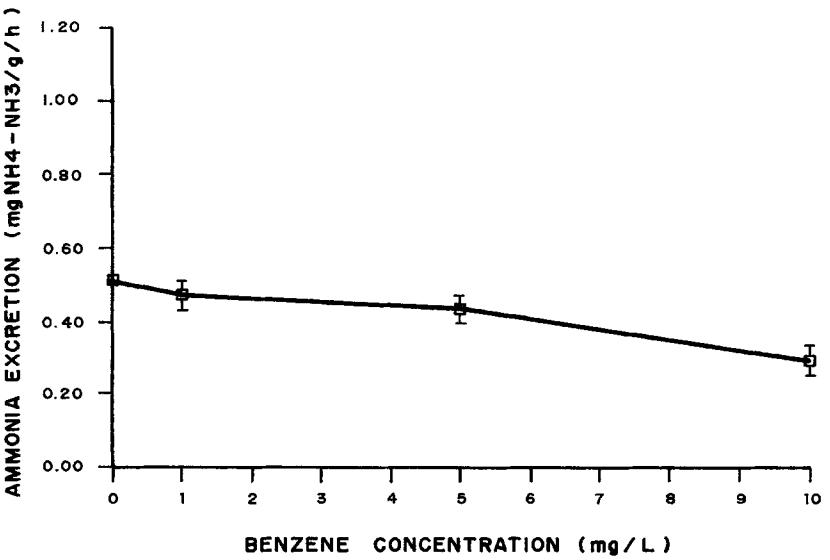

Figure 4. Relationship between ammonia excretion rate for the white mullet Mugil curema exposed to indicated concentration of benzene during 24h. at 20 ± 2°C

activity of the excretory mechanisms will be necessary to understand the physiological impact of benzene on this species.

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