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Acute Toxicity of Cadmium, Copper, and Mercury to Larval American Lobster *Homarus americanus*

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The American lobster, Homarus americanus, is a highly valued and commercially important crustacean along its entire range (WILDER 1970). Because of the fishery's importance, the biology of the lobster has been exhaustively researched since the late 1890's. The bibliographies of LEWIS (1970) and NOWAK (1972) are replete with studies on lobster physiology, biochemistry, reproduction, distribution, migration and fishery management. It has only been in most recent years, however, that researchers have begun to assess the American lobster's sensitivity to pollutants. Motivated by heightened awareness of coastal water contamination and a heavily pressured fishery with diminishing stocks, researchers have sought to find the most sensitive life history stage of the lobster for toxicity evalution. Recent work by WELLS (1976) and FORNS (1977) strongly suggest that the meroplanktonic larval lobster is the most sensitive stage of Homarus americanus.

The objective of this study was to determine the acute toxicity of Mercury, Copper and Cadmium to Stage I larval lobsters.

EXPERIMENTAL

Ovigerous lobsters were obtained offshore during the winter months by a local lobsterman through permits supplied by the Rhode Island Department of Natural Resources. These animals were held in divided brood troughs similar to the system described by HUGHES (1973). The eggbearing females were kept for 3 weeks in flowing natural seawater from Narragansett Bay at ambient temperature. By addition of heated seawater, the females and eggs were slowly acclimated to 20°C over the following three weeks (approximately 2.5°C/72 hours). This temperature regime was performed to stimulate natural egg maturation (PERKINS 1972) and to approximate the temperature planned for the bioassays.

As hatching occurred, the larvae were collected in removable baskets at the end of each trough. The baskets were made from 6 mm transparent lucite and 250 μ mesh nytex plankton netting. As larvae accumulated in the baskets they were transferred to 20°C heated open-system rearing tanks (HUGHES et al. 1974) for holding. At the time of bioassay, 24 hr old larvae were transported from the holding system to the laboratory in

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Pyrex[1] crystalizing dishes (100 mm x 190 mm). Each dish contained 150 ml of multi-media filtered 15 μ seawater. Only those larvae that were freely swimming near the surface were used in the bioassay. Those settling to the bottom of the dish were usually damaged by cannibalism or handling. To prevent cannibalism during the bioassay, individual larvae were exposed in 150 ml polypropylene specimen containers (Falcon Plastics) containing 100 ml of appropriately dosed, filtered seawater.

Cadmium nitrate, copper nitrate, and mercuric chloride stock solutions were prepared by diluting 1000 ppm primary stock solutions (Fisher Certified Atomic Absorption Standards, Fisher Scientific Co.) with deionized water (18 megohm at 20°C) in linear polyethylene volumetric flasks (Nalgene Co.). One milliliter of working stock solution, when diluted to 100 ml with filtered seawater, gave the desired toxicant concentration in the test containter. Final toxicant concentrations reported are nominal values. No attempts were made to analyze final dosed concentrations nor determine rate of loss due to adsorption or volatilization.

Static bioassays using larvae from three separate spawns were performed on Stage I larvae using cadmium, copper, and mercury. Toxicant concentrations choosen for each metal were determined from range finding bioassays using ten animals per concentration. Concentrations determined for mercury and copper were 3.3, 10.0, 33, 100 and 330 μg/l. Those for cadmium were 10, 33, 100, 330 and 1000 μg/l. Each assay included five toxicant concentrations and controls with 30 (stage I) larvae exposed per concentration.

Transfer of larvae from the crystalizing dish to each individual dosed assay container was accomplished using a capture pipette. This capture pipette consisted of a 10 ml graduated plastic pipette with its tip removed. A 450 mm long piece of surgical rubber tubing (6 mm O.D. x 3 mm I.D.) and a blood pipette mouthpiece.

Individual larvae were slowly drawn into the transfer pipette and the index finger immediately placed over the pipette tip. The finger was covered with a surgical rubber sheath (cot) and rinsed with deionized water to reduce contamination. Excess seawater was slowly drained until the larva rested on the finger in 0.5 ml of liquid. The pipette tip and finger were then placed slightly below the surface of the bioassay medium and the larva released. This transfer method gently released the larva into the medium without contacting air. The sheathed finger was rinsed in deionized water at each change of toxicant to avoid cross-contamination. Transferring the larvae in this manner has resulted in less handling mortalities than other methods employed.

When transfer of the larvae was complete, 0.1 ml of concen-

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[1]Use of product or trade names mentioned throughout text does not constitute endorsement by the U.S. Environmental Protection Agency.

trated newly hatched Artemia salina nauplii was added to each container. This resulted in a food ration of approximately 500 nauplii/larva/day. Because exposed larvae were observed to feed less than controls, daily feeding was adjusted accordingly to maintain food ration at 500/day. Each container was covered with a 76 mm diameter Pyrex watchglass to keep airborne contaminants at a minimum during the assays.

Water temperature during hatching, holding, and bioassay was 20° + 2°C since this closely approximated the surface temperature of Narragansett Bay during the height of the natural hatching season (HUGHES and MATTHIESSEN 1972). Salinity was 30.5°/oo + 1°/oo, and dissolved oxygen 8.1 ppm + 0.5 ppm. Illumination during the assay period was approximately 1000 lux, cool-white, on a 14L:10D cycle.

Bioassays were observed and mortalities recorded at 24 hour intervals for 96 hours. Death was determined when no heartbeat was visible during microscopic examination. Toxicant concentrations producing 50 percent mortality were estimated graphically (STANDARD METHODS, 1975) for each observation interval. Corrections for control mortality were made according to ABBOTT's formula.

RESULTS AND DISCUSSION

Control mortalities in the assays were: 13 percent for mercury, zero for copper and 3 percent for cadmium after 96 hours' exposure.

Mercury was tested at concentrations of 3.3, 10.0, 33, 100, and 330 µg/l. Mortalities at 3.3 and 10 µg/l were similar to the control after 96 hours exposure. However, within twenty-four hours of exposure mortalities of 30%, 97%, and 100% were observed at 33, 100, and 330 µgs Hg/l, respectively. Significant mortality continued to occur at the 33 µg Hg/l concentration for the duration of the assay reaching 73% at 96 hours. The LC-50 isopleth for mercury shows that there was a rapid change in mortality during the first two days' exposure with subsequent leveling off (Fig. I).

The toxicity values for mercury in this study were probably conservative. This was principally the result of the static nature of the test method and the potential of mercury to volatilize. Nevertheless, the 96 hour LC-50 of 20 µg Hg/l is comparable to studies reported by SOSNOWSKI and GENTILE (In Press) on the calanoid copepod, Acartia tonsa, with a 96 hour LC-50 of 11 µg Hg/l. GREEN et al. (1976) found 96 hour LC-50 values of 17-20 µg Hg/l for postlarval stages of the commercially important white shrimp, Penaeus setiferus. CONNER (1972) reported a 48 hr LC-50 range of 33-100 µg Hg/l for European lobster larvae, H. gammarus. If one compares the mean of this range (66 µg Hg/l) to the interpolated 48 hr LC-50 for H. americanus larvae of 25 µg Hg/l (Fig. I), it would indicate that H. americanus larvae are more than twice as sensitive to mercury as H. gammarus larvae. Even with this higher range for H. gammarus; however, it would appear from review of the literature

(EISLER 1973, EISLER and WAPNER 1975) that very few marine organisms are as sensitive to mercury as the crustaceans.

Copper was tested over an identical range of concentrations as mercury. Mortalities at 3.3 and 10 µgs Cu/l were identical to the controls after 96 hours exposure. However, unlike mercury, a more graded mortality pattern emerged at 33 and 100 µg/l. At 330 µg Cu/l, 100% mortality was observed after only 24 hours exposure. The estimated LC-50 concentration was 48 µg/l for 96 hours.

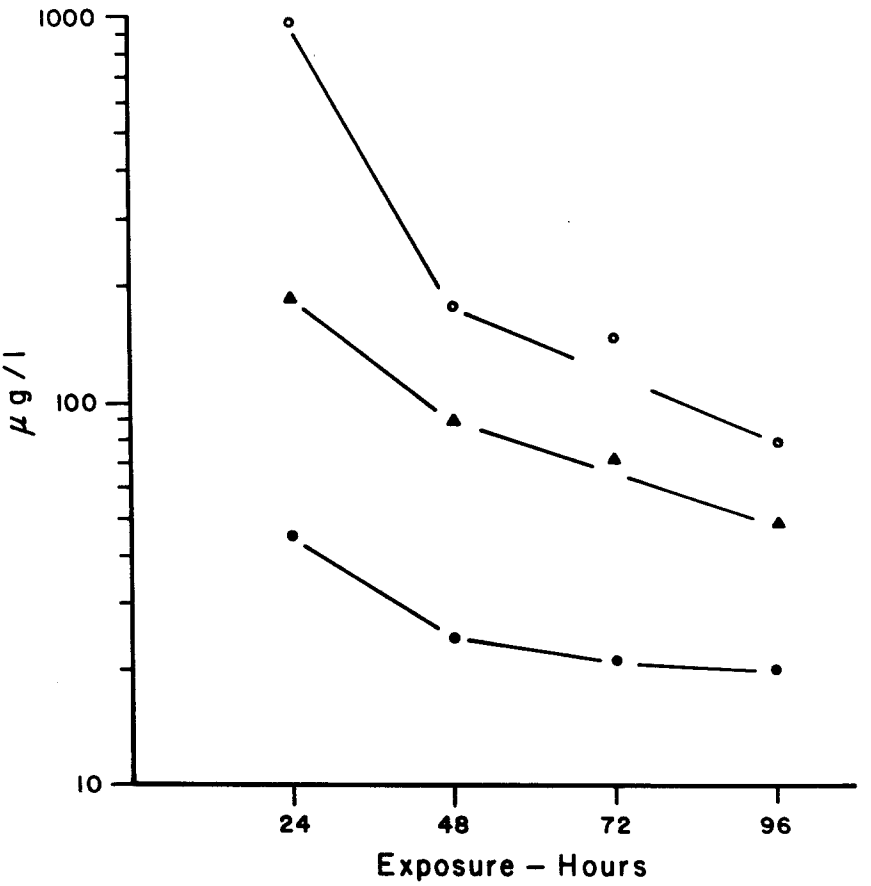

Figure 1. Fifty-percent mortality isopleths for larval lobster (Stage I) exposed to cadmium (O), copper (▲), and mercury (●).

The sensitivity of larval lobsters to copper compares favorably with data from other sources. The 96 hour LC-50 of

48 µg Cu/l is comparable to data for sea urchin larvae, Paracentrotus lividus, of 50 µg Cu/l (BOUGIS, 1965), Acartia tonsa of 31 µg Cu/l, (SOSNOWSKI and GENTILE, In Press), and soft shell clam adults, Mya arenaria at 39 µg Cu/l (EISLER, 1977). CONNER (1972) reported a 48 hr LC-50 range of 100-330 µg Cu/l for H. gammarus larvae. The mean of this range, 215 µg Cu/l, when compared to the interpolated 48 hr LC-50 of 90 µg Cu/l (Fig. I) again indicates a more than two fold sensitivity differential between the American and European lobster larvae.

Cadmium was the least toxic of the metals tested with 96 hour LC-50 concentration of 78 µg Cd/l. Tested concentrations were 10, 33, 100, 330, and 1000 µg Cd/l. Mortalities at 10 and 33 µg Cd/l were 3 and 10%, respectively, after 96 hours (control = 3%). Unlike mercury and copper, the highest level tested (1000 µg Cd/l) produced only 50% mortality after 24 hours exposure, reaching 100% after 48 hours. Mortalities at 100 and 330 µg Cd/l continued to increase with exposure time in a manner similar to copper.

The LC-50 isopleth for cadmium is very steep during the 24 to 48 hour period, reflecting the high mortalities occurring during this time interval. The interval from 48 to 96 hours shows a mortality pattern very similar to copper (Fig. I).

Cadmium toxicity, 96 hour LC-50 = 78 µg Cd/l, to lobster larvae is similar to that of 90 µg Cd/l for A. tonsa (SOSNOWSKI and GENTILE, In Press). However, lobster larvae appeared to be three times more sensitive to cadmium than adults of two other decapod crustaceans from the same environment; the hermit crab, Pagurus longicarpus, and the sand shrimp, Crangon septemspinosa, both with 96 hour LC-50 values of 320 µg/l (EISLER, 1971). Lobster larvae were considerably more sensitive to cadmium than adult grass shrimp, Palaeomonetes vulgaris (96 hr LC-50 = 760 µg/l) and juvenile pink shrimp, Panaeus duorarum (96 hr LC-50 = 3500 µg/l) (NIMMO et al., 1977).

Three significant points have emerged from this study. The first concerns the use of Stage I lobster larvae. WELLS (1976) does not recommend the use of Stage I larvae because of the high natural mortality during molt. The low control mortalities of this study do not reflect the suspected natural pattern described by WELLS (1976). This may be due to the special selection and handling of the larvae outlined in the experimental section of this paper. Stage I lobster larvae are the most vulnerable and possibly the most sensitive of the larval stages. It would be unwise to exclude their use in toxicant testing without further study of comparative stage sensitivities.

The second point deals with the sensitivity of lobster larvae. With the exception of copper toxicity there are no comparative metals data for Homarus americanus adults and larvae. MCLEESE (1974) determined that the 96 hr LC-50 to adult lobster was 100 µg Cu/l. The larval studies reported here resulted in a 96 hr LC-50 of 48 µg Cu/l. Thus, the larvae appear to be twice as sensitive to copper as the adults. Studies with other toxicants confirm the sensitivity of lobster larvae. SPRAGUE and MCLEESE (1968) found adult lobsters more resistant to bleached

kraft pulp mill wastes. WELLS and SPRAGUE (1976) state that Stage I lobster larvae have the lowest published value for acute toxicity of crude oil to an aquatic animal.

Toxicity tests reported on other (decapod) crustaceans also confirm the sensitivity of the larvae. CONNER (1972) reported that the larvae of sand shrimp (Crangon crangon), the green crab (Carcinus maenas), and the European lobster (Homarus gammarus) were 14 to 1,000 times more sensitive to mercury, copper and zinc than the adults of the same species. Studies on polychlorinated biphenyl toxicity to the fiddler crab, Uca pugilator, (VERNBERG et al., 1977) and to the pink shrimp, Penaeus duorarum, (NIMMO et al., 1971) demonstrated that the larvae were 3 to 10 times more sensitive than the adults.

In view of this well documented sensitivity of crustacean larvae and the diminishing returns of the American lobster fishery, it would seem unnecessary to use the adult population for toxicant testing. The authors agree with WELLS (1976) that protective levels of pollutants set for the larvae will protect the adults; and since the lobster larvae are such sensitive zooplankters, levels set for them may result in protection for other species at the same or other trophic levels.

The final point of emphasis is the lobster larvae's susceptability to mercury, copper and cadmium. These metals enter the marine environment primarily through municipal sewage and industrial wastes. Copper is additionally contributed through weathering of natural deposits, corrosion of power plant condenser systems, and antifouling paints. The behavioral (migratory) and reproductive characteristics of a large portion of the population of H. americanus create the potential for increased exposure to these pollutants. Planktonic lobster larvae released in coastal and estuarine waters can be carried through areas of high pollutant load where deleterious effects may occur.

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